9th International Colloquium on Invertebrate Pathology and Microbial Control, 39th Annual Meeting of the Society for Invertebrate Pathology and 8th International Conference on *Bacillus thuringiensis*

Wuhan, China

Aug. 27-Sep. 1, 2006

Sponsored by:

- Society of Invertebrate Pathology
- Chinese Society of Microbiology
- Hubei Society of Microbiology&Wuhan Society of Microbiology, China

Organized by:

- Huazhong Agricultural University, State Key Laboratory of Agricultural Microbiology, National Engineering Research Center for Microbial Pesticides
- State Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academic of Sciences

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Meeting web site: http://sip2006.hzau.edu.cn/

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Program Overview

SIP 2006

Sunday, August 27	Monday, August 28	Tuesday, August 29
8:30-17:00 **SIP Council Meeting** (*Jinxiu Meeting Room*)	8:00-10:00 **Registration** (***Lobby***) 8:30-10:00 **Opening Ceremony** (*Yangchun Hall*) Welcome: SIP 2006 Chairman SIP President Leader of Wuhan and Hubei province / President of Huazhong Agriculture University **Founders' Lecture** (*Yangchun Hall*) Honoree-Dr. Shangying Gao Lecturer- Dr. Just M. Vlak	8:00-10:00 **Symposia:** **i) Genetics and characterization of mechanisms of Bt-resistance (Bacteria Division)** (*Meeting Center*) **ii) Field Performance of Insect Viruses (Virus Division)** (*Multifunctional Hall*) **Contributed papers**: **Fungi 2** (*Nanyuan Meeting Room*) **Microsporidia** (*Xiyuan Meeting Room*)
10:00-21:00 **Registration** (*Lobby*) **Loading up powerpoint presentations** (*Lobby*)	10:00-10:30 Coffee Break	10:00-10:30 Coffee Break
	10:30-12:30 **Plenary Lectures:** **Microbial control in Asia** (*Yangchun Hall*)	10:30-12:30 **Symposium:** **Novel approaches for dealing with difficult data (Microbial Control Division)** (*Nanyuan Meeting Room*) **Contributed papers**: **Bacteria 1** (*Meeting Center*) **Viruses 1** (*Multifunctional Hall*)
	12:30-14:00 Lunch (*Haitiangong Dining-Room*) Setting up Posters (*Meeting Center*)	13:00-17:30 **Excursion** (light lunch included) Buses leave at 13:00 from the hotel, end at Wuhan Botanic Garden for 5K Race and BBQ 18:00-18:50 **5K Race** (*Wuhan Botanic Garden*) **<u>For those who do not participate in the excursion</u>**: 12:30-14:00 Lunch (*Haitiangong Dining-Room*) 16:00 Bus leaves from hotel to Wuhan Botanic Garden for 5K Race and BBQ
	14:00-16:00 **Symposia** i) **Monitoring and managing for Bt-resistance: The challenges for the next decade (Cross-Divisional)** (*Meeting Center*) ii) **Diseases of aquatic invertebrates (Virus Division)** (*Multifunctional Hall*) **Contributed Papers** **Fungi 1** (*Nanyuan Meeting Room*) **Nematodes** (*Xiyuan Meeting Room*)	
	16:00-16:30 Coffee Break **Setting up Posters** (*Meeting Center*)	
	16:30-18:30 **Poster Session I** (*Meeting Center*) **Bacteria I**: BP1-BP17 **Fungi I**: FP1-FP10 **Microbial Control I**: MCP1-MCP13 **Microsporidia**: MP1-4 **Viruses I**: VP1-VP17	
19:00-21:00 Mixer (*Haitiangong Dining-Room*)	18:30-20:00 Dinner (*Haitiangong Dining-Room*)	19:00-22:00 **BBQ and Entertainments** (*Wuhan Botanic Garden*)
	20:00-22:00 **Business Meetings** i) **Bacteria** (*Meeting Center*) ii) **Fungi** (*Nanyuan Meeting Room*) *iii)* **Microsporidia** (*Multifunctional Hall*) iv) **Nematodes** (*Xiyuan Meeting Room*) v) **Viruses** (*Wuhan Institute of Virology*) (Bus leaves from the hotel at 19:40)	

Wednesday, August 30	Thursday, August 31	Friday, September 1
8:00-10:00 **Symposia:** i) **Ecology of Entomophthorales (Fungi Division)** (*Nanyuan Meeting Room*) *ii)* **Emerging pest targets for Entomopathogenic nematodes (Nematode Division)** (*Xiyuan meeting Room*) **Contributed papers:** **Bacteria 2** (*Meeting Center*) **Viruses 2** (*Multifunctional Hall*)	8:00-10:00 **Symposium:** **Bt-performance enhancement (Bacterial Division)** (*Meeting Center*) **Contributed papers:** **Microbial Control 2** (*Nanyuan Meeting Room*) **Viruses 5** (*Multifunctional Hall*)	7:00-9:00 **Tail-end breakfast** **再见 (Goodbye)** **See you in Canada**
10:00-10:30 Coffee Break **Setting up posters** (*Meeting Center*)	10:00-10:15 Coffee Break 10:15-10:30 **Lecture:** **Edward A. Steinhaus, Instigator, Catalyst, and Founder** (*Meeting Center*)	
10:30-12:30 **Symposium:** **Microsporidia in silk moth (Microsporidia Division)** (*Xiyuan meeting Room*) **Contributed papers** **Bacteria 3** (*Meeting Center*) **Microbial Control 1** (*Nanyuan Meeting Room*) **Viruses 3** (*Multifunctional Hall*)	10:30-12:30 **SIP Annual Business Meeting** (*Meeting Center*)	
12:30-14:00 Lunch (*Haitiangong Dining-Room*) **Setting up Posters** (*Meeting Center*)	12:30-14:00 Lunch (*Haitiangong Dining-Room*) Student Awards Committee Meeting	13:00 **Post meeting excursions**
14:00-16:00 **Symposium:** **Bacteria in Bio-control in Asia: natural and Bio-tech strains (Bacteria Division)** (*Meeting Center*) **Contributed Papers:** **Fungi 3** (*Nanyuan Meeting Room*) **Viruses 4** (*Multifunctional Hall*)	14:00-16:00 **Symposium:** **Nematodes and Bacteria: from Pathogenicity to Mutualism (Cross-Divisional)** (*Meeting Center*) **Contributed Papers:** **Microbial Control 3** (*Nanyuan Meeting Room*) **Viruses 6** (*Multifunctional Hall*)	
16:00-16:30 Coffee Break **Setting up Posters** (*Meeting Center*)	16:00-16:30 Coffee Break	
16:30-18:30 **Poster Session II** (*Meeting Center*) **Bacteria II**: BP18-BP32 **Fungi II**: FP11-FP21 **Microbial Control II**: MCP14- MCP27 **Nematodes**: NP1-NP4 **Viruses II**: VP18-VP35	16:30-18:30 **Contributed Papers** **Bacteria 4** (*Meeting Center*)	
18:30-20:00 Dinner (*Haitiangong Dining-Room*)	19:00 **Banquet** (*Yangchun Hall*)	
20:00-21:00 **Microbial Control Business Meeting** (*Multifunctioanl Hall*) 21:00-22:00 **Workshop**: **New Products and Upgrades for Microbial Control: an Industry Update** (*Multifunctioanl Hall*)		

Program

SIP 2006

Society for Invertebrate Pathology 39th Annual Meeting
Wuhan-China-August 27 to September 1, 2006

PROGRAM

Sunday, August 27

8:30-17:00 **SIP Council Meeting** *Jinxiu Meeting Room*

10:00-21:00 **Registration** *Hotel Lobby*
10:00-21:00 **Loading up presentations** *Hotel Lobby*

19:00-21:00 **Mixer** *Haitiangong Dining-Room*

Monday, August 28

8:00-10:00 **Registration** *Hotel Lobby*

8:30-10:00 **Opening Ceremony** *Yangchun Hall*

Welcome: Ziniu Yu, SIP 2006 Chairman
Just M. Vlak, SIP President
Leader of Wuhan and Hubei province / President of Huazhong Agriculture University

Founders' Lecture: Chair: Dudley Pinnock
Honoree-Dr. Shangying Gao
Lecturer- Dr. Just M. Vlak

10:00-10:30 **Coffee Break**

10:30-12:30 Monday ***Yangchun Hall***
Plenary Lectures: **Microbial control in Asia**
Chair: Wendy Gelernter

10:30 Microbial control and biotechnology research on Bacillus thuringiensis (Bt) in China. **Da-Fang Huang.** Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100081, China

11:00 Microbial control in Japan. **Yasuhisa Kunimi.** Department of Bioregulation and Biointeraction, Graduate School of Agriculture, Tokyo University of Agriculture and Technology, Saiwai, Fuchu, Tokyo 183-8509, Japan

11:30 Microbial control in South East Asia. **Ole Skovmand**. Intelligent Insect Control, 118 Ch Alouettes, 34170 Castelnau le Lez, France

12:00 Fungal biocontrol agents for arthropod pest control in India & Pakistan. **Tariq M Butt[2], Len Copping[1].** [1]Department of Biological Sciences, University of Wales, Swansea, SA2 8PP, UK; [2]LGC Consultants, Saffron Walden, Essex, CB11 4EG, UK

12:30-14:00 **Lunch** *Haitiangong Dining-Room*
Setting up Posters *Meeting Center*

14:00-16:00 Monday ***Meeting Center***
Symposium: Monitoring and managing for Bt-resistance: The challenges for the next decade (Cross-Divisional)
Convenors: Juan Ferre and Carlos Blanco

14:00 Resistance monitoring for Bt crops: A US EPA perspective. **John A Glaser[2], Sharlene R Matten[1].** [1]United States Environmental Protection Agency, Office of Pesticide Programs, Biopesticides and Pollution Prevention Division (7511C), 1200 Pennsylvania Ave., NW, Washington D.C. 20460; [2]United States Environmental Protection Agency, Office of Research & Development, National Risk Management Research Laboratory, Sustainable Technology Division, 26 W King Dr. Cincinnati, Ohio 45268

14:17 Monitoring pests of large geographies: How to get the best information when two countries are involved? **Carlos A. Blanco[1], Antonio P. Terán-Vargas[2], Craig Abel[1] and Omaththage P. Perera[1]**. [1]USDA - Agricultural Research Service, 141 Experiment Station Road, Stoneville, Mississippi, 38776, U.S.A.; [2]INIFAP, CESTAM, Km. 55 Carretera Tampico-Mante, Cuauhtemoc, Tamaulipas, 89610, Mexico

14:34 Monitoring and Management Strategy of *Helicoverpa armigera* Resistance to Bt Cotton in China. **Kongming Wu**. State Key Laboratory for Biology of Plant Diseases of Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, 100094 China.

14:51 What is the current situation in Australia for resistance to Bt cotton by *Helicoverpa armigera*? **Sharon Joy Downes[1], Rod Mahon[2].** [1]CSIRO Entomology, Australian Cotton Research Institute, Locked Bag 59,

Narrabri, NSW 2390, Australia; [2]CSIRO Entomology, PO Box 1600, Canberra, ACT 2601, Australia

15:08 Insect Baseline susceptibilities to Bt Cry toxins and the Bt resistance management in India. **Govind T Gujar, V. Kalia, A. Kumari, B.P. Singh, R. Nair and A. Mittal.** Division of Entomology, Indian Agricultural Research Institute, New Delhi 110012, India

15:25 Monsanto's global approach to resistance monitoring. **Graham P Head, Sakuntala Sivasupramaniam, Vaughn T Ty.** Monsanto Company, 700 Chesterfield Parkway West, Chesterfield, MO 63017

15:42 Effective IRM for the novel insect-control cotton, VipCot™. **Ryan W. Kurtz**. Syngenta Biotechnology, Inc., 3054 E. Cornwallis Rd. RTP, NC 27709 USA

14:00-16:00 Monday ***Multifunctional Hall***
Symposium: Diseases of aquatic invertebrates (Virus Division)
Convenors: Zhengli Shi and Just M.Vlak

14:00 Viral diseases of aquatic invertebrates: Introduction to the theme. **Just M. Vlak**. Laboratory of Virology, Wageningen University, Binnenhaven11, 6709 PD Wageningen, The Netherlands

14:15 Biology, genetics and ecology of taura syndrome virus. **Jeffrey M. Lotz.** Department of Coastal Sciences, Gulf Coast Research Laboratory, University of Southern Mississippi, Ocean Springs, Mississippi 39564, USA

14:45 Biology, genetics and ecology of the YHV complex. **Jeff A. Cowley[2], Peter J. Walker[1,2] and Priyanjalie Wijegoonawardane[2]** [1]CSIRO Livestock Industries, Australian Animal Health Laboratory, Geelong, Victoria 3220 Australia; [2]CSIRO Livestock Industries, Queensland Bioscience Precinct, St Lucia, Queensland 4067, Australia.

15:15 Biology and molecular genetics of white spot syndrome virus. **Zhengli Shi.** State Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, P.R. China

14:00-16:00 Monday ***Nanyuan Meeting Room***
Contributed Papers: Fungi 1
Moderators: Michael Brownbridge and Zengzhi Li

14:00 Population dynamics of *Beauveria bassiana* introduced in forest and fresh water**. Bin Wang[1,2], Zengzhi Li[1], Mitsuaki Shimazu[2], Meizhen Fan[1] and Fan Peng[1].** [1]Anhui Provincial Key Laboratory of Microbial Control, Anhui Agricultural University, Hefei 230036, Anhui, P. R. China; [2] Department of Forest Entomology, Forestry and Forest Products Research Institute, 1 Matsunosato, Tsucuba, Ibaraki 305-8687, Japan

14:15 Survival of *Beauveria caledonica* spores in biopolymer-based formulations for control of the *Hylastes ater* (Coleoptera: Scolytidae)**. Michael Brownbridge[1], Tracey N Nelson[1], Steven D Reay[2], Jyanthi Swaminathan[1] and Travis R Glare[1].** [1]AgResearch Ltd., Biocontrol & Biosecurity, AgResearch, PO Box 60, Lincoln, New Zealand; [2]Silver Bullet Forest Research, Silver Bullet Forest Research, Auckland, New Zealand

14:30 Winter survival and germination of aphid-pathogenic Entomophthorales**. Charlotte Nielsen[1], Anselme Fournier[2], Annette B. Jensen[1], Jürg Enkerli[2], Franco Widmer[2] and Jørgen Eilenberg[2].** [1]The Royal Veterinary and Agricultural University, Department of Ecology, Thorvaldsensvej 40, 1871 Frederiksberg C., Denmark; [2]Agroscope FAL Reckenholz, Swiss Federal Research Station for Agroecology and Agriculture, Reckenholzstrasse 191, CH-8046 Zürich, Switzerland

14:45 Disease transmission and chalkbrood control in the alfalfa leafcutting bee**. Rosalind R. James.** USDA-ARS Bee Biology & Systematics Lab, Dept Biol UMC 5310, Utah State Univ., Logan, UT 84333-5310

15:00 Host range of a fungus associated with epizootic in elongate hemlock scale. **Jose Marcelino, Rosanna Giordano, Svetlana Gouli and Vladimir Gouli**. Entomology Research Laboratory, University of Vermont, 661 Spear St., Burlington, Vermont 05405-0105, USA

15:15 **STU** Effects of combining the fiber bands impregnated with *Beauveria bassiana* cultures with attractants for control of *Monochamus alternatus* Hope. **Sibao Wang[1,2], Yongping Huang[2], Meizhen Fan[1] and Zengzhi Li[1].** [1]Provincial Key Laboratory of Microbial Pest Control, Anhui Agricultural University, Hefei, Anhui 230036, P.R. China; [2]Institute of Plant

Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 300 Fenglin Road, Shanghai 200032, P.R. China

15:30 **STU** Relationship of trehalose and polyol accumulation to increased conidial heat and UV-B tolerance in *Metarhizium anisopliae* conidia produced under sub-lethal stresses. **Drauzio E.N. Rangel, Anne J. Anderson and Donald W. Roberts**. Deparment of Biology, Utah State University, 5305, Old Main Hill, Logan, UT. 84322-5305 USA

14:00-16:00 Monday ***Xiyuan Meeting Room***
Contributed Papers: Nematodes
Moderators: Patricia Stock and Mike Wilson

14:00 Field evaluation of *Heterorhabditis indica* with entomopathogens and botanicals against *Helicoverpa armigera* (Hübner)**. Aralimarad Prabhuraj, Patil B.V., Girish K.S. and Shivaleela.** Department of Entomology, College of Agriculture, Raichur 584 101, Karnataka, India

14:15 Performance of *Heterorhabditis indica* with neem against *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae)**. Aralimarad Prabhuraj, Patil B.V., Girish K.S. and Shivaleela.** Department of Entomology, College of Agriculture, Raichur 584 101, Karnataka, India

14:30 A new entomopathogenic nematode, *Steinernema hebeiense* sp. n. (Rhabditida: Steinernematidae), from North China**. Shulong Chen[1], Xiuhua Li[1], Aihua Yan[1], Sergei E. Spiridonov[2] and Maurice Moens[3].** [1]Institute of Plant Protection, Hebei Academy of Agricultural and Forestry Sciences, Dong Guan Street 437, Baoding, Hebei, 071000, China; [2]Institute of Parasitology, Russian Academy of Sciences, Leninskii prospekt, 33, Moscow, 119071, Russia; [3]Agricultural Research Centre, Burg. Van Gansberghelaan, 96, 9820 Merelbeke, Belgium

14:45 A Comparative Study on the Morphology and Ultrastructure of the Bacterial Receptacle in *Steinernema* Nematodes. **S. Patricia Stock[1] and Yolanda Flores-Lara[1,2]**. [1]Department of Entomology, University of Arizona, Forbes 410. 1140 E South Campus Dr. Tucson AZ 85721-0036, USA; [2]Universidad de Sonora, Unidad Santa Ana, Santa Ana, Estado de Sonora, Mexico

15:00 The indigenous Peruvian entomopathogenic nematode and the Andean potato weevil. **Harry K. Kaya[1], Soroush Parsa[1] and Jesus Alcázar[2]**. [1]Department of Nematology, University of California, Davis, California 95616 USA; [2]International Potato Center, Lima 12, Peru

15:15 Molecular characterization of the symbiotic bacteria of entomopathogenic nematodes isolated from China**. Lihong Qiu, Shaoming Peng, Lianlian Liu and Yi Pang.** State Key Laboratory of Biocontrol, Sun Yat-sen [Zhongshan] University, 135 Xin Gang Road, Guangzhou, Guangdong510275, China

15:30 **STU** Effectiveness of entomopathogenic nematodes in the control of oilseed rape pests In Finland**. Melita Zec-Vojinovic, Heikki M.T. Hokkanen and Ingeborg Menzler-Hokkanen.** University of Helsinki, Box 27, FIN-00014, Helsinki, Finland.

16:00-16:30 **Coffee Break**
Setting up posters *Meeting Center*

16:30-18:30 Monday ***Meeting Center***
Poster Session I

Bacteria I

BP1 Cytocidal actions of parasporin-2, an antitumoral crystal protein targeting mammalian cells from *Bacillus thuringiensis* A1547. **Sakae Kitada[1], Yuichi Abe[1], Hiroyasu Shimada[1], Osamu Kuge[1], Eiichi Mizuki[2], Michio Ohba[3] and Akio Ito[1]**. [1]Faculty of Science, Kyushu University, Fukuoka 812-8581, Japan; [2]Faculty of Agriculture, Kyushu University, Fukuoka 812-8581, Japan; [3]Biotechnology and Food Research Institute, Fukuoka Industrial Technology Center, Fukuoka 839-0861, Japan

BP2 Parasporin-2, an oligomerizing and pore-forming toxin from *Bacillus thuringiensis*, is assembled into supramolecular complexes in target human cell membranes. **Hiroyasu Shimada, Yuichi Abe, Osamu Kuge and Sakae Kitada.** Department of Chemistry, Faculty of Science, Kyushu University, Fukuoka 812-8581, Japan

BP3 GPI-anchored proteins are involved in the cytocidal actions of parasporin-2, a mammalian cell-targeting crystal protein from *Bacillus thuringiensis* A1547. **Yuichi Abe[1], Hiroshi**

Inoue[1], Hiroyasu Shimada[1], Michio Ohba[2], Hisashi Ashida[3], Taroh Kinoshita[3], Osamu Kuge[1] and Sakae Kitada[1]. [1]Department of Chemistry, Faculty of Science, Kyushu University, Fukuoka 812-8581, Japan; [2]Department of Applied Genetics and Pest Management, Faculty of Agriculture, Kyushu University, Fukuoka 812-8581, Japan; [3]Research Institute for Microbial Diseases, Osaka University, Suita, Osaka 565-0871, Japan

BP4 Baseline susceptibility to the Cry 1Ac protein and validation of diagnostic concentration in Indian populations of eggplant fruit and shoot borer. **Dattatray K Shirale, Srinivas Parimi and Usha B Zehr.** Maharashtra Hybrid Seeds Company Limited, PO Box 76, Jalna-Aurangabad Road, JALNA-431203, INDIA

BP5 Rear *Anomala corpulenta* (Coleoptera: Scarabaeidae) and bioassay of insecticidal activity of *Bacillus thuringiensis* against its larvae. **Rong-yan Wang, Jin-yao Wang, Shu-liang Feng, Wei-ping Cao, Li-xin Du, jian Song and Rui-hua Wu.** Feng Shu-liang, Institute of Plant Protection, Hebei Academy of Agricultural and forestry Sciences, Baoding 071000,China

BP6 Studies on the Use of Three *Bacillus* Species Against The Date Palm Fruit Stalk Borer , *Oryctes elegans* (Coleoptera: Scarabaeidae). **Mohammad Abdulaziz Al-Doghairi.** Qassim University, College of Agriculture and Veterinary Medicine, Qassim University, Buraydah P.O. Box 1482, Saudi Arabia

BP7 Identification of a novel *Bacillus thuringiensis* strain WZ-9. **Ping Song, Huixian Wu, Wenjie Mao, Xudong Su and Qinying Wang.** College of Plant Protection, Agricultural University of Hebei, Biocontrol Centre of Plant Diseases and Plant Pests of Hebei Province, Baoding, Hebei 071001, P. R. China

BP8 Binding analysis in Cry1Ac-selected populations of *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae). **Ana Rodrigo[1], Konasale J. Anilkumar[2], William J. Moar[2] and Juan Ferré[1].** [1]Departamento de Genética, Universidad de Valencia, Dr. Moliner 50, 46100 Burjassot (Valencia). SPAIN; [2]Department of Entomology and Plant Pathology, Auburn University, Auburn, AL-36849, U.S.A

BP9 Mode of action of *Bacillus thuringiensis* toxins active against *Sesamia nonagrioides* (Lefebvre). **J. González-Cabrera[1], G. P. Farinós[2], S. Caccia[3], M. Díaz-Mendoza[2], P. Castañera[2], M. G. Leonardi[3], B. Giordana[3] and J. Ferré[1].** [1]Dep. Genética, Universidad de Valencia, 46100-Burjassot, Spain; [2]Dep. Biología de Plantas, Centro de Investigaciones Biológicas - C.S.I.C., Madrid, Spain.; [3]Dipart. Biologia, Università degli Studi di Milano, Milano, Italy

BP10 Antibody blocking of putative receptors inhibits the binding of Cry1Ab in *Bombyx mori*. **M. Sales Ibiza-Palacios[1], Satoshi Higurashi[2], Kazuhisa Miyamoto[3], Ryoichi Sato[2] and Baltasar Escriche[1].** [1]Dep. Genética, Universitat de València, 46100 Burjassot, Spain; [2]Grad. School of Bio-Applications and Syst. Eng., Tokyo University of Agr. and Tech., Tokyo 184-8588, Japan; [3]Inst. Insect and Animal Sciences, Natl. Inst. Agrobiol. Sci., Tsukuba Ibaraki 305-8634, Japan

BP11 Effect of Cyt1A yield reduction on *Bacillus sphaericus* Bin toxin synthesis in *Bacillus thuringiensis*. **Yuko Sakano[1], Hyun-Woo Park[3] and Brian A Federici[1,2].** [1]Department of Entomology, University of California Riverside, Riverside, California 92521, USA; [2]Graduate Program in Genetics, University of California Riverside, Riverside, California 92521, USA; [3]Public Health Research & Education Center, Florida A & M University, Panama City, Florida 32405, USA

BP12 A Novel Lysogenic Bacteriophage MZTP02 from *Bacillus thuringiensis* Strain MZ1. **Wei Liao[1, 2], Shaoyun Song[1], Fan Sun[1], Kai Yang[1], Yuncan Ai[1] and Yi Pang[1].** [1]State Key Laboratory of Biocontrol, School of life Science, Sun Yat-sen (Zhongshan) University, Guangzhou, Guangdong, 510275, P. R. China; [2]Guangxi Vacational Technology College, Nanning, Guangxi, 530226, P. R. China

BP13 What is the mechanism of resistance to *Bacillus thuringiensis* toxin Cry1Ac in a greenhouse population of cabbage looper, *Trichoplusia ni*? **Ping Wang[1], Jian-zhou Zhao[1], Ana Rodrigo-Simón[2], Wendy C. Kain[1], Alida F. Janmaat[3], Anthony M. Shelton[1], Juan Ferré[2] and Judith Myers[3].** [1]Department of Entomology, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456, USA; [2]Department of Genetics, University of Valencia, Dr. Moliner 50, 46100 Burjassot (Valencia), Spain; [3]Department of Zoology, University of British Columbia, Vancouver, British Columbia, V6T

1Z4, Canada

BP14 **STU** An approach to the directed evolution of the insecticidal protein from *Bacillus thuringiensis*. **Yasushi Hoshino, Horoshi Ishikawa, Kozue Chayahara, Chinatsu Morimoto, Mika Kitajima and Ryoichi Sato.** Graduate School of Bio-Applications and Systems Engineering, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184-8588, Japan

BP15 **STU** *Bacillus thuringiensis* Cry toxin's domain III, Galactose-binding Domain-like binds specifically to various proteins. **Madoka Kitami, Kazuko Nakanishi, Shogo Atsumi, Fumiaki Obata and Ryoichi Sato**. Graduate School of Bio-Application and Systems Engineering, Tokyo University of Agriculture and Technology, Naka-cho 2-24-16, Koganei, Tokyo 184-8588, Japan

BP16 **STU** Polymorphism of Fatty Acid from *Ralstonia solanacearum* and Its Classificatory Application on Subspecies. **QiuHong Wang[1], Liang Chen[1], YingZhi Lin[2], SuFang Huang[2] and Bo Liu[2*]**. [1]Academy of Life Sciences, Xiamen University, Xiamen 361005, China; [2]Biotechnology Institute, Fujian Academy of Agricultural Sciences, Fuzhou 350003, China

BP17 **STU** Insecticidal Toxicology of HBF-1 Strain from *Bacillus thuringiensis* on *Anomala corpulenta* and *A. exoleta* larvae. **Jian Song, Shu-liang Feng, Rong-yan Wang, Jin-yao Wang, Wei-ping Cao, Li-xin Du, Jie Zhang and Fu-ping Song.** Feng Shu-liang. Institute of Plant Protection, Hebei Academy of Agricultural And Forestry Sciences1, Baoding 071000

Fungi I

FP1 Reduction of feeding by the Japanese pine sawyer, *Monochamus alternatus* infected with *Beauveria bassiana*. **Mitsuaki Shimazu, Noritoshi Maehara and Xueyou He.** Forestry and Forest Products Research Institute, Tsukuba, Ibaraki 305-8687, JAPAN

FP2 Research on Cordyceps and its application in AHAU. **Chun-Ru Li[1], Bo Huang[1], Feng-Lin Hu[1], Sung-Hee Nam[1,2], Mei-Zhen Fan[1] and Zeng-Zhi Li[1].** [1]Anhui Agricultural University, Anhui Provincial Key Laboratory for Microbial Control, Anhui Agricultural University, Hefei, Anhui, PR China; [2]National Institute of Agricultural Science and Technology, National Institute of Agricultural Science and Technology, Rural Development Administration, Suwon, Korea

FP3 **STU** Thermotolerance and cold activity of sixty *Beauveria* spp. isolates. **Everton K.K. Fernandes[1,2], Drauzio E.N. Rangel[1], Vania R.E.P. Bittencourt[2], Aurea M.L. Moraes[2] and Donald W. Roberts[1].** [1]Department of Biology, Utah State University, 5305 Old Main Hill, Logan, UT 84322-5305, USA.; [2]Departamento de Parasitologia Animal, Universidade Federal Rural do Rio de Janeiro, Rio de Janeiro, Brasil

FP4 Evaluation of *Beauveria bassiana* for the control of glassy-winged sharpshooter, *Homalodisca coagulata* (Homoptera: Cicadellidae). **Surendra K Dara[1], Michael R McGuire[2] and Harry K Kaya[3]**. [1]Shafter Research and Extension Center, University of California, Davis, 17053 N Shafter Ave, Shafter, CA 93263; [2]USDA-ARS, 17053 N Shafter Ave, Shafter, CA 93263; [3]Department of Nematology, University of California, One Shields Ave, Davis, CA 95616

FP5 **STU** Is isolate ARSEF 3609 a *Metarhizium anisopliae* var. *anisopliae* or var. *acridum*? **Everton K.K. Fernandes, Drauzio E.N. Rangel, John Orwin, Mark P. Miller and Donald W. Roberts**. Department of Biology, Utah State University, 5305 Old Main Hill, Logan, UT 84322-5305, USA

FP6 **STU** Biological control of soybean cyst nematode using *Verticillium lecanii* (*Lecanicillium* spp.) and fungi isolated from cyst. **Ai Watanabe, Daigo Aiuchi, Ryoji Shinya and Masanori Koike**. Department Agro-environmental Science, Obihiro University of Agriculture and Veterinary Medicine, Hokkaido 0808555, Japan

FP7 **STU** Evaluation of pathogenicity against cotton aphid and greenhouse whitefly, and viability on the leaf to use hybrid strains of *Verticillium lecanii*. **Daigo Aiuchi, Yukiko Baba, Sayaka Horie, Keigo Inami, Katuhisa Kuramochi, Masayuki Tani and Masanori Koike.** Department of Agro-environmental Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, 080-8555, Japan

FP8 **STU** Thermotolerance of germlings and mycelium of *Metarhizium anisopliae* var. *anisopliae* and *acridum*. **Drauzio E.N. Rangel,**

Everton K.K. Fernandes, Seth J. Dettenmaier and Donald W. Roberts. Department of Biology, Utah State University, 5305 Old Main Hill, Logan, UT 84322-5305, USA

FP9 Efficiency of aerial conidia and submerged propagules of *Paecilomyces fumosoroseus* (Wise) Brown and Smith against *Bemisia* (Gennadius) spp nymphs in laboratory. **A. G. Osuna Paez[1], R. Castro Montoya[2] and H. M. Cárdenas Cota[3]**. [1]Consejo Estatal de Ciencia y Tecnología, Avenida Insurgentes s/n Centro. CP 80129, Unidad Administrativa del Gobierno del Estado de Sinaloa. Culiacán, Sinaloa, México; [2]Universidad Autónoma de Sinaloa, Escuela de Ciencias Físico Matemáticas, Ciudad Universitaria. Culiacán, Sinaloa, México; [3]Centro de Ciencias de Sinaloa, Avenida de las Américas No. 2771 CP 80010. Culiacán, Sinaloa, México

FP10 **STU** Efficacy of *Beauveria bassiana* (Bals.) Vuill. against the tarnished plant bug, *Lygus lineolaris* L., in strawberry field. **Rachid Sabbahi and Claude Guertin**. INRS-Institut Armand Frappier, Laboratoire de recherches sur les entomopathogènes, 531 boul. des Prairies, Laval (Québec) H7V 1B7 Canada

Microbial Control I

MCP1 The resistance of *Anopheles sinensis* from southern and central China to *Bacillus thuringiensis* subsp. *israelensis*. **Hongyu Zhang, Jingye Huang and Lin Lü**. Institute of Urban Pest, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, The People's Republic of China

MCP2 Characterization and toxicity to coleopteran insects of *Bacillus thuringiensis* isolates from warehouses. **Hongyu Zhang[1,2] and Ziniu Yu[2]**. [1]Institute of Urban Pest, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, The People's Republic of China; [2]National key laboratories of Agri-microbiology, Wuhan 430070, The People's Republic of China

MCP3 Susceptibility of *Pyrausta sticticalis* to *Bacillus thuringiensis*-based formulations depending on host plant. **Margarita Shternshis, Irina Andreeva and Bibinur Baitasova**. State Agrarian University, Dobrolubov 160, Novosibirsk, 630039, Russia

MCP4 Diagnosis of Arthropod Diseases – Since more than 50 years in the "Institute for **Biological** Control" of the "Federal Biological Research Centre for Agriculture and Forestry". **Regina G. Kleespies, Alois M. Huger and Gisbert Zimmermann.** Federal Biological Research Centre for Agriculture and Forestry, Institute for Biological Control,, Heinrichstr. 243, D-64287 Darmstadt, Germany

MCP5 Inheritance of Resistance and Effect of PM on Toxicity of *Bacillus thuringienesis* toxin Cry1Ac in Cabbage looper, *Trichoplusia ni*. **Wei Guo[1,2], Guoxun Li[3] and Ping Wang[2].** [1]Agricultural University of Hebei, Biocontrol Center of Plant Diseases and Pests of Hebei, Baoding, Hebei 071001, P. R. of China; [2]Department of Entomology, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456,USA; [3]College of Plant Protection, Laiyang Agricultural University, Qingdao, Shandong 266109, China

MCP6 Analysis of midgut ESTs from *Costelytra zealandica* to identify candidate genes involved in amber disease. **Sean DG Marshall[1], Laurence N Gatehouse[2], S Anette Becher[3], Drion G Boucias[4], Mark RH Hurst[1], Darren J Smalley[1] and Trevor A Jackson[1].** [1]Biocontrol and Biosecurity, Agresearch, PO Box 60, Lincoln, New Zealand; [2]Insect Science, HortResearch, Private Bag 11030, Palmerston North, New Zealand; [3]Bioinformatics, Maths and Statistics, AgResearch, Private Bag 50034, Mosgiel, New Zealand; [4]Department of Entomology and Nematology, University of Florida, Gainesville, Florida 32611

MCP7 Biotic and abiotic factors affecting performance of *Serratia entomophila* as a biopesticide for grass grub (*Costelytra zealandica*) in New Zealand. **Trevor A. Jackson[1], Mark R. McNeill[2], Colin M. Fergusson[1], Maureen O'Callaghan[1] and Richard J. Townsend[1]**. [1]Biocontrol and Biosecurity, AgResearch, PO Box 60, Lincoln New Zealand; [2]Invermay Research Centre, Private Bag 50034, Mosgiel, New Zealand

MCP8 Induced *Serratia entomophila* Sep proteins show activity against larvae of the New Zealand grass grub *Costelytra zealandica*. **Mark R.H. Hurst, Sandra M. Jones, Binglin Tan and Trevor A. Jackson.** Biocontrol Technologies, AgResearch, Canterbury Agricultural and Science Centre,

PO Box 60, Lincoln, New Zealand

MCP9 Inheritance of Resistance and Effect of PM on Toxicity of *Bacillus thuringienesis* toxin Cry1Ac in Cabbage looper, *Trichoplusia ni*. **Wei Guo[1,2], Guoxun Li[3] and Ping Wang[2].** [1]Agricultural University of Hebei, Biocontrol Center of Plant Diseases and Pests of Hebei, Baoding, Hebei 071001, China; [2]Department of Entomology, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456,USA; [3]College of Plant Protection, Laiyang Agricultural University, Qingdao, Shandong 266109, China

MCP10 A chitin deacetylase-like protein identified from cabbage looper, *Trichoplusia ni*. **Wei Guo[1,2], Guoxun Li[3] and Ping Wang[2]**. [1]Agricultural University of Hebei, Biocontrol Center of Plant Diseases and Pests of Hebei, Baoding, Baoding, Hebei 071001, China; [2]Department of Entomology, Cornell University, New York State Agricultural Experiment Station,, Geneva, NY 14456,USA; [3]College of Plant Protection, Laiyang Agricultural University, Qingdao, Shandong 266109, China

MCP11 A cDNA-AFLP differential gene expression of the entomopathogenic fungi *Beauveria bassiana* during growth on different insect cuticles. **Akbar Ali khan Pathan[1], Annette Reineke[2] and Uma Devi Koduri[1]**. [1]Andhra University, Department of Botany,Andhra University,Visakhapatnam,India-530003.; [2]Max-Planck Institute of Chemical Ecology, Department of Entomology,Max-Planck Institute of Chemical Ecology, Hans Knoell Str. 8, D-07745, Jena, Germany

MCP12 Cloning and Expression of *cry3Aa8* gene from a *Bacillus thuringiensis* isolate against Coleoptera *Leptinotarsa decemlineata*. **Chengfeng Lei, Meiying Gao, Shunying Dai and Rongsen Li.** Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, 430071, China

MCP13 Mycoinsecticides: comprehensive list and current status. **Marcos R. de Faria[1,2] and Stephen P. Wraight[3].** [1]Embrapa Recursos Genéticos e Biotecnologia, Brasília, DF, 70849-970, Brazil; [2]Cornell University, Comstock Hall, Ithaca, NY 14853-2601, USA; [3]USDA, ARS, Plant, Soil and Nutrition Laboratory, Ithaca, NY, 14850-2901, USA

Microsporidia

MP1 Eleven antimicrobials tested *per os* against a grasshopper pathogenic microsporidium (Fungi: Microsporidia). **S. Johny[1], Carlos E. Lange[2], Leellen F. Solter[3], Amber Merisko[4] and Douglas W. Whitman[4].** [1]Laboratory of Molecular Genetics, Centre for DNA Fingerprinting and Diagnostics, ECIL Road, Nacharam, Hyderabad 500 076, India; [2] La Plata National University, Center for Parasitological Studies (CEPAVE), CONICET, La Plata, Argentina; [3]Illinois Natural History Survey, 1816 S. Oak St., Champaign, IL 61820, USA; [4]Illinois State University, Department of Biology, Normal, IL 61790, USA

MP2 Isolation and partial characterisation of a spore wall protein from *Nosema bombycis*. **Fan Zhang[1], Meng Xing Lu[1], Shyam V Kumar[2], Song Hong Chen[1], Xian Zheng Chen[1] and Yan Hai Zhang[1]**. [1]Laboratory of Invertebrate Pathology, Zhejiang Unversity, Hangzhou 310029, People's Repubic of China; [2]P. G. Department of studies and Research in Sericulture Karnatak University, Dharwad-580 003, INDIA

MP3 STU The phylogenetic analysis of *Endoreticulatus* sp. Taiwan by gene sequences. **Chih Yuan Wang[1], Leellen F. Solter[2] and Chung Hsiung Wang**[1]. [1]Department of Entomology, National Taiwan University, NO. 27, Lane 113, Sec. 4, Roosevelt Rd., Taipei, Taiwan (ROC) 106; [2]Center for Entomology, Illinois Natural History Survey, East Peabody Drive, Champaign, IL 61820, USA 607

MP4 An evaluation on the factors influencing on shelf life of Verticillium lecanii conidia at room temperature. **Zhangyan Shi, Xiaoyu Yang and Long Zhang.** College of Agronomy and Biotechnology, China Agricultural University, Beijing 100094

Studies on *Nosema* sp. (Microsporida) from Beet armyworm *Laphygma exigua* in China. **Chen Guang-Wen[1], Chen Qu-Hou[2].** [1]College of Life Sciences , Henan Normal University , Xinxiang 453002 , Henan , China, [2]College of Life Sciences ,Central China Normal University,Wuhan 430070 , China

Viruses I

VP1 A novel direct cloning system for making recombinant baculoviruses. **Olga Lihoradova[1], Irina Ogay[1], Jeffrey Morley Slack[2] and**

Abdusattor Abdukarimov[1]. [1]Institute of Genetics and Plant Experimental Biology, Uzbek Academy of Science, Tashkent, 702151, Uzbekistan; [2]USDA/ARS, Insect Biocontrol Laboratory, Beltsville MD, 20852-2350 USA

VP2 SV40 polyadenylation (pA) sequence is redundant in baculovirus expression system. **Craig P Seaborn, Jianli Xue and Xiao-Wen Cheng**. Miami University, Department of Microbiology, 32 Pearson Hall, Miami University, Oxford, Ohio 45056 USA

VP3 Influence of Cytochrome C on Apoptosis Induced by SfaMNPV in Insect *Spodoptera litura* Cells. **Lijun Liu, Jianxin Peng, Kaiyu Liu, Hong Yang, Yi Li and Huazhu Hong**. Institute of Entomology, Central China Normal University, Institute of Entomology, Central China Normal University, Wuhan 430079

VP4 Over expression of *Pfu* DNA polymerase by recombinant baculovirus infected silkworm. **Yin Chen, Xu'ai Lin, Yongzhu Yi, Guifang Shen and Zhifang Zhang.** Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing, 100081, China

VP5 Characteristic of *Autographa californica* nucleopolyhedrovirus *Ubiquitin* gene promoter. **Xu'ai Lin, Yin Chen, Yongzhu Yi and Zhifang Zhang**. Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing, 100081, China

VP6 Interactions between subunits of the *Autographa californica* nucleopolyhedrovirus-encoded RNA polymerase. **Erin A. Crouch, Kristy G. Morales and A. Lorena Passarelli**. Division of Biology, Kansas State University, Manhattan, KS 66506 USA

VP7 Cloning and sequencing of *Epinotia aporema* granulovirus (EpapGV) *gp37*-like gene. **Ricardo Salvador[1,2], Leticia Ferrelli[2], Marina Biedma[2], Alejandro Parola[2], Victor Romanowski[2] and Alicia Sciocco-Cap[1].** [1]IMYZA-CICVyA, Instituto Nacional de Tecnología Agropecuaria (INTA), CC 25, (1712) Castelar, Buenos Aires, Argentina; [2]IBBM, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, 115 y 49, (1900) La Plata, Argentina

VP8 Expression of human renin using two baculovirus systems. **Dulcyane Neiva Mendes[1,2], Francisco De Assis Rocha Neves[2], Luiz Alberto Simeoni[2], William Sihler[1] and Marlinda Lobo de Souza[1]**. [1]Embrapa Genetic Resources and Biotechnology, NTCB, Brasília, DF, Brazil, 70700-900; [2]Laboratory of Molecular Pharmacology, University of Brasilia, Brasília, DF, Brazil, 70910-900

VP9 Production of human interferon-gamma by a novel bi-cistronic baculovirus expression vector. **Tzong-Yuan Wu**. Department of Bioscience Technology, Chung Yuan Christian University, Department of Bioscience Technology, Chung Yuan Christian University, Chung-Li 320, Taiwan

VP10 **STU** Characterization of MacoNPV enhancin and its interaction with *Mamestra configurata* peritrophic matrix proteins. **Umut Toprak[1,2], Martin Erlandson[1], Cedric Gillott[2], Dwayne Hegedus[1] and Qianjun Li[1]**. [1]Agriculture and Agri-Food Canada, Saskatoon Research Centre, Saskatoon, 107 Science Place, Saskatoon, Saskatchewan, Canada S7N 0X2; [2]Department of Biology, University of Saskatchewan, 112 Science Place Saskatoon, Saskatchewan, Canada S7N 5E2

VP11 **STU** Functional studies on *Spodoptera litura* multiple nucleopolyhedrovirus anti-apoptotic genes. **Tiehao Lin, Mei Yu, Wenbi Wu, Yingxue Gong, Yi Pang and Kai Yang.** State Key Laboratory of Biocontrol, Sun Yat-sen University, Guangzhou 510275, People's Republic of China

VP12 **STU** Analysis of the immediate early *me53* gene from the baculovirus AcMNPV. **Jondavid De Jong[1], Theilmann A David[2], Arif M Basil[3] and Krell John Peter[1]**. [1]Department of Molecular and Cellular Biology, University of Guelph, 488 Gordon Street, Guelph, Ontario Canada N1G 2W1; [2]Agriculture and Agrifood Canada, Biotechnology, Summerland BC, Canada V0H 1Z0; [3]Great Lakes Forestry Centre, 1219 Queen Street East, Sault Ste Marie, Ontario, Canada P6A 2E5

VP13 **STU** Expression of anti-apoptotic *p35* gene in tobacco enhances tolerance to abiotic stresses and increases the virulence of AcMNPV. **Jianhua Song, Zhihua Wang, Changyong Liang, Shiyun Chen and Xinwen Chen.** State Key Laboratory of Virology, Wuhan Institute of Virology, the Chinese Academy of Sciences, Wuhan, 430071, the People's Republic of China

VP14 **STU** Ha135, a unique nonstructural protein of

HearNPV, is not essential for viral propagation. **Xiaoyu Pan[1], Gang Long[1,2], Marcel Westenberg[2], Songwang Hou[1], Fei Deng[1], Hualin Wang[1], Just M Vlak[2] and Zhihong Hu[1].** [1]State key laboratory of virology, Wuhan institute of Virology, Chinese Academy of Sciences, Wuhan, Hubei, 430071, P.R.China; [2]Laboratory of Virology, Wageningen University and Research Center, 6709 PD Wageningen, the Netherlands

VP15 STU Construction of Bac-to-Bac system of *Bombyx mori* NPV. **Jinshan Huang[1], Bifang Hao[1,2], Xiulian Sun[1], Fei Deng[1], Hualin Wang[1] and Zhihong Hu[1]**. [1]State Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, Hubei, 430071, P.R.China; [2]Northwest A & F University, Yangling, Shanxi, 712100, P.R.China

VP16 STU Functional role of aspartic proteinase cathepsin D in *Bombyx mori* metamorphosis. **Kwang Sik Lee[1], Byung Rae Jin[1], Zhong Zheng Gui[1], Bo Yeon Kim[1], Young Soo Choi[1], Ya Dong Wei[1], Young Moo Choo[1], Yeon Ho Je[2], Xijie Guo[3] and Hung Dae Sohn[1]**. [1]College of Natural Resources and Life Science, Dong-A university, Busan, 604-714, Korea; [2]School of Agricultural Biotechnology, Seoul National University, Seoul, Korea; [3]Sericultural Research Institute, Chinese Academy of Agricutural Sciences, Zhenjiang, China

VP17 STU Construction of the herpes simplex virus 1 ICP0 eukariotic expression vector and its effects of macrophage function. **Huang Liang**. Department of Microbiology and Immunology, Nanhua University, Hengyang Hunan 421001 , China

18:30-20:00 **Dinner** *Haitiangong Dining-Room*

20:00-22:00 Monday, *Meeting Center*
Bacteria Division Business Meeting

20:00-22:00 Monday, *Nanyuan Meeting Room*
Fungi Division Business Meeting

20:00-22:00 Monday, *Multifunctional Hall*
Microsporidia Division Business Meeting

20:00-22:00 Monday, *Xiyuan Meeting Room*
Nematodes Division Business Meeting

19:40-22:00 Monday, *Wuhan Institute of Virology*
(Bus leaves at 19:40 from the hotel)
Virus Division Business Meeting

Tuesday, August 29

8:00-10:00 Tuesday ***Meeting Center***
Symposium: Genetics and characterization of mechanisms of Bt-resistance (Bacteria Division)
Convenors: Juan Ferre and William Moar

8:00 Mutant alleles of a cadherin gene and Cry1Ac resistance in the cotton bollworm, *Helicoverpa armigera*. **Yidong Wu, Haiyan Chen, Yajun Yang, Yihua Yang and Shuwen Wu**. Nanjing Agricultural University, Nanjing, 210095, Jiangsu Province, China

8:30 Resistance and hypersensitivity to Bt crystal toxins. **Raffi Aroian, Brad Barrows, Larry Bischof and Danielle Huffman**. University of California, San Diego, 9500 Gilman Drive, Mail Code 0349 La Jolla, CA 92093-0349 USA

9:00 The diversity of Bt-resistance genes in Lepidoptera. **David G. Heckel**. Max Planck Institute for Chemical Ecology, Hans-Knoell-Str. 8, D-07745 Jena, GERMANY

9:30 A proteomic approach to study resistance to *Bacillus thuringiensis* Cry toxins in *Heliothis virescens* larvae. **Juan L. Jurat-Fuentes[1] and Michael J. Adang[2]**. [1]Department of Entomology and Plant Pathology, The University of Tennessee, 205 Ellington Plant Sciences Building, Knoxville, TN 37996; [2]Department of Entomology, University of Georgia, 413 Biological Sciences Building, Athens, GA 30602

8:00-10:00 Tuesday ***Multifunctional Hall***
Symposium: Field Performance of Insect Viruses (Virus Division)
Convenors: Zhihong Hu and Basil M. Arif

8:00 New developments in the use of codling moth granulovirus. **Juerg Huber.** BBA, Institute for Biological Control, D-64287 Darmstadt, Germany

8:30 Abietiv: Field efficacy and registration of the balsam fir sawfly nucleopolyhedrovirus in Canada. **Christopher John Lucarotti.** Canadian Forest Service - Atlantic Forestry Centre, 1350 Regent Street, P.O. Box 4000, Fredericton, NB E3B5P7, Canada

9:00 New strategies of using viruses to control agricultural and forest pests in China. **Xiulian Sun, Zhihong Hu and Huiying Peng**. State

Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, 430071, Hubei, China

9:30 Preliminary greenhouse trials with indigenous TnSNPV and AcMNPV isolates for cabbage looper (*Trichoplusia ni*) control in greenhouse vegetable production. **Martin A. Erlandson[1, 2], Dave Gillespie[3], Melissa Strom[2], Don Quiring[3] and David Theilmann[4]**. [1]Agriculture and Agri-Food Canada, Saskatoon Research Centre, 107 Science Place, Saskatoon, SK, S7N 0X2 Canada; [2]Department of Applied Microbiology and Food Science, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK, S7N 5A8 Canada; [3]Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, 6947 Hyw 7, Agassiz, BC, V0M 1A0 Canada; [4]Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, 4200 Hyw 97, Summerland, BC, V0H 1Z0 Canada

8:00-10:00 Tuesday ***Nanyuan Meeting Room***
Contributed Papers: Fungi 2
Moderators: Jørgen Eilenberg and Keqin Zhang

8:00 Does each host species harbour its own genotype of *Strongwellsea?* **Jørgen Eilenberg and Annette Bruun Jensen.** Department of Ecology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK 1871 Frb C, DENMARK

8:15 Regulator of G protein signaling pathway gene *cag8* is involved in conidiation of *Metarhizium anisopliae***. Weiguo Fang and Michael J Bidochka.** Department of Biological Sciences Brock University, St. Catharines, Ontario L2S 3A1, Canada

8:30 Isolation and insecticidal property of toxins from *Verticillium lecanii*. **Liande Wang[1,2], Jian Huang[2], Minsheng You[1], Xiong Guan[2] and Bo Liu[3].** [1]Institute of Applied Ecology, Fujian Agriculture & Forestry University, Fuzhou, 350002, P.R. China; [2]Key Laboratory of Biopesticide and Chemical Biology, MOE.Fujian Agriculture & Forestry University, Fuzhou, 350002, P.R. China; [3]Institute of Biotechnology, Fujian Academy of Agricultural Science, Fuzhou, 350001, P.R. China.

8:45 A PCR-RFLP approach for three endochitinase genes from glycohydrolase family 18 for the characterization and identification of *Metarhizium* strains. **Vandana Ghormade, Franco Widmer and Juerg Enkerli.** Molecular Ecology, Agroscope FAL Reckenholz, Reckenholzstrasse 191, CH-8046 Zürich, Switzerland.

9:00 **STU** Sequence comparison of a hydrophobin-like protein involved in conidial thermotolerance of different *Beauveria bassiana* strains. **Sheng-Hua Ying and Ming-Guang Feng**. Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, 310058, China

9:15 Cloning and characterization of a gene encoding a cuticle-degrading protease from the nematophagous fungus *Lecanicillium psalliotae* (syn. *Verticillium psalliotae*). **Jinkui Yang, Lianming Liang, Ying Zhang, Juan Li and Keqin Zhang*.** Laboratory for Conservation and Utilization of Bio-resources, Yunnan University, No.2 of north cuihu road, Kunming, 650091, Yunnan Province, P. R. China

9:30 **STU** Purification and cloning of extracellular enzymes from *Clonostachys rosea* and their potential as pathogenic factors. **Lianming Liang, Jinkui Yang, Zhongwei Gan, Ying Zhang and Keqin Zhang***. Laboratory for Conservation and Utilization of Bio-resources, Yunnan University, No.2 of north Cuihu road, Kunming, 650091, Yunnan Province, P.R. China

9:45 **STU** Variation in the activities of superoxide dismutase among twenty *Beauveria bassiana* strains. **Bao-Fu Huang and Ming-Guang Feng**. Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, 310058, China

8:00-10:00 Tuesday ***Xiyuan Meeting Room***
Contributed Papers: Microsporidia
Moderators: Andreas Linde and James Becnel

8:00 Discovery of an *Encephalitozoon* sp. (Fungi: Microsporidia) in an invertebrate host. **Carlos E. Lange[1], Leellen F. Solter[2], Michael D. Baker[3], S. Johny[4], Douglas W. Whitman[5] and Ann Cali[6]**. [1]Center for Parasitological Studies (CEPAVE), La Plata National University, CONICET, La Plata, Argentina; [2]Illinois Natural History Survey, 1816 S. Oak St., Champaign, IL 61820, USA; [3]Iowa State University, 1184 Molecular Biology Bldg., Ames, IA 50011 USA; [4]Laboratory of Molecular Genetics Centre for DNA Fingerprinting and Diagnostics, ECIL Road,

Nacharam, Hyderabad 500 076, India; [5]Illinois State University, Department of Biology, Normal, IL 61790, USA; [6]Rutgers University, Dept. of Biological Sciences, Smith Hall, 101 Warren St., Newark, NJ 07102, USA

8:15 The pathogenicity of Nosema apis, from Apis mellifera ligustica, to worker of Apis cerana cerana. **Huang Shaokang, Ye Shengzhu, Dong Je.** Bee Sciences College, Fujian Agriculture and Forestry University, 350002

8:30 Quantifying transmission of microsporidia in the gypsy moth, *Lymantria dispar*. **Gernot Hoch[1], Vincent D'Amico[2], Dörte Goertz[1] and Leellen F. Solter[3].** [1]BOKU – University of Nat. Res. and Appl. Life Sci., Hasenauerstrasse 38, 1190 Vienna, Austria; [2]USDA FS – NERS RWU 4502 / Univ. of Delaware, Townsend Hall, Newark, DE 19716, U.S.A.; [3]Illinois Natural History Survey, 1816 S. Oak St., Champaign, IL 61820, U.S.A

8:45 Strategies and tactics for control of locust (*Locusta migratoria manilensis*) in China. **Long Zhang, Yuhua Yan.** Key Lab for Biocontrol of Pests, Ministry of Agriculture China Agricultural University, Beijing 100094, China

9:00 **STU** Infections experiments with different spore types and different microsporidian isolates of *Lymantria dispar*. **Thomas Kolling and Andreas Linde**. Fachhochschule Eberswalde, Dept. of Forestry, Applied Ecology, Alfred-Moeller-Str. 1, 16225 Eberswalde, Germany

9:15 **STU** Effects of an anti-exospore monoclonal antibody on microsporidial (*Nosema bombycis*) germination in *vitro*. **Fan Zhang[1], Meng Xing Lu[1], Shyam V Kumar[2], Jie Hong Zhu[1], Song Hong Chen[1], Xian Zheng Chen[1] and Jian Hong[1].** [1]Laboratory of Invertebrate Pathology , Zhejiang Unversity, Hangzhou 310029,People's Repubic of China; [2]P.G.Department of studies and Research in Sericulture Karnatak University, Dharwad-580 003,INDIA

9:30 **STU** The comparison of rDNA of *Nosema ceranae* isolates**. Wei-Fone Huang[1], Michel BOCQUET[2], Ker-Chang Lee[1] and Chung-Hsiung Wang[1].** [1]Department of Entomology, National Taiwan University, 106, Taipei, Taiwan; [2]APIMEDIA, BP22, Pringy, France

10:00-10:30 **Coffee Break**

10:30-12:30 Tuesday ***Nanyuan Meeting Room***
Symposium: Novel approaches for dealing with difficult data (Microbial Control Division)
Convenor: Surendra Dara

10:30 Analysis, interpretation, and avoidance of difficult data in bioassay. **S. P. Wraight**. USDA-ARS Plant Protection Research Unit,U.S. Plant, Soil and Nutrition Laboratory, Tower Road, Cornell University, Ithaca, New York 14853 USA

11:00 Top reasons why papers have been rejected for publication. **Mark S. Goettel[1], Quirico Migheli[2] and Charles H. Pickett[3]**. [1]Lethbridge Research Centre, Agriculture & Agri-Food Canada, 5403 1st Avenue South, Lethbridge, AB, Canada T1J 4B1; [2]Dipartimento di Protezione delle Piante, Universita degli Studi di Sassari, Via E. De Nicola 9, I-07100 Sassari, Italy; [3]Biological Control Program, California Department of Food and Agriculture, 3288 Meadowview Road, Sacramento, CA, 95832, USA

11:30 Lost to industrial secrecy, statistical insignificance and short attention span: dark, dead, and dated data. **Jeff Lord.** US Department of Agriculture, Agricultural Research Service, Manhattan, Kansas 66502, USA

12:00 Hard lessons and perspectives of laboratory bioassays and field trials with entomopathogenic fungi. **Jarrod E. Leland[1] and Debbie Boykin[2]**. [1]USDA-ARS, SIMRU, NBCL, 59 Lee Road, Stoneville, MS 38776; [2]USDA-ARS, Midsouth Area Statistician, 141 Experiment Station Road, Stoneville, MS, 38776

10:30-12:30 Tuesday ***Meeting Center***
Contributed papers: Bacteria 1
Moderator: Didier Lereclus

10:30 The *Bacillus thuringiensis* InhA metalloproteases*:* conclusive weapons for infection**. Christina Nielsen-LeRoux[1,2], Myriam Ellouze-Hajaij[1], Nalini Ramarao[1], Christophe Buisson[1], Elisabeth Guillemet[1], Michel Bréhelin[3], Michel Gohar[1] and Didier Lereclus[1].** [1]INRA, Unité Génétique Microbienne et Environnement, INRA, la Minière, 78285 Guyancourt; [2]Institut Pasteur, Departement of Micrbiology Institut Pasteur, 75724 Paris Cedex 15, France2; [3]INRA ,

Université de Montpellier II, 3Ecologie Microbienne des Insectes et Interactions Hôte-Pathogène Université Montpellier II, 34095 Montpellier Cedex 05, France

10:45 **STU** *Bacillus nematocida* kills nematodes with two coordinated pathogenic factors: Bae16 and Bace16**. QiuHong Niu, XiaoWei Huang, Lin Zhang and Keqin Zhang*** . Laboratory for Conservation and Utilization of Bio-resources, Yunnan University, Kunming 650091

11:00 **STU** Requirement of *spoIIIAE* gene and *spoIVF* operon for sporulation and producing δ-endotoxins in *Bacillus thuringiensis* G03**. Changpo Sun[1], Fuping Song[2], Jie Zhang[2] and Dafang Huang[1].** [1]Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100081,China; [2]State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100094, China

11:15 The organic composition of silk gland of silkworm, *Bombyx mori* L infected with *Bacillus* thuringiensis**. Bharathi Depuru[1] and Yungen Miao[2].** [1]Prof.D.Bharathi, Dept of Sericulture, Sri Padmavati Women's University, Tirupati-517502, A.P., INDIA; [2]Prof.Miao Yungen, Dept of Sericulture, College of Animal Sciences, Zhejiang University,Hangzhou, China

11:30 **STU** Molecular characterization of the plasmid genome from *Bacillus thuringiensis* subsp. *Tenebrionsis* YBT-1765. **Junyan Huang, Suxia Guo, Dongmei Han, Li Wang, Ziniu Yu and Ming Sun**. State Key Laboratory of Agricultural Microbiology, College of Life Science and Technology, Huazhong Agricultural University, Wuhan 430070, Hubei, People's Republic of China

11:45 **STU** Anthrax virulence plasmid pXO1 conservative fragments within *Bacillus cereus* group and their phylogenic relatedness with pathogenicity. **Xiaomin Hu[1,2], Bjarne Munk Hansen[2], Niels Bohse Hendriksen[2] and Zhiming Yuan[1].** [1]Wuhan Institute of Virology, Chinese Academy of Science, Wuhan 430071, China; [2]National Environmental Research Institute, 4000 Roskilde, Denmark

12:00 **STU** Toxicity analysis of truncated insecticidal crystal protein Cry1Ah from *Bacillus thuringiensis*. **Jing Xue[1], Fuping Song[1], Dafang Huang[2] and Jie Zhang[1*]**. [1]State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100094, P. R China; [2]Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100081, P. R China

12:15 Molecular dynamics simulations of the Bt toxin Cyt1A: the model and its validation by fluorescence resonance energy transfer. **Xiaochuan Li[1], Kerrick Nevels[1], Dexuan Xie[2], Marianne P. Carey[3] and Peter Butko[1]**. [1]University of University of Southern Mississippi, Department of Chemistry and Biochemistry, Hattiesburg, MS 39406, USA; [2]University of Wisconsin, Department of Mathematical Sciences, Milwaukee, WI 53211, USA; [3]Case Western Reserve University, Department of Biochemistry, Cleveland, OH 44106, USA

10:30-12:30 Tuesday ***Multifunctional Hall***
Contributed papers: Viruses 1
Moderators: Linda King and Xinwen Chen

10:30 *Helicoverpa armigera* nucleopolyhedrovirus *orf80* encodes a late, nonstructural protein. **Dun Wang[1,2] and Chuan-xi Zhang[1].** [1]Institute of Applied Entomology, Zhejiang University, Hangzhou, 310029, P.R. China.; [2] College of Forestry, Northwest A&F University, Shaanxi Yangling, 712100, P.R. China.

10:45 **STU** The *Helicoverpa armigera* nucleopolyhedrovirus FGF is essential for BV infection. **Xiang Li**, **Changyong Liang, Jianhua Song, Xinwen Chen.** State Key Laboratory of Virology, Wuhan Institute of Virology, the Chinese Academy of Sciences, Wuhan, 430071, the People's Republic of China

11:00 **STU** Functional analysis of baculovirus DNA photolyase genes. **Fang Xu[1], Margit Lampen[1,2], Christina Van Houte[1], André P.M. Eker[2], Just M. Vlak[1] and Monique M. Van Oers[1]**. [1]Laboratory of Virology, Wageningen University, Binnenhaven 11, 6709 PD Wageningen, the Netherlands; [2]Department of Cell Biology and Genetic, Erasmus University Medical Center, Dr Molewaterplein 50, 3015 GE Rotterdam, the Netherlands

11:15 **STU** The Baculovirus P10 protein forms two distinct cytoskeletal-like structures with different cellular localisation properties. **David CJ Carpentier, Caroline M Griffiths and**

Linda A King. Insect Virus Research Group, School of Biological and Molecular Sciences, Oxford Brookes University, Headington, Oxford, OX3 0BP, United Kingdom

11:30 **STU** 38K is required for *Autographa californica* multiple nucleopolyhedrovirus nucleocapsid Assembly. **Wenbi Wu, Tiehao Lin, Lijing Pan, Mei Yu, Zhaofei Li, Yi Pang and Kai Yang**. State Key Laboratory of Biocontrol, Sun Yat-sen University, Guangzhou 510275, People's Republic of China

11:45 **STU** HA2 from the *Helicoverpa armigera* nucleopolyhedrovirus: A WASP-related protein that activates Arp2/3-induced actin filament formation**. Qian Wang[1,2], Changyong Liang[1], Jianhua Song[1] and Xinwen Chen*[1].** [1]State Key Lab of Virology,Wuhan Institute of Virology, Chinese Academy of Sciences., Wuhan 430071 ,People's Republic of China; [2]Graduate School of the Chinese Academy of Sciences, Beijing, 100039, People's Republic of China

12:00 **STU** The cytoplasmic tail domain of baculovirus group II F proteins is essential for viral infectivity**. Gang Long[1,2], Xiaoyu Pan[1], Marcel Westenberg[2], Zhihong Hu[1] and Just M. Vlak[2].** [1]Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, Hubei, 430071, China; [2]Laboratory of Virology, Wageningen University, Binnenhaven 11, 6709 PD Wageningen, The Netherlands

12:15 **STU** The unique functions of a marvelous gene p13 from type II baculoviruses. **Enqi Du[1], Feng Yan[1], Weixin Jin[2], Wenke Zhou[1], Yipeng Qi[1].** [1]Key laboratory of Virology, Wuhan University, Wuhan, P. R. China, [2]Institute of Microbiology, KIM IL SUNG University, Daesung, Pyongyang, P. R. Korea

13:00-17:30 **Tuesday, Excursion** (light lunch included, tickets required)
Buses leave at 13:00 from the hotel, end at Wuhan Botanic Garden for 5K Race and BBQ

18:00-18:50 **Tuesday, 5K Race** *Wuhan Botanic Garden*

For those who do not participate in the excursion:
12:30-14:00 **Lunch** *Haitiangong Dining-Room*

16:00 Buses leave from the hotel to Wuhan Botanic Garden for 5K Race and BBQ

19:00-22:00 **BBQ and Entertainments** *Wuhan Botanic Garden*

Wednesday, August 30

8:00-10:00, Wednesday ***Nanyuan Meeting Room***
Symposium: Ecology of Entomophthorales (Fungi Division)
Convenor: Ming-Guang Feng

8:00 Host-pathogen interaction in Entomophthorales in agro-ecosystems: initiation of epizootics and relationship between host species and fungal genotype. **Jørgen Eilenberg, Annette Bruun Jensen and Charlotte Nielsen.** Department of Ecology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK 1871 Frb C, DENMARK

8:30 Persistence and spread of *Entomophaga maimaiga* infecting *Lymantria dispar*. **Ann E. Hajek[1], Charlotte Nielsen[2] and Patrick C. Tobin[3]**. [1]Department of Entomology, Cornell University, Ithaca, New York 14853-2601 USA; [2]Royal Veterinary & Agricultural University, Copenhagen 1870 Denmark; [3]USDA, Forest Service, Morgantown, West Virginia 26505-3180 USA

9:00 Intraguild interactions involving Entomophthorales. **Judith K. Pell[1], Jason Baverstock[1], Ariel W. Guzman Franco[1] and Helen E. Roy[2]**. [1]Plant and Invertebrate Ecology Division, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK; [2]Department of Life Sciences, Anglia Ruskin University, Cambridge, Cambridgeshire, CB1 1PT, UK

9:30 Transmission of obligate aphid pathogens (Entomophthorales) with host dispersal flight: from biological hypothesis to confirmation. **Ming-Guang Feng.** Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, 310058, China

8:00-10:00, Wednesday ***Xiyuan Meeting Room***
Symposium: Emerging pest targets for Entomopathogenic nematodes (Nematode Division)
Convenors: David Shapiro-Ilan and Richou Han

8:00 Emerging pests targets for Entomopathogenic nematodes in China. **Richou Han, Li Cao, Guohong Wang, Jinghua Chen and Xuehong Qiu**. Guangdong Entomological Institute, Guangzhou, China

8:24 Entomopathogenic nematodes and emerging pests in Latin America: the quest for a sustainable world. **S.Patricia Stock[1], Jesus Alcazar[2], Juan Carlos Lopez-Nunez[3], Luis Leite[4] and Mayra Rodriguez Hernandez[5].** [1]Dept. Entomology, University of Arizona, USA. [2]Centro International de la Papa, Peru. [3] IB/Lab. Controle Biolóico. CP 70 Cep 13001-970. Campinas, Brasil. [4]Cenicafe, Manzinales, Chinchina, Colombia. [5]Centro Nacional de Sanidad Agropecuaria, Cuba

8:48 New and upcoming target pests for entomopathogenic nematodes in North America. **David I. Shapiro-Ilan[1] and Parwinder Grewal[2]**: [1]USDA-ARS, SAA Byron, GA USA. [2]Ohio State University, Wooster, OH USA

9:12 Current and future uses of nematodes in Western Europe. **Michael J Wilson[1], Cyrille Verdun[2], Ralf Udo Ehlers[3].** [1]University of Aberdeen, Aberdeen UK. [2]Becker Underwood Ltd, West Sussex, United Kingdom. [3]Christian-Albrechts University Kiel, Raisdorf, Germany

9:36 Emerging pest targets for Entomopathogenic nematodes in Asia outside of China. **Ho Yul Choo[26], Dong Woon Lee[2], Sang Myeong Lee[3], Satoshi Yamanaki[4], Sudershan Ganguly[5] and Vacheree Somsook[6]**. [1]Dept. of Applied Biology & Environmental Sciences, Gyeongsang National University, Jinju, 660-701, Gyeongnam, Korea; [2]Dept. of Applied Biology, Sangju National University, Sangju, 742-711. Kyungpook; [3]Southern Forest Research Center, Forest Research Institute, Jinju, 660-300, Gyeongnam; [4]Arysta Lifescience Cooperation, St. Luke's Tower, Akashi-cho 8-1, Chuo-ku, Tokyo, 104-6591, Japan; [5]Division of Nematology, Indian Agricultural Research Institute, New Delhi-110012, India; and [6]Biological Control Research Section, Entomology & Zoology Group, Plant Protection Research & Development Office, Dept. of Agriculture, Bangkok 10900, Thailand.

8:00-10:00, Wednesday ***Meeting Center***
Contributed Papers: Bacteria 2
Moderator: Zhiming Yuan

8:00 What is the mechanism of resistance to *Bacillus thuringiensis* toxin Cry1Ac in a greenhouse population of cabbage looper, *Trichoplusia ni*? **Ping Wang[1], Jian-zhou Zhao[1], Ana Rodrigo-Simón[2], Wendy C. Kain[1], Alida F. Janmaat[3], Anthony M. Shelton[1], Juan Ferré[2] and Judith Myers[3].** [1]Department of Entomology, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456, USA; [2]Department of Genetics, University of Valencia, Dr. Moliner 50, 46100 Burjassot (Valencia), Spain; [3]Department of Zoology, University of British Columbia, Vancouver, British Columbia, V6T 1Z4, Canada

8:15 **STU** Production of Bt Cry1Ac resistance in cotton bollworm, *Helicoverpa zea* (Boddie)**. Konasale J Anilkumar and William J Moar.** Department of Entomology and Plant Pathology, 301 Funchess Hall, Auburn University, Auburn, Alabama-36849, USA

8:30 Lack of irreversible binding as a novel mechanism of resistance to *Bacillus thuringiensis* Cry1Ab toxin**. M. Sales Ibiza-Palacios[1], Juan Ferré[1], Satoshi Higurashi[2], Kazuhisa Miyamoto[3], Ryoichi Sato[2] and Baltasar Escriche[1].** [1]Dep. Genética, Universitat de València, 46100-Burjassot, Spain; [2]Grad. School of Bio-Applications and Syst. Eng., Tokyo University of Agr. and Tech., Tokyo 184-8588, Japan; [3]Inst. Insect and Animal Sciences, Natl. Inst. Agrobiol. Sci., Tsukuba Ibaraki 305-8634, Japan

8:45 Cross-resistance between Bt and non-Bt insecticides in *Plutella xylostella*. **Ali H Sayyed[1,2], Graham Moores[3], Denis J Wright[2] and Neil Crickmore[1]**. [1]Imperial College, Faculty of Life Sciences, Silwood Park, Ascot, Berkshire, SL5 7PY, UK; [2]University of Sussex, School of Life Sciences, Falmer, Brighton, BN1 9QG, UK; [3]Rothamsted Research, Harpenden, Herts, AL5 2JQ, UK

9:00 **STU** Cellular mode of action of the *Bacillus sphaericus* binary toxin. **Onya Opota[1], Nils Gauthier[2], Emmanuel Lemichez[2], Colin Berry [3], David Pauron[1]**. [1]Institut National de la Recherche Agronomique, UMR 1112 INRA/UNSA, 400 Route des Chappes, BP 167, 06903 Sophia Antipolis Cedex, France; [2]INSERM U 627, Faculté de Médecine, 06107 NICE Cedex 2, France; [3]Cardiff School of Biosciences, Cardiff University, Cardiff CF10 3US, United Kingdom

9:15 **STU** A second GTPase modifying toxin, named LopT2, is encoded by a remnant prophage in *Photorhabdus luminescens* and produced in insect specific organs. **Sonia C. P. Costa[1, 2], Karine Brugirard-Ricaud[1], Michel Brehelin[1], Alain Givaudan[1] and Robert**

Zumbihl[1]. [1]Laboratoire EMIP - Unité INRA UMII 1133, Université de Montpellier II, Place Eugène Bataillon, Montpellier, France; [2]Departamento de Biologia, Universidade dos Açores, Ponta Delgada, Portugal

9:30 **STU** Mutations of residues in three domains of *Bacillus thuringiensis* Cry1C δ-endotoxin affect insecticidal activity. **Yu Ren[1], Fuping Song[1], Dafang Huang[2] and Jie Zhang[1*]**. [1]State Key Laboratory for Biology of Plant Diseases and Insect Pests,Chinese Academy of Agricultural Sciences, Beijing 100094,P.R.China; [2] Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100081, P.R.China

9:45 **STU** Cry1Ac N-terminal mutants with increased toxicity towards the diamondback moth. **Mark Bruce and Neil Crickmore**. University of Sussex, Department of Biochemistry, School of Life Science, University of Sussex, Falmer. Brighton. BN1 9QG. UK

8:00-10:00, Wednesday ***Multifunctional Hall***
Contributed Papers: Viruses 2
Moderators: Johannes A. Jehle and Chuanxi Zhang

8:00 Salivary gland hypertrophy virus (SGHV) as a threat to the success of SIT eradication programs for tsetse flies. **Adly ABD-ALLA[1,3], Hervé Bossin[1], François Cousserans[2], Andrew Parker[1], Max Bergoin[2] and Alan Robinson[1].** [1]International Atomic Energy Agency, Entomology Unit FAO/IAEA Agriculture and Biotechnology Laboratory, A-2444 Seibersdorf, Austria; [2]Université Montpellier II, Laboratoire de Pathologie Comparée, France; [3] National Research Centre, Department of Pests and Plant Protection, Dokki, Giza, Egypt.

8:15 Nucleopolyhedrosis virus introduction in Australia**. Patrick Buerger[1], Caroline Hauxwell[2] and David Murray[3].** [1]Ag Biotech Australia Pty Ltd, P.O.Box 537, Richmond, NSW Australia 2753; [2]Queensland Department of Primary Industries and Fisheries, 80 Meiers Road, Indooroopilly, QLD Australia; [3]Queensland Department of Primary Industries and Fisheries, 203 TorSt, Toowoomba, QLD Australia

8:30 Protection mechanism by lignin additives for baculoviruses against the negative effect of uv radiation. **S. Elnagar[1], M.A.K. El- Sheikh[1], A. Amin[1], G. Fédière[1], A. A. Atwaand[1] and M. Khattab[1]**. [1]Department of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo University, Giza, Egypt.; [2]Plant Protection Research Institute, Agricultural Research Center,, Ministry of Agriculture, Dokki, Giza.; [3]*Center of Virology, Institut de Recherche pour Le Dèveloppement (IRD)- Faculty of Agriculture, Cairo University, Egypt.

8:45 Field resistance of codling moth to Cydia pomonella granulovirus: Occurrence, genetics and breaking. **J. A. Jehle[1], K. E. Eberle[1], S. Asser[1], S. M. Sayed[1] and M. R. Rezapanah[2]**. [1]Laboratory of Biotechnological Crop Protection, Department of Phytopathology, Agricultural Service Center Palatinate (DLR Rheinpfalz), Neustadt a. d. Wstr., Germany; [2]Dept. of Biological Control, Plant Pests Diseases Research, Tehran, Iran

9:00 Virulence of a Nucleopolyhedrovirus to Balsam Fir Sawfly (Hymenoptera: Diprionidae). **Shiyou Li.** Canadian Forest Service, Natural Resources Canada, Building 57, 960 Carling Ave. Ottawa, ON, Canada K1A 0C6

9:15 **STU** Competition and transmission rate of wild type and recombinant HaSNPV in *Helicoverpa armigera* larvae**. Liljana Georgievska[1,3], Jenny Cory[2], Wopke van der Werf[3] and Just M. Vlak[1].** [1]Laboratory of Virology, Wageningen University, Binnenhaven 11, 6709 RD Wageningen, The Netherlands; [2]Great Lakes Forestry Research Centre, Sault Ste. Marie, Ontario P6A 2E5, Canada; [3]Laboratory of Crop and Weed Ecology, Haarweg 333, 6709 RZ Wageningen, The Netherlands

9:30 **STU** Quantitative Relationship of two viruses Viruses (MrNV and XSV) in White Tail Disease of *Macrobrachium rosenbergii* de Man. **Hua Jun Zhang[1], Jian Min Wang[1], Jun Fa Yuan[1], Li Juan Li[1], Jian Hong Zhang[1], Jean Robert Bonami[2] and Zheng Li Shi[1]**. [1]State Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, 430071 Wuhan, China; [2]Pathogens and Immunity, UMR5119, ECOLAG, CNRS/UM2, Université Montpellier 2, Montpellier, France

9:45 Building up standard of cotton bollworm (*Helicoverpa armigera* (Hübner)) Nuclear Polyhedrosis Viruses wettable powder. **Jiang Hui, Wang Xiaojun, Lin Ronghua, Han Xianguo, Chen Kun, Chen Hongying and Liu Qiong.** Institue for the control

Agrochemicals, Ministry of Agriculture，Beijing 100026

10:00-10:30 Wednesday, **Coffee Break**
Setting up Posters *Meeting Center*

10:30-12:30 Wednesday ***Xiyuan Meeting Room***
Symposium: Microsporidia in silk moth (Microsporidia Division)
Convenors: Gernot Hoch and Leellen Solter

10:30 *Nosema bombycis* and the silkworm industry**. James J. Becnel**. U. S. Department of Agriculture, Agriculture Research Service, Center for Medical, Agricultural and Veterinary Entomology, 1600 S. W. 23rd Drive, Gainesville, Florida 32608

11:00 Diversity among microsporidian parasites causing silkworm Pebrine disease. **Ji-Ping Liu[1], Judith E. Smith[2] and Ling Zeng[1].** [1]South China Agricultural university, Wushan, Tianhe, Guangzhou 510642, China; [2]Leeds university, Leeds, LS2 9JT,UK

11:30 From *Nosema bombycis* rDNA organization to revise the *Nosema* isolates in Taiwan. **Chih-Yuan Wang, Wei-Fone Huang and Chung-Hsiung Wang.** Department of Entomology, National Taiwan University, 106, Taipei, Taiwan

12:00 Impact of *Nosema sp.* infection on nutritional physiology and growth of the tasar silkworm *Antheraea mylitta*. **Sudhansu Sekhar Rath, Mrinal Kanti Singh and Suryanarayana N.** Central Tasar Research and Training Institute, Piska Nagri, Ranchi-835 303, Jharkhand, INDIA

10:30-12:30, Wednesday ***Meeting Center***
Contributed Papers: Bacteria 3
Moderator : Dafang Huang

10:30 Identification of *Bacillus cereus* internalin and other candidate virulence genes specifically induced during infection in insects. **Nadine Daou, Sinda Fedhila, Christina Nielsen-LeRoux and Didier Lereclus.** Unité Génétique Microbienne et Environnement, INRA, La Minière, 78285 Guyancourt cedex, France

10:45 **STU** Preliminary Characterization of a thermostable DNA polymerase I from a mesophilic *Bacillus sphaericus* strain C3-41**. Han Bei, Hu Xiaomin, Liu Haizhou, Cai Ya jun, Yuan Zhiming**. Wuhan Institute of Virology, Chinese Academy of Science, Wuhan 430071, China

11:00 **STU** Identification of three zwittermicin A Biosynthesis-Related Genes from *Bacillus thuringiensis* YBT-1520. **Changming Zhao, Yi Luo, Chunxu Song, Hui Zeng, Ziniu Yu and Ming Sun**. State Key Laboratory of Agricultural Microbiology, College of Life Science and Technology, Huazhong Agricultural University, Wuhan 430070, Hubei, People's Republic of China

11:15 Amino acid substitutions in aA and aC of Cyt2Aa2 alter hemolytic activity and host specificity**. Boonhiang Promdonkoy[1], Amporn Rungrod[1], Patcharee Promdonkoy[1], Wanwarang Pathaichindachote[1], Chartchai Krittanai[2] and Sakol Panyim[2].** [1]National Center for Genetic Engineering and Biotechnology, 113 Paholyothin Road, Klong 1, Klong Luang, Pathumthani 12120, Thailand; [2]Institute of Molecular Biology and Genetics, Mahidol University, Salaya Campus, Nakhonpathom 73170, Thailand

11:30 **STU** Cloning and expression of *gabT* and *gabD* of *Bacillus thuringiensis* YBT1520**. Li Zhu[1], FuPing Song[2], Jie Zhang[2] and DaFang Huang[1].** [1]Biotechnology Research Institute,Chinese Academy of Agricultural Sciences, Beijing 100081, People's Republic of China; [2]State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100094, People's Republic of China

11:45 **STU** Expression of *Vitreoscilla* hemoglobin gene in *Bacillus thuringiensis* improve the cell density and insecticidal crystal proteins yield. **Feng Liang, Chen Shouwen, Sun Ming, Yu Ziniu.** State Key Laboratory of Agricultural Microbiology, National Engineering Research Center for Microbial Pesticides, Huazhong Agricultural University, Wuhan, 430070, P.R.China

12:00 **STU** The prepation of the HBF-1 polyclonal antibody and its application to detection of the protoxin in soil**. Rui-hua Wu[1,2], Shu-liang Feng[2], Guo-xun Li[3], Rong-yan Wang[2], Jin-yao Wang[2], Wei-ping Cao[2], Lin-xin Du[2] and Jian Song[2].** [1]Feng Shu-liang, Institute of Plant Protection Hebei Academy of Agriculture and Forestry Sciences, Baoding 071000; [2]College of Plant Protection, Agricultural University of Hebei,Baoding 071001,China;

[3]Laiyang Arricultural College,Qingdao 266109,China

12:15 **STU** Insecticidal toxicology of HBF-1 strain from *Bacillus thuringiensis* on *Anomala corpulenta* and *A. exoleta* larvae**. Jian Song[1], Shu-liang Feng[1], Rong-yan Wang[1], Jin-yao Wang[1], Wei-ping Cao[1], Li-xin Du[1], Jie Zhang[2] and Fu-ping Song[2].** [1]Feng Shu-liang, Institute of Plant Protection, Hebei Academy of Agricultural And Forestry Sciences, Baoding 071000

10:30-12:30, Wednesday ***Nanyuan Meeting Room***
Contributed Papers: Microbial Control 1
Moderators: Wendy Gelernter and Svetlana Gouli

10:30 Microbial insecticides: some thoughts on history, commercialization and the future. **Wendy Gelernter**[1]. [1]PACE Consulting, San Diego, CA 92109 USA

10:45 Lessons Learned from LUBILOSA. **Roy Bateman[1].** [1]IPARC, Imperial College London, Silwood Park Campus, Ascot, Berks, SL5 7PY, UK

11:00 Ecological mechanism of sustainable pest control in pine plantation ecosystem. **Zengzhi Li, Meizhen Fan, Degui Ding, Bin Wang and Baoyu Han.** Department of Forestry, Anhui Agricultural University, Hefei, Anhui 230036, P. R. China

11:15 **STU** Production of biomass and shelf life screening of the lepidopteran specific entomopathogenic fungi *Nomureae rileyi*. **Akbar Ali khan Pathan, Narasimha Reddy Parine and Uma Devi Koduri.** [1]Andhra University, Department of Botany,Andhra University, Visakhapatnam, India-530003

11:30 **STU** Expression and Characterization of a novel vegetative insecticidal protein gene of *Bacillus thuringiensis*. **Liang Xiao, Yuehua Chen and Jun Cai**. Tianjin Key Laboratory of Microbial Functional Genomics, Department of Microbiology, College of Life Sciences, Nankai University, Tianjin 300071, China

11:45 **STU** A structured model for the entire fermentation of *Bacillus thuringiensis* var. *kurstaki*. **Ana Karin Navarro and Fermín Pérez-Guevara**. CINVESTAV, Department of Biotechnology, Av. IPN 2508, San Pedro Zacatenco, Mexico City, México. PC 07360

12:00 **STU** Screening and breeding of *Bacillus thuringiensis* subsp. *kurstaki* with high toxicity against *Spodoptera exigua*. **Zhang Xiao-peng, Gong yu-hua, Chen Shou-wen*, Yu Zi-niu**. State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan, 430070, China

10:30-12:30, Wednesday ***Multifunctional Hall***
Contributed Papers: Viruses 3
Moderators: David A Theilmann and Linda Guarino

10:30 Baculovirus immediate early 1 protein is a broad-spectrum bridge for enhancer function *in trans***. Yin Chen, Xu'ai Lin, Yongzhu Yi and Zhifang Zhang.** [1]Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing, 100081, China

10:45 Baculovirus genes involved in BmNPV *ubiquitin* gene expression in transient expression assays. **Xu'ai Lin, Yin Chen, Yongzhu Yi and Zhifang Zhang**. [1]Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing, 100081, China

11:00 **STU** Functional analysis of the AcMNPV budded virus regulatory protein EXON0. **Minggang Fang[1], Xiaojiang Dai[2] and David A Theilmann[1,2]**. [1]Department of Plant Science, Faculty of Land and Food System, University of British Columbia, Vancouver, B.C., Canada V6T 1Z4, Vancouver, B.C., Canada V6T 1Z4; [2]Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Summerland, B.C., Canada V0H 1Z0

11:15 **STU** Functional comparison of the *Autographa californica* multiple nucleopolyhedrovirus transcription factors IE0 and IE1. **Yingchao Nie[1] and David A Theilmann[1,2]**. [1]Department of Plant Science, Faculty of Land and Food System, University of British Columbia, Vancouver, B.C., Canada V6T 1Z4; [2]Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Summerland, B.C., Canada V0H 1Z0

11:30 **STU** *Lef*-2 dual role in DNA replication and late gene expression during baculovirus-infection. **Clare Allen[1,2], Linda King[2] and Robert Possee[1].** [1]NERC Centre for Ecology & Hydrology, Mansfield Road, Oxford, OX1 3SR, UK; [2]School of Biological and Molecular Sciences, Oxford Brookes University,

Headington, Oxford, OX3 0BP UK)

11:45 Role of the RNA triphosphatase domain of LEF-4 in late gene expression and viral replication. **Yi Li and Linda Guarino.** [1]Texas A&M University, Department of Entomology, 2475 TAMU, Texas A&M University, College Station, TX 77840

12:00 **STU** Characterizing the region of the polyhedrin promoter affected by a few polyhedra mutant baculovirus**. Carolyn Pritchard[1,2], Barbara Kelly[1], Linda King[2], Rosie Hails[1] and Robert Possee[1].** [1]NERC Centre of Ecology and Hydrology, Mansfield Road, Oxford, OX1 3SR, UK; [2]Oxford Brookes University, Headington Campus, Gipsy Lane, Oxford, OX3 0BP, UK

12:15 Unique expression strategy of cricket densonucleosis (AdDNV) genome. **Peter Tijssen[1], Yi Li[2], Zoltan Zadori[1], Francoise-Xaviere Jousset[3], Jozsef Szelei[1], Mohamed El-Far[1], Joseph Woodring[4], Regina G. Kleespies[5] and Max Bergoin[3].** [1]INRS-Institut Armand-Frappier, Laval QC, Canada H7V 1B7; [2]Huazhong Normal University, Wuhan, 430079 P.R. China; [3]Université Montpellier II, Montpellier, 34095 France; [4]Universität Bayreuth, Bayreuth, 95440 Germany; [5]Federal Biological Research Centre for Agriculture and Forestry, 64287 Darmstadt, Germany

12:30-14:00 Wednesday, **Lunch** *Haitiangong Dining-Room*
Setting up Posters *Meeting Center*

14:00-16:00 Wednesday ***Meeting Center***
Symposium: Bacteria in Bio-control in Asia: natural and Bio-tech strains (Bacteria Division)
Organizer: Ray Akhurst
Moderator: D.H. Dean

14:00 Application of mosquitocidal *Bacillus sphaericus* and the resistance management in China. **Zhiming Yuan**. Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, 430071, China

14:30 Transgenic bacteria expressing combinations of genes from *Bacillus thuringiensis***. Arieh Zaritsky and Eitan Ben-Dov.** Ben-Gurion University of the Negev, Department of Life Sciences, Ben-Gurion University of the Negev, POB 653, Be'er-Sheva 84105, Israel

15:00 Microbial control of scarabs in Japan. **Shin-ichiro Asano[1], Hisanori Bando[1], Noriko Shisa[2], Katsuyoshi Takeuchi[2] and Toshihiko Iizuka[3].** [1]Division of Applied Biosciences, Graduate School of Agriculture, Hokkaido University, Sapporo, Hokkaido 060-8589, Japan; [2]SDS-Biotech KK, Tukuba Research and Development Center, Tsukuba, Ibaraki 300-2646, Japan; [3]Hokuren Agricultural Research Institute, Naganuma, Hokkaido 069-1317, Japan

15:30 Toxicity of *Bacillus thuringiensis* crystal proteins against plant root-knot nematode. **Ziquan Yu, Suxia Guo, Ziniu Yu, Ming Sun.** State Key Laboratory of Agricultural Microbiology, College of Life Science and Technology, Huazhong Agricultural University, Wuhan 430070, PR China

14:00-16:00 Wednesday ***Nanyuan Meeting Room***
Contributed Papers: Fungi 3
Moderators: Tariq M Butt and Mingguang Feng

14:00 Laboratory bioassays of entomopathogenic fungi for control of western flower thrips *Franklinella occidentalis* in horticultural growing media**. Minshad Ali Ansari[1], Michael Brownbridge[2], Farooq Ali Shah[1], Mark Whittaker[3], Munoo Prasad[4] and Tariq M Butt[1].** [1]Department of Biological Science, University of Wales, Swansea, SA2 8PP, UK; [2]AgResearch Ltd, PO Box 60, Lincoln, New Zealand; [3]Koppert Biological Systems, Suffolk, CB9 8QP, UK; [4]Board Na Mona Horticulture Division, Research Centre, Main Street, Newbridge, Co. Kildare, Ireland

14:15 Field evaluation of *Beauveria bassiana* isolates from *Lygus* spp. for control of *Lygus* spp. (Hemiptera: Miridae)**. Jarrod E. Leland[1], Michael R. McGuire[2], Tina G. Teague[3], Jennifer Lund[3], Steinkraus C. Donald[4] and Gore Jeff[1].** [1]USDA-ARS, SIMRU, NBCL, 59 Lee Road, Stoneville, MS 38776, USA; [2]USDA-ARS, Northern Plains Area, NRRC 2150 Centre Avenue, Building D, Suite 300, Fort Collins, CO 80526-8119, USA; [3]College of Agriculture, Arkansas State Univeristy, P.O. Box 2340, State University, AR, 72467; [4]Department of Entomology, University of Arkansas, 319 Agricultural Building, University of Arkansas, Fayetteville, AR 72701

14:30 Grain-Based Production of the Entomopathogenic fungus Nomuraea rileyi**. David Holdom and Hauxwell Caroline.**

[1]Department of Primary Industries and Fisheries, 80 Meiers Road, Indooroopilly, Queensland, Australia 4068

14:45 Evaluation of the potential of native fungal isolates and *Metrahizium anisopliae* var. *acridum* for the greater wax moth, *Galleria mellonella* (L). **Namusana Hellen, Emiru Seyoum and Bekele Jembere**. Department of Biology, Addis Ababa University, P.O.Box 1176, Addis Ababa, Ethiopia

15:00 Assessment toxicity of *Beauveria bassiana* blastospores against colding moth *Cydia pomonella* (Lepidoptera: Tortricidae) in laboratory**. García-Gutiérrez Cipriano[1], Solis-Soto Aquilés[2], Galán-Wong Luis J.[3], González-Maldonado Ma. Berenice[1] and Medrano-Roldán Hiram[2].** [1]CIIDIR-COFAA-IPN, Sigma s/n Fracc. 20 de Nov. II. C. P. 34220. Durango, Dgo. México.; [2]ITD, Blvd. Felipe Pescador No. 1830 C. P. 34080. Durango, Dgo. México.; [3]UANL, San Nicolás de los Garza, N. L. C.P. 66450. México

15:15 **STU** Enhanced efficiency of *Beauveria bassiana* blastospore-based transformation system by restriction enzyme-mediated integration**. Qiong Jiang, Sheng-Hua Ying and Ming-Guang Feng.** [1]Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, 310058, China

15:30 **STU** Pathogenicity of *Beauveria bassiana* towards Fuller's rose weevil larvae in soil. **Carolyn Mander[1], Trevor Jackson[2] and Bruce Chapman[1]**. [1]Bio-Protection and Ecology Division, PO Box 84, Lincoln University, Canterbury, New Zealand; [2]AgResearch, PO Box 60, Lincoln, Canterbury, New Zealand

15:45 **STU** Variation in carbendazim resistance and ovicidal activity of *Paecilomyces fumosoroseus* strains against *Tetranychus cinnabarinus*. **Wei-Bing Shi[1] and Ming-Guang Feng[1, 2]**. [1]Institute of Insect Science, College of Agriculture and Biotechnology, Hangzhou, Zhejiang, 310029, China; [2]Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, 310058, China

14:00-16:00 Wednesday ***Multifunctional Hall***
Contributed Papers: Viruses 4
Moderators: Robert R Granados and Yi Pang

14:00 **STU** Identification and analysis of a viral-like chitinase gene isolated from *Spodoptera exigua*. **William Ian Tyne, Xiahong Yu and Robert D Possee**. Centre for Ecology and Hydrology, Oxford, CEH Oxford, Mansfield Road, Oxford, United Kingdom, OX1 3SR

14:15 Insect cell culture as protein factories: progress and challenges. **Robert R Granados[1] and Guoxun Li[2].** [1]Boyce Thompson Institute, Tower Road, Cornell Univ., Ithaca, NY 14853-1801; [2]Liayang Agricultural College, Qingdao, Shandong Province, 266109, P.R. China

14:30 Advanced baculovirus expression vectors enabling easy and fast purification of recombinant proteins**. Jae Young Choi[1], Yang-Su Kim[1], Heekyu Choi[1], Jong Yul Roh[1], Joong Nam Kang[1], Yong Wang[1], Soo Dong Woo[2], Byung Rae Jin[3] and Yeon Ho Je[1]**. [1]School of Agricultural Biotechnology, Seoul National University, Seoul 151-742, Korea; [2]College of Agriculture, Life Sciences, Chungbuk National University, Cheongju 361-763, Korea; [3]College of Natural Resources and Life Science, Dong-A University, Busan 604-714, Korea

14:45 The establishment of a controllable expression vector system in baculovirus. **Jui-Ching Wang[1] and Yu-Chan Chao[1,2].** [1]Institute of Molecular Biology, Academia Sinica, No. 128, Sec. 2, Academia Rd. Nankang, Taipei 115, Taiwan, ROC; [2]College of Life Sciences, National Chung Hsing University, Taichung 40227, Taiwan, ROC

15:00 Antiviral effect of extracts of Spondias mombin and Newbouladi laevis on the infectivity of cowpea aphid borne mosaic virus (CABMV) genus potyvirus. **Chinwe C. Ukoha[1], Godfrey E. Ezeifeka[2] and Chinyere N. Umeaku[1].** [1]Dept. of Microbiology, Anambra State, University of Science and Technology, Uli, Nigeria; [2] Dept. of Applied Microbiology & Brewing,, Nnamdi Azikiwe University, Awka, Nigeria.

15:15 **STU** A Cell Line (NTU-MV) established from *Maruca vitrata* (Lepidoptera: Pyralidae): characterization, viral susceptibility, and polyhedra production**. Shih Chia Yeh[1], Song Tay Lee[2], Chih Yu Wu[1] and Chung Hsiung Wang[1].** [1]Department of Entomology, National Taiwan University, Room 105, No. 27, Lane 113, Sec. 4, Roosevelt Rd., Taipei, Taiwan

(ROC) 106; [2]Department of Biotechnology, Southern Taiwan University of Technology, No.1,Nantai St,Yung-Kang City, Tainan Taiwan 710 Roc

15:30 **STU** Study the infectivity of budded viruses of wild type and recombinant HearNPVs by quantitative PCR. **Huiyuan Wang, Manli Wang, Wentao Dai, Fei Deng, Zhihong Hu and Hualin Wang**. [1]State Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, Hubei, 430071, P. R. China

15:45 A cytoplasmic polyhedrosis virus isolated from the pine processionary caterpillar, *Thaumetopoea pityocampa***. Ikbal Agah Ince, Remziye Nalcacioglu, Ismail Demir and Zihni Demirbag.** [1]Karadeniz Technical University, Department of Biology, Faculty of Arts and Sciences, Karadeniz Technical University, 61080, Trabzon, Turkey

16:00-16:30 Wednesday, **Coffee Break**
Setting up posters *Meeting Center*

16:30-18:30 Wednesday *Meeting Center*
Poster Session II

Bacteria II

BP18 Cloning and characterization of the STAT gene from *Hyphantria cunea*. **Hong Ja Kim[1], Yong Min Kwon[1], Yong Il Kim[1], Yeon Soo Han[2], In Hee Lee[3], Beong Rae Jin[4], Young Jin Kang[5] and Sook Jae Seo[1].** [1]Division of Applied Life Science, Gyeongsang National University, Jinju, Gyeongnam, 660-701, Korea; [2]Department of Agricultural Biology, Chonnam National University, Bukgu, Gwangju, 500-757, Korea; [3]Department of Bio-Technology, Hoseo University, Asan, Chungnam, 336-795, Korea; [4]College of Natural Resources and Life Science, Dong-A University, Busan, 604-714, Korea; [5]Department of Pharmacology, Yeungnam University, Gyeongsan, Gyeongbuk, 712-749, Korea

BP19 Comparative analysis of two attacin genes of *Hyphantria cunea*. **Yong Min Kwon[1], Hong Ja Kim[1], Yong Il Kim[1], Yeon Soo Han[2], In Hee Lee[3], Young Jin Kang[4], Hyang Mi Cheon[1] and Sook Jae Seo[1].** [1]Division of Applied Life Science, Gyeongsang National University, Jinju, Gyeongnam, 660-701, Korea; [2]Department of Agricultural Biology, Chonnam National University, Gwangju, 500-757, Korea; [3]Department of Bio-Technology, Hoseo University, Asan, Chungnam, 336-795, Korea; [4]Department of pharmacology, Yeungnam University, Gyeongsan, Gyeongbuk; [5]Southern Forest Research Center, Korea Forest Research Institute, Jinju, Gyeongnam, 660-300, Korea

BP20 Migration of *Bacillus thuringiensis* towards bean leaves. **Pau Maduell[1,2], Gemma Armengol[1], Montserrat Llagostera[2], Steve Lindow[3] and Sergio Orduz[1,4].** [1]Biotechnology and Biological Control Unit, Corporación para Investigaciones Biológicas, Carrera 72A No. 78B-141, Medellin, Colombia; [2]Microbiology Unit, Department of Genetics and Microbiology, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain; [3]Department of Plant and Microbial Biology, University of California, Berkeley, California; [4]Facultad de Ciencias, Universidad Nacional de Colombia sede Medellín, Medellín, Colombia

BP21 A Mini-Replicon from pBtoxis of *Bacillus thuringiensis* subsp. *Israelensis*. **Mujin Tang[1], Dennis K Bideshi[1], Hyun-Woo Park[2] and Brian A Federici[1].** [1]Department of Entomology, University of California, Riverside, Riverside, California, USA, 92521; [2]John A. Mulrennan, Sr., Public Health Entomology Research and Education Center, Florida A & M University, Panama City, Florida, USA, 32405

BP22 Trisodium citrate influenced productions of thuringiensin, PHB and heat in cultivation of *Bacillus thuringiensis* YBT-032 cells. **Zhi Wang[1], Shouwen Chen[1], Jun Yao[2], Ziniu Yu[1].** [1]Huazhong Agricultural University, College of Life Science and Technology, State Key Laboratory of Agricultural Microbiology, National Engineering Research Center for Microbial Pesticides, Wuhan, 430070, P.R. China; [2]China University of Geosciences, School of Environmental Studies, Wuhan, 430074, P.R. China

BP23 Cloning a Novel Crystal Protein Gene from a "non-insectidical" Bacillus thuringiensis strain YBT978. **Zhenyu Zhang, Suxia Guo, Ziniu Yu, Ming Sun.** State Key Laboratory of Agricultural Microbiology, College of Life Science and Technology, Huazhong Agricultural University, Wuhan 430070, P. R. China

BP24 Synergism between Thuringiensin and Cry1Aa, Cry1Ac, Cry1B and Cry1C against *Helicoverpa armigera* and *Spodoptera exigua*. **Dong Chunming, Sun Ming, Ruan Lifang, Yu Ziniu***. State Key Laboratory of

Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070, China; National Engineering Research Centre for Microbial Pesticides, Huazhong Agricultural University, Wuhan 430070, China

BP25 Characterization of the replication of plasmid pBMB2062 from the YBT1520 strain of *Bacillus thuringiensis*. **Xiaojin Liu, Ming Sun, Ziniu Yu.** The State Key Laboratory of Agricultural Microbiology Huazhong Agricultural University, Wuhan 430070, P.R.China

BP26 Physiological Characterization of Accumulated Poly-β-hydroxybutyrate(PHB) in *Bacillus thuringiensis*. **Chen Deju, Yan Jin, Chen Shouwen, Sun Ming, Yu Ziniu**. State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan, China

BP27 Protective Effect of Poly (γ-glutamic acid) on *Bacillus thuringiensis* Active Components Against High Temperature and UV Irradiation**. Wu Guangtao, Ji Zhixia, Chen Shouwen* and Yu Ziniu**. State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan, China

BP28 Study on reconstruction of flexible polypeptide linker about scFv **Zheng Zeng-jie and Wang Shi-hua** Key Laboratory of Biopesticide and Chemical Biology, Ministry of Education, and College of Life Science, Fujian Agriculture and Forestry University, Fuzhou, China, 350002

BP29 **STU** *Spodoptera exigua* selection using a marginally toxic Cry protein provided a wide range of toxin resistance. **Patricia Hernadez-Martinez and Baltasar Escriche.** Departmento de Genetica, Universitat de Valencia, Dr. Moliner 50, 46100-Burjassot (Valencia), Spain

BP30 **STU** Characterization of Mn superoxide dismutase cDNA from *Hyphantria cunea* . **Yong Il Kim[1], Hong Ja Kim[1], Yong Min Kwon[1], Yeon Soo Han[2], In Hee Lee[3], Yong Jin Kang[4] and Sook Jae Seo[1].** [1]Division of Applied Life Science, Gyeongsang National University, Jinju, Gyeongnam, 660-701, Korea; [2]Department of Agricultural Biology, Chonnam National University, Buk-gu, Gwanguj, 500-757, Korea; [3]Department of Bio-Technology, Chonnam National University, Asan, Chungnam, 336-795, Korea; [4]Department of Pharmacology, Yeungnam University, Gyeongsan, Gyeongbuk, 712-749, Korea

BP31 **STU** **Transferrin inhibits stress-induced apoptosis in a beetle*.** Bo Yeon Kim[1], Hung Dae Sohn[1], Byung Rae Jin[1], Kwang Sik Lee[1], Hong Ja Kim[2], Sook Jae Seo[2], Yong Soo Choi[1], Young Moo Choo[1], Young Joo Kim[1], Yeon Ho Je[3] and Doh Hoon Kim[1]. [1]College of Natural Resources and Life Science, Dong-A university, Busan, 604-714, Korea; [2]Division of Applied Life Science, Gyeongsang National University, Jinju, Korea; [3]School of Agricutural Biotechnology, Seoul National University, Seoul, Korea

BP32 **STU** Plasmid transfer among *Bacillus cereus* group strains within lepidopteran larvae. **Yuan Yongming[1], Hu Xiaomin[1], Zheng Dasheng[1], Bjarne Munk Hansen[2], Yuan Zhiming[1]**. [1]Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, China; [2]National Environmental Research Institute, 4000 Roskilde, Denmark

Study on the isolation of endogenous fungus producing anti-termite compounds from *Juniperus virginiana L.* and *Chamaecyparis lawsoniana*（*A.Murr.*）*Parl.* **Dong-Po Zhou[1], Wen-Xiang Ping[1], Kai Zhao[1], Qiang Li[1], Jun Liu[1], Ya-Hong Han[1], Chung Y. Hse[2].** College of life Science[1], Hei Longjiang University, 74 Xuefu Road Harbin 150080 China; Southern Research Station Headquarters[2], Forest Service, United States Department of Agriculture, Louisiana 70803

Location of the *thu* gene responsible for synthesis of extrotoxin (thuringiensin) in *Bacillus thuringiensis* CT43**. Lifang Ruan, Chunming Dong, Xiaoyan Liu, Ming Sun, Ziniu Yu.** State Key Laboratory of Agricultural Microbiology, College of Life Science and Technology, Huazhong Agricultural University, Wuhan 430070, Hubei, People's Republic of China

Fungi II

FP11 Identification of insect pathogenic fungus, *Cordyceps sphecocephala*, and its cultivation on host insect, drone of honey bee(*Apis mellifera*). **Sung Hee Nam[1,2], Chun Ru Li[1], In Pyo Hong[2], Kyu Byoung Sung[2], Mei-Zhen Fan[1] and Zeng-Zhi Li[1].** [1]Anhui Agricultural University, Hefei, Anhui 230036, P.R.China; [2]National Institute of Agricultural Science and Technology, R.D.A., 441-100, Sedun-Dong,

Suwon, Korea

FP12 Isolation and PCR-based detection of Entomopathogenic Fungus, *Ascosphaera apis* from honey bee (*Apis mellifera*) larva and their breeding environment. **Sung Hee Nam[1,2], Myeong Lyeol Lee[2], Ji Young Choi[2], Young Soo Kim[2], Chun-Ru Li[1], Mei-Zhen Fan[1] and Zeng-Zhi Li[1]**. [1]Anhui Agricultural University, Hefei, Anhui 230036, P.R.China; [2]National Institute of Agricultural Science and Technology, R.D.A., 441-100, Sedun-Dong, Suwon, Korea

FP13 Fungi associated with Hemlock Woolly Adelgid, *Adelges tsugae*, and development of the most active isolates for pest control. **Bruce L. Parker, Margaret Skinner, Svetlana Y. Gouli, Brenton H. Teillon, Vladimir V. Gouli and Cheryl Frank**. Entomology Research Laboratory, University of Vermont, 661 Spear Street, Burlington, Vermont 05405-0105, USA

FP14 Understanding and assessing the complex of fungi impacting the Elongate Hemlock Scale, *Fiorinia externa*, in New England. **Svetlana Y. Gouli, Bruce L. Parker, Margaret Skinner, Rosanna Giordano, Jose Marselino and Vladimir V. Gouli**. Entomological Research Laboratory, University of Vermont, 661 Spear Street, Burlington, Vermont 05405-0105, USA

FP15 Genetic diversity of Japanese isolates of *Verticillium lecanii* (*Lecanicillium* spp.). **Masanori Koike, Midori Sugimoto and Daigo Aiuchi.** Department of Agro-environmental Science, Obihiro University of Agriculture & Veterinary Medicine, Hokkaido 080-8555, Japan

FP16 Host range of a fungus associated with epizootic in elongate hemlock scale. **Jose Marselino, Rosanna Giordano, Svetlana Y. Gouli and Vladimir V. Gouli.** Entomological Research Laboratory, University of Vermont, 661 Spear Street, Burlington, Vermont 05405-0105, USA

FP17 Studies on the infective characters of Nomuraea viridulus**. Ho, Shu -Yi[1] and Wen -Feng Hsiao[2].** [1]Graduate Institute of Biopharmaceutics, National Chiayi Unviersity, Chiayi, Taiwan; [2]Department of Bioresources,, National Chiayi Unviersity, Chiayi, Taiwan

FP18 STU Thermal adaptation of *Metarhizium anisopliae* strains in association with components of their cell wall hydrophobin-like proteins. **Jun Li and Ming-Guang Feng.** Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, 310058, China

FP19 STU Variable benzimidazole resistance and thermotolerance of *Beauveria bassiana* are associated with mutations of its beta-tubulin sequence. **Gen Zou and Ming-Guang Feng.** Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, 310058, China

FP20 STU Isolation of ESTs expressed by *Metarhizium anisopliae* in the invasion process of *Plutella xylostella* using RDA. **Israel E Padilla-Guerrero[1], Dessire Vargas-Gamez[1], Jose M Zamudio Arroyo[1], Angélica González-Hernández[1], Eduardo Salazar-Solis[2] and Juan Carlos Torres-Guzmán[1].** [1]Instituto de Investigación en Biología Experimental. Facultad de Quimica. Universidad de Guanajuato, Ap. Postal 187, Noria Alta s/n, Guanajuato, Gto., México; [2]2Instituto de Ciencias Agricolas. Universidad de Guanajuato, Ap. Postal 311, C. P. 36500, Irapuato , Gto., México

FP21 STU Pathogenicity of hybrid strains of *Verticillium lecanii* (*Lecanicillium spp.*) to eggs of the soybean cyst nematode. **Ryoji Shinya[1], Ai Watanabe[1], Daigo Aiuchi[1], Masanori Koike[1] and Atsuhiko Kushida[2].** [1]Department of Agro-Environmental Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan; [2]National Agricultural Research Center for Hokkaido, Shinsei, Hokkaido 080-0071, Japan

Histopathological studies of spruce budworm infected by *Hirsutella longicolla* and *Toypocladim niveum*. **Liande Wang[1,2], Minsheng You[1], Jian Huang[2], Xiong Guan[2] and Doug Strongman[3].** ([1]Institute of Applied Ecology, Fujian Agriculture & Forestry University, Fuzhou, 350002, P.R. China; [2]& Key Laboratory of Biopesticide and Chemical Biology, MOE.,Fujian Agriculture & Forestry University, Fuzhou, 350002, P.R. China; [3]Dept. of Biology, Saint Mary's University, Halifax, B3H3C3, Canada)

Survival of *Beauveria bassiana* on cadavers of *Monochamus alternatus* adults. **Xue-You HE[1] and Shimazu Mitsuaki[2].** ([1]Fujian Academy of Forestry, Fuzhou, Fujian, 350012, P.R.China;

[2]Forestry and Forest Products Research Institute, Tsukuba, Ibaraki 305-8687, Japan)

Microbial Control II

MCP14 Characterisation of a *Bacillus thuringiensis* isolate that is highly toxic to *Eldana saccharina* (Lepidoptera: Pyralidae). **Gustav Bouwer**. School of Molecular and Cell Biology, University of the Witwatersrand, Johannesburg, Private Bag 3, Wits 2050, South Africa

MCP15 Experimental use of *Epinotia aporema* granulovirus (EpapGV) in Argentina. **Marina Biedma[1], Leticia Ferrelli[1], Ricardo Salvador[1,2], Victor Merlo[2], Graciela Quintana[2], Victor Romanowski[1] and Alicia Sciocco-Cap[2]**. [1]IBBM, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, 115 y 49, (1900) La Plata, Argentina; [2]IMYZA-CICVyA, Instituto Nacional de Tecnología Agropecuaria (INTA), CC 25, (1712) Castelar, Buenos Aires , Argentina

MCP16 Viability studies for a Brazilian isolate of SfMNPV production in suspension cultures. **Andrea Farias de Almeida[1], Gorete Ribeiro de Macedo[1], Zilda Maria A. Ribeiro[2], Maria Elita B. de Castro[2], Marlinda Lobo de Souza[2] and Márcia Regina da Silva Pedrini[1].** [1]PPGEQ/Federal University of Rio Grande do Norte, Natal, RN, Brazil; [2]EMBRAPA Genetic Resources and Biotechnology, Brasília-DF, Brazil

MCP17 Kaolinite effect on spouted bed drying process and baculovirus biopesticide hygroscopicity. **Yvson Costa e Silva[1], Wilton Menezes Andrade Jr.[1], Carlos Henrique Xavier[1], Flávio Moscardi[2], Marlinda Lobo de Souza[3], Maria de Fátima Dantas de Medeiros[1] and Márcia Regina Da Silva Pedrini[1]**. [1]PPGEQ/Universidade Federal do Rio Grande do Norte, Natal, RN, Brazil; [2]EMBRAPA Soya, Londrina, PR, Brazil; [3]EMBRAPA Genetic Resources and Biotechnology, Brasília, DF, Brazil

MCP18 The use of a nucleopolyhedrovirus for the suppression of its natural host, the balsam fir sawfly (*Neodiprion abietis* Harris). **Roger W Graves[1], Dan T Quiring[1] and Christopher J Lucarotti[1,2]**. [1]Faculty of Forestry and Environmental Management, P.O. Box 44555, University of New Brunswick, Fredericton, New Brunswick, E3B 6C2, Canada; [2]Canadian Forest Service, Atlantic Forestry Centre, P.O. Box 4000, Fredericton, New Brunswick, E3B 5P7, Canada

MCP19 Susceptibility of *Pyrausta sticticalis* to *Bacillus thuringiensis*-based formulations depending on host plant. **Margarita Shternshis, Irina Andreeva and Bibinur Baitasova**. Department for Biological Control, State Agrarian University, Dobrolubov 160, 630039, Novosibirsk, Russia

MCP20 Development of PCR-RFLP approach using three chitinase genes for the genetic characterization and identification of *Metarhizium* strains. **Vandana Ghormade, Franco Widmer and Juerg Enkerli.** Molecular Ecology, Agroscope FAL Reckenholz, Swiss Federal Research Station for Agroecology and Agriculture, Reckenholzstrasse 191, CH-8046 Zürich, Switzerland

MCP21 Potential of *Lecanicillium* species for dual microbial control of aphids and the cucumber powdery mildew fungus, *Sphaerotheca fuliginea*. **Jeong Jun Kim[1], Mark S Goettel[1] and Dave Gillespie[2]**. [1]Lethbridge Research Centre, 5403 1st Ave S., Lethbridge, AB, Canada, T1j 4B1; [2]Pacific Agriculture Research Centre, 6947 Number 7 Highway, Agassiz, BC V0M 1A0

MCP22 Impact of SDS in baculovirus occlusion body purification buffer on biological activity. **Hilal Susurluk[1], Umut Toprak[1,2] , Oktay Gürkan[1].** [1]University of Ankara, Faculty of Agriculture, Department of Plant Protection, 06110 Dışkapı/Ankara Turkey; [2]Agriculture and Agri-Food Canada, Saskatoon Research Centre, Saskatoon, 107 Science Place, Saskatoon, Saskatchewan, Canada S7N 0X2

MCP23 **STU** The distribution and expression of chitinolytic enzymes from *Bacillus thuringiensis*. **Wei Lu, Qiuming Zhao, Yanling Chen, Qingqing Chen, Jun Cai and Yuehua Chen.** Key Laboratory of Microbial Functional Genomics, Department of Microbiology, College of Life Sciences, Nankai University, Tianjin, 300071, China

MCP24 **STU** Effects of sublethal nucleopolyhedrovirus infection on the metabolic rate of *Helicoverpa armigera* (Lepidoptera: Noctuidae). **Luisa Nardini[1], Gustav Bouwer[1] and Frances D Duncan[2]**. [1]School of Molecular and Cell Biology, University of the Witwatersrand, Private Bag 3,

2030, Wits, Johannesburg, South Africa; [2]School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Private Bag 3, 2030, Wits, Johannesburg, South Africa

MCP25 STU Isolation and Characterization of Novel Insecticidal *cry1*-Type Genes from *Bacillus thuringiensis* K1 Strains. **MingShun Li[1], Zi Niu Yu[1], Jae Young Choi[2], Jong Yul Roh[2], Hee Jin Shim[2], Joong Nam Kang[2], Yong Wang[2], Yang-Su Kim[2], Hee Kyu Choi[2] and Yeon Ho Je[2].** [1]State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Lion Street, Hongshan district, Wuhan, China, 430070; [2]School of Agricultural Biotechnology, College of Agriculture and Life Sciences, Seoul National University, San 56-1, Shillimdong, Gwanakgu, Seoul 151-742, Korea

MCP26 STU Cloning and heterogeneous expression of a *mel* gene from a wild-type melanin-yielding *Bacillus cereus* strain Bt799. **Zhang Jingtao, Yan Jianping, Zheng Dasheng, Cai Quanxin, Yuan Zhiming.** Wuhan institute of Virology, Chinese Academy of Sciences, Wuhan 430071 , China

MCP27 Study on the Bioactivities of Plant Extracts against *Lasioderma serricorne*. **Zhao Haigang[1], Song Jizhen[2], Xie Jianping[2], Je Yeon Ho[3], Jin Byung Rae[4], Li Jianhong[1]*.** 1.College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, China; 2. Zhengzhou Tobacco Research Institute, China Tobacco General Company, Zhengzhou 450001, China; 3. School of Agricultural Biotechnology, Seoul National University, Seoul 151-742, Korea; 4. College of Natural Resources and Life Science, Dong-A University, Busan 604-714, Korea

Identification of an insect intestinal mucin from the Lepidopteran peritrophic membrane of *Helicoverpa armigera*. **Junping Wang[1], Fan Yang[1], Guoxun Li[1], Shupeng Gai[1], Ping Wang[2], Changyou Li[1], Hongxu Zhou and Linyou Cheng[1].** [1]Laiyang agricultural College, Qingdao 266109, P.R. China; [2]Department of Plant Protection, Laiyang agricultural College, Qingdao 266109, P.R. China

Nematodes

NP1 Gene clone of insecticidal protein from *Xenorhabdus nematophila* HB310. **Qinying Wang, Ping Song, Ziyan Nangong and Long Cui.** College of Plant Protection, Agricultural University of Hebei, Biocontrol Centre of Plant Diseases and Plant Pests of Hebei Province, Baoding, Hebei 071001, P. R. China

NP2 Insecticidal activity and midgut histopathological effects of *Xenorhabdus nematophila* on *Pontia daplidice*. **Qinying Wang, Ping Song, Jun Yang, Ziyan Nangong and Long Cui.** College of Plant Protection, Agricultural University of Hebei, Biocontrol Centre of Plant Diseases and Plant Pests of Hebei Province, Baoding, Hebei 071001, P. R. China

NP3 Insecticidal activity of the toxins from entomopathogenic nematode symbiotic bacteria**. Huan Wang[1], Bin Cong[*2] and hui Dong[2].** [1]College of biological science and technology ，Shenyang Agri. Univ, Shenyang，Liaoning， 110161，China; [2] College of Plant Protection，Shenyang Agri. Univ, Shenyang，Liaoning，110161 ， China

NP4 STU The application of *Ovomermis sinensis* in cooperation with Bt (Bacillus thurigiensis) to the control of *Helicoverpa armigera*. **Jiang-Yi Wang, Hua-Mei Yue, Guo-Xiu Wang and Hong-Tao Wang.** College of Life Science, Central China Normal University, Wuhan, 430079, China

Viruses II

VP18 Genome sequence and genome organization analyses of *Trichoplusia ni ascovirus 2c (Ascoviridae)*. **Lihua Wang[1], Jianli Xue[1], Basil M. Arif[2] and Xiao-Wen Cheng[1].** [1]Department of Microbiology, 32 Pearson Hall, Miami University, Oxford, Ohio, 45056 USA; [2]Great Lakes Forestry Center, 1219 Queen St. E., Sault Ste. Marie, Ontario P6A 2E5 Canada

VP19 Presence of nuclear polyhedrosis virus in *Neodiprion sertifer* populations in Latvia. **Liga Jankevica.** Department of Experimental Entomology, Institute of Biology, University of Latvia, Miera iela 3, Salaspils, Riga district, LV 2169, Latvia

VP20 *Choristoneura fumiferana* defective nucleopolyhedrovirus spindlin is a superior model for studying baculovirus GP37-type proteins. **Cailing Liu[1,2], Peter J Krell[2] and Basil M Arif[1].** [1]Great Lakes Forestry Centre, Sault Ste. Marie, Ontario, Canada P6A 2E5; [2]Department of Microbiology, University of Guelph, Guelph, Ontario, Canada N1G 2W1

VP21 Sequence analysis of the genome of *Maruca vitrata* multicapsid nucleopolyhedrovirus (MaviNPV). **Chih-Yu Wu[1], Song-Tay Lee[2] and Chung-Hsiung Wang[1]**. [1]Department of Entomology, National Taiwan, 106, Taipei, Taiwan; [2]Department of Biotechnology, Southern Taiwan University of Technology, 710, Tainan, Taiwan

VP22 Replication of *Bombyx mori* nucleopolyhedrovirus in nonpermissive insect cell lines. **Soo-Dong Woo[1], Yeon-Ho Je[2] and Byung-Rae Jin[3]**. [1]Department of Plant Medicine, Chungbuk National University, Cheongju 361-763, Korea; [2]School of Agricultural Biotechnology, College of Agriculture & Life Sciences, Seoul National University, Seoul 151-742, Korea; [3]College of Natural Resources and Life Science, Dong-A University, Busan 604-714, Korea

VP23 Sequence analysis on the genome of the *Chroristoneura biennis* entomopoxvirus. **Zhen Li[1], Peter Krell[2], Christopher Lucarott[3] and Basil Arif[1]**. [1]Laboratory for Molecular Virology, Great Lakes Forestry CentreLakes Forestry, Sault Ste. Marie, Ontario, P6A 2E5 , Canada; [2]Dept. of Molecular Cellular Biology, University of Guelph, Guelph, Ontario, N1G 2W1, Canada; [3]Atlantic Forestry Centre, Fredericton, New Brunswick, E3B 5P7, Canada

VP24 Characterization of a new baculovirus isolated from *Iragoides fasciata* and its infection of TN-5B1-4 cell line. **Li-Rong Yang, Zhang-Nv Yang and Chuan-Xi Zhang**. Institute of Insect Sciences, Zhejiang University, Hangzhou, 310029, China

VP25 Salivary gland hyperplasia virus of the house fly, *Musca domestica* (Diptera: Muscidae). **Christopher J. Geden[1], Verena Ulricke Blaeske-Lietze[2] and Drion G. Boucias[2].** [1]USDA, ARS, CMAVE, 1600 SW 23rd Dr., Gainesville, FL 32607 USA; [2]Univ. of Florida Dept. of Entomol. and Nematol., Building 970, Natural Area Dr, Gainesville, FL 32611 USA

VP26 Ascertaining the efficiency of granulovirus based bio-pesticides in *Cydia pomonella* and *Adoxophyes orana* control, using PCR based techniques**. Jiban Kumar Kundu, Jitka Stará, Dita Bohdanecká and František Kocourek.** Division of Plant Medicine, Research Institute of Crop Production, Drnovská 507, Prague 6, 161 06 Czech Republic

VP27 Promoter analysis of *Bombyx mori* nucleopolyhedrovirus Ubiquitin gene. **Xu'ai Lin, Yin Chen, Yongzhu Yi and Zhifang Zhang**. Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing, 100081, China

VP28 **STU** Identification of baculovirus genes for activation of the *hhi1* promoter of Hz-1 virus. **Yueh-Lung Wu[1], Song-Tay Lee[2] and Yu-Chan Chao[3].** [1]Institute of biotechnology, National Cheng Kung University, No.1, Ta-Hsueh Road, Tainan 701, Taiwan; [2]Department of Biotechnology, Southern Taiwan University of Technology, No.1,Nantai St,Yung-Kang City,Tainan Taiwan 710; [3]Institute of Molecular Biology, Academia Sinica, 128 Sec.2, Academia Rd, NanKang, Taipei 115 Taiwan

VP29 **STU** Cloning of a gene encoding *Lymantria xylina* nucleopolyhedrovirus fusion protein and its expression in LD cells. **Hsiu-Wen Pien, Chu-Min Lo and Chung-Hsiung Wang.** Department of Entomology,National Taiwan University, No.1, 4, Sec, Roosevelt Road, Taipei, Taiwan 10617, R.O.C.

VP30 **STU** Influence of fetal bovine serum on the growth of insect cell cultures and baculovirus. **Jae-Kyung Lee and Soo-Dong Woo.** Department of Plant Medicine, College of Agriculture, Chungbuk National University, Cheongju 361-763, Korea

VP31 *Spodoptera litura* multicapsid nucleopolyhedrovirus inhibits *Microplitis bicoloratus* polydnavirus-induced host granulocytes apoptosis. **Kaijun Luo[1,2] and Yi Pang[1].** [1]State key Laboratory of Biocontrol & Institute of Entomology, Sun Yat-sen (Zhongshan), Guangzhou 510275, P. R. China; [2]Agriculture Environment and Resource Research Institute, Yunnan Academy of Agriculture Sciences, Kunming 650205, P.R. China

VP32 **STU** Expression of a *Microplitis bicoloratus* polydnavirus-encoded protein causes disruption of actin cytoskeleton in lepidopteran insect cells. **Kaijun Luo[1,2] and Yi Pang[1]**. [1]State key Laboratory of Biocontrol & Institute of Entomology, Sun Yat-sen (Zhongshan) university, Guangzhou 510275, P. R. China; [2]Agriculture Environment and Resource Research Institute, Yunnan Academy of Agriculture Sciences, Kunming 650205, P.R. China

VP33 **STU** Genome analysis of *Cotesia plutellae* bracovirus. **Yang-Su Kim[1], Jae Young Choi[1], Jong Yul Roh[1], Joong Nam Kang[1], Yong Wang[1], Heekyu Choi[1], Soo Dong Woo[2], Byung Rae Jin[3] and Yeon Ho Je[1]**. [1]School of Agricultural Biotechnology, Seoul National University, Seoul 151-742, Korea; [2]College of Agriculture, Life Sciences, Chungbuk National University, Cheongju 361-763, Korea; [3]College of Natural Resources and Life Science, Dong-A University, Busan 604-714, Korea

VP34 **STU** The evolutionary analysis of *baculoviruses* based on variety evolution rates and function constraint. **Yue Jiang, Fei Deng, Zhihong Hu and Hualin Wang**. State Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, Hubei, 430071, P. R. China

VP35 Putative promoters isolated from infectious hypodermal and hematopoietic necrosis virus (IHHNV) of shrimp direct expression of a reporter gene in bacteria, insects and fish cells, and shrimp. **Arun K. Dhar1, Nikolai A. Van Beek, Robert A. Bullis, Robert J. Moss and Thomas C. Allnutt.** Advanced Bionutrition, 7155 Columbia Gateway Dr., Ste H, Columbia, MD 21046, USA

18:30-20:00 **Dinner** *Haitiangong Dining-Room*

20:00-21:00 Wedensday *Multifunctioanl Hall*
Microbial Control Business Meeting

21:00-22:00 Wedensday *Multifunctioanl Hall*
Workshop: New Products and Upgrades for Microbial Control: an Industry Update (Microbial Control Division)
Convenors: Jeff Lord, Dr Ziwen Yang and Michael Brownbridge

Thursday, August 31

8:00-10:00 Thursday ***Meeting Center***
Symposium: Bt- performance enhancement (Bacteria Division)
Convenor: Yu Cheng Zhu

8:00 Synergistic Effect of Inorganic salts to Improve the Biological activity of *Bacillus thuringiensis* subsp. *aizawai* NT0423 against *Plutella xylostella*. **Jae Su Kim**. Dongbu Hannong Co. Ltd., Daejon 305-708, Korea; 2Seoul National University, Seoul 151-742, Korea1.

8:30 A novel function of *Bacillus thuringiensis* Cry1C toxin on insect peritrophique matrix. **Christina Nielsen-LeRoux[1,2], Christophe Buisson[1] and Didier Lereclus[1]**. [1]INRA, Unité Génétique Microbienne et Environnement, INRA, la Minière, 78285 Guyancourt; [2]Pasteur Institute, Département de Microbiologie, Institut Pasteur, 75724 Paris Cedex 15, France

9:00 Potential use of proteinase inhibitors for insect control and Bt resistance management. **Yu Cheng Zhu**. USDA-ARS-JWDSRC, PO Box 346, 141 Experiment Station Road, Stoneville, Mississippi 38776, USA

9:30 Improved genetically engineered bacteria for controlling mosquito larvae. **B. Federici, D. Bideshi, H. W. Park, J. Johnson, M. Tang, M. Wirth, Y. Sakano**. Department of Entomology and Interdepartmental Graduate Programs in Genetics and Microbiology, University of California, Riverside, California 92521

8:00-10:00 Thursday ***Nanyuan Meeting Room***
Contributed Papers: Microbial Control 2
Moderators: Carlos A. Blanco and Bo Liu

8:00 Association of the components of the binary toxin from *bacillus sphaericus* in solution and with model lipid bilayers. **Panadda Boonserm[1], Seangduen Moonsom[1], Chanikarn Boonchoy[2], Boonhiang Promdonkoy[3], Krupakar Parthasarat[4] and Jaume Torres[4]**. [1]Institute of Molecular Biology and Genetics, Mahidol University, Salaya, Phuttamonthol, Nakornpathom 73170, Thailand; [2]Institute of Science and Technology for Research and Development, Mahidol University, Salaya, Phuttamonthol, Nakornpathom 73170, Thailand; [3]National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, 113 Paholyothin Road, Klong 1, Klong Luang, Pathumthani 12120, Thailand; [4]School of Biological Sciences, Nanyang Technological University, 60 Nanyang Drive 637551, Singapore

8:15 Mechanism of *Bacillus brevis* against *Fusarium oxysporum* Schl. and Microcapsule approach for its formulated product. **Lan Jiang-lin, Huang Su-fang, Che jian-mei and Liu Bo***. Biocide Center，Institute of Biotechonology, FAAS, Fuzhou 350003，China

8:30 Single Nucleotide Polymorphisms (SNPs) in Bt Toxin Binding Genes in Natural Populations of

Heliothis virescens. **Omaththage P. Perera and Carlos A. Blanco.** Southern Insect Management Research Unit, USDA-ARS, 141 Experiment Station Road, Stoneville, MS 38776, USA

8:45 STU Secondary structure Analysis of a highly mosquitocidal mutant strain of *Bacillus thuringiensis LDC-9* from Madurai, South India. **Poornima K Kani, Mahalakshmi Ayyasamy, Sujatha Kabilan and Shenbagarathai Rajaiah**. Lady Doak College, PG Department of Zoology & Research Centre, Lady Doak College, Chinnachokikulam, Madurai, Tamilnadu, India-625002

9:00 STU Location and identification of *cry* genes in *Bacillus thuringiensis* strain 4.0718. **Zujiao Fu[1], Yunjun Sun[2], Xuezhi Ding[3], Shengbiao Hu[4], Xiaohong He[5] and Liqiu Xia[*]**. [1]College of life science, College of Life Science, Hunan Normal University, Changsha 410081, P R China

9:15 STU Diversity of *B. thuringiensis* strains from Madurai with insecticidal activity against different mosquito species. **Mahalakshmi Ayyasamy, Poornima K Kani, Sujatha Kabilan and Shenbagarathai Rajaiah.** Lady Doak College, PG Department of Zoology & Research Centre, Lady Doak College, Chinnachokikulam, Madurai, Tamilnadu, India, 625002

9:30 STU Production of thuringiensin by fed-batch culture of *Bacillus thuringiensis* subsp. *darmstadiensis* 032 with an improved pH-control glucose feeding strategy. **Zhou Jing-Wen, Chang Ya-Fei , Yu Zi-Niu, Chen Shou-Wen.** State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070, P.R. China

8:00-10:00 Thursday *Multifunctional Hall*
Contributed Papers: Viruses 5
Moderators: Jim Maruniak and Peter J. Krell

8:00 Identification of Structural Proteins of *Culex nigripalpus* Nucleopolyhedrovirus (CuniNPV) . **Omaththage P. Perera[1], Terry B. Green[2], Stanley M. Stevens, Jr.[3], Susan E. White[2] and James J. Becnel[2].** [1]Southern Insect Management Research Unit, USDA-ARS, Stoneville, MS 38776, USA; [2]Center for Medical, Agricultural, & Veterinary Entomology, USDA-ARS, 1600 SW 23rd Ave., Gainesville, FL 32608, USA; [3]Proteomics Core, Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, FL 32610, USA

8:15 The partial genome sequence of *Oryctes rhinoceros* virus. **Yongjie Wang[1], Monique M. Van Oers[2], Allan M. Crawford[3], Just M. Vlak[2] and Johannes A. Jehle[1].** [1]Laboratory for Biotechnological Crop Protection, Department of Phytopathology, Agricultural Service Centre Palatinate (DLR), 67435 Neustadt an der Weinstrasse, Germany; [2]Laboratory of Virology, Wageningen University, Binnenhaven 11, 6709 PD Wageningen, The Netherlands; [3]AgResearch Invermay Agricultural Centre, Mosgiel, New Zealand

8:30 The genomic sequence of the *Gryllus bimaculatus* virus. **Yongjie Wang[1], Regina G. Kleespies[2], Alois Huger[2] and Johannes A. Jehle[1].** [1]Laboratory for Biotechnological Crop Protection, Department of Phytopathology, Agricultural Service Centre Palatinate (DLR Rheinpfalz), 67435 Neustadt an der Weinstrasse, Germany; [2]Federal Biological Research Center for Agriculture and Forestry, Institute for Biological Control, Heinrichstr. 243, 64287 Darmstadt, Germany

8:45 STU Identification of the Structural Proteins of the Occlusion-derived Virus of HearNPV. **Fei Deng, Ranran Wang, Minggang Fang, Hualin Wang, Xushi Xu, Hanzhong Wang, Xinwen Chen and Zhihong Hu**. State Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, Hubei, 430071, P. R. China

9:00 An investigation on biochemical variation of two isolates of potato tuber moth granulovirus, Phthorimaea operculella granulovirus**. Mohammadreza Rezapanah[1], Amir Amiri Yekta[1] and Ahmad Dezianian[2].** [1]Biological Control Research Department, Plant Pests and Diseases Research Institute, Tehran 19395, Iran; [2]Plant Pests & Diseases Research Dept, Semnan Agricultural & Resources Research Center, Semnan, Iran

9:15 Transcriptional analysis of *Choristoneura fumiferana* nucleopolyhedrovirus (CfMNPV) genes using an oligonucleotide-based DNA microarray. **Dan-Hui Yang[1], Basil M Arif[2] and Peter John Krell[1]**. [1]Department of Molecular and Cellular Biology, University of Guelph, 488 Gordon Street, Guelph, Ontario Canada N1G 2W1; [2]Great Lakes Forestry Centre, 1219 Queen Street East, Sault Ste

Marie, Ontario, Canada, P6A 2E5

9:30 Identification of baculovirus transactivator for early promoters using viral genomic library. **Yin Chen[1,2], Xu'ai Lin[2], Yiyu Lu[1], Yongzhu Yi[2] and Zhifang Zhang[2]**. [1]Virus Reaserch Institute, Zhejiang Provincial Center for Disease Prevention and Control, Hangzhou, Zhejiang, 310009, China; [2]Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing, 100081, China

9:45 **STU** Characterization of a bacmid-derived defective baculovirus with a large deletion in the genome**. Yi Huang[1,3], Minggang Fang[1], Xinwen Chen[1], Ting Li[1], Just M Valk[2], Zhihong Hu[1] and Hanzhong Wang[1].** [1]Wuhan Institute of Virology, Chinese Academy of Sciences, State Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, 430071, People's Republic of China,; [2]Laboratory of Virology, Wageningen University,, Laboratory of Virology, Wageningen University, Binnenhaven 11, 6709 PD Wageningen, The Netherlands; [3]Graduate School of Chinese Academy of Sciences, Graduate School of Chinese Academy of Sciences, Beijing, 100039, People's Republic of China.

10:00-10:15 **Coffee Break**

10:15-10:30 Thurdsay ***Meeting Center***

Lecture: Edward A. Steinhaus, Instigator, Catalyst, and Founder.

Elizabeth W. Davidson. School of Life Sciences, Arizona State University, Tempe, AZ 85287-4501

10:30-12:30 Thurdsay *Meeting Center*

SIP Annual Business Meeting

12:30-14:00 Thurdsay, **Lunch** *Haitiangong Dining-Room*

Student Awards Committee Meeting

14:00-16:00 Thurdsay ***Meeting Center***

Symposium: Nematodes and Bacteria: from Pathogenicity to Mutualism (Cross-Divisional)

Convenors: Raffi Aroian and Parwinder Grewal

14:00 Bacterial toxin - nematode interactions: using Bt toxins to control parasitic nematodes. **Anderson Tan, Raffi Aroian, Xiang-Qian Li.** University of California, San Diego, 9500 Gilman Drive, Mail Code 0349 La Jolla, CA 92093-0349 USA

14:30 Elucidating the molecular mechanisms of bacteria-host interactions using the *C. elegans* pathogenesis model. **Man-Wah Tan.** Departments of Genetics, and of Microbiology and Immunology, Stanford University School of Medicine, Stanford CA 94305-5120, USA

15:00 Virulence of *Moraxella osloensis*, a bacterium associated with the slug-parasitic nematode *Phasmarhabditis hermaphrodita*, to the slug *Deroceras reticulatum.* **P. S. Grewal.** Department of Entomology, Ohio State University, Wooster, OH 44691, USA

14:00-16:00 Thurdsay ***Nanyuan Meeting Room***

Contributed Papers: Microbial Control 3

Moderators: Roy Bateman and Ping Cheng

14:00 Design and evaluation of the 'MycoHarvester' for separation of powdery fungal conidia from substrates. **Roy Bateman, Sylvia Mermelstein, Belinda Luke, Emma Thompson and Adrian Arnold.** IPARC, Imperial College London, Silwood Park Campus, Ascot, Berks, SL5 7PY, UK

14:15 The effect of water quantity, added during mass production, on *Beauveria bassiana* conidia yield and pathogenicity against *Oryzaephilus surinamensis*. **Belinda Luke[1] and Maureen Wakefield[2].** [1] CABI, Silwood Park, Buckhurst Road, Ascot , Berkshire, SL5 7TA UK; [2]Central Science Laboratory, Sand Hutton, York, UK YO41 1LZ

14:30 Development of a mycoinsecticide for the control of *Helicoverpa armigera* infestation on pulses: Significance of back-up strains in the commercial production. **S. Chavan[1], V. Ghormade[1], G. Kulkarni[2], A. Gondhalekar[1], A. Rajendran[1], M. Taranekar[1], S. Kulkarni[1], Y. Shauche[2] and M.V. Deshpande[1]**. [1]Biochemical Sciences Division, National Chemical Laboratory, Pune –411008, India; [2]Molecular Biology Unit, National Centre for Cell Science, Pune-411007, India

14:45 Comparison of two different methods for quality of spray deposits after application of fungal formulations. **Vladimir V. Gouli, Svetlana Y. Gouli, Carolina Provost, Bruce L. Parker and Margaret Skinner**. Entomological Research Laboratory, University of Vermont, 661 Spear Street, Burlingtonm

Vermont 05405-0105, USA

15:00 A phenologically based programme for season-long control of false codling moth on citrus, with particular use of a granulovirus and entomopathogenic nematodes. **Sean Douglas Moore[1,2], Antoinette P Malan[3] and Wayne Kirkman[2]**. [1]River Bioscience, PO Box 20388, Humewood 6013, Port Elizabeth, South Africa; [2]Citrus Research International, PO Box 20285, Humewood 6013. Port Elizabeth, South Africa; [3]University of Stellenbosch, P/Bag X1, Matieland 7602, Stellenbosch, South Africa

15:15 Preparation of scFv and monoclonal antibody against HrpA. **Shi Hua Wang and Zong Hua Wang.** Key Laboratory of Biopesticide and Chemical Biology, Ministry of Education, China, College of Life Science, Fujian Agriculture and Forestry University, Fuzhou, Fujian 350002

14:00-16:00 Thurdsay ***Multifunctional Hall***
Contributed Papers: Viruses 6
Moderators: Rollie Clem and Monique M. Van Oers

14:00 Characterization of early events during infection of TN368 cells with AcMNPV lacking *p35***. Bart Bryant and Rollie J. Clem.** Molecular, Cellular, and Developmental Biology Program, Division of Biology, Kansas State University, Manhattan, KS 66506 USA

14:15 Activation pathways and signal-mediated upregulation of the insect *Spodoptera frugiperda* caspase-1. **Qingzhen Liu[1, 2] and Nor Chejanovsky[1]**. [1] Entomology Department, Institute of Plant Protection, Agricultural Research Organization, The Volcani Center, POB 6, Bet Dagan, 50250 Israel; [2] State Key laboratory of Virology and Modern Virology Research Center, College of Life Sciences, Wuhan University, Wuhan 430072, P.R. China

14:30 Functional analysis of *Helicoverpa armigera* single nucleopolyhedrovirus *inhibitor of apoptosis* genes. **Marcel Westenberg[1], Job De Lange[1], Fei Deng[2], Hualin Wang[2], Zhihong Hu[2] and Just M. Vlak[1].** [1]Laboratory of Virology, Wageningen University, Binnenhaven 11, 6709 PD Wageningen, The Netherlands, marcel.westenberg@wur.nl; [2]State Key Laboratory of Virology, Key Laboratory of Molecular Virology and Joint Laboratory of Invertebrate Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, People's Republic of China

14:45 Indirect and direct evidence for the role of trypsin in baculovirus infection. **Jeffrey M Slack[1], Susan D Lawrence[2], Peter J Krell[3] and Basil M Arif[1]**. [1]Great Lakes Forestry Centrel, Natural Resources Canada, Sault Sainte Marie, Ontario, P6A 2E5, Canada; [2]Insect Biocontrol Laboratory, US Department of Agriculture, Beltsville, Maryland, 20852-2350, USA; [3]Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario, N1G 2W1, Canada..

15:00 The 5' nontranslated region of *Varroa destructor virus* 1 (*Iflavirus*): Structure prediction and IRES activity in insect cells. **Juliette R. Ongus[1], Els C. Roode[1], Cornelis W.A. Pleij[2], Just M. Vlak[1] and Monique M. Van Oers[1].** [1]Laboratory of Virology, Wageningen University, Binnenhaven 11, 6709 PD Wageningen, The Netherlands; [2]Leiden Institute of Chemistry, Leiden University, Einsteinweg 55, 2333 CC Leiden, The Netherlands

15:15 siRNA injection induce sequence-independent protection in *Penaeus monodon* against White Spot Syndrome Virus**. Marcel Westenberg, Bas Heinhuis[1] and Just M. Vlak.** Laboratory of Virology, Wageningen University, Binnenhaven 11, 6709 PD Wageningen, The Netherlands, marcel.westenberg@wur.nl

15:30 The *Aedes albopictus* Inhibitor of Apoptosis protects vertebrate cells from Bluetongue virus-induced apoptosis. **Qianjun Li.** Department of Medicine, University of Alabama at Birmingham, BBRB 559, 1530 3RD AVE S, Birmingham, AL 35294, USA

16:00-16:30 **Coffee Break**

16:30-18:30 Thursday ***Meeting Center***
Contributed Papers: Bacteria 4
Moderator: Brian Federici

16:30 Diversity of toxin gene from *Bacillus thuringiensis* against scarab larvae. **Shu-liang Feng[1], Da-fang Huang[2], Rong-yan Wang[1], Jin-yao Wang[1], Wei-ping Cao[1], Li-xin Du[1], Jian Song[1], Rui-hua Wu[1], Fu-ping Song[2] and Jie Zhang[2]**. [1]Feng Shu-liang, Institute of Plant Protection, Hebei Academy of Agricultural and Forestry Sciences, Baoding 071000, China; [2]Song Fu-ping, State key laboratory for biology of plant diseases and insect pests. Institute of Plant Protection, China Academy of

Agricultural Sciences, Beijing 100094, China

16:45 A novel insecticidal factor from *Bacillus sphaericus* with no mosquitocidal activity. **Hisashi Nishiwaki, Kenta Nakashima, Tadayuki Kawamura and Kazuhiko Matsuda**. Department of Applied Biological Chemistry, School of Agriculture, Kinki University, 3327-204 Nakamachi, Nara 631-8505, Japan

17:00 Molecular studies on Iranian native dipteran active *Bacillus thuringiensis* isolates**. Gholamreza Salehi Jouzani.** Agricultural Biotechnology Research Institute of Iran (ABRII), Microorganisms and Biosafety Department, Agricultural Biotechnology Research Institute of Iran (ABRII), Mahdasht road, P.O. Box 21525-1897, Karaj, Iran

17:15 Studying of natural strains of *Bacillus cereus-B.thuringiensis* from Siberia and Far East. **Galina Kalmykova[1], Ljudmila Burtseva[1], Ivan Dybovskiy[1], Victor Glupov[1], Irina Andreeva[2], Anna Mokeeva[2], Svetlana Oreshkova[2] and Vladimir Repin[2].** [1]Institute of Animal Systematics and Ecology Siberian Branch Russian Academy of Sciences, Frunze str. 11, Novosibirsk, 630091 Russia; [2]State Research Center of Virology and Biotechnology "Vector", Kol'tsovo, Novosibirsk region, 630559 Russia

17:30 Symbiosis in mosquitoes and its potential application in vector control. **Akinkurolere Rotimi Oluwafemi and Hongyu Zhang.** Institute of Urban Pest, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070. P.R.China

17:45 Susceptibility of Legume Pod Borer (LPB), *Maruca vitrata* to the δ-endotoxins of *Bacillus thuringiensis* (Bt). **Srinivasan Ramasamy.** AVRDC-The World Vegetable Center, 60 Yi Ming Liao, Shanhua, Tainan 74151, Taiwan

18:00 A 1.1 kb fragment downstream from the *bin* operon in *Bacillus sphaericus* 2362 affects Bin yield and crystal size. **Hyun-Woo Park[1], Yuko Sakano[2] and Brian A Federici[2,3]**. [1]John A. Mulrennan, Sr., Public Health Entomology Research & Education Center, CESTA, Florida A & M University, Panama City, Florida 32405, USA; [2]Department of Entomology, University of California, Riverside, Riverside, California 92521, USA; [3]Interdepartmental Graduate Programs in Genetics and Molecular Biology, University of California, Riverside, Riverside, California 92521, USA

18:15 Effect of Ciprofloxacin on Pathogenicity of Resistant *Escherichia coli***. Liancheng Lei and Wenyu Han.** Veterinary College, Jinlin University, Jilin ,130062, China

19:00 **Banquet** ***Yangchun Hall***

Book of Abstracts

SIP 2006

STU indicates papers being judged for graduate student presentation awards

Monday, August 28, 10:30-12:30, *Yangchun Hall*
Plenary Lectures: Microbial Control in Asia
Chair: Wendy Gelernter

10:30

Microbial Control and Biotechnology Research on *Bacillus thuringiensis* (*Bt*) in China

Da-Fang Huang
(*Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing100081, China*)

More than 300 products of microbial insecticide have been registered, including 276 *Bacillus thuringiensis* (Bt) products for the control of plenty of Lepidopteran pests, 26 products of NPV or GV for the control of pests in various crops, and 2 products of *Metarhizium anisopliae* for the control of various locusts. Besides, a number of microbial insecticides including *Beauveria bossiana*, microsporidia and entomopathogenic nematodes have been successfully used on a large scale application though the formal registrations have not been accomplished. The discovery and application of novel Bt insecticidal crystal protein genes is potential to biological control of insect pests. Since 1997, more than 50 Bt genes, which covered nearly one forth of the genes recorded worldwide at the same time period have been isolated by Chinese scientists and named by the International Bt Insecticidal Protein Gene Nomenclatural Committee. For example, *cry8Ca2*, *cry8Ea1*, *cry8Fa1* and *cry8Ga1* genes were successively identified and verified respectively to be specifically toxic to 4 major grubs: *Anomala exoleta*, *A. corpulenta*, *Holotrichia parallela* and *H. oblita*. In order to enhance the toxicity to lepidopteran pests, the *cry1Ac10* gene in conjunction with a chaperone gene *p20* were transferred to Bt strain YBT1520. Consequently, the engineered Bt strain WG-001 as the first approved recombinant Bt agent has started to commercial production in China. For the effective control of lepidopteran and coleopteran pests in simultaneously occurrence, *cry3Aa7* gene was transferred into Bt strain G03 harboring *cry1Ca7* gene and Strain UV17 harboring *cry1Ba3* gene, respectively. Both engineered strains, G033A and UV173A have been permitted to conduct the productive experiment on a large scale in China. The research and development of Bt cotton with own independent patents is one of the typical examples of agricultural biotechnology advances in China. The total cultivated area of Bt cotton with the modified *cry1A* and *CpTI* genes in 2005 was 3.3 million hectares, occupying 66% of the national cotton area in China. Planting Bt cotton has consistently delivered significant benefits, not only economical but also ecological and environmental. Only the reduction in chemical pesticides of active ingredient was over 260000 tons, which was equivalent to a 21% reduction in the associated environmental impact of pesticide use on cotton. Together with the labor-saving and the yield increasing, Chinese peasants have totally reaped more than $4.2 billion profits from Bt cotton since 1997. Transgenic Bt rice with modified *cry1A* or *cry1Ab* gene has also been developed and shown excellent result in the control of various species of rice stem borers. A serious transgenic restorer lines and hybrid combinations with high yield and good quality have been approved for large scale productive experiment and in the process of the final biosafety assessment.

11:00

Microbial control in Japan

Yasuhisa Kunimi
(*Department of Bioregulation and Biointeraction, Graduate School of Agriculture, Tokyo University of Agriculture and Technology, Saiwai, Fuchu, Tokyo 183-8509, Japan*)

Historically in Japan, studies on the diseases of the silkworm, *Bombyx mori*, as a factor affection the well-being of the silk industry, have dominated insect pathology. However, work by Hidaka (1933) demonstrated the possibility of controlling the pine moth, *Dendrolimus spectabilis,* with the fungus *Beauveria bassiana.* Since then, various attempts have been made to develop a method to control insect pests using insect pathogens. Cypovirus product, Matsukemin, was the first to be registered as an agricultural chemical in 1974, and inactive and live *Bacillus thuringiensis* (Bt) products were also registered and put on the market as agricultural chemicals in 1980 and 1981, respectively. Since then, various other products have been registered; in 1990, fungus *Monacrosporium phymatophagum* product, Nemahiton, was developed to control *Meloidogyne incognita* (nematode), in 1993, entomopathogenic nematode *Steinernema carpocapsae* product, Bio Safe, to control grass insect pests, and in 1995, fungus *Beauveria brongniartii* product, Biolisa Kamikiri, to control longicorn beetles. Recently, there is a significant increase in the number of microbial insecticides registered as agricultural chemicals (currently, there are 32 microbial insecticides on the market). However, the shipments in value terms have fallen since the 1996 agricultural chemical fiscal year when it peaked

at 1.9 billion yen. In the 2002 agricultural chemical year, the value decreased to approximately 0.9-1 billion yen; given the benefits of using microbial insecticides, a broader use of the products is desired. Prospects of the use of microbial insecticides in Japan will be discussed.

11:30

Microbial Control in South East Asia

Ole Skovmand

(*Intelligent Insect Control, 118 Ch Alouettes, 34170 Castelnau le Lez, France*)

South East Asia is the Small Dragon region, a region with rapid industrial development, but also with a traditional and modernizing agricultural sector partly turned toward export. The use of pesticides and biopesticides is directed by a complex of factors including prices, traditions, understanding of their mode of action, how the extension service of national and private companies reach farmers and of external factors as import regulations in other countries especially for pesticide residues. Only vegetable production can pay for microbial pesticides and most is directed to home markets. Periodically, rough violations of pesticides residues in food get consumer and authority attention and encourage research institutes working on alternative solutions. Beside the agricultural use, Bt israelensis is used for vector control. The storage of water in big jars and containers creates breeding places for Aedes (Stegomyia) species that are vector of dengue, the most important vector disease in the region. Success is limited even for dedicated products like Bti tablets that are successful in Germany for control of container breeders. Research on microbial control is intensive in the region, but focused on more basic aspects and much less on application areas like formulation, application and advice aspects. One interesting exemption is the IPM program of FAO for rice and vegetables. Experience has shown that a system of farmers school and farmers self-learning systems has a very good local effect where established, and farmers adapt classic biological control and microbial control when they understand how to use them. To improve the economy of this sort of pest control, the program in Thailand includes farmer's production of entomopathogene fungi and virus based on stock cultures provided by government institutions. The presentation will show some examples of successful use of microbial control in rice and vegetables and hopefully create some debate on barriers for the use of microbial pesticides and how to overcome these, inspired by work in the region.

12:00

Fungal biocontrol agents for arthropod pest control in India & Pakistan

Len Copping[2], Tariq M Butt[1]

([1]*Department of Biological Sciences, University of Wales, Swansea, SA2 8PP, UK;* [2]*LGC Consultants, Saffron Walden, Essex, CB11 4EG, UK*)

Biopesticides (i.e. microbial and microbial biocontrol agents) currently account for 2.5% of the global crop protection market. However, the biopesticide sector has a projected average growth rate of 9.9% per annum, significantly higher than the relatively flat to declining rate projected for conventional chemical pesticides. According to the Business Communications Company, the world biopesticide market will expand by about 60% from $672 M to $1.075 billion by 2010. The conventional pesticide market, currently worth $26.1 billion, is forecast to contract by 7.2%. The Indian subcontinent is one region where has been an expansion of the biopesticide market. Presumably, one of the driving forces for the interest in biopesticides has been the withdrawal of conventional pesticides, which may be harmful to humans and the environment, and increasing reports of insecticide resistance in key pest (e.g. *Bemisia* spp., *Thrips* spp., *Helicoverpa* spp., *Spodoptera* spp.) populations. A wide range of microbial agents (Bt, viruses, entomogenous fungi) of native and exotic origin, are being sold or developed, particularly in India. Virtually all the major genera of insect and mite-pathogenic fungi (e.g. *Nomuraea, Metarhizium, Beauveria, Paecilomyces, Verticillium, Hirsutella*) are available at highly competitive prices. The relatively low prices may be due to the low costs of R&D, labour, overheads, and culture media. The fact that so many products are available for sale suggests that registration is faster than in the West. However, there are many issues that still need to be resolved which will influence future sales. In particular there is a need to develop: (1) quality control parameters to ensure the end product is efficacious and uncontaminated, thereby reassuring end users of their effectiveness and safety; (2) formulations to enhance fungal efficacy at low humidities and to protect the inoculum against harmful UV and solar radiation; and (3) strategies to optimise the impact of the biological control agent and ensure compatibility with other crop protection agents and pest management systems.

Monday, August 28, 14:00-16:00, *Meeting Center*
Cross-Divisional Symposium: Monitoring and Managing for Bt-resistance: The Challenges for the next Decade
Convenors: Juan Ferre and Carlos Blanco

14:00

Resistance Monitoring for Bt crops: A US EPA Perspective

John A Glaser[2], Sharlene R Matten[1]
([1]*United States Environmental Protection Agency,Office of Pesticide Programs, Biopesticides and Pollution Prevention Division* (*7511C*)*,1200 Pennsylvania Ave., NW, Washington D.C. 20460;* [2]*United States Environmental Protection Agency,Office of Research & Development, National Risk Management Research Laboratory, Sustainable Technology Division,26 W King Dr. Cincinnati, Ohio 45268*)

Resistance monitoring for *Bacillus thuringiensis* (*Bt*) plant-incorporated protectants (PIPs) expressed in corn and cotton has been part of the US registration requirements since 1996, when the first *Bt* PIP was registered. U.S. EPA requires that each *Bt* PIP seed registrant conduct an annual resistance monitoring program and provide results to EPA annually. The goal of resistance monitoring is to detect resistance (significant changes in susceptibility) before widespread field failure so that modifications to the insect resistance management plan can be made to ensure the longevity of the product. They may also permit field validation of certain genetic parameters used in predictive insect resistance management models. The resistance monitoring programs include both testing insects for potential resistance and collection of information from growers about events that may indicate resistance. Plans include the following: the sampling plan (numbers, locations, and methodology), bioassay methodology, detection technique(s), and sensitivity. A discussion of the resistance monitoring programs and new research directed to early detection of resistance development for *Bt* corn and *Bt* cotton will be provided.

14:17

Monitoring pests of large geographies: How to get the best information when two countries are involved?

Carlos A. Blanco[1], Antonio P. Terán-Vargas[2], Craig Abel[1] and Omaththage P. Perera[1]
([1]*USDA - Agricultural Research Service, 141 Experiment Station Road, Stoneville, Mississippi, 38776, U.S.A.;* [2]*INIFAP, CESTAM, Km. 55 Carretera Tampico-Mante, Cuauhtemoc, Tamaulipas, 89610, Mexico*)

Heliothis virescens and *Helicoverpa zea* are know to migrate between Mexico and the United States. Both insects are targets of transgenic cotton (Bt cotton) and *H. zea* of transgenic corn (Bt corn). These crops constantly express *Bacillus thuringiensis* proteins and this can be a source of Bt-resistance selection. Since 1996 both countries have planted these crops at different adoption rates. In the US Bt cotton and Bt corn represent approximately 50% of the planted area, while in Mexico Bt cotton reaches nearly 90%. The area that these crops cover in each country also vary greatly, Bt cotton area in the US is nearly 600 times more than in Mexico. These agroecosystem differences can create very different Bt selection scenarios and in order to understand the potential resistance sources, if any, both countries should have a coordinated Bt monitoring program. Standardized methodologies, regulatory requirements, identification of logistical pitfalls and future challenges will be discussed.

14:34

Monitoring and Management Strategy of Helicoverpa armigera Resistance to Bt Cotton in China

Kongming Wu
(*State Key Laboratory for Biology of Plant Diseases of Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, 100094 China. E-mail: kmwu@ippcaas.cn*)

Transgenic cotton expressing the Cry1Ac toxin from *Bacillus thuringiensis* has been widely planted in China since 1998. Susceptibility of *H. armigera* field populations to the Bt insecticidal protein Cry1Ac was monitored from 1997 to 2005. The results indicated that the field populations sampled were still susceptible to Cry1Ac protein and that a shift toward resistance among *H. armigera* populations was not apparent. The evaluations on natural refuges showed that a planting system consisting of wheat, soybean (peanut), corn, and Bt cotton could supply refuges for cotton bollworm throughout the year. Adequate provision of refuges on an area-wide basis, and successful production

of susceptible insects could increase the probability that a rare resistant homozygote mated with a susceptible individual to produce heterozygous progeny susceptible to Cry1Ac. This strategy has been recommended for areas where farmers exclusively grow cotton without natural refugia from other crops.

14:51

What is the current situation in Australia for resistance to Bt cotton by *Helicoverpa armigera*?

Sharon Joy Downes[1], Rod Mahon[2]

(*[1]CSIRO Entomology, Australian Cotton Research Institute, Locked Bag 59, Narrabri, NSW 2390, Australia; [2]CSIRO Entomology, PO Box 1600, Canberra, ACT 2601, Australia*)

In 1996/97 the Australian industry adopted an insect-resistant variety of cotton (Ingard) which expresses the Bt toxin Cry1Ac that is specific to the group of insects including the target *Helicoverpa* spp., but excluding predators and parasitoids of this pest. To prolong the efficacy of transgenic cotton against *Helicoverpa* spp., a resistance management plan (RMP) that included a restriction on the area planted to transgenic cotton was implemented. The RMP was conservative in order to preserve the efficacy of the Cry1Ac gene in Ingard until two-gene transgenic cotton was available. In 2004/05 Bollgard II replaced Ingard as the transgenic variety of cotton available to Australian growers. It improves on Ingard by incorporating an additional insecticide protein (Cry2Ab) to combat Helicoverpa. As long as an appropriate refuge is grown, there is no restriction on the proportion of Bollgard II that can be grown. In 2004/05, more than 200,000 hectares were planted to Bollgard II. This high uptake of transgenic cotton is expected to continue in the 2005/06 season. The Bollgard II acreage will represent around 80% of the total area planted to cotton in Australia. The sensitivity of field-collected populations of *Helicoverpa* spp. to Bt products was assayed before and subsequent to the widespread deployment of Ingard cotton expressing Cry1Ac in the mid-1990's. Over the past three seasons baseline levels of susceptibility to Cry2Ab were established in preparation for replacement of Ingard with Bollgard II. There have been no reported field failures of Bollgard II due to resistance. However, our work shows that while alleles that confer high level resistance to *H. armigera* in the field are rare for Cry1Ac, they are surprisingly common for Cry2Ab. Individuals that carry a resistance allele for Cry2Ab are killed by Cry1Ac. We discuss some possible explanations for the high baseline frequency of Cry2Ab genes in field populations in the absence of prolonged selection to this toxin, as well as the implications of our frequency information for the current RMP.

15:08

Insect Baseline susceptibilities to Bt Cry toxins and the Bt resistance management in India

Govind T Gujar, V. Kalia, A. Kumari, B.P. Singh, R. Nair and A. Mittal

(*Division of Entomology, Indian Agricultural Research Institute, New Delhi 110012, India*)

In India, *Bacillus thuringiensis* (Bt) transgenic cotton with insecticidal *cry1Ac* gene, only approved transgenic crop, was grown over 1.3 million hectares in 2005 and cumulatively on about 2 million hectares, since its introduction in 2002. It, along with sprayable Bt formulations, poses the potential risk of evolution of Cry toxin resistance in the target insects under field conditions, similar to the insecticide resistance. Bt resistance management is therefore considered an essential pre-requisite for development of Bt crops. The studies on baseline susceptibilities of insects and monitoring of resistance to Bt crops are the essential component of Bt resistance management. The baseline susceptibilities of the bollworm, *Helicoverpa armigera* collected from different locations to Bt strains and toxins showed wide variation. The 96-hr LC_{50} of Cry1Ac to the neonates of *H. armigera* from different localities varied from 0.023 to 4.50 µg/ml over 1999-2005. It did not show any significant increase in insect resistance. The variation was influenced by various factors like temperature, host crops, prior xenobiotic-exposure and genetic diversity of the populations. Bt resistance management prescribes growing of 20% area or five border rows of non-Bt cotton as refuge crops whichever is maximum. However, the farmers sparingly practice it. The Cry1Ac toxin in the Indian cotton hybrids, though not high, has given satisfactory control of cotton bollworms like *H. armigera*, *Earias vitella* and *Pectinophora gossypiella*. It, however, does not control occasional defoliator, *Spodoptera litura*. The dual stacked cotton hybrids of the Bollgard™ II (Monsanto) series are in advanced stage of development. There is no known well-documented case of Bt cotton failure under field conditions due to Bt resistance in target insects so far. Nevertheless, Bt resistance management remains high on agenda for Bt cotton cultivation in India.

15:25

Monsanto's global approach to resistance monitoring

Graham P Head, Sakuntala Sivasupramaniam, Vaughn T Ty

(*Monsanto Company, 700 Chesterfield Parkway West, Chesterfield, MO 63017*)

Transgenic crops producing insecticidal toxins from the bacterium *Bacillus thuringiensis* (*Bt*) have been grown in many parts of the world since 1996. In the United States, the Environmental Protection Agency (EPA) has required that industry submit insect resistance management (IRM) plans for each *Bt* corn and cotton product commercialized. A coalition of stakeholders including the EPA, USDA, academic scientists, industry, and grower organizations have cooperated in developing specific IRM strategies. Resistance monitoring (requiring submission of annual reports to the EPA), and a remedial action plan addressing any contingency if resistance should occur, are important elements of these strategies. At a global level, Monsanto conducts baseline susceptibility studies, followed by monitoring studies on target pest populations, for all of its commercialized *Bt* crop products. To date, we have conducted baseline/monitoring studies in Argentina, Australia, Brazil, Canada, China, Colombia, India, Mexico, the Philippines, South Africa, Spain and the United States. Examples of pests on which resistance monitoring has been conducted include cotton bollworm (*Helicoverpa zea*), European corn borer (*Ostrinia nubilalis*), pink bollworm (*Pectinophora gossypiella*), Southwestern corn borer (*Diatraea* grandiosella), tobacco budworm (*Heliothis virescens*) and western corn rootworm (*Diabrotica virgifera virgifera*) in the United States, cotton bollworm (*H. armigera*) in China, India and Australia, and *Helicoverpa zea* in Mexico. No field-selected resistance to *Bt* crops has yet been documented.

15:42

Effective IRM for the novel insect-control cotton, VipCot™

Ryan W. Kurtz

(*Syngenta Biotechnology, Inc., 3054 E. Cornwallis Rd. RTP, NC 27709 USA*)

VipCot™ is a new product offering from Syngenta which provides a novel choice for transgenic control of lepidopterans in cotton. What makes VipCot™ unique is the novel insecticidal protein Vip3A. Vip3A's name originates from the acronym for Vegetative Insecticidal Protein since it is produced & secreted during vegetative growth and stationary stages of *Bacillus thuringiensis* (Bt). Vip3A is highly selective and effective against several economically important lepidopteran pests as well as structurally and functionally distinct from all other currently registered Bt cotton plant-incorporated protectants. By combining the effectiveness of Vip3A with the proven efficacy and performance of Cry1Ab, Syngenta's VipCot™ exhibits outstanding control of *Helicoverpa zea, Heliothis virescens, Pectinophora gossypiella* and a number of other lepidopteran species. Furthermore, from an insect resistance management (IRM) perspective, the distinct modes of action in this protein combination provide exceptional benefits which we will discuss along with our proposed IRM strategy. Currently, Syngenta is investigating the dose status of VipCot™ and its component proteins against the target pests, the potential for cross resistance between Vip3A and Cry toxins, and performing resistance risk assessment modeling to guide our IRM strategy. We are also initiating baseline susceptibility studies and developing plans for future resistance monitoring efforts.

Monday, August 28, 14:00-16:00, *Multifunctional Hall*
Virus Division Symposium: Diseases of Aquatic Invertebrates
Convenors: Zhengli Shi and Just M.Vlak

14:00

Viral diseases of aquatic invertebrates: Introduction to the theme

Just M. Vlak

(*Laboratory of Virology, Wageningen University, Binnenhaven11, 6709 PD Wageningen, The Netherlands [just.vlak@wur.nl]*)

Shrimp farming has developed over the last two decades from a very extensive culture into an industrial scale and has become of major economic importance in many countries in Asia and Central and South America. Concomitant with this enormous growth the industry suffered from a negative impact due to the pollution of the environment, destruction of pristine habitats, chemical pollution and inadequate waste disposal. As a consequence of the intensification of shrimp culture, the increased

trade of brood stock, inadequate sanitation and poor management, diseases became prominent at an early stage, in particular those caused by viruses and bacteria. Annual economic losses are estimated at about 2 billion USD. Since the emergence of viral diseases in shrimp culture in the early 1980s an array of viruses have appeared, some from already known virus families such as Baculoviridae (Monodon baculovirus, BP) and Parvoviridae (Infectious hypodermal and hematopoietic necrosis virus, IHHNV) but most of these are entirely new to the virus community. The most notable viruses affecting shrimps and related crustaceans are White spot syndrome virus (WSSV, Nimaviridae), Taura syndrome virus (TSV, Dicistroviridae) and Yellow head virus (YHV, Roniviridae) and unknown before. They are also notifiable viruses for the World Organization for Animal Health (OIE). Another at least ten viruses or diseases with a viral aetiology have been reported and no-doubt more will appear in the future. The 'success' of these viruses is mainly due to their quick spread in shrimp populations through suitable cultivation practices and trade of animals, but also because of their potential to adapt quickly to new environments and new hosts. WSSV for example has a very wide host range and is able to replicate in at least 30 species of shrimp, 36 species of crabs, 8 species of lobster and 6 species of crayfish. These species not always get the disease but are carriers or reservoirs of the virus as well. Also variants of these viruses (WSSV and TSV) quickly appear and evolve to higher virulent types due to the ever availability of unaffected shrimp cultures and movement of the virus to new territories. The aim of this symposium is to understand the biology, genetics and pathology of these viruses better in order to develop successful intervention strategies to control virus diseases in shrimp.

14:15

Biology, genetics and ecology of taura syndrome virus

Jeffrey M. Lotz

(*Department of Coastal Sciences, Gulf Coast Research Laboratory, University of Southern Mississippi, Ocean Springs, Mississippi 39564, USA*)

Taura syndrome was first reported from shrimp farms along the Taura River of Ecuador in the early 1990s. The original outbreaks were associated with increased use of fungicides in banana farms and a toxic etiology was suspected. Subsequently investigators confirmed a viral pathogen. Taura syndrome virus (TSV) spread soon to most shrimp faming areas of Latin America where it has become established. Sporadic outbreaks of TSV have occurred in the USA but TSV has been eradicated after each epidemic. In 1999 TSV was introduced into Taiwan with *Litopenaeus vannamei*. Therefore TSV is now of great concern world-wide. TSV is a 30 to 32 nm, icosahedral virus particle containing positive-sense, single-stranded RNA of about 10.2 kb in length. TSV is closely related to the cricket paralysis-like viruses (CrPV-like viruses) and is probably a member of the "picornavirus superfamily", the family *Dicistroviridae* and the genus *Cripavirus*. TSV has only been reported from penaeid shrimp. The genome codes for three structural coat proteins and several nonstructural proteins. The RNA viruses lack a nucleic acid replication proofreading mechanism and therefore have some of the highest mutation rates known. The genetic structure of a population of virus results from a balance between mutation rate and natural selection. There is a considerable genetic component to TSV resistance in *L. vannamei* and TSV genomic variability is associated with biological characteristics of geographical viral isolates. Pathogenicity is positively correlated with host viral load. In addition there is a positive correlation between viral load and transmission.

14:45

Biology, genetics and ecology of the YHV complex

Jeff A. Cowley[2], Peter J. Walker[1,2] and Priyanjalie Wijegoonawardane[2]

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Yellow head virus (YHV) and *Gill-associated virus* (GAV) are invertebrate nidoviruses classified in the new family *Roniviridae*. YHV first emerged in farmed black tiger shrimp (*Penaeus monodon*) in Thailand in 1990 and has since been reported in most Indo-Pacific shrimp farming countries. YHV is one of the most virulent of shrimp pathogens, causing rapidly accumulating mortalities during the mid to late stage of grow-out. GAV is a closely related virus associated with disease in *P. monodon* in Australia. GAV infections may be acute or chronic. Chronic infections occur commonly in healthy *P. monodon* in Australia and several other countries. The virus is transmitted horizontally by injection, ingestion or immersion. GAV is also transmitted vertically during mating from chronically infected

male or female parents. The 26.235kb (+) RNA genome of GAV shares sequence motifs, structural organization and a strategy of gene expression with other nidoviruses including coronaviruses, toroviruses and arteriviruses. Comparisons in several regions of the replicase (ORF1b), nucleoprotein (ORF2) and glycoprotein (ORF3) genes indicate that GAV and YHV are closely related but distinct genotypes. Phylogenetic analysis of sequences amplified from the ORF1b gene of viruses from *P. monodon* from throughout the Indo-Pacific region indicates the isolates cluster into at least six genotypes. Genotype 1 corresponds to the lineage of the original YHV isolate and was only detected in shrimp from Thailand and Chinese Taipei with yellow head disease. Genotype 2 corresponds to the lineage of the original GAV isolate and was detected in both healthy and diseased shrimp from Australia and healthy broodstock and post-larvae from Fiji, Indonesia, Thailand and Malaysia. Other genotypes were detected only in healthy shrimp. Genotype 3 was detected in shrimp from Vietnam, Indonesia and Thailand, genotype 4 was detected in shrimp from India, genotype 5 was detected in Malaysia, Thailand and Philippines, and genotype 6 was detected in shrimp from Mozambique. Phylogenies generated for the same isolates using an amplified region of the ORF3 gene indicated 10 of 28 isolates clustered differently from genotype assignments in the ORF1b gene, indicating a high frequency of genetic recombination. Sequence analysis of several isolates confirmed a recombination "hot spot" in the 3' region of the ORF1b gene. The data suggests that the vast international trade in broodstock and seed is providing adequate opportunities for co-infection and recombination, with potentially significant implications for the emergence and spread of disease.

15:15

Biology and molecular genetics of white spot syndrome virus

Zhengli Shi

(*State Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, 430071 Wuhan, People's Republic of China.*)

The white spot syndrome virus (WSSV) has been the most important shrimp virus worldwide since its appearance in the early of 90s of the last century. The wide host rang and the international trade made this virus spread quickly to all shrimp cultured countries. Because of the high mortality of this virus, the shrimp aquaculture has suffered important economic losses during the last 15 years. WSSV virions are ovoid-to-bacilliform in shape with a tail-like appendage at one end. The virions contain a rod-shaped nucleocapsid with hatched appearance, typically measuring 65-70 nm in diameter and 300-350 nm in length. The genome characterization showed that this is a double-stranded DNA virus with a genome of about 300 kb and a low identity of gene products to other known viral family. The unique characteristics of the morphology and the genome made WSSV to be classified into a new viral family Nimaviridae. The successive studies on WSSV gene function was slow down because of lacking the permissive cell lines. By virtue of the powerful proteomics techniques, the most progresses were achieved in the analysis of the structure proteins and their function during the entry into the host cells. At least 39 structural proteins were characterized to the component of viral structure. These proteins were divided into envelope protein, tegument protein and nucleocapsid protein by using different concentrations of dissolvent and NaCl, among them 8 were identified as envelope protein, 5 as tegument protein and 4 as nucelocapsid protein. All envelope proteins were reported to be involved in the virus infection. The major envelope protein VP28 was used as a potential vaccine against WSSV infection. However, most of gene function analysis was based on only *in vitro* virus neutralization with antibodies against different envelope proteins and experimental infection *in vivo*. Thus the real role played by these envelope proteins need to be further investigated.

Monday, August 28, 14:00-16:00, *Nanyuan Meeting Room*
Contributed papers：Fungi 1
Moderators: Michael Brownbridge and Zengzhi Li

14:00

Population dynamics of *Beauveria bassiana* introduced in forest and fresh water

Bin Wang[1, 2], Zengzhi Li[1], Mitsuaki Shimazu[2], Meizhen Fan[1] and Fan Peng[1]

([1]*Anhui Provincial Key Laboratory of Microbial Control, Anhui Agricultrual University, Hefei 230036, Anhui, P. R. China;* [2] *Department of Forest Entomology, Forestry and Forest Products Research Institute, 1 Matsunosato, Tsucuba, Ibaraki 305-8687, Japan*)

To study dynamics of pathogen population, one of the most important factors on the study of epizootiology of insect disease, two selective media, dodine-oat medium and DOC2 medium were chosen for monitoring *B. bassiana* introduced in forest and in fresh water, respectively. Surveys on dynamics of *B. bassiana* in 12 experimental plots inoculated at different doses and frequencies showed that: 1. the inoculum density of *B. bassiana* surviving in pine plantations was correlated to released inoculum at the beginning of the *B. bassiana* release. 2. At the beginning period, the consequence of surviving inoculum densities at different niches of the plantations was litter layer>crown layer>soil layer. A month later, inoculum densities in X and Y plots, inoculated with *B. bassiana* twice and once one year, respectively, decreased by 44%~99%, and the inoculum densities decreased from 37.5~300g/ hm^2 to 1.72~21.08g/ hm^2 (1.0×10^{11} spores/g). Four months later, inoculum densities in X and Y plots as well as Z plots inoculated with *B. bassiana* once one year ago ranged from 0.07~7.18g/ hm^2. 3. The decline rate of *B. bassiana* surviving in the crown layer was the most rapid, and that in the soil layer was the slowest. Density dynamics of *Beauveria bassiana* in fresh water was investigated to consider possible influences of this fungus on aquatic ecosystems. Conidia of *B. bassiana* were placed in non-sterilized lake water, sterilized lake water, non-sterilized distilled water, and sterilized distilled water in the laboratory and their densities were monitored. The conidia decreased sharply in all experimental waters in a short time. More than 90% of the live conidia were lost within 28d, especially only 0.03% survived in the non-sterilized lake water. No germinating conidium was found in non-sterilized waters after 27d, while they had been observed in sterilized waters until 84d after inoculation. It was thought that the fungus was inhibited for germination by other microorganisms existing in natural water and reduced the densities of live conidia. The result testified difficulty of multiplication of *B. bassiana* when this fungus accidentally contaminates in natural fresh waters, but it was still with great ecological meanings since the conidia of the fungus can be dispersed by a stream of river for a long distance.

14:15

Survival of *Beauveria caledonica* spores in biopolymer-based formulations for control of the *Hylastes ater* (Coleoptera: Scolytidae)

Michael Brownbridge[1], Tracey N Nelson[1], Steven D Reay[2], Jyanthi Swaminathan[1] and Travis R Glare[1]

(*[1]AgResearch Ltd., Biocontrol & Biosecurity, AgResearch, PO Box 60, Lincoln, New Zealand; [2]Silver Bullet Forest Research, Silver Bullet Forest Research, Auckland, New Zealand*)

Hylastes ater was first recorded in New Zealand in 1929 and is now established in all exotic pine plantations. During maturation feeding adults damage and may kill young pine seedlings, which can significantly impact regenerative plantings. The insect-pathogenic fungus *Beauveria caledonica* is frequently isolated from individual beetles and frass collected from breeding populations of *H. ater* in *Pinus radiata* stumps, and is being evaluated as a biological control agent for this and other bark beetles. As part of this investigation, novel formulations are being developed for delivery of fungal inoculum to the target insect. Survival of inoculum in a formulation is obviously critical for it to be functionally effective, and studies were done to assess the suitability of different materials for this purpose. Data are presented on survival of *B. caledonica* conidia in biopolymer-clay formulations (incorporating pine cambium or vermicultite), biopolymer gel, and biopolymer-coated rice.

14:30

Winter survival and germination of aphid-pathogenic Entomophthorales

Charlotte Nielsen[1], Anselme Fournier[2], Annette B. Jensen[1], Jürg Enkerli[2], Franco Widmer[2] and Jørgen Eilenberg[2]

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Pandora neoaphidis, Entomophthora planchoniana and *Conidiobolus obscurus* are well-known pathogens of many aphid species. These fungal species regularly cause epizootics among aphid pests suggesting great potential for use in microbial control of aphids either by inoculation or conservation biological control strategies. However, regardless of the strategy, a better understanding of the epizootiology is, essential for success. So far, most attention has been given to the effects of entomophthoralean fungi on aphid populations in economically important crops during the summer months, whereas knowledge concerning performance during the winter and initiation of infections in spring is limited. Soil samples originating from organically-grown winter wheat fields in Denmark

were collected before egg hatch of holocyclic aphids. The samples are being examined for the presence of inoculum by applying molecular methods and by baiting with aphids. Furthermore, the effects of light as well as host-induced factors on germination of survival structures are tested. Our findings will be discussed in relation to possibilities for biological control.

14:45

Disease transmission and chalkbrood control in the alfalfa leafcutting bee

Rosalind R. James

(*USDA-ARS Bee Biology & Systematics Lab, Dept Biol UMC 5310, Utah State Univ., Logan, UT 84333-5310*)

Disease establishment and transmission in a host population is very important in the study of insect pathology. For microbial control, an understanding of the factors that lead to establishment and transmission can be used to enhance the effectiveness of an introduced pathogen. In bee pathology, such an understanding can be used to control disease problems. The alfalfa leafcutting bee (*Megachile rotundata*) is used extensively for pollination of alfalfa grown for seed, but propagation of the bee is hampered in the U.S. by a disease of the larvae called chalkbrood, the causative agents are Ascomycetes in the genus *Ascosphaera*. We demonstrate that disinfecting nesting blocks does not significantly reduce chalkbrood levels, and thus is not the primary source of transmission. Adult bees emerging from "loose cells" (nest cells that have been removed from the nesting blocks) were found to be contaminated with large numbers of spores, spores which potentially could get transferred to the larvae during nesting. To test this potential, loose cells were treated before incubation with the fungicide Rovral (iprodione) at three rates using two different formulations. We included two control groups: untreated and formula-only treated (no active ingredient). Bees were then incubated and released into an alfalfa field with new nesting boards. Adult emergence was reduced by a flowable formulation, but a dry powder formulation increased emergence. The highest rates of emergence were at intermediate levels of active ingredient, thus high rates of the fungicide had some toxicity no matter what the formulation. Chalkbrood levels were significantly reduced by the iprodione treatments, but also by the blank formulation. In summary, Rovral treatments significantly decreased chalkbrood, increased nesting and brood production, and increased the percent of progeny that were healthy. Thus, we provide further evidence that the disease is transmitted by emerging female bees, and this source of contamination can be controlled with fungicides.

15:00

Host range of a fungus associated with epizootic in elongate hemlock scale

Jose Marcelino, Rosanna Giordano, Svetlana Gouli and Vladimir Gouli

(*Entomology Research Laboratory, University of Vermont, 661 Spear St., Burlington, Vermont 05405-0105, USA*)

The northeastern US hemlock forests [*Tsuga canadensis* (L.) Carrière] are in alarming decline due to two exotic Homopteran insect pests, the hemlock woolly adelgid, *Adelges tsugae* Annand (Homoptera: Adelgidae) (HWA) and the elongate hemlock scale, *Fiorinia externa* Ferris (Homoptera: Diaspididae) (EHS). In 2002 an epizootic was reported in an EHS population found in the Mianus River Gorge Preserve in Bedford, NY, USA. We investigated this epizootic as it promised to be an opportunity to identify a natural control agent of EHS. Up to 85% of the insects sampled in this area were partially or completely covered with sclerotic formations. We molecularly characterized this fungus, and determined its biology and host range. Phylogenetic analysis showed that the epizootic-causing fungi had 100% similarity with phytopathogenic strains of the widely known genus, *Colletotrichum*. Using five different genes, no genetic variation was seen in several isolates tested from various localities within the region of the epizootic. This lack of variation across the epizootic range, suggests that the epizootic spread from a single focus of dispersion. Fungal host range trials reveal a propensity of the fungus to infect and cause massive mycosis in Homopteran insects. However, endophytic fungal growth, morphologically identical to *Colletotrichum,* was observed in Rosaceae (Morus sp.) and Lauraceae (Sassafras) bushes, and Magnoliaceae (Magnolia and Liriodendron) and Rosaceae trees (apple) present in several areas of the epizootic. In addition, we have also inoculated and endophytically infected a Gramineae species (barley) and a Legumisosae species (bush beans) and biotrophically infected a Rosaceae species (strawberries). Given that this fungal strain is capable of infecting insects and establish mainly endophytic growth in plants, we propose two possible pathways that could have led to its present day distribution: a) outbreak of a cryptic, previously unreported entomopathogenic strain either already in the environment or introduced with the insect, or b) a new

recombinant strain, with a selective advantage towards arthropods rather than plant infection. Currently, this entomopathogenic fungus and the epizootic that it causes appear to be of critical importance in the reduction of EHS on hemlock and its rapid spread.

15:15 STU

Effects of combining the fiber bands impregnated with *Beauveria bassiana* cultures with attractants for control of *Monochamus alternatus* Hope

Sibao Wang[1,2], Yongping Huang[2], Meizhen Fan[1] and Zengzhi Li[1]

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The pine wilt disease which is caused by the pine wood nematode, *Bursaphelenchus xylophylus* Nickle has been one of the most serious epidemic diseases for the pine (*Pinus* spp.) forests in the south of China. The pine sawyer, *Monochamus alternatus* Hope is the primary vector of the pine wood nematode. *Monochamus alternatus* is difficult to control by spraying chemical insecticides because most of the life stages are present deep inside host trees, and the young larvae develop just under the bark but later larvae develop in the xylem. Biological control, therefore, provides a viable alternative for controlling the tree-boring beetles. The entomopathogenic fungus *Beauveria bassiana* was considered to be the most important natural pathogen and the most promising control agent of the pine sawyer. The attractant trapping played an important role in monitoring the population dynamics and suppressing the adult populations of pine sawyer. In order to integrate the merits of aggregative function of attractant and massive natural epizootic potential of *B. bassiana*, investigations were carried out to evaluate the effects of combination of fiber bands impregnated with *Beauveria bassiana* cultures with attractants against *Monochanus alternatus*. The results of the 3 years of field experiments showed that the larvae number, tree mortality, the beetle population density and infestation rate were significantly decreased in the all combined applications compared with the sole treatment and untreated control, while the infectivity of adult and larval beetles and the *B. bassiana* inoculum in forest were significantly higher in the combination treatments than in the sole treatment and untreated control. Among them, the tree mortality was up to 65.66 %, the beetle population declined by nearly 50% in the plot of bait tree and the fungal band. It was demonstrated that the combined application of *Beauveria bassiana* fiber bands and attractants displayed very unique synergistic effect for control *Monochamus alternatus*, which offering a new way for against the tree-boring beetles, *Monochamus alternatus*. The population genetic structure and ecological effects of the released strain were also investigated by simple sequence repeat (SSR).

15:30 STU

Relationship of trehalose and polyol accumulation to increased conidial heat and UV-B tolerance in *Metarhizium anisopliae* conidia produced under sub-lethal stresses

Drauzio E.N. Rangel, Anne J. Anderson and Donald W. Roberts

(*Deparment of Biology, Utah State University, 5305, Old Main Hill, Logan, UT. 84322-5305 USA*)

Acquisition of increased conidial tolerance to stresses in fungi may be induced by exposing them to a sub-lethal stress during growth. Several mechanisms are associated with this increased conidial tolerance, the most important of which is conidial accumulation of mannitol and trehalose. In this report, conidia of the insect-pathogenic fungus *Metarhizium anisopliae* var. *anisopliae* (isolate ARSEF 2575) were produced on mycelia which had been subjected to nutritive, heat-shock, osmotic, and oxidative stresses. The amounts of trehalose and polyols accumulated in conidia produced under these stresses were quantified, and the tolerance responses of the conidia to UV-B radiation and heat were evaluated. Conidia produced under nutritive stress [minimal medium = Czapek without saccharose (MM), or MM plus lactose] had a 2-fold increase in tolerance to UV-B radiation and half-fold increase in thermotolerance when compared to non-stressed control conidia produced on rich medium [potato dextrose agar + yeast extract (PDAY)]. These conidia also had high concentrations of trehalose and mannitol in comparison to conidia produced on non-stressed (rich medium) controls and under all other stresses; but they had the lowest concentrations of erythritol and arabitol. Conidia produced on heat-shocked mycelium had increased thermotolerance over that of conidia produced under non-stressed conditions. The trehalose and mannitol concentrations in conidia produced under heat shock were lower than conidia produced under nutritive stress; but, as compared with control, heat shocked mycelium 72 h after inoculation had higher levels of mannitol and trehalose. Conidia produced under osmotic stress had 2-fold increased thermotolerance and UV-B tolerance, but they had

the lowest level of mannitol and trehalose. On the other hand, conidia produced under osmotic stress had very high concentrations of arabitol and erythritol. Conidia produced under oxidative stress (H_2O_2 or UV-A irradiation) had UV and heat tolerances similar to that of their non-stressed controls, yet (with an exception of the medium with H_2O_2 5 mM) similar amounts of polyols or trehalose were found in both non-stressed controls and conidia produced under oxidative stress.

Monday, August 28, 14:00-16:00, *Xiyuan Meeting Room*
Contributed papers：Nematodes
Moderators: Patricia Stock and Mike Wilson

14:00

Field evaluation of *Heterorhabditis indica* with entomopathogens and botanicals against *Helicoverpa armigera* (Hübner)

Aralimarad Prabhuraj, Patil B.V., Girish K.S. and Shivaleela
(*Department of Entomology, College of Agriculture, Raichur 584 101, Karnataka, India*)

Field experiments were conducted for two seasons, viz., 2003-05 to evaluate local isolate of *Heterorhabditis indica* in combination with other entomopathogens and botanicals against *Helicoverpa armigera* (Hübner) in chickpea ecosystem. In the first field experiment, 22 treatments consisting of different combinations of nematode with Bt, NPV, three entompathogenic fungi and four botanicals were evaluated and compared with a chemical treatment. Pooled data on per cent larval reduction and total yield after two sprays revealed that sequential application of *H. indica* with *Pongamia pinnata*, *Prosopis juliflora, Vitex nigundo* and Bt recorded highest larval reduction with maximum yield and were comparable with chemical treatment. Thus, in the second year, only the above treatments were evaluated in large area. Among the combination treatments, insecticidal spray and sequential application of *H. indica* + *P. juliflora* (@ 1 lakh infectives/l + 10%) were significantly superior over other combinations recording highest per cent larval reduction (26.49 and 23.47 on 4th day, lowest per cent pod damage (17.19 and 11.27), highest pod yield (19.43 and 19.24 q/h) and highest additional income (Rs8926 and Rs. 8624/h). These two treatments also recorded the highest incremental cost benefit ratio of 1:11.6 and 1:7.63. Thus, sequential application of 10% aqueous leaf extract of *P. Juliflora* followed by *H. indica* @ one lakh per liter proved to be an economical, eco-friendly and alternative control strategy against chickpea pod borer. Performance of other combinations is discussed in detail.

14:15

Performance of *Heterorhabditis indica* with neem against *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae)

Aralimarad Prabhuraj, Patil B.V., Girish K.S. and Shivaleela
(*Department of Entomology, College of Agriculture, Raichur 584 101, Karnataka, India*)

Studies were undertaken to evaluate the effect of combination of an entomopathogenic nematode, *Heterorhabditis indica* and two neem products, viz., Neem seed extract (NSE) and neem oil against two instars of *Helicoverpa armigera* under laboratory condition. In a preliminary study, the compatibility of neem products with *H. indica* was carried out. The results revealed that, both the neem products were highly detrimental to the infective juveniles (IJs) at higher concentrations resulting in more than 90 per cent mortality after 48 hours of exposure. However, as the concentration of the botanicals was reduced the survivability increased indicating the scope for integration of the above neem products with *H. indica*. In a second set of experiment, *H. indica* was combined with NSE and neem oil at lower concentrations and tested their efficacy against third and fourth instar larvae. The results revealed that, sequential application of NSE and *H. indica* (1% + 50 IJs larva-1) and simultaneous application (0.25 % + 50 IJs larva^{-1}) resulted in highest mortality of 99.67% and 98%, respectively against third instar larva. However, sequential application of neem oil and *H. indica* (2% + 50 IJs larva^{-1}) was found to be highly effective resulting in cent per cent mortality of third instar larva at 24 hour and fourth instar larva at 48 hours. It was evident that the integration of neem with *H. indica* either sequentially or simultaneously resulted in significantly higher mortality as compared to alone. Increased mortality in simultaneous application might be attributed to the synergistic action.

14:30

A new entomopathogenic nematode, *Steinernema hebeiense* sp. n. (Rhabditida: Steinernematidae), from North China

Shulong Chen[1], Xiuhua Li[1], Aihua Yan[1], Sergei E. Spiridonov[2] and Maurice Moens[3]

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A new species of *Steinernema* (Rhabditida), *Steinernema hebeiense* n. sp. was isolated from sandy soil in Hebei province of China. Diagnostic characters of infective juveniles of *S. hebeiense* n. sp. include total body length (610-710 µm), distance from anterior end to excretory pore (43-51µm), tail length (63-71 µm), and E% ratio (65-80). The lateral field has eight ridges in mid-body (two very thin submarginal ridges, poorly discernible in light microscope, but visible in SEM); the anterior end is slightly offset and flattened. First generation males have body length between 1036 and 1450 µm, spicular length 51-63µm, gubernaculum length 38-50µm, spicules are of light brown colour and slightly curved; the manubrium is longer than wide. Tail mucron is present in second generation males. These morphometrical and morphometric features classify *S. hebeiense* n. sp. within the '*feltiae-kraussei-oregonense*' group. Also molecular data classify the new species within this group. Within this group, the smallest difference in *S. hebeiense* sp. n. sequence is with *S. weiseri*: 73 bp in the ITS rDNA and 13 bp in the studied partial sequence of D2D3 expansion segment of LSU rDNA.

14:45

A Comparative Study on the Morphology and Ultrastructure of the Bacterial Receptacle in *Steinernema* Nematodes

S.Patricia Stock[1] and Yolanda Flores-Lara[1, 2]

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Third-stage infective juveniles (IJ) of entomopathogenic nematodes *Steinernema* spp. are colonized by a monoculture of *Xenorhabdus* bacteria at a discrete structure located in the anterior portion of the intestine known as the 'bacterial vesicle' or 'intestinal vesicle'. The nature and structure of this receptacle is presently not well understood. Early transmission electron microscopy (TEM) studies on the vesicle suggested that this vesicle is a modification of the ventricular region of the intestine that lies immediately beneath the esophago-intestinal valve in the nematode IJ. We examined structural and ultrastructural features of the bacterial vesicle in a selection of *Steinernema* spp., which represent distinctive evolutionary clades in this nematode's molecular (multigene) phylogenetic framework. Differential interference contrast optics (DIC) were used to examine the shape of the vesicle and its esophageal-intestinal connections among different taxa. Transmission electron microscopy was also considered to assess ultrastructural variation in colonized and no-colonized receptacles. Vesicle lining (presence *vs.* absence of microvilli), cells and organelles were compared in each of the examined species. Hypothesis on the evolution of these morphological and ultrastructural traits will be discussed.

15:00

The indigenous Peruvian entomopathogenic nematode and the Andean potato weevil

Harry K. Kaya[1], Soroush Parsa[1] and Jesus Alcázar[2]

(*[1]Department of Nematology, University of California, Davis, California 95616 USA; [2]International Potato Center, Lima 12, Peru*)

Andean potato weevils (*Premnotrypes* spp.) are the most important insect pests of potatoes throughout the Andean region. Although they are endemic to the Andes, no significant natural enemies of these pests have been reported until recently. An undescribed entomopathogenic nematode in the genus *Heterorhabditis* designated Alcázar-1 was isolated from potato weevil pre-pupae in a potato storage unit in Peru. The potential of this nematode to suppress the Andean potato weevil, *Premnotrypes suturicallus*, was investigated. The tolerance of this nematode to the cold Andean temperatures, its infectivity against different stages of *P. suturicallus*, and its recycling potential within this host were evaluated. *Heterorhabditis* sp. Alcázar-1 was infectious to *Galleria mellonella* larvae at temperatures (mean 11.1°C; range 8.6-14°C) similar to those recorded for daily fluctuations in the Andes. In

laboratory bioassays, the nematode protected potato tubers from infestations by neonate *P. suturicallus* larvae and killed weevil larvae within the tubers before significant damage was incurred. Median lethal concentration for *P. suturicallus* pre-pupae was 5.9 infective juveniles/host reflecting high susceptibility. At this concentration, mortality increased over a 7-day period, resulting in 66% pre-pupae, 65% pupae and 52% teneral adults killed by the nematode. Alcázar-1 has a high reproductive potential in *P. suturicallus* pre-pupae, producing an average of 97,817 infective juveniles per host. These results suggest that Alcázar-1 has excellent potential for biological control of the Andean potato weevil.

15:15

Molecular characterization of the symbiotic bacteria of entomopathogenic nematodes isolated from China

Lihong Qiu, Shaoming Peng, Lianlian Liu and Yi Pang

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Xenorhabdus and *Photorhabdus* are symbiotic bacteria (SB) of entomopathogenic nematodes (EN) of *Steinernema* and *Heterorhabditis*, respectively. EN-SB complex are effective biopesticides for controlling many insect pests. They are also becoming an ideal biological model for studying the molecular mechanism of symbiosis and pathogenicity. The bioactive by-products of SB, such as insecticidal toxins, antibiotics, anticancer and nematicides may make significant contribution to the society. A well established culture collection of EN and SB is critical for all EN and SB related studies and applications. The State Key Laboratory of Biocontrol has carried out a systematic study on survey of EN in Guangdong and Yunnan provinces of China since 2001 and has recovered more than 400 isolates of EN. Most EN obtained have been identified, of which four new species have been published and at least ten new species are under description. In the present study, the taxonomic status of some SB of the EN obtained from the survey was determined preliminarily using 16S rDNA as molecular marker. The results indicated that the symbiotic bacteria of *S. aciari*, *S. akhursti*, *S. guangdongense* and YNd72, YNc215, YNd37, YNd393 and GDc328 are likely to be eight new species of *Xenorhabdus*; while those of *S. beddingi*, YNb33 and YNb59 are strains of *X. bovienii*. The phylogeny of *Xenorhabdus* and the co-evolution between *Steinernema* and *Xenorhabdus* are discussed.

15:30 STU

Effectiveness of entomopathogenic nematodes in the control of oilseed rape pests In Finland

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Nematodes can be highly effective in controlling pest insects in many cropping systems (Grewal et al. 2005). Detailed studies in the rapeseed ecosystem have been conducted in Finland, on the target impacts of inundating the nematode *Steinernema feltiae* with some additional data on *S. carpocapsae*. The aim of this study was to investigate the potential of entomopathogenic nematodes (EPN) in controlling oilseed rape (OSR) pests, in particular the pollen beetle (*Meligethes aeneus*) as the model target pest, EPN persistence and factors that influence their persistence. As the timing and method of EPN application appeared to play a crucial role along with the EPN dose applied and impact of some biotic factors, this study focused on determining the effect of these factors on pollen beetle mortality. Effects on cabbage flea beetle (*Phyllotreta* spp.) were also assessed. Two application methods were compared: application on the soil surface with a watering can (simulating spray treatment), and placement of the EPNs about 2–3 cm into the soil within an experimental protective slow-release system (NemaBag™), two different versions. Two application times were tested for the surface treatment: about one week after sowing of the spring turnip rapeseed (end of May), and at the time of the start of pollen beetle pupation (mid-July, 'optimum timing'). Slow-release bag treatments were done only at the earlier date. Tested EPN application densities ranged in these experiments from 15,000 to 1,000,000 infective juveniles (IJ) per m^2, for both application methods. In most experiments, the EPN *Steinernema feltiae* was used; additionally *S. carpocapse* was tested at the density of 150,000 nematodes/m^2. Control plots were always included, and treated with clean water. Additionally, the impact of the fungus *P. fumosoroseus* on EPN survival and reproduction was assessed. Results from two study years showed that the treatment with watering can, applied at pollen beetle pupation time, lowered the pest population by 80–90%, and populations of cabbage flea beetles by about 50%, regardless of nematode application density. Application 8 weeks before the optimum time yielded first

disappointing results, regardless of the delivery method (watering can or slow-release bag, nylon mesh material). In 2005, however, the treatment with watering can at pollen beetle pupation time at the application densities of 0.5 M/m^2 and 0.1 M/m^2 unexpectedly showed no lowering of pest populations, whereas the treatment with slow-release bags (biodegradable material) 4 weeks prior to pollen beetle pupation lowered pollen beetle numbers by 35%, and the numbers of cabbage flea beetles by 25%. The natural occurrence of the entomopathogenic fungus *P. fumosoroseus* in all plots could be the reason that pest populations showed no additional reduction in plots treated with *S. feltiae* (applied with watering can) in 2005, except in case of slow release system application. *P. fumosoroseus* showed negative impact on EPN multiplication and their emergence from bait larval body. The challenge remains now to find out ways of suitable conservation and enhancement methods for EPN.

Monday, August 28, 16:30-18:30, *Meeting Center*
Poster Session I

Bacteria I

BP1

Cytocidal actions of parasporin-2, an antitumoral crystal protein targeting mammalian cells from *Bacillus thuringiensis* A1547

Sakae Kitada[1], Yuichi Abe[1], Hiroyasu Shimada[1], Osamu Kuge[1], Eiichi Mizuki[2], Michio Ohba[3] and Akio Ito[1]

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The crystal (Cry) proteins produced in *Bacillus thuringiensis*, a Gram-positive bacterium, are known to show high cytotoxicity against insects. Although a number of *B. thuringiensis* strains producing insecticidal toxins have been identified, many other *B. thuringiensis* strains containing non-insecticidal inclusion proteins have also been ubiquitously discovered in natural environments and are rather more widely distributed than the insecticidal strains. Through a wide screening of non-insecticidal Cry protein cytotoxicities toward several human cancer cell lines, we have identified novel *B. thuringiensis* toxins, the parasporins that possess antitumoral and non-hemolytic activities against a wide range of human cells. Parasporin-2 (Cry31Aa), a new crystal protein derived from *B. thuringiensis* A1547, recognizes and kills human liver and colon cancer cells as well as some classes of mammalian cultured cells. Here, we report that a potent proteinase K-resistant parasporin-2 toxin shows specific binding to and a variety of cytocidal effects against human hepatocyte cancer cells. Cleavage of the N-terminal region of parasporin-2 was essential for the toxin activity, while C-terminal digestion was required for rapid cell injury. Protease-activated parasporin-2 induced remarkable morphological alterations, cell blebbing, cytoskeletal alterations and mitochondrial and endoplasmic reticulum fragmentation. The plasma membrane permeability was increased immediately after the toxin treatment and most of the cytoplasmic proteins leaked from the cells, whereas mitochondrial and endoplasmic reticulum proteins remained in the intoxicated cells. Parasporin-2 selectively bound to cancer cells in slices of liver tumor tissues and susceptible human cultured cells, and became localized in the plasma membrane until the cells were damaged. Thus, parasporin-2 is a cell-discriminating, membrane-targeting and pore-inducing toxin that subsequently causes irreversible intracellular decay in cancer cells.

BP2

Parasporin-2, an oligomerizing and pore-forming toxin from *Bacillus thuringiensis*, is assembled into supramolecular complexes in target human cell membranes

Hiroyasu Shimada, Yuichi Abe, Osamu Kuge and Sakae Kitada

(*Department of Chemistry, Faculty of Science, Kyushu University, Fukuoka 812-8581, Japan*)

Bacillus thuringiensis strains are well-known bacteria used in agriculture, forestry management and vector controls, since individual strains produce various species-specific insecticidal toxins as parasporal crystals. Recently, we identified a novel *B. thuringiensis* crystal toxin, designated parasporin-2 (Cry31Aa), which is non-insectidal and non-hemolytic, but shows potent cytotoxicities

against several cultured human cancer cells. Active mature parasporin-2 (30 kDa) is obtained via *in vitro* processing of the N- and C-terminal regions of the protoxin (37 kDa) by proteinase K. The active parasporin-2 binds to the target cell surface, and induces the formation of large pores in the cell membrane. Here, we report that parasporin-2 forms SDS-resistant membrane-integrated oligomers in the plasma membrane and, furthermore, that the toxin is assembled into supramolecular complexes. When human hepatocyte (HepG2) cells were intoxicated with parasporin-2, the 30 kDa parasporin-2 monomers in the target cell membrane transformed into oligomers whose size was estimated to be approximately 200 kDa by SDS-PAGE and western blotting. The toxin oligomerization appeared to be correlated to the plasma membrane permeability. Fractionation of toxin-treated membranes by sucrose-gradient centrifugation revealed that parasporin-2 was localized in the plasma membrane. However, the physical states in the membrane differed between the monomeric and oligomeric toxins. The monomer bound to the cell surface peripherally, whereas the oligomer was embedded in the membrane. When parasporin-2 oligomers were solubilized from intoxicated cell membranes by mild detergent treatment and their mobilities were analyzed by non-denaturing gel electrophoresis, molecular complexes of at least 600 kDa were detected. These results suggest that parasporin-2 binds to the plasma membrane via a putative receptor and forms quite large toxin complexes that probably punch pores in the plasma membrane. These results imply that other pore-forming toxins which resemble parasporin-2 in their cytocidal actions and structures, such as aerolysin and e-toxin from *Aeromonas hydrophila* and *Clostridium perfringens*, respectively, may also form larger toxin complexes than the pentameric oligomers detected under denaturing conditions.

BP3

GPI-anchored proteins are involved in the cytocidal actions of parasporin-2, a mammalian cell-targeting crystal protein from *Bacillus thuringiensis* A1547

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Non-insecticidal and non-hemolytic *Bacillus thuringiensis* strain A1547 produces parasporin-2, which exhibits cytolytic activities against mammalian cells. The cytotoxicity of the toxin varies considerably from cell to cell. For example, it is highly cytotoxic toward MOLT-4, Jurkat and HepG2 cells, but less so toward normal hepatocyte, HeLa and COS-7 cells. The toxin forms SDS-resistant oligomers on a target cell and induces permeabilization of its plasma membrane. Immunofluorescence microscopy analysis revealed that parasporin-2 binds to the surface of highly toxin-sensitive cells. Thus, putative parasporin-2 receptors could exist on these toxin-sensitive cells. Here, we report that glycosylphosphatidylinositol (GPI)-anchored proteins mediate the cell-targeting and oligomerization of parasporin-2. When toxin-treated cells were solubilized with Triton X-100 and fractionated by floatation-centrifugation, the toxin was recovered in the detergent-resistant membrane fraction, indicating that parasporin-2 bound to the lipid rafts. We further examined the effects of biosynthesis inhibition, degradation or suppression of molecules located in the lipid rafts on the actions of parasporin-2. Through various investigations, we found that treatment of cells with phosphatidylinositol-specific phospholipase C (PI-PLC), which releases GPI-anchored proteins from the plasma membrane surface, decreased the cytotoxicity, cell-binding and oligomerization of parasporin-2. Moreover, Chinese hamster ovary (CHO) cells deficient in the biosynthesis of GPI-anchored proteins were resistant to parasporin-2, since the cells remained viable at very high toxin concentrations. Cell-binding and oligomerization of the toxin were not detected on GPI-anchored protein-deficient cells. From these results, we conclude that GPI-anchored proteins are required for the specific cytotoxic actions of parasporin-2.

BP4

Baseline susceptibility to the Cry 1Ac protein and validation of diagnostic concentration in Indian populations of eggplant fruit and shoot borer

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Eggplant fruit and shoot borer (EFSB), *Leucinodes orbonalis* (Gueńee), is the most destructive insect

pest of eggplant in South Asia. The EFSB larvae cause shoot damage reducing fruit set and fruit damage, making the fruit inedible and unfit for market. Damage from the EFSB starts soon after crop transplanting and continues until harvest, leading to 50-90% yield losses. The heavy use of insecticides for EFSB management results in long-term harmful effects on the environment and human health. With the intent of dveloping effective and environment friendly management option for the EFSB, eggplant has recently been engineered with cry1Ac gene by Maharashtra Hybrid Seeds Company Ltd. (MAHYCO). The sustainability of the EFSB-resistant eggplant depends on information generated from standard mortality and diagnostic bioassays through regular monitoring. This study resulted in generating baseline information which forms a strong basis for resistance monitoring after commercialization of Bt eggplant and in the development of a proactive resistance management strategy. Suscpetibility to the Cry1Ac protein from *B. thuringiensis* was determined for 22 populations of EFSB collected from eggplant growing regions of India during 2004-'05. Neonate EFSB larvae from the field-collected populations were exposed to artificial diet incorporated with Cry1Ac protein at different concentrations, and mortality was recorded after 7d. The bioassays revealed 12- and 8-fold variability in LC_{50} and LC_{95} values, respectively, among the twenty-two populations. The interpopulation variation in susceptibility indicated by moult inhibition was 13-fold at MIC_{95} (MIC-moult inhibitory concentration). The baseline data was used to estimate a candidate diagnostic concentrations, LC_{99}, that causes 99% mortality. Validation experiments using field-collected EFSB populations from across India showed that, a concentration corresponding to the upper limit of the 95% confidence interval of the LC_{99}, produced >99% for all populations tested.

BP5

Rear *Anomala corpulenta* (Coleoptera: Scarabaeidae) and bioassay of insecticidal activity of *Bacillus thuringiensis* against its larvae

Rong-yan Wang, Jin-yao Wang, Shu-liang Feng, Wei-ping Cao, Li-xin Du, jian Song and Rui-hua Wu
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Anomala corpulenta adult was collected with Light-Gray 3650A from the field. Adult group were put into in rearing cage (40cm×60cm×20cm) that placed soil (8-10 cm thick) with humidity about 18-20% under the condition of room temperature 26-28℃ and photoperiod 16:8(L: D). The adults feed fresh leaf of elm or corn. The humidity of soil and the oviposition were observed every 3-5 day. Larvae were picked up and placed into soil on the day of egg hatch in temperature 26-28℃. Newly hatched larvae were put into wet soil to rear in temperature 26-28℃. Five 1st or 2nd instar larvae were put into one little box (Φ9cm, H6cm), because they can not killed one another. The larvae of *A. corpulenta* feed potato or root of corn. The soil was replaced at the period of 2nd instar larvae. The 3rd instar larvae were put into one little box alone, as they were killed each other, the soil and the food was replaced once a month. At the end of 3rd instar larvae feed nothing and prepare to pupate. The pupae were not usually turned into wet soil. The adults were put into the rearing cage (40cm×60cm×20cm) bottom soil after emergence, finished their life history. The humidity of soil, food and pathogenic microorganism affect the growth of larvae. The average survival rate of eggs was up to 90%, the survival rate of 1-instar,2-instar and 3-instar larvae averages at 82%,76% and 60%, respectively. The 10d and 15d larvae are the best larvae for bioassay because their mortality 14 days after incubation in the soil mixed with potatoes is 8.8±1.6% and 4.0±1.6%, respectively, obviously lower than the mortality of the newly hatched larvae and 5d larvae. The larvae grow normally in the UV-irradiated loam and sandy loam, their mortality is lower than that in untreated and autoclaved loam and sandy loam. The crystal-shapes of *Bt* with insecticidal activity against *A. corpulenta* larvae are all round crystals, account for 7.30% of all screened strains. The strains causing 100% mortality of larvae were account for 0.83% of all the strains of round crystals.

BP6

Studies on the Use of Three *Bacillus* Species Against The Date Palm Fruit Stalk Borer, *Oryctes elegans* (Coleoptera: Scarabaeidae)

Mohammad Abdulaziz Al-Doghairi
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An interdisciplinary approach program has been implemented for the control of the date palm fruit stalk borer, *Oryctes elegans*, in date palm plantations in Al-Qassim. The program included the use of different tactics including biological pesticides. 3 species of entomopathogenic bacteria (*Bacillus*

popilliae, B. thuringiensis var. israealensis and *B. t. kurstaki*) were used against The Date Palm Fruit Stalk Borer, *Oryctes elegans* (Coleoptera: Scarabaeidae).

BP7

Identification of a novel *Bacillus thuringiensis* strain WZ-9

Ping Song, Huixian Wu, Wenjie Mao, Xudong Su and Qinying Wang

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Bacillus thuringiensis is a bacterium known for producing protein crystals with insecticidal activity. In this study, we isolated a novel Bt strain WZ-9 which had high insecticidal activity against *Henosepilachna vigintioctomaculata* larvae, no insecticdal activity to larvae of Lepidoptera insects including *Helicoverpa armigera* , *Plutella xylotella*, *Pieris rapae* , *Ostrinia furnacalis* and *Spodoptera exigua*. The strain contained Cry toxin proteins that exhibited two bands (about 130kDa and 76kDa) on SDS-PAGE. The crystal protein was bipyramidal crystal. Purified Cry toxin treated by midgut enzyme or trypsin lost its activity against *H. vigintioctomaculata* larvae. WZ-9 strain has two plasmids, about 1.6Kb and 200bp. By several pairs of universal oligonucleotide primers, WZ-9 strain was detected including *cry1* gene and *cry7* gene.

BP8

Binding analysis in Cry1Ac-selected populations of *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae)

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Helicoverpa zea is the most important secondary pest of cotton in the USA (after *Heliothis virescens* and *Pectinophora gossypiella*). Contrarily to the principal insect pests of cotton, *H. zea* is relatively tolerant to *B. thuringiensis* toxins. It is for this reason that an increase in the level of tolerance of *H. zea* populations is so important, because a relatively moderate increase in tolerance could confer the insects a complete resistance against Bt-cotton. The aim of the present study was to select, in the laboratory, a *H. zea* population for resistance to Cry1Ac (the toxin expressed in first generation Bt-cotton) and to determine whether binding site alteration is responsible for such resistance. Selection of a laboratory population was performed with Cry1Ac via diet incorporation of either MVP II or Cry1Ac activated toxin. The strain selected with MVP II and activated toxin are referred as MR and AR strain respectively. The increase in tolerance was evident after 4 generations of selection, and significant difference in resistance ratios (RR) was observed after 7 generatios. It was previously known that *H. zea* has common binding sites for Cry1Aa, Cry1Ab, and Cry1Ac. To determine if binding site alteration was involved in the increase of resistance in the selected populations, *in vitro* binding assays have been performed to obtain binding parameters. ^{125}I-labeled Cry1A toxins were incubated with brush border membrane vesicles prepared from the midgut (after 7 generations of selection) of resistant and susceptible insects in the absence and in the presence of competitor. Since in some Cry1A-resistant populations binding site alteration is better observed with one of the Cry1A toxins but not others (due to the occurrence of multiple binding sites, not all of them involved in the toxic action), we have performed the assays with the three above mentioned Cry1A toxins. The results showed that early generations of selection do not confer a significant change in the binding parameters of either toxin.

BP9

Mode of action of *Bacillus thuringiensis* toxins active against *Sesamia nonagrioides* (Lefebvre)

J. González-Cabrera[1], G. P. Farinós[2], S. Caccia[3], M. Díaz-Mendoza[2], P. Castañera[2], M. G. Leonardi[3], B. Giordana[3] and J. Ferré[1]

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Sesamia nonagrioides, also known as the Mediterranean corn borer, is one of the most damaging pests of corn in Spain and other Mediterranean countries. Bt-corn expressing the *Bacillus thuringiensis* Cry1Ab toxin, that was designed to control *Ostrinia nubilalis*, has shown good control of *S. nonagrioides* populations. Here we have tested the effect of several Bt toxins against neonate larvae of

S. nonagrioides. Furthermore, we have studied the mode of action of those Bt toxins that were found active: Cry1Ab, Cry1Ac, Cry1Ca, and Cry1Fa. Binding assays were performed with ^{125}I- or biotin-labeled toxins and larval brush border membrane vesicles (BBMV). Competition experiments indicated that these toxins bound specifically to the BBMV and that Cry1Aa, Cry1Ab, and Cry1Ac shared their binding site. Cry1Ca and Cry1Fa bind to different sites. In addition, Cry1Fa binds to the Cry1A's binding site with very low affinity and vice versa, indicating that these toxins act on different binding sites. Binding of Cry1Ab and Cry1Ac was found stable over time, which indicates that the observed binding is irreversible. Pore forming activity of Cry proteins on BBMV was determined using the voltage sensitive fluorescent dye DiSC3 (5). Membrane permeability increased in the presence of the active toxins Cry1Ab and Cry1Fa, but not with the non-active toxin Cry1Da. In terms of resistance management, and considering that Cry1Ca is non-toxic to *O. nubilalis*, our results support the strategy of pyramiding Cry1Ab and Cry1Fa in the same Bt-corn plant for better long-term control of corn borers.

BP10

Antibody blocking of putative receptors inhibits the binding of Cry1Ab in *Bombyx mori*

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The mode of action of *Bacillus thuringiensis* (Bt) insecticidal toxins is not completely clear. Binding of the toxin to midgut receptors is considered a key step in toxicity. In fact, a large reduction in binding is thought to be the most common basis of resistance. Two kinds of protein (Aminopeptidases, APNs, and Cadherin-like compound) are possible receptors for one class of Bt toxins (Cry1A). However, little is known about the involvement of these proteins in Cry1A binding to membranes in solution. The susceptibility of larvae of *Bombyx mori* strain N65 to Cry1Ab was determined in toxicity experiments and studies of the effects of the toxin on midgut cells. Brush border membrane vesicles (BBMVs) of strain N65 were incubated with labeled ^{125}I-Cry1Ab in a dissociation experiment, revealing that about 30% of the binding was irreversible. Highly specific antibodies raised against APN1, APN3, and BtR175 (cadherin-like) proteins of *B. mori* were used to pretreat BBMVs in association kinetics experiments. A significant reduction in specific binding was observed when anti-BtR175 and -APN1 were used in the association experiments. The dissociation experiments revealed a marked reduction in reversible and irreversible binding when anti-BtR175 was used. By contrast, the anti-APN1 specifically affected the amount of irreversible binding, while reversible binding was not affected. High resistance to Cry1A toxins has been associated with reduced binding levels. Consequently, to improve the management of resistant pests, it has been proposed that populations be screened for alleles that can confer resistance. Our experiments indicate that the *cadherin-like* and *apn1* genes are strong candidates for the screening of resistance alleles.

BP11

Effect of Cyt1A yield reduction on *Bacillus sphaericus* Bin toxin synthesis in *Bacillus thuringiensis*

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The mosquitocidal binary (Bin) toxin of *Bacillus sphaericus* (Bs) is composed of two proteins, a 42-kDa toxic domain (BinA) and a 51-kDa binding domain (BinB) that co-crystallize forming a parasporal inclusion that adheres to the internal surface of the exosporium. Although commercial formulations based on strain 2362 are used in many countries, significant resistance to Bs has already been reported in field populations of *Culex* mosquitoes. Cyt1A produced by *B. thuringiensis* subsp. *israelensis* (Bti) masks or delays resistance to Bs Bin and, therefore, can be used to develop recombinant bacteria to overcome or avoid resistance. Previously, we developed a recombinant Bt strain that synthesizes Bin, Cyt1A and Cry11B, and exhibits very high toxicity against *Culex* mosquitoes. In this strain, however, Cyt1A synthesis is unnecessarily high for resistance management, as *cyt1A* expression is controlled by its three strong wild type promoters. Thus, if Cyt1A yield can be

reduced by using a single promoter, the conserved energy and nutrients might be used to increase the yield of other toxins and perhaps increase potency. To test this hypothesis, we constructed a Bt strain in which *bin* transcription was driven by three *cyt1A* promoters and *cyt1A* by only one of its promoters. This strain produced 7.7- and 7.8-fold more BinB and BinA per spore, respectively, compared to that synthesized by a control strain in which transcription of *bin* and *cyt1A* was driven by three *cyt1A* promoters. Though there was an increase in the amount of Bin toxin produced per spore, no significant differences were detected in toxicity on a weight basis between the constructs that used either one or three *cyt1A* promoters to drive Cyt1A synthesis.

BP12

A Novel Lysogenic Bacteriophage MZTP02 from *Bacillus thuringiensis* Strain MZ1

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A new isolated bacteriophage, designated as MZTP02, was induced by mitomycin C, H_2O_2 and UV treatment from *Bacillus thuringiensis* fermentative strain MZ1 and was proved to be a lysogenic phage by indicator *B. thuringiensis* strain ZK1. The plaques lysised by this phage were round and about 3 mm in diameter. Electron microscopic analysis showed that MZTP02 had a long tail (220nm×18nm) and an icosahedral head (82nm×85nm), however, no tail spikes were observed. Experiments of protease and exonucleased analysis revealed that some kind of proteins bound to 5'-extremity of MZTP02 genome. Six different *B. thuringiensis* strains were found to be sensitive to this phage. The MZTP02 genome was determined to be a linear dsDNA containing 15,717 bp with 37.55% G+C content. The genome is flanked by 40 bp terminal inverted repeats sharing 65% identity. Twenty putative open reading frames (ORFs) existed in MZTP02 genome, and nine predicted proteins of them showed similarity to other phage proteins including two terminase subunits, portal protein, minor head protein, scaffold protein, two putative membrane proteins, tail component, and minor structural protein, which were all associated with phage system. Six ORFs were unique to MZTP02. Phylogenetic analysis suggested that MZTP02 might belong to B1 morphotype of family *Siphoviridae* and was closely related to phage *phi* LC.

BP13

What is the mechanism of resistance to *Bacillus thuringiensis* toxin Cry1Ac in a greenhouse population of cabbage looper, *Trichoplusia ni*?

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The cabbage looper, *Trichoplusia ni*, is one of only two insect species that have evolved resistance to *Bacillus thuringiensis* (Bt) in agricultural production situations. To study the mechanism of Bt-resistance in a *T. ni* population that evolved resistance in commercial greenhouses, the genetic trait of resistance to the Bt toxin Cry1Ac from a greenhouse population was introgressed into a highly inbred susceptible laboratory *T. ni* strain. The *T. ni* introgression strain, GLEN-Cry1Ac-BCS, and its near-isogenic susceptible strain were used in this study to identify the mechanism of Cry1Ac resistance evolved in the greenhouse population. The Cry1Ac-resistance in *T. ni* is a monogenic, autosomal and incompletely recessive trait. Biochemical analyses indicated that the activities and composition of midgut proteases, immune response, and the activity and composition of midgut esterase were not altered in the GLEN-Cry1Ac-BCS strain. The pattern of cross-resistance of the GLEN-Cry1Ac-BCS strain to 11 Bt Cry toxins suggested that the resistance is correlated with the Cry1Ab/Cry1Ac binding site in the larval midgut. Further analysis of specific binding of Bt toxins Cry1Ab and Cry1Ac to the midgut brush border membranes confirmed the loss of the binding site for Cry1Ab and Cry1Ac in the midgut of the resistant larvae. We conclude that the mechanism for the Cry1Ac resistance that evolved in the greenhouse population of *T. ni* is alteration of the midgut binding site shared by Cry1Ab and Cry1Ac.

BP14 STU

An approach to the directed evolution of the insecticidal protein from *Bacillus thuringiensis*

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A new method that can develop highly active Cry toxins easily has long been sought. By the way, the activity of AB-type toxin molecules such as the Cry toxin, which have one active site and two binding site, is theoretically improved in parallel with the binding affinity to its receptor. Consequently, the affinity maturation of Cry toxin to the insect receptor is one strategy for developing new Cry toxins with high activity. In this experiment, we tried to devise a method of phage display and screening for the directed evolution of Cry toxins to increase the binding affinity to the insect receptor. Using a commercial T7 phage-display system, we expressed Cry1Aa and Cry1Ab toxin on the phage surface as fusions with the capsid protein 10B. These recombinant phages bound to a cadherin-like protein (BtR175) that is one of the Cry1Aa toxin receptors in the model target insect *Bombyx mori.* Magnetic beads coated with BtR175 were used to assess the binding ability of Cry1Aa-expressing phage (Cry1Aa phage) and Cry1Ab-expressing phage (Cry1Ab phage) to it. The apparent affinity of Cry1Aa phage for the receptor was higher than that of Cry1Ab phage. To confirm the usefulness of beads selection for concentrating phages with higher binding ability to BtR175, the Cry1Aa and Cry1Ab phages were mixed and a total 1.0 x 10^9 pfu phages were selected serially for nine rounds using BtR175-coated beads. When selection was started at a ratio of 0.0001%, the Cry1Aa phage was concentrated up to 13% on average by round nine, meaning that it was concentrated 1.3 x 10^5 times. This result indicates that very few phages that have higher apparent affinity to BtR175 can be concentrated from a mixture using beads selection. To get toxin mutants possessing higher binding affinity to BtR175 and higher insecticidal activity, experiments are currently underway. We are constructing and screening phage libraries of Cry1Aa domain II loop mutants and domain II random mutants.

BP15 STU

***Bacillus thuringiensis* Cry toxin's domain III, Galactose-binding Domain-like binds specifically to various proteins**

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The galactose-binding domain-like fold binds to several types of molecules, including phospholipid, carbohydrate, and DNA. Cry toxins have been reported to bind not only to receptors on larval midgut epithelial cells but also to several unrelated proteins. In this study, we investigated the binding properties of *Bacillus thuringiensis* Cry toxins, focusing on domain III, galactose-binding domain-like fold. In ligand-blot experiments, Cry1Aa, Cry 1Ac, and Cry 8Ca specifically bound to several proteins unrelated to insect midgut cells, although their binding spectra are a little different from each other. N-acetylgalactosamine (GalNAc) did not inhibit binding of Cry1Aa to *Bombyx mori* aminopeptidase N (BmAPN) or other Cry toxin-binding proteins, and also did not inhibit binding of Cry1Ac to Cry toxin-binding proteins. Cry1Aa binding to the Cry toxin-binding proteins was inhibited by a monoclonal antibody, 2C2, which binds domain III of Cry1Aa and inhibits its binding to BmAPN, indicating that Cry1Aa binds to these Cry toxin-binding proteins through domain III. Cry1Aa binding to BmAPN and other Cry toxin-binding proteins was inhibited by bovine erythrocytic carbonic anhydrase, a Cry toxin-binding protein. While, binding regions of bovine erythrocytic carbonic anhydrase and BmAPN were specified to less than 20-amino-acids but they did not have any similarity, suggesting that the galactose-binding domain-like fold of Cry toxin has a binding pocket for multiple proteins or has several protein-binding pockets in near proximity.

BP16 STU

Polymorphism of Fatty Acid from *Ralstonia solanacearum* and Its Classificatory Application on Subspecies

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Ralstonia solanacearum is a soil-borne plant pathogen, which normally invades several hundred

species belonging to at least 50 different plant families. It has been leading to a great economic losing in agriculture and forest. There exist many kinds of *R. solanacearum* strains, which show significant difference and obvious polymorphism on their host-plant species, geography distributing, virulent characteristics, subspecies differentiation and so on. Subspecies differentiation of *R. solanacearum* is complex and the methods to identify them are mostly from physiology, biovar type, serotype, lysotype, virulent type, gene type, etc. Fatty acid is a rich and important component of microbe cells, which has proved to have a consanguineous relativity with the heredity variance, virulence and drug tolerance of bacteria. Microbiology study indicates that: there are accurate homoeologies among fatty acids, largely in bacteria cells, and bacterial DNAs. Every bacterium has its own specific fatty acid chromatogram, so the kind and quantity of whole-cell fatty acid can be used as an index for chemistry taxonomy. The whole-cell fatty acid can be quickly and exactly checked out by using Gas Chromatography technique, and the classification, datamation and automatization can be can make by computer program. In this study, the whole-cell fatty acids of 40 strains of *R. solanacearum* were analyzed and their subspecies were discussed according to the whole-cell fatty acid results. The results showed that C16: $1^{\Delta 7}$ (21%~34%), C16:0 (21%~32%) and C18: $1^{\Delta 7}$ (12%~23%) were the most plentiful fatty acids in *R. solanacearum*. After clustering the results by LGS software, three groups were present: subspecies I (avirulent strain), subspecies Ⅱ (transition-virulent strain) and subspecies III (virulent strain). There was an obvious relativity between the virulence of the bacteria and the kind and quantity of the whole-cell fatty acids, which could be used as an index for chemistry taxonomy of *R. solanacearum*.

BP17 **STU**

Insecticidal Toxicology of HBF-1 Strain from *Bacillus thuringiensis* on *Anomala corpulenta* and *A. exoleta* larvae

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This study is to get more information on the toxicology of HBF-1 strain to *Anomala corpulenta* and *A. exoleta* larvae and its interaction with chemical pesticides. After a series of experiments, the following results were got: The survival rate of *A corpulenta* larvae and *A.exoleta* larvae was decreased, the antifeeding and growth inhibition of *A. corpulenta* larvae were increased, the feeding selectitity to feedstuff of *A. corpulenta* larvae enhanced as the concentration of HBF-1 strain was increased and the time of infected with HBF-1 strain was prolonged. When the *A. corpulenta* larvae treated with HBF-1 strain at the concentration of 10 μg/g soil for seven days, the antifeeding rate and growth inhibition rate were 4.74% and 9.84%, 30.75% and 73.87% for fourteen days. When the *A. corpulenta* larvae treated with HBF-1 strain at the concentration of 160 μg/g soil for seven days, the antifeeding rate and growth inhibition rate were 39.01% and 83.61%, 86.10% and 97.75% for fourteen days. *A. corpulenta* larvae were more repellent to the feedstuff containing HBF-1 strain of high concentration. The sensitivity of *A.corpulenta* to the chemical insecticides increased gradually with increasing exposure time to HBF-1 strain and HBF-1 strain concentration. After the larvae were treated by a mixture of HBF-1 strain and chemical insecticides, its sensitivity were significantly increased by comparing to the larvae treated with chemical insecticides alone. When the larvae treated with a mixture of HBF-1 strain and chemical insecticiedes at the ratio of 1:50 and 1:200, its sensitivity to Phoxim increased 6.06 and 4.00 times while the sensitivity to Chlorpyrifos increased 16.05 and 12.78 times respectively. The activity of AchE in larvae was decreased slightly compared to the blank control and the activity of GsTs and CarE increased in some extent. The histopathology of *A corpulenta* larvae and *A.exoleta* larvae infected by HBF-1strain were studied by means of optical instrument The results showed that the primary symptom of *Anomala corpulenta* and *A. exoleta* larvae after HBF-1 strain infection is not visible, with the increasing time, the infected insect appears inactive, paralysis and lost its sensitivity to the environment, finally the insect become blackish, straight or shrinked until to dead. The histopathology of scarabaeid larvae infected by HBF-1 strain was studied, malformation and vacuolization of the mid-gut cell appeared three days after HBF-1 strain infection, the mid-gut cells were serious damaged and even destroyed completely after 7 days, while the epithelium disappeared after 10 days.

Fungi I

FP1

Reduction of feeding by the Japanese pine sawyer, *Monochamus alternatus* infected with *Beauveria bassiana*

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Monochamus alternatus vectors the pinewood nematode, which causes the pine wilt disease by infecting pine trees through wounds of maturation feedings by the adult beetles. To prevent prevalence of this disease, microbial control of *M. alternatus* has been attempted. *Beauveria bassiana* could bring high mortality on the adult beetles when emerged adults were inoculated. However, since the fungus could not kill the adult beetles immediately, transmission of the nematodes during maturation feedings had been worried about. Therefore, to discuss the possibility of transmission of the nematodes, changes in amounts of maturation feedings by *M. alternatus* adults infected with *B. bassiana* were investigated. Feeding areas by the inoculated beetles were greatly reduced 2 to 4 d after the inoculation. The inoculated beetles did not feed on twigs a few days before their death and died 8 to 10 d in average after the inoculation. On the other hand, feeding areas by non-inoculated beetles increased in 8 d after emergence and then gradually decreased, but were still more than 4 cm^2/d in average on day 10 when most of the inoculated beetles were killed, and kept 1.7 cm^2/d in average even on day 30. Consequently, total area fed by the non-inoculated beetles up to day 30 was 104.3 to 116.7cm^2/individual, whereas that by the inoculated beetles was only 12 to 14% of the non-inoculated beetles. Feeding areas by the inoculated beetles began to decrease at least 9 d before the death and were less than 1/2 of the non-inoculated beetles 6 d before the death. Most of the nematodes began to depart from the inoculated beetles 9 d after emergence and many nematodes were transmitted to branches by non-inoculated beetles whereas only few nematodes were transmitted by inoculated beetles. There were some cases of departure of the nematodes from the inoculated beetles before their death, but even in such cases, the nematodes could not enter the branches, because the beetles did not wound branches. The results numerically indicated that inoculation of *B. bassiana* affected not only to the survivability but also to the activity during their life to decrease the amount of maturation feeding, and low possibility of the nematode transmission by *Beauveria*-infected beetles was demonstrated.

FP2

Research on Cordyceps and its application in AHAU

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Since 1994, we have got some progress in research on *Cordyceps* and its application including: (1) Resource of *Cordyceps* in Anhui province of China. About 40 *Cordyceps* spp. were collected from Anhui province and identified. At the same time, more than 20 anamorphic species were isolated and the relationships between teleomorph and anamorph were established by morphological and molecular methods. 10 new species or varieties or combinations of *Cordyceps* and its anamorphs were identified, such as *Cordyceps bassiana* Z. Z. Li, C. R. Li, B. Huang & M. Z. Fan and its *Beauveria bassiana* anamorph, *C. phymatospora* C. R. Li, M. Z. Fan & Z. Z. Li, *C. gunnii* var. *minor* and *Paecilomyces gunnii* var. *minor* Z. Z. Li, C. R. Li, B. Huang, M. Z. Fan & M. W. Lee, *Hirsutella longissima* C. R. Li, B. Huang, M. Z. Fan & Z. Z. Li as the anamorph of *C. longissima* Kobayasi, *H. huangshanensis* C. R. Li, M. Z. Fan & Z. Z. Li as the anamorph of *C. formosana* Kobayasi & Shimizu, *Hirsutella heteropoda* C.R. Li, M.J. Chen, M.Z. Fan & Z.Z. Li and its teleomorph *C. heteropoda* var. *langyashanensis* C.R. Li, M.J. Chen, M.Z. Fan & Z.Z. Li, *Lecanicillium militare* (Kobayasi) C. R. Li, M. Z. Fan & Z. Z. Li as the anamorph of *C. militaris*, and *Paraisaria gracilioides* (Kobayasi) C. R. Li, M. Z. Fan & Z. Z. Li as the anamorph of *C. gracilioides* Kobayasi. (2) Application of anamorphic strains of *Cordyceps* and its allies in food or medicine development. Extracts from more than 20 species of *Cordyceps* and its allies including *Hirsutella sinensis*, *Paecilomyces cicadae*, *P. farinosus*, *P. gunnii* var. *minor*, *P. tenuipes*, *Acremonium implicatum*, *Lecanicillium militare*, *Beauveria amorpha*, *B. bassiana* and so on were proved by several kinds of pharmacological models in vivo or in vitro to possess various functions, such as immunological regulation, anti-rheumatic arthritis, hormone regulation, anti-senescence, liver protection, sedation and hypnosis，anti-oxidation，anticancer and

antidepressant effects. Up to now, we have obtained 5 Chinese patents related with *Cordyceps* and its allies from National Bureau of Intellectual Property Rights of China and 1 Certificate of Permission of health food from Ministry of Public Health of China.

FP3 STU

Thermotolerance and cold activity of sixty *Beauveria* spp. isolates

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Heat and cold are two abiotic factors of the environment that restrict the use of entomopathogenic fungi as agents for biological control of insects. To aid in selecting isolates of *Beauveria* spp. with promise for field use, we studied the thermotolerance and cold activity of 60 isolates, including six species, of *Beauveria* from various geographic regions, arthropod hosts and/or substrates. High variability in conidial thermotolerance was observed among the isolates after 2 h heat exposure at 45 °C, ranging from low (0 - 20%), to medium (20 – 60%), to high germination (60 – 80%). Less than 40% of conidia from the three most thermotolerant isolates of *B. bassiana* (CG138, GHA and ARSEF 252) remained viable after exposure to 47 ºC for 2h. The thermal death point of conidia of these isolates was between 44°C and 45°C after 6 h of heat exposure. At cold temperatures, with few exceptions (viz. UFPE 3138 and CG 66) most of the isolates grew at 5°C. Interestingly, the one isolate of *Beauveria alba* (UFPE 3138) was the most susceptible isolate to both heat and cold stress. Isolates ARSEF 252 and GHA, on the other hand, were the most thermotolerant and had the highest cold activity. Some isolates with high cold activity, however, were thermosensitive (e.g. ARSEF 1682). An attempt to correlate the latitude of origin with thermotolerance or cold activity indicated that isolates from higher latitudes were more cold active than isolates from nearer the equator. There was not a similar correlation, however, for heat.

FP4

Evaluation of *Beauveria bassiana* for the control of glassy-winged sharpshooter, *Homalodisca coagulata* (Homoptera: Cicadellidae)

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Various isolates of *Beauveria bassiana* recovered from the *Homalodisca coagulata* habitats and insect hosts in southern California were evaluated along with the commercial (GHA) isolate and those recovered from natural infections in *H. coagulata* in Texas and Mississippi. The radial growth of these isolates was evaluated at 15, 23, 28 and 32 ^{o}C. Genetic relatedness of these isolates was also determined through molecular characterization. Based on the virulence and ability to grow at a range of temperatures suitable for California climate, two California isolates – one from a soil sample and the other from the three-cornered alfalfa hopper, *Spissistilus festinus*, and the Texas isolate from *H. coagulata* were selected for further evaluation. Virulence of these three isolates was similar at each of the three concentrations (10^5, 10^7 and 10^9 conidia/ml) evaluated in the additional assays.

FP5 STU

Is isolate ARSEF 3609 a *Metarhizium anisopliae* var. *anisopliae* or var. *acridum*?

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In a recent study on characterization of 100 Metarhizium cultures the isolate ARSEF 3609 (= IIBC 191—614) was identified as *M. anisopliae* var. *anisopliae* (Driver *et al*. 2000. Mycol. Res. 104: 134-150). This isolate, however, has physiologic features (e.g. host range, culture aspect, dodine susceptibility, and UV-B and heat tolerance) more compatible with those of *M. anisopliae* var. *acridum* (Braga *et al*. 2001. J. Invertebr. Pathol 78: 98-108; Rangel *et al*. 2005. J. Invertebr. Pathol. 88, 116-125; Alston et al. 2005. Biol. Control 35, 163-171). The study reported here examined the genotypic similarity of ARSEF 3609 to several isolates of *M. anisopliae* varieties *anisopliae* and *acridum*. Ten isolates were used for AFLP analysis: four isolates known to be *M. anisopliae* var. *acridum* (ARSEF 3341, ARSEF 324 plus its parent "FI985" and its commercial product "Green

Guard") and six isolates of *M. anisopliae* var. *anisopliae* (ARSEF 23, 2575, 4343, 5626, 7847, and F52). There were 123 polymorphic bands produced by three primers pairs. A combined dendrogram for all three banding patterns revealed two clusters of isolates. The within-group similarity in the first group was 90%, and 65% in second group. The *M. anisopliae* var. *acridum* isolates were clustered in the first group and the *M. anisopliae* var. *anisopliae* isolates in the second group. Isolates FI985, Green Guard and ARSEF 324, supposedly are the same isolate with different names due to different culture histories. The AFLP results suggest that they are indeed the same isolate. Isolate ARSEF 3609 clustered with the variety *acridum* isolates. Analysis by Driver et al. (2000) using ITS and ribosomal DNA sequence data placed their culture of this fungus within *M. anisopliae* var. *anisopliae*. Use of the same primers with our subculture of ARSEF 3609 placed the fungus in *M. anisopliae* var. *acridum*, and this is in accordance with its biological traits, e.g. host range, heat and UV-B tolerance, spore size and color, and dodine susceptibility.

FP6 STU

Biological control of soybean cyst nematode using *Verticillium lecanii* (*Lecanicillium* spp.) and fungi isolated from cyst

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Soybean cyst nematode (SCN), *Heterodera glycines,* is widely distributed in soybean producing areas, and causes great yield loss in worldwide. Suppression of plant parasitic nematodes with nematode parasites, predators or antagonists is an eco-friendly approach than the chemical nematicides. In this study, three strains of *V. lecanii* (Vertalec, Mycotal and B-2), 162 *V. lecanii* hybrid strains obtained for protoplast fusion among three strains of *V. lecanii,* and 31 fungi isolated from cyst were assayed in greenhouse for control against of SCN. First experiment was conducted using all fungal isolates. Seeds of soybean were sown into 6 cm diameter pots containing fungus-inoculated soil (95% potting compost, 5% wheat bran). After 5 days, seeds were germinated and transplanted to potting compost (20% contaminating soil included cysts/g) 12 cm diameter pots. These plants were harvested after a further 8 weeks, and roots or foliages were estimated visually. 20 out of 196 isolate were showed inhibition in parasitism of SCN. In the second experiment, 20 fungal strains showed good result in first screening were selected, and seeds were sown into 6 cm diameter pots (97% potting compost, 3% wheat bran which containing fungal strains). 5 days later, the three seedlings were transplanted into 18 cm diameter pots (10% contaminating soil included). After 8 weeks, population level of cyst, flesh weight, root weight, foliages and number of seed pod were evaluated. These result indicated that it could be possible that isolate of *V. lecanii* (AaF17, AaF28, AaF42, AaF80, AaF88, and AaF103) and fungi isolated from cyst (W10, W13) have beneficial effect as an alternative to control of SCN. Further study would be required to develop better methodology to quantify population dynamics of these fungi and their effects on nematode populations in fields, and t determine their potential as biological control agents.

FP7 STU

Evaluation of pathogenicity against cotton aphid and greenhouse whitefly, and viability on the leaf to use hybrid strains of *Verticillium lecanii*

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Protoplast fusion among three strains of the entomopathogenic fungi *Verticillium lecanii* (Vertalec, Mycotal and B-2) was conducted to obtain hybrid strains that have wide host range and long-term effect (Aiuchi *et al*., 2004). Vertalec and Mycotal were commercial biological control agents which have high virulence against aphids, high virulence against whiteflies, respectively. Strain B-2, isolated from green peach aphid in Obihiro (northern Japan), and was found recently to have high colonization ability on cucumber leaves under low humidity conditions (Koike *et al*., 2004). This study has aimed to screen the favorable strains that have the characteristics described above, from hybrid strains. These hybrid strains, first of all, were applied to bioassay for cotton aphid, *Aphis gossypii* and 38 hybrid strains in 44 showed equal or more mortality compared to Vertalec (22.2%). Secondly, these 44 hybrid strains were applied to bioassay of greenhouse whitefly, *Trialeurodes vaporariorum* and 37 hybrid strains showed equal or higher infection rate compared to Mycotal (36.2%). Finally, 44 hybrid strains

were applied to colonization test to evaluate the viability on cucumber leaves under low humidity condition (c.a. 13% r.h.). Two weeks after spraying, 12 hybrid strains showed equal or higher colonization ability compared to B-2 (1494.7cfu/cm^2). In these hybrid strains, the highest mortality against cotton aphid was 84.5% (2aF31), and highest infection rate against whitefly was 94.7% (2aF1). Furthermore 4 hybrid strains (2aF1, 2aF4, 2aF26, 2aF43) showed more than 70% mortality and infection rate to both noxious insects. Interestingly, these data indicates that parents' characteristics not only just combined but also a virulence itself was improved. In addition to high virulence against both insect, 2aF26 and 2aF43 also have high viability, therefore these hybrid strains can be expected the highly effect of control on a practical level.

FP8 STU

Thermotolerance of germlings and mycelium of *Metarhizium anisopliae* var. *anisopliae* and *acridum*

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High temperature may limit in some environments the effectiveness of *Metarhizium* spp. for insect control. This is particularly true with control of orthopterous insects (e.g. grasshoppers, locust, and katydids) that bask in sunlight to raise their hemolymph temperature to 40°C or higher (behavioral fever) and thereby impede growth of most insect-pathogenic fungi. Dormant conidia of *Metarhizium anisopliae* var. *acridum* isolate ARSEF 324 had high levels of survivability after a 12-h exposure to 45°C; and this was the highest thermotolerance noted in a comparison of 17 *Metarhizium* isolates (Rangel et al., 2005. J. Invertebr. Pathol. 88, 116-125). The upper limit temperature for conidial germination or mycelial growth of *M. anisopliae*, however, is from 37 to 40°C (e.g. Hallsworth and Magan 1999. J. Invertebr. Pathol. 74, 261-266; Milner, 1997. Mem. Entomol. Soc. Canada. 171, 287-300). Several physiological changes are associated with germination, such as trehalose degradation and change in the cell membrane fatty acids ratios from saturated to increasingly unsaturated. These factors alter membrane permeability and consequently severely reduce the stress resistance of germ tubes and mycelia. In this study, the upper temperature limits for germination and growth of three isolates of *M. anisopliae* var. *acridum* (ARSEF 324, 3341 and 3609) and two isolates of var. *anisopliae* (ARSEF 2575 and 5749) were examined and compared with a thermophilic fungus, *Aspergillus nidulans* (ATCC 10074), that has an unusually high (near 40°C) optimum growth temperature (Pontecorvo et al., 1953. Adv. Genetics 5, 141-238). Germination was strongly impaired at 38 and 40°C for both varieties of *M. anisopliae*. Isolate ARSEF 324 was the most thermotolerant, i.e., approximately 40% germinated at 38°C, and less than 10% at 40°C. When the radial growth rates were measured, however, none of the *M. anisopliae* isolates grew at 38°C during the study period (10 days). Therefore, conidia that germinated at 38 and 40°C discontinued growth soon after germination. At 35-36°C, most of *Metarhizium* isolates germinated well, but their radial growth was very slow as compared with at 28°C (except ARSEF 5749 from Mexico, which did not grow). All isolates kept at 38 and 40°C for 10 days resumed growth soon after they were transferred to 28°C, and they all eventually sporulated. However, when the plates were kept at 42°C, only the isolates of *M. anisopliae* var. *acridum* (i.e. ARSEF 324 and 3609) were able to resume growth at 28°C. The results indicate that, as compared to the thermophilic fungus *A. nidulans* that germinated and grew well at 42°C, both *M. anisopliae* var. anisopliae and *M. anisopliae* var. *acridum* are mesophilic (optimum for growth between 25 and 35°C).

FP9

Efficiency of aerial conidia and submerged propagules of *Paecilomyces fumosoroseus* (Wise) Brown and Smith against *Bemisia* (Gennadius) spp nymphs in laboratory

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The whitefly has been a problem for crops in northwest México, even when successful endeavors have been in the application of technologies; there is enough work to realize together. In México, Sinaloa State Government reported 40,000 ha of bean, soybean and vegetables crops affected by whitefly during last agriculture cycle. Strategies for controlling whitefly have used *Paecilomyces*

fumosoroseus because is its natural enemy, producing strong epizootic in greenhouse like in field. So, aerial conidia and submerged propagules of *Paecilomyces fumosoroseus* has been used in whitefly control without to establish what is better. Lacey *et al*. (1999) found no significative difference between virulence of blastospores and aerial conidia of *P. fumosoroseus* strain Pfr42 (applying 3.8 x 10^3 propagules/cm^2) against nymphs of *Bemisia argentifolii* Bellows and Perring. However, blastospores of strain Pfr97 were significantly more virulent than aerial conidia, with mortalities of 77 ± 5 and 27 ± 7 %, respectively. Our objective was to determine the efficiency of aerial conidia and submerged propagules of *P. fumosoroseus* strain Pfrd against *Bemisia* spp nymphs in laboratory to compare with our field trials (Osuna *et al*., 2003). Bioassays were done using 8 treatments with three replicates using 2^{nd} -instar whitefly nymphs grew on young plants of *Gossypium* spp. Propagules were obtained in solid and submerged media. Evaluations of dead and health whitefly nymphs were made by changes in coloration at 10 days after applications. Average temperature and relative humidity were 27° C and 74%, respectively. Growing degree-day was used to understand behavior of whitefly (*Bemisia* spp). Dose quantification was determinated by counting under an optical microscope. There was no significative difference between average mortalities produced by fermentation broth, submerged propagules, aerial conidia, sugmerged propagules plus fermentation broth of *P. fumosoroseus* strain Pfrd against (*Bemisia* spp) nymphs (75 ± 25, 78 ± 16, 75 ± 23 and 80 ± 25 %, respectively) but they were significatly upper than control (F = 5.510, P = 0.00409). These results were similar to our field trials with same plague, strain and treatments but with different host (*Solanum melongena*).

FP10 STU

Efficacy of *Beauveria bassiana* (Bals.) Vuill. against the tarnished plant bug, *Lygus lineolaris* L., in strawberry field

Rachid Sabbahi and Claude Guertin

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The entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin has a high potential to control the populations of the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), an important pest of strawberries. *B. bassiana* isolates INRS-IP and INRS-CFL were highly infective against *L. lineolaris* populations under laboratory conditions. LC_{50} values of theses isolates were respectively of $7.8x10^5$ and $5.3x10^5$ conidia/ml for adults at 7 days post treatment, and LT_{50} values were respectively of 4.46 and 4.37days at a concentration of $1x10^7$ conidia/ml. The efficacy and the infectivity persistence of *B. bassiana* based formulation against the *L. lineolaris* populations were evaluated in strawberry fields. Conidia of isolates INRS-IP and INRS-CFL were applied at two rates of $1x10^{11}$ and $1x10^{13}$ conidia/ha. Using a randomized block design with four replicates, plants were treated weekly for a month. The viability of *B. bassiana* propagules on foliage was investigated by measuring the number of colony forming units (CFUs) recovered after treatments from discs sampled on strawberry leaves at intervals of 3 days. The pathogenicity test carried on *L. lineolaris* adults fed with Leaves harvested from treated plants every 3 days were fed to *L. lineolaris* adults in laboratory, pathogenicity tests were conducted to assess the persistence of conidial infectivity in the field. Field evaluation of *B. bassiana* isolates INRS-IP and INRS-CFL efficacy against *L. lineolaris* populations revealed the presence of viable and infective conidia up to 6 days on strawberry foliage. The temporal changes in insect population densities was also investigated. The multiple sprays of *B. bassiana* at weekly intervals resulted in a reduction in nymph populations. Furthermore, a trend towards a significant reduction of 26.3 % in *L. lineoalris* injury of strawberries was observed in the treated plots with application rate of $1x10^{13}$ conidia/ha compared to the control. This study is the first to highlight that multiple treatments must be taken into account in foliar application of *B. bassiana* conidia in strawberry crops to control *L. lineolaris* populations.

Microbial Control I

MCP1

The resistance of *Anopheles sinensis* from southern and central China to *Bacillus thuringiensis* subsp. *israelensis*

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In this experiment, lethal and sublethal effects of *Bacillus thuringiensis* subsp. *israelensis* on *Anopheles sinensis* was evaluated through investigating 50% lethal concentrations (LC_{50}) value, development and mortality in responses to sublethal doses of *B. thuringiensis*. The LC_{50}s for populations from Zhongshan, Hengxian, Wuchang, Xinzhou, Laboratory and Ruichang were 146.4-, 50.4-, 57.9-, 50.0-, 24.6- and 41.6 ng/ml, respectively. Five field populations displayed susceptible differences to *Bacillus thuringiensis* subsp. *israelensis* from 1.7- to 5.9-fold compared to the laboratory strain with zhongshan strain having lowest susceptibility. After 24 h exposure to the sublethal concentration, cumulative corrected mortalities of larvae increased daily although the surviving larvae were rinsed with distilled water, removed to clean distilled water, and reared on the standardized procedure. *B. thuringiensis* subsp. *israelensis* had residual effects extending beyond 6 - 7 days with mortality peaks from 1st to 4th day. Sublethal exposure to *B. thuringiensis* subsp. *israelensis* made the duration of larval development longer and the duration of pupal development shorter, indicting that *B. thuringiensis* subsp. *israelensis* influenced also the development of larvae and pupae besides killing the mosquitoes.

MCP2

Characterization and toxicity to coleopteran insects of *Bacillus thuringiensis* isolates from warehouses

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Bacillus thuringiensis was isolated from 122 of 413 samples obtained from warehouses. Most isolates were not toxic to *Phaedon brassicae*. Mortality from 78 isolates (64%) were less than 4%, and 98 isolates (80%) were distributed in 0 to 10% mortality group; only isolate 165 proved toxic to larvae of *P. brussicae* with more than 90% mortality. Then toxicity of isolate 165 to several stored production beetles were evaluated. The results showed that its corrected mortalities (%) to *Rhizoperta dominica* adult、*Tribolium castaneum* adult、*T. castaneum* larvae、*Oryzaephilus*、*surinamensis* adult、*Callosobruchus chinensis* adult、*Cryptolestes minutus* adult、*Henosepilachua vigintioctopunctata* larvae、*Sitophilus zeamais* adult、*Tenebrio molitor* larvae、*Derestes maculatus* larvae、*Aulacophora femoralis* larvae、*Phyllodecta vulgatissima* larvae and *P. brassicae* larvae were 59.55-、8.89-、26.67-、2.22-、4.65-、0-、60.95-、4.44-、52.44-、13.33-、56.32-、90.36- and 92.00%. Isolates 165 belonged to serotype $H_{8,}$ and were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and polymerase chain reaction (PCR). The results showed that they contained ~135 and 65 kDa proteins and harbored *cry3* without *cry1* and *cry2*, which were similar with reference strain *B. thuringiensis* subsp. *tenebrionis* strain Te.

MCP3

Susceptibility of *Pyrausta sticticalis* to *Bacillus thuringiensis*-based formulations depending on host plant

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The beet web worm, *Pyrausta sticticalis* L., is a serious pest insect damaged to various crops in Russia. Susceptibility of *P.sticticalis* larvae to *Bacillus thuringiensis* (*Bt*)- based formulations registered in Russia was shown in our earlier research. However, some recent data have discovered the significance of tritrophic interactions (plant - phytophagous insect - biocontrol agent) for microbial insect control. Therefore, the aim of this study was to evaluate the susceptibility of *P, stisticalis* larvae to two *Bt*-based formulations on two species of host plants. Lucerne and carrot were chosen as common plants strongly damaged by the beet web worm. After emeging from eggs, larvae were reared on these plants under laboratory conditions. Larval development was rather quick both on carrot and lucerne (12-14 days). Third instar larvae were exposed to plant treated with suspension of *Bt*- formulation of different concentration. Lepidocid containing spores and crystals of *Bt* subsp. *kurstaki* and Bitiplex based on hydrolysed crystals of the same subspecies were used for insect treatment. Data for corrected mortality showed greater susceptibility of the insect to the Lepidocid when beet web worm larvae were fed on lucerne compared to to larvae fed on carrot. More than 90% larvae fed on lucerne plant died whereas the most part of larvae fed on carrot plant was survived at 3 day after treatment with Lepidocid (o.5%). LC_{50} for this preparation was 10 times greater when larvae

were fed on carrot compared with LC_{50} obtained for larvae fed on lucerne. The influence of host plant on Bitiplex activity toward beet web worm was almost the same despite this preparation caused the less rate of larval mortality both on carrot and lucerne compared with mortality caused by Lepidocid. Further research on the effect of host plant on mortality of *P.sticticalis* larvae induced by *Bt*-based formulations is required.

MCP4

Diagnosis of Arthropod Diseases – Since more than 50 years in the "Institute for Biological Control" of the "Federal Biological Research Centre for Agriculture and Forestry"

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In the "Institute for Biological Control", diagnosis of arthropod diseases is conducted since more than 50 years. In the middle of the 1950ies, the "Laboratory for diagnosis, histo- and cytopathology of arthropod diseases" was founded, and in the following it has developed to a worldwide acknowledged diagnostic service. All known pathogenic groups of arthropods, like viruses, bacteria, rickettsiae, fungi, and protozoa are diagnosed and investigated. These basic studies are aimed at developing methods of biological and integrated control and at broadening knowledge on the biodiversity of microbial pathogens of arthropods. The importance of diagnostic research is continuing, especially in view of the following tasks: (1) Detection and description of new pathogens for biological and integrated pest management. (2) Prognostic monitoring of pest populations with special reference to the presence and efficacy of natural antagonists. (3) Sanitary supervision of arthropod rearings. Up to this year, altogether 1880 accessions of pest or beneficial arthropods sent for diagnosis have been investigated. Pathogens were isolated from about 400 different arthropod species of 15 orders. Details of these investigations will be presented. More informations on our diagnostic laboratory can be found at: http://www.bba.de/english/bbaeng.htm

MCP5

Inheritance of Resistance and Effect of PM on Toxicity of *Bacillus thuringienesis* toxin Cry1Ac in Cabbage looper, *Trichoplusia ni*

Wei Guo[1,2], Guoxun Li[3] and Ping Wang[2]

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Two colonies of cabbage looper, *Trichoplusia ni*, received from British Columbia, Canada in 2001, were selected with *Bt* Cry1Ac in the laboratory. After ten generations of selection, a highly resistant strain, GIP-Cry1Ac was obtained with a resistance ratio of 3,688 fold compared to a susceptible laboratory strain. Another strain, GLEN-Cry1Ac showed a resistance ratio of 981 fold after seven generations of selection with *Bt* Cry1Ac The progeny from diverse crosses were used to determine the mode of inheritance of resistance to *Bt* Cry1Ac. The F_1 progeny from reciprocal crosses between GIP-Cry1Ac and susceptible laboratory strains has resistance ratios of 9.9 and 10.0, respectively, with a degree of dominance of –0.44. The results suggested that the inheritance of resistance to Cry1Ac in the GIP-Cry1Ac strain was autosomal and incompletely recessive. Observed mortality did not significantly deviate from expected mortality of progeny from the backcrosses (F_1 x resistant strain) at ten testing Cry1Ac concentrations using the single locus test. This demonstrated that the resistance was controlled by one major locus; therefore inheritance of resistance was monogenic. Resistant GLEN-Cry1Ac strain showed the similar inherited traits of resistance. Interstrain complementation tests displayed that the F_1 progeny from crossing the GIP-Cry1Ac and GLEN-Cry1Ac strains were resistant to a diagnostic concentration of Cry1Ac (10μg/ml), which suggested that GIP-Cry1Ac and GLEN-Cry1Ac strains shared the same genetic locus conferring resistance to Cry1Ac. Bioassays of larvae treated with Cry1Ac and enhancin or Calcofluor showed that peritrophic membrane affected the activity of *Bt* Cry1Ac.

MCP6

Analysis of midgut ESTs from *Costelytra zealandica* to identify candidate genes involved in amber disease

Sean DG Marshall[1], Laurence N Gatehouse[2], S Anette Becher[3], Drion G Boucias[4], Mark RH Hurst[1],

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The endemic grass grub, *Costelytra zealandica*, is an important pasture pest in New Zealand. With the declining use of chemical control methods, alternative methods for managing *C. zealandica* have been explored. The bacteria *Serratia entomophila* and *S. proteamaculans* cause amber disease in *C. zealandica* larvae and strains of *S. entomophila* have been used to successfully control *C. zealandica* in the field. Symptoms of amber disease in *C. zealandica* larvae include: cessation of feeding; rapid clearance of midgut contents and a near elimination of proteolytic activity associated with the midgut; amber colouration of the midgut; and eventual death of larvae. The disease causing genes are plasmid bourne and both the antifeeding and the gut clearance gene cassettes have now been identified. While the bacterial genes contributing to amber disease are now known, how the disease disrupts *C. zealandica* midgut function remains largely unknown. To enable the identification of potential gene targets within *C. zealandica*, an EST library from healthy third instar larval midgut tissue was constructed and analysed. Genes encoding proteolytic enzymes are of particular interest as amber disease results in rapid elimination of midgut proteolytic activity. At least fifteen different serine protease (SP; e.g. trypsin, chymotrypsin) genes are expressed in the midgut of *C. zealandica*. Sequence analysis demonstrates that these putative *C. zealandica* SP sequences share conserved sequence motifs characteristic of SPs. Phylogenetic analysis of the *C. zealandica* SPs, together with those from other insects (including Coleoptera, Diptera, and Lepidoptera species), distinguishes approximately four major subgroups of *C. zealandica* SPs. Quantitative PCR (qPCR) experiments examining the transcriptional regulation of SPs and other candidate genes within the gut of *C. zealandica* are also being conducted. Initial qPCR results suggest differential patterns of SP regulation in the anterior and posterior portions of the midgut. A model for regulation of SP synthesis by *Serratia* spp. will be discussed based on the results from both the phylogenetic and the qPCR analyses.

MCP7

Biotic and abiotic factors affecting performance of *Serratia entomophila* as a biopesticide for grass grub (*Costelytra zealandica*) in New Zealand

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The native grass grub (*Costelytra zealandica*) is a major pest of pastures in New Zealand. It is host to a wide range of pathogens, including *Serratia* spp. causing amber disease. Strains of *Serratia entomophila* have been developed for use as biopesticides against the grass grub. The bacterial product has been applied directly to the soil in liquid or granule formulations. Bacterial persistence is influenced by both biotic and abiotic factors. The key biotic factor is density of the host insect as the applied bacteria will recycle through the target population. Abiotic factors are harder to define. Severe drought can affect bacterial persistence but other management factors may also influence long term survival. Factors involved in performance and persistence of *S. entomophila* will be discussed in relation to the biopesticide programme carried out in New Zealand for more than a decade.

MCP8

Induced *Serratia entomophila* Sep proteins show activity against larvae of the New Zealand grass grub *Costelytra zealandica*

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The *Serratia entomophila* pathogenicity (*sep*) genes are the genetic determinants of amber disease of the New Zealand grass grub *Costelytra zealandica*. Each of the *sep* genes were placed as either separate entities or as various combinations under the control of an arabinose expression vector allowing their inductive expression. Western analysis confirmed production of the Sep proteins. Bioassays showed that the phenotypes of amber coloration and gut clearance were only produced when *sepA, sepB* and *sepC* were co-expressed. Transferring the arabinose expression cassettes to a *S. proteamaculans* strain resulted in the production of Sep proteins with increased activity, coinciding

with the production of a mature SepB protein in relation to its truncated *E. coli* counterpart. After 10-11 days from ingestion of the toxin filtrates, some of the larvae reverted from a diseased to a healthy phenotype suggesting that continuous presence of Sep proteins is necessary to produce the disease effect.

MCP9

Inheritance of Resistance and Effect of PM on Toxicity of *Bacillus thuringienesis* toxin Cry1Ac in Cabbage looper, *Trichoplusia ni*

Wei Guo[1,2], Guoxun Li[3] and Ping Wang[2]

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Two colonies of cabbage looper, *Trichoplusia ni*, received from British Columbia, Canada in 2001, were selected with *Bt* Cry1Ac in the laboratory. After ten generations of selection, a highly resistant strain, GIP-Cry1Ac was obtained with a resistance ratio of 3,688 fold compared to a susceptible laboratory strain. Another strain, GLEN-Cry1Ac showed a resistance ratio of 981 fold after seven generations of selection with *Bt* Cry1Ac. The progeny from diverse crosses were used to determine the mode of inheritance of resistance to *Bt* Cry1Ac. The F_1 progeny from reciprocal crosses between GIP-Cry1Ac and susceptible laboratory strains has resistance ratios of 9.9 and 10.0, respectively, with a degree of dominance of –0.44. The results suggested that the inheritance of resistance to Cry1Ac in the GIP-Cry1Ac strain was autosomal and incompletely recessive. Observed mortality did not significantly deviate from expected mortality of progeny from the backcrosses (F_1 x resistant strain) at ten testing Cry1Ac concentrations using the single locus test. This demonstrated that the resistance was controlled by one major locus, therefore inheritance of resistance was monogenic. Resistant GLEN-Cry1Ac strain showed the similar inherited traits of resistance. Interstrain complementation tests displayed that the F_1 progeny from crossing the GIP-Cry1Ac and GLEN-Cry1Ac strains were resistant to a diagnostic concentration of Cry1Ac (10μg/ml), which suggested that GIP-Cry1Ac and GLEN-Cry1Ac strains shared the same genetic locus conferring resistance to Cry1Ac. Bioassays of larvae treated with Cry1Ac and enhancin or Calcofluor showed that peritrophic membrane affected the activity of *Bt* Cry1Ac.

MCP10

A chitin deacetylase-like protein identified from cabbage looper, *Trichoplusia ni*

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A novel midgut peritrophic membrane (PM) protein, TnPM-P42, was identified from the cabbage looper, *Trichoplusia ni*. TnPMP42 was shown as a 42 kDa protein by SDS-PAGE analysis and appeared to be associated with the PM throughout its entire length. In *T.ni* larvae, the midgut is the only tissue where TnPM-P42 could be detected during the feeding period of the larvae. TnPM-P42 has chitin-binding activity and is strongly associated with the PM, which is similar to the currently known peritrophin type PM proteins. However, TnPM-P42 represents a unique family of proteins distinctly different from the peritrophin type PM proteins in its sequence characteristics. TnPM-P42 does not contain the peritrophin domain which is present in all the currently known PM proteins, but instead has a chitin deacetylase-like domain. Sequence similarity search of the GenBank database did not result in identification of any known proteins with a significant overall sequence similarity to the TnPM-P42. However, expressed sequence tags (ESTs) from various arthropods were identified to code for proteins with high sequence similarities to TnPM-P42, indicating the presence of TnPM-P42 homologs in other arthropods. Consistent with the identification of various ESTs from arthropods, Western blot analysis demonstrated the presence of a TnPM-P42-like protein in the PMs from *Heliothis virescens*, *Helicoverpa zea* and *Spodoptera exigua*larvae. The sequence characteristics of TnPM-P42 indicate that TnPM-P42 represents a novel family of insect proteins. However, its biochemical and physiological functions require further investigation.

MCP11

A cDNA-AFLP differential gene expression of the entomopathogenic fungi *Beauveria bassiana* during growth on different insect cuticles

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The filamentous fungus *Beauveria bassiana* is considered to be an opportunist, endophyte or saprophyte and turn out to be a pathogen when brought into a new environment. Several attempts have been made to address the question of what makes fungi a parasite, in different host pathogen systems. In the present study an attempt is made to know more salient features revealed by analysis of the genome, including the search for candidate pathogenecity and virulence genes and the switch to a pathogenic lifestyle while adapting to a successful parasitic life. A cDNA-AFLP (cDNA-amplified fragment length polymorphism) differential gene expression technique is used to cave the molecular basis of the process of infection and invasive growth of the insect host cuticle. Penetration and growth of *B.bassiana* on the four different insect cuticles belonging to different taxonomic order was tested towards a nutrient rich Sabouraud dextrose broth (SDB) as control invitro. The overall expression patterns on cuticle and SDB was assessed and the critical difference in the transcriptional control during pathogenic and growth on the nutrient media was recorded. The nucleotide sequence of 48 transcripts was analyzed using 4 primer combinations and the amino acid sequences of their predicted products were compared with entries in databases. Most of the unregulated genes were involved in metabolism, signal transduction and invasive growth. Two of them showed homology with the fungal proteases and one with the esterase and one with amino peptidase. There were also many genes of unknown function.

MCP12

Cloning and Expression of *cry3Aa8* gene from a *Bacillus thuringiensis* isolate against Coleoptera *Leptinotarsa decemlineata*

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cry3Aa8 gene (Accession number in GenBank: AY572010) coding insecticidal crystal protein Cry3A was cloned by PCR technique from *Bacillus thuringiens* subsp. *Morrisoni,* strain YM-03, which was isolated from soil in China and highly toxic to coleopteran (e.g.*Leptinotarsa decemlineata*, *Phaedon brassicae*). *cry3Aa8* contains an open reading frame (ORF) of 1956 bps encoding a protein of 652 amino acid residues and exhibited 99% similarity to the known *cry3Aa7* in sequences. A potential ribosome binding site (RBS) (gaaagggagg) and STAB-SD sequence were found at upstream of the proposed ATG start codon and downstream of the -10 sequence (tataaatt), respectively. -35 sequence (gattaaga) lied at upstream of the -10 sequence. The structure of *cry3Aa8* promoter is similar to other *cry3A* genes. Two recombined plasmids, *pHT304/3Aa8* and *pHT304/*P_{1Ac}*3Aa8* were constructed. The former contains *cry3Aa8* and its own promoter while the later contains *cry3Aa8* and the promoter of *cry1Ac* from *Bt* strain C-33 with highly toxic to lepidopteran. Two recombined plasmids were transferred into *Bacillus thuringiensis* acrystalliferous (*Cry*$^-$) BMB171, and two engineering strains BMB171/*3Aa8* and BMB171/P_{1Ac}*3Aa8* were obtained. Analysis of insecticidal protein Cry3Aa8 indicated that the expression level of *cry3Aa8* was higher in BMB171/*3Aa8* than in wild-type *Bt* strainYM-03, and the lowest in BMB171/P_{1Ac}*3Aa8.*

MCP13

Mycoinsecticides: comprehensive list and current status

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This study aimed to assemble a comprehensive list of mycoinsecticides developed worldwide. A variety of sources, including scientific publications, personal communications and websites from manufacturers and regulatory agencies was accessed. During the last four decades, approximately 80 companies worldwide developed and/or manufactured 144 mycoinsecticides (including products with activity against mites), among which 66.7% are currently registered, under registration or marketed (in some circumstances without registration), 16.7% are no longer available, and the status of 16.7%

of listed products could not be determined. To date, at least 13 species or subspecies of fungi have been employed as active ingredients of mycoinsecticides, the most common being *Metarhizium anisopliae* (53 products), *Beauveria bassiana* (46), *Lecanicillium* spp. (11), *Paecilomyces fumosoroseus* (8), and *B. brongniartii* (7) . Targets are distributed among 9 insect orders, mainly in Hemiptera (59.0%), Coleoptera (42.4%), Lepidoptera (20.1%), Thysanoptera (14.6%), and Orthoptera (10.4%). South American products represent 47.9% of all developed mycoinsecticides, followed by USA (16.7%), Central America and Mexico (14.6%), Europe (11.1%), Asia (4.9%), Oceania (2.8%), and Africa (2.1%). In South America, the most common fungus is *M. anisopliae*, and spittlebugs (Hemiptera: Cercopidae) in sugar-cane and pastures are the main target species. Although the formulation type could not be determined for 31.3% of products, and despite conflicting data and lack of standardization, at least 12 different formulation types were identified. The most common formulation types were Technical Concentrates - used for preparation of formulations, but in many countries used as the end-product and sometimes referred to as fungus-colonized substrates (29.2%), Wettable Powders (16.0%) and suspensions based on emulsifiable oils (11.1%). Although incomplete and likely with imperfections, the list of products to be presented represents a database that could be updated periodically, providing the scientific community with a valuable source of state-of-the-art information on fungal-based insecticides.

Microsporidia

MP1

Eleven antimicrobials tested *per os* against a grasshopper pathogenic microsporidium (Fungi: Microsporidia)

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Microsporidia are unique obligate pathogens related to fungi that, as a group, parasitize a wide range of invertebrate and vertebrate hosts, including humans. They cause epizootics in wild arthropod populations and can be a particular problem in insect laboratory colonies. We tested 11 commercial antibiotics against an *Encephalitozoon*-like species that infects the grasshopper *Romalea microptera*. Treatment with fumagillin or thiabendazole significantly reduced spore counts, whereas, spore counts of grasshoppers fed with albendazole, ampicillin, chloramphenicol, griseofulvin, metronidazole, quinine, streptomycin, sulfadimethoxine, or tetracycline, were not significantly different from the infected controls. Quinine, however, reduced the number of spores but the reduction was not significant. Streptomycin also exhibited a non-significant antagonistic trend. Although two antibiotics significantly reduced spore counts, in no case was the pathogen totally eliminated. This study reaffirms the difficulty researchers and physicians have had in treating microsporidia infection. It also suggests that quinine and related alkaloid compounds should be further examined as possible therapeutic agents against this group of ubiquitous pathogens. In addition, streptomycin and related compounds should be tested to determine if this widely used antibiotic enhances microsporidiosis.

MP2

Isolation and partial characterisation of a spore wall protein from *Nosema bombycis*

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The microsporidian *Nosema bombycis*(*N.bombycis*) is an obligate intracellular parasite that can cause heavy losses in sericulture. We prepared Monoclonal antibodies (mAbs) for the exospore of the anti-microsporidia which recognize *N.bombycis*. After immunocytochemical identification of spore wall proteins (SWPs) in *N. bombycis*, one SWP involving the invasion of the spore was isolated from immunoaffinity chromatography and two-dimensional gels. The molecular mass of 34 kDa is well identical to the value given by SDS–PAGE, Westem blotting analysis showed that it could be

specifically recognized by anti-*N.bombycis* antibody and the pI in isoelectric focusing (IEF) is about 4.7. So far, only a chitin deacetylase-like protein has been shown to localize to the *Encephalitozoon cuniculi* endospore and either one or two proteins have been clearly assigned to the exospore in two *Encephalitozoon* species: SWP1 in E. cuniculi, SWP1 and SWP2 in *Encephalitozoon intestinalis*. Here, we report the isolation of one new spore wall proteins in *N.bombycis*, SWP, these results will lead to clarification of the targeting peptide and the active mature protein of SWP and it will be helpful to lead the development of novel strategies controlling the microsporidians.

MP3 **STU**

The phylogenetic analysis of *Endoreticulatus* sp. Taiwan by gene sequences

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The taxonomic position of *Endoreticulatus* sp. Taiwan, isolated from the *Ficus* pest (*Ocinara lida*), had been determined by the morphological characters and the sequence of small subunit rRNA gene (SSUrRNA). The sequences of conserved regions of large subunit rRNA (LSUrRNA), alpha-, and beta-tubulin genes were selected and used for this analysis. These partial gene sequences of *Endoreticulatus* sp. Taiwan together with those of other two genera, *Encephalitozoon* and *Nosema*, were analyzed. In the analyses of LSUrRNA and alpha-tubulin genes, *Nosema* were more closely related to *Encephalitozoon* than *Endoreticulatus.* Whereas *Endoreticulatus* closer to *Encephalitozoon* than *Nosema* was found in the analysis of beta-tubulin gene, this result was similar to previous SSUrRNA analysis. Theses analyses using the sequences of individual gene for phylogenetic analysis resulted in the occurrence of diverse phylogenetic relationships among these genera. Combined the sequences of SSUrRNA, four genes were used to analyze simultaneously. This analysis gave a convinced result: *Endoreticulatus* is closer to *Encephalitozoon* than *Nosema*.

MP4

An evaluation on the factors influencing on shelf life of *Verticillium lecanii* conidia at room temperature

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Entomophagous fungus is a group of potential microbial control agents against insect pests. One of the key factors limiting its commercialization is shelf life at room temperature. The investigations of the temperature tolerances, moisture content, formulation and the harvest time of *V. lecanii* conidia were carried out, after evaluation of effects of drying methods and procedures on conidia of *V. lecanii*, in order to optimize the storage methods at room temperature. Vacuum-freeze drying (VFD) was found more suitable for drying of the conidia of *V. lecanii*, after 24-hour VFD, leading to a powdery product with a moisture content about 5.4%, the water content of control conidia powder without drying is higher than 19%, and viability (16h germination rate) of >90.3% and the infection to greenhouse whitefly at 94.7%, which are not significantly different from the control without drying. In contrast, vacuum drying at room temperature leads the water content of conidia significant lower than the control after drying 12h, 24h and 36h at 30℃, 35℃ and 40℃, but the viabilities significantly decreased with length of drying and increase of temperature. Water content of *V. lecanii* conidia is major factor influencing on the viability of conidia, the dried conidia germinated as much as 50% in clay and activated carbon, significant higher than the control conidia without drying stored at 25℃ for 6 months. Carrier can significantly improve the conidia viability after storage, though the viabilities of spores stored in dry powder were considerably higher than in oil represented by germination and infection of *V. lecanii*. Clay and activated carbon were suitable as carriers for *V. lecanii* conidia storage. Both germination and infection of conidia decreased with storage temperature increasing from 15℃ to 35℃, declined rapidly for *V. lecanii* conidia stored at 35℃ for 6 months. The comparison of harvest time of *V. lecanii* conidia on 15 day and 20 day, the germination and infection of the stored conidia have no significant difference at 25℃and at 15℃ in clay and activated carbon carriers for 6 months though there is scarcely germination at 35℃.

Studies on *Nosema* sp. (Microsporida) from Beet armyworm *Laphygma exigua* in China

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A microsporidian pathogen was first isolated from the larvae of Beet armyworm (*Laphygma exigua*). The microsporidian invaded primarily midgut, fat body and Malpighian tubules of the host. The fresh spores were generally long oval in shape and measured (3.98±0.43) μm×(1.65±0.33) μm (n=50). The surface of spores was smooth in scanning electron microscope. The ultrastructure of extrusion apparatus was observed by transmission electron microscope. The result showed that the extrusion apparatus consisted of three distinct parts: the polaroplast, the polar filament with associated organelles (anchoring disc, polar aperture, manubroid) and the posterior vacuole. The anterior part of the spore was occupied by the polaroplast which in mature spores occupied about 25～30 percent of the total spore volume. The polaroplast composed of tightly packed electron dense lamellae between which less electron dense layer was interposed. The polaroplast was typical of the polaroplast reported from most species of *Nosema*. The pathogen was highly virulent to three lepidopteron, *Laphygma exigua, Pieris rapae* and *Helicoverpa armigera*, and could be transmitted vertically to their offspring by either contamination of the surface of eggs (transovum)or via an ovarian infection (transovarian) in the female host, and the horizontal transmission was by the food contaminated by the faeces and cadavers of infected larvae. The changes in the ultrastructrue of the infected host cells in *Laphygma exigua* were investigated by electron microscopy in this study. Our observation showed that cytoplasm of uninfected cells contained normal round nucleus, parallel rough endoplasmic reticulum and typical metazoan mitochondria. The ultrastructure of the infected host cells was markedly changed due to hypertrophy and elongation of the nucleus, showing decrease in the size.

Viruses I

VP1

A novel direct cloning system for making recombinant baculoviruses

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Baculoviruses have been used with some success for several decades for the biocontrol of insect pests. Progress has been made improving these viruses by genetically engineering them with insect-specific toxin genes. Most of these recombinant baculoviruses have been made by homologous recombination into the UDP-ecdyglycolsyl transferase (*egt*) gene locus. Furthermore, recombinant baculoviruses are primarily made by either homologous recombination or bacmid transposition. In both cases, foreign genes must be cloned into a bacterial plasmid transfer vector prior to introduction into a baculovirus genome. This has limited the numbers of candidate genes that can be screened recombinant baculoviruses. We have developed a universal direct cloning method to insert foreign genes into the *egt* loci of group I and group II baculovirus species. We call this method, the “Homingbac system” as the key technology of this system is a group of DNA cutting enzymes called homing endonucleases. We have engineered the Homingbac system into the baculoviruses, *Ac*MNPV, *Bm*NPV, *Px*MNPV, *Rou*MNPV *Hear*SNPV and *Hz*NPV. We demonstrate the Homingbac system can be used to directly clone a polymerase chain reaction (PCR)-amplified betta-glucuronidase gene cassette into the *egt* loci of the baculoviruses, *Ac*MNPV and *Px*MNPV. The time from PCR amplification of target gene to detection of recombinant virus was six days. We found the resulting virus free of wild type virus and we could easily detect the recombinant product four days after transfection. The Homingbac system is a significant improvement to baculovirus cloning and will accelerate the future development of improved baculovirus insecticides.

VP2

SV40 polyadenylation (pA) sequence is redundant in baculovirus expression system

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Baculovirus expression system has been widely used for eukaryotic protein production for pharmaceutical and research application. Many of the expression vectors use SV40 polyadenylation

sequence (SV40 pA) downstream of the gene of interest, anticipating to producing more stable mRNA and eventually increasing protein yields. We investigated the effect of SV40 pA on the expression of green fluorescence protein (GFP) under the control of polyhedrin promoter in the baculovirus expression system. GFP gene with and without the SV40 pA sequence was constructed at the *polh, egt* and *gp37* loci of baculovirus, *Autographa californica* nucleopolyhedro-viruses (AcMNPV). Recombinants viruses carrying the desired sequences were obtained and the viruses were used to infect SF-21 insect cells at 10 MOI. After an incubation period of 48 hours, the cells were harvested and combined with 0.1% SDS, which lyse the cells. The extracted GFP in 0.1% SDS was then analyzed by a UV spectrophotometer for UV absorption to compare GFP expression yields. Transcripts with and without SV40 pA were also quantified and compared. Termination of transcripts with and without SV40 pA was further mapped by 3'RACE method. We concluded from the similar UV absorption (GFP expression yield), and amount of transcripts between the virus pairs that the SV40 pA termination sequence does not increase GFP expression and is redundant in the Baculovirus Expression System.

VP3

Influence of Cytochrome C on Apoptosis Induced by SfaMNPV in Insect *Spodoptera litura* Cells

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Our previous results showed that an elevation of cytochrome c in the cytosol and a corresponding decrease in the mitochondria occurred in *Spodoptera litura* cell line (SL-1) induced by *Syngrapha falcifera* multiple nuclear polyhedrosis virus (SfaMNPV). In the present study we further investigate the influence of cytochrome c on apoptosis induced by this virus. Microscopic observation revealed that infection of SL-1 cells with SfaMNPV resulted in the induction of apoptosis, displaying apoptotic bodies in fluorescent-stained nuclei of SfMNPV-infected SL-1cells. Western blot analysis demonstrated that SfaMNPV-induced apoptosis in insect SL-1 cells was significantly inhibited by cyclosprin A which blocked a translocation of cytochrome c from the mitochondria to the cytosol. As determined by using AC-DEVD-AFC as substrate, the activity of caspase-3 in SfaMNPV-induced cells was detected as early as 4h post infection and gradually increased with time extension, and reached a highest level after 16h of infection. However, activity of caspase-3 in apoptotic cells decreased in the presence of cyclosprin A (30μm), indicating that activation of caspase-3 in SfaMNPV-induced cells was dependent on the release of cytochrome c from the mitochondria. In addition, cyclosprin A could markedly inhibit mitochondrial transmembrane potential ($\Delta\Psi m$) reduction in undergoing apoptotic cells. These data indicate that cytochrome c plays a key role in SfaMNPV-induced apoptosis in *S. litura* cells and may be required for caspase activation during the induction of apoptosis.

VP4

Over expression of *Pfu* DNA polymerase by Recombinant Baculovirus Infected Silkworm

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The *Pfu* DNA Polymerase is a thermostable DNA polymerase for high fidelity PCR from the hyperthermophilic archaeum *Pyrococcus furiosus*. It catalyzes the incorporation of nucleotides into duplex DNA in the 5'-3' direction in the presence of Mg^{2+} resulting in blunt-ended PCR products. Unlike *Taq* DNA polymerase, *Pfu* DNA polymerase exhibits 3'-5' exonuclease activity (proofreading), that enables the polymerase to correct mis-incorporation. Commercial available *Pfu* DNA polymerases are purified form native or recombinant enzyme expressed in *E.coli*. In this study, the coding region of *Pfu DNA polymerase* gene (*PfuPol*) was amplified from the genome of *P. furiosus* (strain JCM8422) using PCR. The sequence confirmed *PfuPol* gene was subcloned into the baculoviral transfer vector pVL1393 under the control of the *polyhedrin* promoter using *Sma* Ⅰ and *Pst* Ⅰ sites. After co-transfection with *Bsu*36 Ⅰ linearized BmBacPAK6 genomic DNA into Bm-5 cells by lipofectin, the recombinant baculovirus harboring *PfuPol* gene was obtained and plaque screening was further conducted. Early 5th instar silkworm larvae were infected with the recombinant virus and further reared for 4~5 days. The hemolymph containing *Pfu* DNA Polymerase was collected on ice from dying larvae. SDS-PAGE analysis revealed that the recombinant enzyme was approximately 91 kDa in size and was purified to about 80% homogeneity in a single heat-treatment step at 85℃ for 10 min. The heat-treated enzyme was sufficient and retained good performance for

amplification in PCR directly. The *Pfu* DNA polymerase was overexpressed up to 200×10^3 U per ml hemolymph as compared with commercial available counterpart in PCR reaction. No contaminations both from viral or cellular genomic DNA were detected by PCR confirming.

VP5

Characteristic of *Autographa Californica* Nucleopolyhedrovirus *Ubiquitin* Gene Promoter

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Baculovirus *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV) encodes an ubiquitin protein, which may involve in the virus infection. The promoter activity analysis indicated that ubiquitin promoter was transcribed during the late phase of virus infection. In the presence of viral factors, transient expression assay demonstrated that the *cis*-acting elements responsive to viral factors are mainly located within the range of – 595 to – 382 bp upstream of ATG, and a 196 bp fragment between -187 bp and -383 bp upstream of ATG, containing another baculovirus late transcriptional initiation site, TAAG motif, at –195 bp from ATG, is essential for the expression of reporter gene. Pointed mutation analysis indicated that the mutations of TATA box and TAAG motif reduced the promoter activity remarkably, while two CAAT motif mutations enhanced the promoter activity obviously compared to the native promoter. Especially the mutation of CAAT distal ATG not only enhanced the reporter gene expression, but also made the promoter transcription independent of viral factor and can transcript not only in *Spodoptera frugiperda* (Sf-21) cells but also in *Bombyx mori* (Bm) cells. All the results suggested that ubiquitin gene transcription is controlled by two continuous promoter regions, the proximal region as basic promoter to startup gene transcription, and the distal region play an important role in escaping the transcriptional regulation of host elements.

VP6

Interactions between subunits of the *Autographa californica* nucleopolyhedrovirus-encoded RNA polymerase

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Autographa californica nucleopolyhedrovirus transcribes genes using two DNA-directed RNA polymerases; early genes are transcribed by the host RNA polymerase II, and late and very late genes are transcribed by a viral encoded multisubunit RNA polymerase. The viral RNA polymerase is composed of four proteins: Late Expression Factor-4 (LEF-4), LEF-8, LEF-9, and P47. The predicted amino acid sequences of *lef-9* and *lef-8* contain motifs also present at the catalytic center of known RNA polymerases in a number of organisms. The requirement for the motif present in LEF-8 in late gene expression has been previously demonstrated. We have assessed the requirement of specific residues within the motif in LEF-9 for late gene expression. We found that conserved aspartic acid residues within the LEF-9 motif, corresponding to those essential for activity of the *Escherichia coli* RNA polymerase largest subunit, were also required for late gene expression. Like the *E. coli* b and b′ RNA polymerase subunits, LEF-8 and LEF-9 may form the catalytic center of the enzyme. We found that LEF-8 and LEF-9 interacted by coimmunoprecipitation experiments as previously suggested for the *Bombyx mori* nucleopolyhedrovirus LEF-8 and LEF-9. We next investigated the interactions of all the RNA polymerase subunits in pairwise combinations. Coimmunoprecipitation experiments indicated associations between LEF-9 and P47, LEF-4 and P47, and LEF-8 and P47. In contrast, LEF-4 and LEF-8 did not coimmunopreciptate, suggesting that they do not associate directly. The association between LEF-4 and LEF-9 was weak or may be absent. Further analysis also suggests that some of the subunits have the ability to form homodimers. Studies on protein-protein interactions may provide insight into the mechanistic aspects affecting late and very late gene expression.

VP7

Cloning and sequencing of *Epinotia aporema* Granulovirus (EpapGV) *gp37*-like gene

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Epinotia aporema (Lep. Tortricidae) and *Anticarsia gemmatalis* (Lep. Noctuidae) are major soybean

pests in Argentina. Studies conducted to evaluate the effect of the interaction between the baculoviruses EpapGV and AgMNPV, on *A. gemmatalis* larvae, showed that the addition of EpapGV occlusion bodies enhanced the virulence of AgMNPV preparations. At present, certain baculovirus encoded proteins have been indentified as infectivity enhancers: *e.g.* VEFs (virus enhancing factor, also known as enhancin) and GP37-like proteins. Enhancin is a metalloproteinase, which digests components of the insect peritrophic membrane facilitating the initiation of infection. GP37 (spindolin) is related to the fusolins of entomopoxviruses. A *gp37* gene was localized in the genome of EpapGV, whereas attempts to find *vef* homologous were unsuccessful. In this work, we cloned the entire EpapGV- *p37* gene. Sequence analysis indicated that the gene is 669 bp long (the smallest gp37 sequenced at present) and encodes a predicted 222 amino acid long protein. A late promoter element was found upstream the ORF. Characteristic N-glycosylation sites, five chitin-binding domains and six cystein residues were present. The pairwise comparison of EpapGV *gp37* gene product with all the baculovirus sequences in GenBank yielded high similarity values, ranging from 45 to 63%, CpGV-gp37 being the most closely related gene. Interestingly, the phylogenetic analysis grouped the GVs in a cluster more closely related to entomopoxviruses than to NPVs. This result, could be interpreted *a priori* as indicating independent events of gene acquisition by different baculovirus lineages. The precise biological function of GP37 has not been elucidated yet. Consequently, further studies are conducted in this direction. We are also aiming to evaluate the capacity EpapGV GP37 to enhance the infection of heterologous baculoviruses, such as AgMNPV. Since no spindle-like crystals were detected in larval tissues or viral preparations, an antiserum against EpapGV gp37 will be raised to characterize its subcellular and structural localization.

VP8

Expression of human renin using two baculovirus systems

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The renin plays an important role in the regulation of blood pressure and salt balance. The major source of active renin is the kidney cells, in which it is produced from prorenin, a larger inactive precursor. In the present work, two different baculovirus systems were used to allow production of higher levels of renin. A fragment of 1.4 kb, containing the preprorenin gene, was initially cloned in the pRHR1100 vector and sequenced by *dye terminator chemistry* method. This fragment was removed by restriction enzymes and inserted in the plasmid pSynXIV VI^{+}X3. This plasmid was co-transfected with the vSyn XIV VI – gal DNA into insect cells to generate, by homologous recombination, a new virus containing the renin gene. The recombinant virus has the polyhedrin gene and was selected by plaque purification, based on the occluded form of the virus. Two cell lines, IPLB SF21AE and Tn5B1-4, were used for virus multiplication and selection. The second expression system is the commercially available Bac-to-bac system (Invitrogen). This method is based on site-specific transposition of an expression cassette into a baculovirus shuttle vector (bacmid) propagated in *E. coli*. The fragment of 1.4 kb was first inserted in the pFastBac HT C donor plasmid and transformed in DH10Bac competent cells. The recombinant bacmid DNA was then extracted and transfected into insect cells to generate an occluded negative recombinant virus. The presence of the gene was confirmed in all steps by PCR. Analysis of the viral proteins was done by acrylamide-SDS electrophoresis. Mock infected cells and AcMNPV purified polyhedra were used as controls. In the occluded form of the recombinant virus, a band of approx. 30 kDa was present in the infected cells and migrated together with the polyhedrin of the control (purified virus). However no extra band, corresponding to renin, was detected in the infected cells. Analysis of proteins synthesized at different times p.i. and pulse labeled with ^{35}-S methionine showed the same results. On the other hand, the analysis of the proteins of the occluded negative virus generated using the Bac-to-Bac system reveled the expression of a protein of approx. 50 kDa. Current studies, using immunological analysis, are being done in order to confirm if this protein is the human renin.

VP9

Production of human interferon-gamma by a novel bi-cistronic baculovirus expression vector

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Interferon-γ (IFN-γ) is one of the most important therapeutic proteins. The purpose of the studies

focused on how to produce bioactive human IFN-γ efficiently. (Ⅰ) Use RhPV (*Rhopalosiphum padi virus*) 5' UTR IRES (internal ribosome entry site) and green fluorescent protein to develop a rapid screening strategy for recombinant virus. (Ⅱ) One step purification of the secreted product from supernatants of virus infected Sf21 cells by IMAC (immunobilized metal affinity chromatography). (Ⅲ) Through lectin blotting, which can detrmine the terminal of the carbohydrates, we also analyzed the glycosylated pattern of purified IFN-γ. (Ⅳ) Based on anti-dengue virus activity assay, we determined the biological activity of IFN-γ produced in different culture conditions and insect cells. In this report, we confirmed the RhPV 5' UTR IRES sequence coupled with green fluorescence could facilitate the isolation of recombinant virus,vAcIFN-γ-Rhir-E. With one step IMAC procedure, we obtained 96% purity of IFN-γ from vAcIFN-γ-Rhir-E-infected insect Sf21 cells. The concentration of the IFN-γ from serum-free cultured condition reached 0.3 mg/l and can inhibit 90% dengue virus productions.

VP10 STU

Characterization of MacoNPV enhancin and its interaction with *Mamestra configurata* peritrophic matrix proteins

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Viral enhancing factor or *enhancin* has been identified in a number of *Granulovirus* species and is characterized by its ability to enhance the oral infectivity of *Nucleopolyhedrovirus* in lepidopteran insects. *Enhancin* has been demonstrated to be a metalloprotease that facilitates baculovirus infection by disrupting the peritrophic matrix (PM) in the midgut of host insects. AcMNPV recombinant viruses expressing the MacoNPV *enhancin* were developed. Bioassays demonstrated that the AcMNPV - MacoNPV *enhancin* recombinants produced mortality approximately 12-24 h sooner than AcMNPV-wt in third-instar *Trichoplusia ni* larvae. Using an antiserum to the *T. ni* insect intestinal mucin (IIM), we demonstrated that feeding of the AcMNPV-enhancin recombinant resulted in increased degradation of IIM relative to the non-recombinant strain. This effect was similar to that observed with the fluorescent brightener, Calcofluor White, which is known to disrupt PM integrity. Furthermore, we observed that when *M. configurata* was fed MacoNPV occlusion bodies, that while the fully glycosylated form of McIIM was resistant to degradation, non- and under-glycosylated forms were degraded. This effect was transient in that the McIIM profile/complement was restored within 24 hours post-ingestion. We also undertook to identify additional protein targets for enhancin residing with the *M. configurata* PM. Genomics and proteomic approaches were employed to comprehensively survey the proteins expressed by the midgut epithelium, as well as those associated with the peritrophic matrix (PM). Our tactic was to couple one and two-dimensional gel resolution of PM proteins with MALDI-mass spectrometry to generate peptide sequence information. In parallel, ca. 4,000 expressed sequence tags (EST) were generated from a midgut-specific cDNA library yielding a database of genes expressed in the midgut. Bioinformatics tools were used to link the data sets (peptide and cDNA sequencing) leading to the identification of genes for corresponding to additional putative PM proteins. A model describing the role of two PM proteins (IIM and peritrophin 1) in maintaining PM structure will be presented.

VP11 STU

Functional Studies on *Spodoptera litura* Multiple Nucleopolyhedrovirus Anti-apoptotic Genes

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Although *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) can replicate in numerous insect species and both distinct apoptotic suppressors genes, *p35* and *iap*, are present in its genome, the virus still induce apoptosis when it infects *Spodoptera litura in vitro* and *in vivo*, indicating that the anti-apoptotic genes in AcMNPV genome are nonfunctional as inhibitors of apoptosis in *S. litura*. However, *S. litura* multiple nucleopolyhedrovirus (SpltMNPV) can successfully replicate in *S. litura* and SpltMNPV genome also possesses two anti-apoptotic suppressors: *p49* and *iap*. In present study, a *p35* knockout bacmid containing the AcMNPV genome was constructed through homologous recombination in *Escherichia coli*. Then, *Ac-p35*, or *Splt-p49*, or *Splt-iap*, or both *Splt-p49* and *Splt-iap* genes were inserted into the polyhedrin locus of this mutant virus by

site-specific transposition, respectively. The resulting recombinant viruses were determined their abilities to replicate in Sf-9 cells. Light microscopic analysis, annexin V assay and DNA fragmentation assay showed that apoptosis induced by infection of the *p35* null mutant virus was suppressed by expression of *Splt-p49* or both *Splt-p49* and *Splt-iap* in Sf-9 cells, but *iap* failed to block the virus-induced apoptosis. Thus, SpltP49 shares sequence and functional similarity with AcMNPV counterpart in AcMNPV-infected Sf-9 cells. Interestingly, Spli-221 (*S. litura*) cells infected with the four recombinant viruses undergo apoptosis respectively, suggesting that only the replacement of anti-apoptotic genes cannot expand the host range of AcMNPV.

VP12 STU

Analysis of the immediate early *me53* gene from the baculovirus AcMNPV

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Baculoviruses encode five major immediate early transcripts corresponding to *ie1, ie-0, ie-2, pe38* and *me53*. Four of these, *ie-1, ie-2, ie-0* and *pe38* have been studied in some detail and were found to play vital roles in viral infection. In the type virus, *Autographa californica* MNPV, *me53* is expressed at high levels from an immediate-early promoter. Although the gene has been defined transcriptionally, the function of *me53* is still unknown. *Me53* encodes a protein containing a putative C-4 zinc-finger and is thought to be involved in transcriptional transactivation. We have generated a me53-null AcMNPV mutant and have compared its infectivity, virus yield and DNA replication to wild-type virus. Results have indicated that although PIBs are seen in transfected cells by 72 hours post-infection, Δ*me53* AcMNPV is severely compromised in the production of budded virions. A *me53* repair virus was also generated and found to behave identically to wild-type AcMNPV in terms of replication and budded virus yield. Using real-time PCR to monitor DNA replication and electron microscopy, we are currently trying to determine if this defect is in DNA replication or production of budded virions.

VP13 STU

Expression of anti-apoptotic *p35* gene in tobacco enhances tolerance to abiotic stresses and increases the virulence of AcMNPV

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The P35 protein from baculovirus is a broad-range caspase inhibitor and can suppress programmed cell death in animals. We tested the effects of expression of the anti-apoptotic baculovirus *p35* gene in transgenic tobacco under various abiotic stresses. Transcription and expression of the *p35* gene in tobacco plants was confirmed by RT-PCR and Western blot. Transgenic tobacco plants were efficiently protected from abiotic stresses, *e.g.* methyl alcohol, H_2O_2 and starvation. Transgenic plants tolerated higher concentrations of methyl alcohol than control plants. Transgenic plants showed tolerance to 10-100 mM H_2O_2 while the control did not. Expression of *p35* in tobacco also efficiently delayed leaf senescence under starvation in the dark. Bioassay by feeding *Spodoptera exigua* larvae with transgenic tobacco leaves showed that the LC_{50} was efficiently reduced compared with the wild-type tobacco plants. Taken together, our results indicated that expression of the anti-apoptotic baculovirus *p35* gene in tobacco provided broad-spectrum tolerance to abiotic stresses and enhanced the virulence of AcMNPV to insect. Our studies implicated that expression of P35 in plants is a potential strategy for engineering broad-spectrum abiotic stresses and insect resistant plants.

VP14 STU

Ha135, a unique nonstructural protein of HearNPV, is not essential for viral propagation

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Helicoverpa armigera single nucleocapsid nucleopolyhedrovirus (HearNPV) contains several genes

with a homologue found only in its closely related variant *H. zea* SNPV. These unique genes warrant further investigation as they may contribute to the unique feature of HearNPV. ORF135 is 585bp in size and has a putative early transcription motif In the present report, the function of HearNPV ORF135 is analyzed. Ha135 was transcribed as a polyadenylated transcript from 12 h post-infection onwards in infected HzAM1 cells. 5'RACE mapping indicated that Ha135 transcription initiates at the A in the consensus early transcription motif CAGT, 36nt upstream of the predicted translation start codon. The transcription termination site was mapped 18nt downstream of the predicted translational stop codon. Western blot analysis demonstrated that the Ha135 encodes a 23kDa protein, which was not present in budded virions (BV) nor in occlusion derived virions (ODV). When C-terminally fused with the green fluorescence protein, the Ha135 protein is predominantly detected in HzAM1 cell nuclei in distinctive aggregates. Gel filtration analysis demonstrated that the Ha135 protein forms predominantly dimers which is compatible with the observation of Ha135 aggregates in the nucleus. A Ha135 deletion recombinant HaBac-135-null and a repair recombinant HaBac-135-repair both containing also a polyhedrin gene were constructed. Growth curve and electron microscope analysis indicated that Ha135 knockout mutation do not affect polyhedra formation or BV production. In summary, our data suggest that Ha135 encodes a non-structural protein of HearNPV, expressed at early times post infection and targeted to the nucleus. The Ha135 gene is not essential for HearNPV viral infectivity in cell culture.

VP15 STU

Construction of Bac-to-Bac system of *Bombyx mori* NPV

Jinshan Huang[1], Bifang Hao[1, 2], Xiulian Sun[1], Fei Deng[1], Hualin Wang[1] and Zhihong Hu[1]

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To construct the Bac-to-Bac expression system of *Bombyx mori* nuclopolyhedrovirus (BmNPV),a transfer vector was constructed which contained an *E. coli* mini-F replicon and lacZ:attTN7:lacZ cassette within the upstream and downstream arms of BmNPV polyhedrin gene. *B. mori* larvae were co-transfected through subcutaneous injection with wild-type BmNPV genome DNA and the transfer vector to generate recombinant viruses by homologous recombination. The genome DNA of budded viruses extracted from the haemolymph of the transfected larvae was used to transform *E. coli* DH10B. Recombinant bacmids were screened by kanamycin resistance. After restriction enzyme analysis of the genome DNAs, one of bacmid colonies, BmBacJS13 which has similar profiles to wild-type BmNPV, was selected for further research. To investigate the infectivity of BmBacJS13, *egfp* gene was introduce into the bacmid and the resulted BmBacJS13-egfp was transfected to BmN cells. The budded viruses were collected for infection of BmN cells and green fluorencence could be observed after 24 hrs post infection. Growth curves analysis indicated that BmBacJS13-egfp has similar growth curve to that of wild-type BmNPV. Bio-assay indicated that the recombinant virus was also infectious to *B. mori* larvae.

VP16 STU

Functional role of aspartic proteinase cathepsin D in *Bombyx mori* metamorphosis

Kwang Sik Lee[1], Byung Rae Jin[1], Zhong Zheng Gui[1], Bo Yeon Kim[1], Young Soo Choi[1], Ya Dong Wei[1], Young Moo Choo[1], Yeon Ho Je[2], Xijie Guo[3] and Hung Dae Sohn[1]

([1]*College of Natural Resources and Life Science, Dong-A university, Busan, 604-714, Korea;* [2]*School of Agricultural Biotechnology, Seoul National University, Seoul, Korea;* [3]*Sericultural Research Institute, Chinese Academy of Agricutural Sciences, Zhenjiang, China*)

Metamorphosis is a complex, highly conserved, and strictly regulated process that involves the programmed cell death of obsolete larval organs. Here we show a novel functional role of the aspartic proteinase cathepsin D during metamorphosis. The silkworm *Bombyx mori* cathepsin D (*BmCatD*) was ecdysone-induced, differentially and spatially expressed in the final instar larval fat body and pupal midgut, and its expression led to programmed cell death. Furthermore, *BmCatD* was highly induced in the fat body of baculovirus-infected *B. mori* larvae, suggesting that this gene has been shown to be involved in the induction of metamorphosis of host insect infected with baculovirus. RNA interference (RNAi)-mediated *BmCatD* knock-down inhibited the programmed cell death of larval fat body, resulting in the arrest of larval-pupal transformation. *BmCatD* RNAi also inhibited the programmed cell death of pupal gut. These results indicate that BmCatD is critically involved in the histolysis of larval fat body and pupal gut during silkworm metamorphosis.

VP17 STU

Construction of the herpes simplex virus 1 ICP0 eukariotic expression vector and its effects of macrophage function

Huang Liang

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Objectives: To construct the eukaryotic expression vector of herpes simplex virus 1 ICP0 (HSV-1 ICP0) and test its expression in macrophage cells. Then study the effects of GFP-ICP0 on LPS induced chang of macrophage function. Methods: Restriction enzyme digest PT7-110 , the fragment of ICP0 was subcloned into eukaryotic expression vector pEGFP-C1，then the recombined vector pEGFP/ICP0 was transfected into macrophage. The expression of GFP-ICP0 was analyzed by fluorescent microscope and Western blotting. TNF-α and IL-1β ELISA kits were respectively used to determine the effects of GFP-ICP0 protein transient expression on cytokines secretion induced by lipopolysaccharide of macrophage. Macrophages were stimulated with lipopolysaccharide to assay the production of NO with Griess Reagents. Results: ①The recombinant pEGFP/ICP0 was digested by SmaI, a approximate 2300bp DNA fragment can be seen in agrose electrophoresis, it indicated that ICP0 fragment was already subcloned into pEGFP-C1 plasmid, namely, the eukaryotic expression vector pEGFP/ICP0 has been constructed successfully, pEGFP/ICP0 was transfected into macrophage, fluorescent microscope and western blotting identified specially the expression of GFP-ICP0 protein, result showed GFP-ICP0 can be expressed in macrophage.②The secretory volume of cytokines from GFP-ICP0-stimulated macrophage was determined by ELISA, the results from analysis of variance showed as following: there is a remarkable difference between pEGFP/ICP0 group and lipopolysaccharide control group and pEGFP-C1 control group, but no remarkable difference between pEGFP-C1 control group and lipopoly－saccharide control group, all of the results indicated transient expression of GFP-ICP0 raises LPS-induced product of TNF-α and IL-1βby macrophage. ③The secretory volume of NO from GFP-ICP0-stimulated macrophage was determined by Griess Reagents, the results from analysis of variance showed as following: there is a remarkable difference between pEGFP/ICP0 group and lipopolysaccharide control group and pEGFP-C1 control group, but no remarkable difference between pEGFP-C1 control group and lipopoly－saccharide control group,all of the results indicated transient expression of GFP-ICP0 raises LPS-induced product of NO by macrophage. Conclusion: ① The gene ICP0 of HSV-1 expressing eukariotic vector pEGFP/ICP0 was successfully constructed, and it can be expressed GFP-ICP0 protein correctly in macrophage. ② Transient expression of GFP-ICP0 up-regulated lipopolysaccharide-induced TNF-α and IL-1β secretion of macrophage. ③ Transient expression of GFP-ICP0 up-regulated lipopolysaccharide-induced NO secretion of macrophage

Tuesday, August 29, 8:00-10:00, *Meeting Center*

Bacteria Division Symposium: Genetics and Characterization of mechanisms of *Bt*-resistance

Organizers: Juan Ferre and William Moar

8:00

Mutant alleles of a cadherin gene and Cry1Ac resistance in the cotton bollworm, *Helicoverpa armigera*

Yidong Wu, Haiyan Chen, Yajun Yang, Yihua Yang and Shuwen Wu

(*Nanjing Agricultural University, Nanjing, 210095, Jiangsu Province, China*)

Cotton bollworm (*Helicoverpa armigera*) is a major insect pest targeted by transgenic *Bt* cotton. The evolution of resistance by this pest to Bt toxins is the main threat to the long-term effectiveness of transgenic Bt cotton. Our previous study showed a mutant allele (*r1*) of a cadherin gene (*Ha-BtR*) is associated with Cry1Ac resistance in a laboratory-selected strain of cotton bollworm. Two novel cadherin alleles (*r2*, *r3*) associated with Cry1Ac resistance were directly detected in field populations of cotton bollworm from China with a biphasic screen strategy. It confirms that *Bt* resistance-associated cadherin mutants are not only present in the laboratory resistant strain, but also in the field populations. After 10 years' planting of transgenic *Bt* cotton, estimated gene frequency of cadherin mutants in two field populations of *Helicoverpa armigera* in China is still low (around 0.0015). Our findings will help the development of a promising DNA-based method for detecting *Bt* resistance alleles of cotton bollworm in field.

8:30

Resistance and Hypersensitivity to *Bt* Crystal Toxins

Raffi Aroian, Brad Barrows, Larry Bischof and Danielle Huffman

(*University of California, San Diego, 9500 Gilman Drive, Mail Code 0349 La Jolla, CA 92093-0349 USA*)

Our laboratory is using the nematode Caenorhabditis elegans to understand two sides of the *Bt* toxin - host interaction equation-- 1) how resistance to *Bt* Cry toxins can develop and 2) how the host protects itself against the pore-forming activities of *Bt* toxins. We have performed genetic screens to identify genes in the host that, when mutated, lead to *Bt* toxin resistance. Five resistance genes were identified in this screen and all have now been cloned and characterized. We will discuss the implications of these genes in the development of resistance to *Bt* toxins. In addition, we are initiating an RNAi screen to identify on a genome-wide level other genes that, when reduced or eliminated, give rise to *Bt* toxin resistance. Several interesting candidated have emerged from this screen. At the other end of the equation, we have identified two pathways that are required for host defense against *Bt* toxins. Elimination of either of these pathways leads to animals that are hypersensitive to the intoxicating effects of *Bt* toxin. We are also in the midst of a genome-wide RNAi screen and have tested roughly half of the genome for genes that call into this class. Several interesting classes of genes have emerged from this study.

9:00

The diversity of *Bt*-resistance genes in Lepidoptera

David G. Heckel

(*Max Planck Institute for Chemical Ecology, Hans-Knoell-Str. 8, D-07745 Jena, GERMANY*)

Although the mode of action of Cry-toxins produced by *Bacillus thuringiensis* is fairly well understood, knowledge of the molecular mechanisms by which Lepidoptera have evolved resistance to them is still in its infancy. The most common type of resistance has been called "Mode 1" and is characterized by recessive inheritance, >500-fold resistance to at least one Cry1A toxin, and negligible cross-resistance to Cry1C. In three Lepidopteran species (*Heliothis virescens*, *Pectinophora gossypiella*, and *Helicoverpa armigera*), Mode 1 resistance is caused by mutations in a toxin-binding 12-domain cadherin protein expressed in the larval midgut. These mutations all disrupt the protein and prevent its normal localization in the membrane, presumably removing the major toxic binding target of the Cry1A toxins. However, membrane preparations of some of these resistant strains still bind to the toxin; implying that not all toxin binding results in a toxic effect. In *Plutella xylostella*, however, Mode 1 resistance appears to be caused by a different genetic mechanism, as Cry1A resistance is unlinked to the cadherin gene. Moreover, membrane preparations from resistant insects may display greatly reduced Cry1A binding. Mapping studies in *Heliothis virescens* have detected another major Cry1A resistance gene, which on the basis of comparative linkage mapping is likely to be distinct from the one in *Plutella*. Additional resistance mechanisms involve a protease in *Plodia interpunctella* and an aminopeptidase in Cry1C-resistant *Spodoptera exigua*. Altered glycolipid patterns, shown to play a central role in Cry5B resistance in the nematode *C. elegans*, are under investigation in insects as well. This diversity of resistance genes greatly complicates efforts to develop general DNA-based diagnostic methods for resistance; and may require species-specific approaches at least.

9:30

A proteomic approach to study resistance to *Bacillus thuringiensis* Cry toxins in *Heliothis virescens* larvae

Juan L. Jurat-Fuentes[1] and Michael J. Adang[2]

([1]*Department of Entomology and Plant Pathology, The University of Tennessee, 205 Ellington Plant Sciences Building, Knoxville, TN 37996;* [2]*Department of Entomology, University of Georgia, 413 Biological Sciences Building, Athens, GA 30602*)

Binding of the *Bacillus thuringiensis* Cry1Ac toxin to specific receptors in the midgut brush border membrane (BBM) is required for toxicity. Alteration of these receptors is the most reported mechanism of resistance. We used a proteomic approach to identify Cry1Ac binding proteins from brush border membrane vesicles (BBMV) prepared from *Heliothis virescens* larvae. BBMV proteins were resolved by two-dimensional (2D) electrophoresis into discrete protein spots. Spots detected on 2D blots by their ability to bind Cry1Ac were digested with trypsin and the resulting peptide fingerprints (PMF) used for database searching and protein identification. Proteins not identified by PMF searching were further fragmented using MS/MS and analyzed by de novo sequencing. Protein

identifications were confirmed using Western blotting. Identification of Cry1Ac binding proteins allowed us to study toxin binding site alterations by comparing BBM proteomes of susceptible and resistant *H. virescens* larvae. Our results validate the use of proteomic approaches to characterize putative toxin receptors and proteome alterations in resistant insects.

Tuesday, August 29, 8:00-10:00, *Multifunctional Hall*
Virus Division Symposium: Field Performance of Insect Viruses
Convenors: Zhihong Hu and Basil M. Arif

8:00

New developments in the use of codling moth granulovirus

Juerg Huber
(*BBA, Institute for Biological Control, D-64287 Darmstadt, Germany*)

When, in fall 1963, a granulovirus was found in codling moth larvae collected in Northern Mexico, nobody could have anticipated that this virus isolate would become one of the most successful insect viruses for use in biological control of an agricultural pest. The isolation and description of the virus by Tanada was the beginning of a long history of research on the virus, involving scientists from all over the world, which eventually led, in 1987 in Switzerland, to the first registration of a viral pesticide for use on a food-crop. Today, there are world wide half a dozen products, all based on the original isolate from Mexico, on the market: Carpovirusine (NPP; Arvesta), Cyd-X (Certis USA), Granupom (Probis), Madex (Andermatt Biocontrol), Virgo (Sipcam) and Virosoft CP_4 (BioTepp). Up to around the year 2000, the market for these preparations was rather small, being restricted to the use in organic farming only. Today, in Europe alone, the virus is used on more than 100'000 ha, mostly in integrated fruit production. The reason for this extension of use was based on special traits of the virus, which were known since the beginning of research about the virus, but so far had never been taken advantage of: (1) the tremendous impact of the virus on codling moth populations (about 10 times higher than the prevention of immediate damage by the larvae), (2) the shallow slope of the dose response curve (even 10 or 100 fold dilutions of the virus show an effect), and (3) the fact that there was no cross resistance with chemical pesticides. Many farmers started to add diluted concentrations of virus to their chemical insecticide and fungicide treatments, and, consequently, the sales of the virus boomed. In spite of this heavy and in many orchards long time use there were no reports about resistance against the virus, though there were a few complaints from farmers maintaining that the virus didn't work properly in some of their orchards. It was only in 2003, that we started a study about susceptibility of field populations to the virus. In the fall of that year, specimens from local codling moth populations were collected in three different orchards in the south of Germany; two of them having been treated with granulovirus of codling moth over many years, and one since two years (only from one of them, the farmer had reported failure of the virus). In autumn 2004, in addition to the three locations from the previous year, specimens from populations in four other orchards with serious CM problems were collected. The susceptibility of the neonate descendents of the overwintering generation to *Cp*GV was investigated in the spring of the following year in bioassays on artificial diet and compared to a laboratory strain of the codling moth. The results indicated significant differences in sensitivity to the virus between the local codling moth populations. The LC_{50}-values showed that two of the populations sampled in 2003 were more than thousand fold less susceptible than the third population and the laboratory strain. The results from the bioassays from the descendents of the diapausing larvae sampled in 2004 confirmed the low susceptibility of the two old populations and showed also for the larvae from the new locations an up to 1000-fold resistance. For the time being, the problem of resistance to the virus seems to be limited to a few orchards in Germany, the majority of orchards being not affected. But the farmers are startled and there are already first reports about resistance from France and Switzerland. On the other hand, there are also first indications that the resistance can be overcome by new isolates of the *Cp*GV.

8:30

Abietiv: Field efficacy and registration of the balsam fir sawfly nucleopolyhedrovirus in Canada

Christopher John Lucarotti
(*Canadian Forest Service - Atlantic Forestry Centre, 1350 Regent Street, P.O. Box 4000, Fredericton, NB E3B5P7, Canada*)

In 1997, we isolated a nucleopolyhedrovirus (NeabNPV) from the balsam fir sawfly (*Neodiprion abietis*). The balsam fir sawfly had become a major defoliator of pre-commercially thinned balsam fir stands, especially in western Newfoundland (Canada), beginning in the early 1990's. With no control measures in hand, NeabNPV was developed as an agent for the biological control of balsam fir sawfly. To this end, research was carried out on a number of fronts including NeabNPV field efficacy, studies on mammalian and non-target invertebrate toxicology, balsam fir sawfly life table, ecology and impact on balsam fir trees and NeabNPV genome sequencing and analysis. As part of the field efficacy trials 22,550 hectares of balsam fir sawfly infested forest were aerially treated with NeabNPV between 2000 and 2005. NeabNPV was found to be both efficacious and economical for the suppression of balsam fir sawfly outbreak populations. Results from all of the research, both basic and applied, were included in the submission to the Pest Management Regulatory Agency (PMRA) (Health Canada) in June 2004 for the registration of NeabNPV under the trade name, Abietiv. Temporary registration of Abietiv was received from the PMRA in April, 2006.

9:00

New strategies of using viruses to control agricultural and forest pests in China

Xiulian Sun, Zhihong Hu and Huiying Peng

(*State Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, 430071, Hubei , China*)

So far, eleven baculoviruses, one densovirus and one cypovirus have been registered as commercial insecticides in China. These viral pesticides play important roles in IPM, however, their efficacy still needs to be improved in the practice of organic farm. We focused on two strategies to improve the control efficacy of the viruses, one is to develop a genetically modified baculovirus for controlling cotton bollworm and another is using *Trichogramma* spp. as vectors to deliver viruses to the target pests.

Helicoverpa armigera nucleopolyhedrovirus (HearNPV) is the most widely used viral insecticide in China. A neurotoxin expressing recombinant HearNPV (HearNPV-AaIT) was constructed and it inactivated host larvae significantly faster than its wild-type parent. A reasonable yield of the recombinant virus could be achieved by inoculating 4th instar of host larvae with an optimal virus dose. Field release experiments indicated that HearNPV-AaIT protected cotton from bollworm damage better than the wild-type virus. Inactivation rates of HearNPV-AaIT on cotton did not differ from the wild-type virus. There was no significant difference in virus persistence in the soil after applications of recombinant and wild-type HearNPV variants. Horizontal and vertical transmission rate of HaSNPV-AaIT was significantly lower than that of the wild-type. No negative impact of HearNPV-AaIT on nontarget parasitoids and predators was found in the laboratory and in the field. Furthermore, the recombinant was not pathogenic for non-target animals (bee, silkworm, bird, fish and rat). Based upon the results mentioned above, a data package of the potential ecological and health risks of the genetically modified virus has been submitted to the Ministry of Agriculture, P.R. China, for the registration of this product as a commercial insecticide. Since 1970's, the parasitoid wasps, such as *Trichogramma* spp., have been reared and released to control forest and agriculture pests in China. We developed a technique to deliver viruses to the target pests by using *Trichogramma* spp. as vector. When *Trichogramma dendrolimi* was released with *Dendrolimus punctatus* cypovirus to control masson pine moth (*D. punctatus*) in forest, it was observed that 20-40% of eggs had been parasitized and 40-47% of *D. punctatus* larvae had been infected with the virus. This technology has been also used for controlling of *Helicoverpa armigera*, *Spodoptera litura*, *Plutella xylostella*, *Ectropis oblique*, *Pieris rapae*, etc. on more than 13,000 hectares since 1991 in China.

9:30

Preliminary greenhouse trials with indigenous TnSNPV and AcMNPV isolates for cabbage looper (*Trichoplusia ni*) control in greenhouse vegetable production.

Martin A. Erlandson[1, 2], Dave Gillespie[3], Melissa Strom[2], Don Quiring[3] and David Theilmann[4]

([1]*Agriculture and Agri-Food Canada, Saskatoon Research Centre, 107 Science Place, Saskatoon, SK, S7N 0X2 Canada;* [2]*2Department of Applied Microbiology and Food Science, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK, S7N 5A8 Canada;* [3]*Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, 6947 Hyw 7, Agassiz, BC, V0M 1A0 Canada;* [4]*Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, 4200 Hyw 97, Summerland, BC, V0H 1Z0 Canada*)

The cabbage looper (*Trichoplusia ni*) (Lepidoptera: Noctuidae) has become a serious pest of greenhouse vegetable production in Canada due in part to the development of populations resistance to *Bacillus thuringiensis* (*Bt*) based bioinsecticides. Much of the Canadian greenhouse vegetable crop is produced with out chemical pesticides but rather using IPM systems relying on biological control agents. Baculoviruses may provide an attractive alternative to *Bt* products for cabbage looper control as they are highly infectious, typically host specific and therefore compatible with IPM systems based on the use of a complex of insect biological control agents. Indigenous strains of TnSNPV and AcMNPV were isolated from cabbage looper populations infesting commercial greenhouse crops and the most active strain for each of TnSNPV and AcMNPV was selected based on laboratory bioassays. Feeding preference studies were conducted with 4th instar *T. ni* larvae and leaf discs of commercial crops and larvae preferred cucumber, pepper and tomato varieties in declining order. Crop plant species also had a significant impact on virus-related mortality in dose uptake studies, cabbage looper larvae feeding on sprayed sweet pepper typically had lower levels of virus-related mortality than those fed on sprayed cucumber plants. A preliminary spray trial in which 2nd and 4th instar larvae were fed on potted cucumber plants sprayed with either TnSNPV or AcMNPV at dose levels ranging from 10^{10} to 10^{12} PIB/Ha equivalents indicated that on a per occlusion body (OB) basis the AcMNPV isolate was more efficacious than the TnSNPV isolate. Interestingly, 4th instars showed higher rates of mortality in 7-day assays than did 2nd instar larvae likely as result of higher virus dose uptake during the 48h feeding period on sprayed plants due to higher absolute plant consumption rates by 4th instars. Based on results from this initial cage trial a larger spray trial was conducted on cucumber plants sprayed with the AcMNPV isolate at $1.0x10^{12}$ OB/Ha, 400L/Ha volume equivalent and seeded with 2nd instar cabbage looper larvae. Treatment and control plants were destructively sampled at 2, 6, 12 and 14 days post treatment and assessed as to number of surviving larvae, virus-infected cadavers, and feeding damage to foliage and fruit. The AcMNPV treatments showed significantly lower numbers of surviving larvae, and lower foliage and fruit damage at 6 days post treatment and onwards. These initial results indicate good potential for the use of indigenous baculovirus isolates as biological control agents of cabbage loopers in greenhouse vegetable production.

Tuesday, August 29, 8:00-10:00, *Nanyuan Meeting Room*
Contributed papers: Fungi 2
Moderators: Jørgen Eilenberg and Keqin Zhang

8:00

Does each host species harbour its own genotype of *Strongwellsea?*

Jørgen Eilenberg and Annette Bruun Jensen
(*Department of Ecology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK 1871 Frb C, DENMARK*)

The genus *Strongwellsea* is very peculiar. Infected hosts from Diptera develop one or two abdominal holes, from which conidia are discharged while the host is still alive. The genus consists of two recognized species, *S. castrans* and *S. magna*, infecting Anthomyiid and Fanniid flies, respectively. Recently, it has been documented that natural infections of *Strongwellsea* do also occur in other dipteran families, for example Muscidae and Scatophagidae. In order to clarify the host-pathogen relationship, we initiated a study to characterize *Strongwellsea* from natural infected hosts from dipteran families. Each infected host was determined to species level, and the ITS II region of *Strongwellsea* was sequenced. The preliminary data suggests a high level of host specificity: within a single host species there was a high degree of sequence similarity, while the genetic profile of *Strongwellsea* from different hosts was different.

8:15

Regulator of G protein signaling pathway gene *cag8* is involved in conidiation of *Metarhizium anisopliae*

Weiguo Fang and Michael J Bidochka
(*Department of Biological Sciences Brock University, St.Catharines, Ontario L2S 3A1, Canada*)

Entomopathogenic fungi, such as *M. anisopliae*, are currently used as biological agents. The conidium of entomopathogenic fungi is the propagule that initiates pathogenesis and is involved in disease transmission. However, the molecular basis of conidiation of entomopathogenic fungi is poorly

understood. Here, we report the cloning and characterization of a regulator of G protein signaling pathway (RGS) gene *cag8* from *M. anisopliae*. *cag8* contained a 1326-bp open reading frame which predicted a protein of 441 amino acids. RT-PCR showed that *cag8* was constitutively expressed throughout various growth stages in broth culture and agar medium as well as emergency of fungal mycelia and conidiation on the infected insect cadavers. The phylogenetic relationship of CAG8 to other fungal RGS proteins was inferred and found to be concordant with species relationship identified in Genbank. *cag8* can resort conidiation in *Aspergillus nidulans* in *DflbA* mutant. *cag8*-deficient mutants of *M. anisopliae* (*Dcag8*) were not able to conidiate on regular media (e.g. PDA, SDAY and M100) and on infected insect cadavers. *Dcag8* produced fewer blastospores in YPD broth than wild type strain. Compared to wild type strain, *Dcag8* showed significantly slower growth speed on PDA agar medium. Real-time PCR showed that the transcript level of hydrophobin gene *ssga* was remarkably reduced (>200 fold) in *Dcag8*. Transcript level of *pr1A* was similar in wild type strain and *Dcag8*. Transformants overexpressing *cag8* (driven by the promoter of translation elongation factor gene) had significantly slower germination speed in YPD broth and lower conidial yield on PDA agar medium than wild type strain. The colony of wild type strain was fluffy, while transformants overexpressing *cag8* had sparse mycelia in the middle of colonies. Our results show that G protein signaling pathway is involved in condiation of *M. anisopliae*.

8:30

Isolation and insecticidal property of toxins from *Verticillium lecanii*

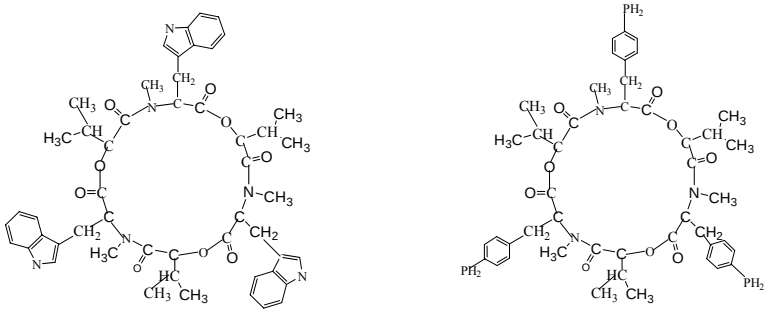
Liande Wang[1,2], Jian Huang[2], Minsheng You[1], Xiong Guan[2] and Bo Liu[3]

([1]*Institute of Applied Ecology,Fujian Agriculture & Forestry University, Fuzhou, 350002, P.R. China;* [2]*Key Laboratory of Biopesticide and Chemical Biology, MOE.Fujian Agriculture & Forestry University, Fuzhou, 350002, P.R. China;* [3]*Institute of Biotechnology, Fujian Academy of Agricultural Science, Fuzhou, 350001, P.R. China*)

Two new beauvericin analogues (toxin-Ⅳ and toxin-VⅡ, Fig.1) and two known fungal metabolites, dipicolinic acid (pyridine-2, 6-dicarboxylic acid) and phospholipid, have been isolated from liquid-substrate fermentation cultures of three strains of *Verticillium lecanii*. All compounds display insecticidal property against *Bemisia tabaci*. Phospholipid and two beauvericin analogues exhibit contact toxicity; moreover, dipicolinic acid shows deterrent and contact toxicity against *B. tabaci* (Tab.1). The compounds were obtained by chromatographic fractionation of the EtOAc culture extract and identified by analysis of IR, NMR and MS data. (Note：Tab. 1 and Fig. 1 will be attached in email)

Tab.1 Toxins from *Verticillium lecanii*.

Compound	Molecular formula	Structure	Insecticidal property
Toxin-Ⅳ	$(C_{17}H_{22}N_2O_3)_3$	Beauvericin analogue (Fig.1- A)	Contact toxicity
Toxin-VⅡ	$(C_{15}H_{24}NPO_3)_3$	Beauvericin analogue (Fig.1- B)	Contact toxicity
Toxin-VIII	$C_7H_5NO_4$	Pyridine-2, 6-dicarboxylic acid	Deterrent and contact toxicity
Toxin-X	$C_{53}H_{76}P_2O_5$	Phospholipid	Contact toxicity



(A) (B)

Fig. 1 Structure of toxin-Ⅳ and toxin-VⅡ

8:45

A PCR-RFLP approach for three endochitinase genes from glycohydrolase family 18 for the characterization and identification of *Metarhizium* strains

Vandana Ghormade, Franco Widmer and Juerg Enkerli

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Switzerland.)

Metarhizium anisopliae, an entomopathogenic fungus infects its hosts by actively penetrating the cuticle using mechanical pressure and enzymic degradation with synergistic action of hydrolases. The host cuticle, composed of a complex of proteins and chitin, is breached by hydrolases such as proteases and chitinases. Chitinases belonging to glycohydrolase family 18, are grouped into two classes, Class III and Class V according to sequence similarities of their genes. Secreted Class III chitinases possess serine/threonine rich domains and chitin binding domains that may be involved in their role during growth and morphogenesis. Class V chitinases are secreted and may contribute to the utilization of exogenous chitin as a source of nutrients. Our goal was to clone and sequence endochitinase genes of Class III and Class V from *M.anisopliae* to design specific primers and develop a PCR-RFLP approach for characterization and identification for different strains. Class III endochitinases have been previously isolated from *M.acridum* and *M.anisopliae* while Class V chitinases have been isolated from *M.anisopliae* and *M.flavovoridae.* We have isolated one Class III (*ChiIII*) and two ClassV (*ChiVa* and *ChiVb*) endochitinase genes from two type strains of *M.anisopliae* from Switzerland and India. Specific primers were designed by aligning the obtained *M.anisopliae* sequences to the sequences available in the database. The amplified *ChiIII* and *ChiVa* fragment lenghts from the two type strains of *M.anisopliae* were 1418bp and 1484bp, respectively. The sequences of *ChiIII* and *ChiVa* for the two type strains showed 91% and 98% similarity respectively, while *ChiVb* showed 98% similarity. We report for the first time a second Class V endochitinase gene (*ChiVb*) in *M.anisopliae* with 70% homology with *B.bassiana* ChitI. Specific primer pairs were designed for each endochitinase based on obtained sequences and a PCR-RFLP approach developed. This approach will be applied to a broader collection of *Metarhizium* strains to evaluate the genetic diversity. The PCR-RFLP method for chitinases may provide tool for detection and identification of *Metarhizium* strains and to monitor the *M.anisopliae* strains released for biological purposes.

9:00 STU

Sequence comparison of a hydrophobin-like protein involved in conidial thermotolerance of different *Beauveria bassiana* strains

Sheng-Hua Ying and Ming-Guang Feng

(*Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, 310058, China*)

In an earlier study, the conidial thermotolerance of fungal biocontrol agent *Beauveria bassiana* was found in an association with the contents of hydrophobin-like or formic-acid- extractable proteins in conidial walls. Of those, a 15.0-kDa component was postulated to take a major part in conidial thermotolerance and its gene was cloned from the wild strain *B. bassiana* 2860. In this study, the upstream sequence of the 15.0-kDa protein was cloned for comparison of the gene sequence to those from other *B. bassiana* strains. The 646-bp upstream sequence from *B. bassiana* 2860 included a 200-bp sequence that might function as putative promoter because seven TATA box motifs and an AT-rich region were located at bases from -107 to -94. Comparison of the genomic and cDNA sequence showed no intron for the gene encoding the protein. The presence of this gene in other *Beauveria* strains (Bb2864, Bb2861 and Bb0201) was also confirmed by amplifying their genomic DNA samples and sequencing the products. A conserved region (HNDRVVGAWDQDVKI) was found by complete alignments of the amino acid sequences from the four strains. This conserved region might be involved in integration of the 15.0-kDa protein into conidial walls. Based on analysis of paired distances, the amino acid sequence from Bb 0201, a strain derived from Asian corn borer in northeast China, had only ~60% identity to those from other three strains derived from cereal aphids in Idaho, USA. However, over 95% sequence similarity was found among the aphid-derived strains. The results indicate that the hydrophobin-like protein in conidial wall could be used as maker for genetic diversity of the fungal strains from different origins as well as for their tolerance to stressed temperature.

9:15

Cloning and characterization of a gene encoding a cuticle-degrading protease from the nematophagous fungus *Lecanicillium psalliotae* (syn. *Verticillium psalliotae*)

Jinkui Yang, Lianming Liang, Ying Zhang, Juan Li and Keqin Zhang*

(*Laboratory for Conservation and Utilization of Bio-resources, Yunnan University, No.2 of north cuihu road, Kunming, 650091, Yunnan Province, P. R. China*)

An isolate of *Lecanicillium psalliotae* (syn. *Verticillium psalliotae*) produced an extracellular protease (Ver112), which was purified to apparent homogeneity giving a single band on SDS-PAGE with a molecular mass of 32 kDa. The optimum activity of Ver112 was at pH 10 and 70 °C (over 5 min). The purified protease can degrade a broad range of substrates including casein, gelatin, and nematode cuticle. 81% of nematodes (*Panagrellus redivivus*) are degraded after treating with Ver112 for 12 h. The protease is highly sensitive to Phenylmethyl Sulfony Fluoride (PMSF) (1 mM) indicating it to be a serine protease. The N-terminal amino acid residues of Ver112 shared a high degree of similarity with other cuticle-degrading proteases from nematophagous fungi. The gene encoding a cuticle-degrading serine protease Ver112 was cloned from three isolates of *Lecanicillium psalliotae* by 3' and 5' RACE (rapid amplification of cDNA ends) method, which encodes for 382 amino acids and shares conserved motifs with subtilisin N and peptidase S8. Comparison of translated cDNA sequences of three isolates revealed one amino acid polymorphism at position 230. The deduced protease sequence shared high degree of similarities to other cuticle-degrading proteases from other nematophagous fungi, which suggests ver112 play a role in nematode infection. * C*orrespondence Author* (*E-mail: kqzhang111@yahoo.com.cn*)

9:30 STU

Purification and cloning of extracellular enzymes from *Clonostachys rosea* and their potential as pathogenic factors

Lianming Liang, Jinkui Yang, Zhongwei Gan, Ying Zhang and Keqin Zhang*

(*Laboratory for Conservation and Utilization of Bio-resources, Yunnan University, No.2 of north Cuihu road, Kunming, 650091, Yunnan Province, P.R. China*)

The filamentous fungus *Clonostachys rosea* (syn. *Gliocladium roseum*) played a role as a pathogenic factor against nematodes. It can parasitize both female nematodes and nematode eggs, and extracellular enzymes including protease and chitinase serve as the key virulence factors in infection process. A protease (PrC) was purified from the supernatant of the *C. rosea* culture apparent homogeneity. 80±5% of nematodes (*Panagrellus redivivus*) were immobilized and degraded after treating with PrC for 48 h. The protease was highly sensitive to PMSF (Phenylmethyl Sulfony Fluoride) (5 mM) indicating it belonged to the serine protease family. The N-terminal amino acid residues of PrC are ATQSNAPWGL, which is highly conservative with other proteases from *Pochonia chlamydosporia* and *paecilomyces lilacinus*. The conservative encode nucleotide sequence of PrC was cloned from *C. rosea* by using degenerate primers, and the unknown sequences of 5' and 3' terminus by using "DNA Walking" strategy. The full-length gene encodes 386 amino acid and the mature protease (277 aa) which has 55% identity to a Cuticle-degrading protease precursor (PR1) (from *Chymoelastase*). The conserved domain Peptidase_S8 of serine protease S8 family was detected in the amino acid sequence when BLAST by BLASTp method. Simultaneously, a chitinase gene about 1.9 kb was cloned with the same strategy. The amino acid sequence has a 70% similarity with endochitinase (from *Hypocrea lixii*). The protein may belong to glycosyl hydrolases family 18 for the conserved domain Glyco_hydro_18 was found in the sequence. These extracellular enzymes will be studied further to determine their functions in the fungi-nematode interactions. *C*orrespondence Author* (*E-mail:* kqzhang111@yahoo.com.cn)

9:45 STU

Variation in the activities of superoxide dismutase among twenty *Beauveria bassiana* strains

Bao-Fu Huang and Ming-Guang Feng

(*Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, 310058, China*)

Anti-oxidation ability of Hyphomycetous biocontrol agents such as *Beauveria* and *Meharizium* is likely to be associated with their tolerance to environmental stress during growth and thus field persistence. This ability can be well represented by their superoxide dismutase (SOD) activity in living cells. In this study, the SOD activities among 20 ARSEF strains of *Beauveria bassiana* were assessed. SOD extracts were made from fungal colonies of all the strains initiated by aerial conidia using phosphate balanced solution (PBS). Their SOD activity was assayed in the reaction system consisting of 1 ml 0.1 mol/L Tris-HCl, 0.1 ml 0.02 mol/L PBS buffer (extract solution) and 40 µl 30 mmol/L pyrogallic acid dissolved in 10 mmol/L HCl (pH 8.0). One SOD unit was defined as the amount of SOD to reduce 50% auto-oxidation rate of pyrogallic acid as reactive substrate. The results show significant variability in SOD activity among 15 strains (F=22.8, P<0.01), ranging from 0.13±0.06 (Bb5965) to 1.46±0.00 U/mg (Bb2861). Most of these strains had an SOD activity around

0.57 U/mg. However, the method did not suit well to five other strains in repeated assays. Based on the profiles from native gel electrophoresis (stained with nitroblue tetrazolium and riboflavin) of the extracts, three protein bands were recognized to differentiate the SOD types (Cu/Zn-, Mn-, or FeSOD). Further study is under way to clarify the structure and function of the different SOD types in the fungal species.

Tuesday, August 29, 8:00-10:00, *Xiyuan Meeting Room*
Contributed papers: Microsporidia
Moderators: Andreas Linde and James Becnel

8:00

Discovery of an Encephalitozoon sp. (Fungi: Microsporidia) in an invertebrate host

Carlos E. Lange[1], Leellen F. Solter[2], Michael D. Baker[3], S. Johny[4], Douglas W. Whitman[5] and Ann Cali[6]

(*[1]Center for Parasitological Studies* (*CEPAVE*)*, La Plata National University, CONICET, La Plata, Argentina; [2]Illinois Natural History Survey, 1816 S. Oak St., Champaign, IL 61820, USA; [3]Iowa State University, 1184 Molecular Biology Bldg., Ames, IA 50011 USA; [4]Laboratory of Molecular Genetics Centre for DNA Fingerprinting and Diagnostics, ECIL Road, Nacharam, Hyderabad 500 076, India; [5]Illinois State University, Department of Biology, Normal, IL 61790, USA; [6]Rutgers University, Dept. of Biological Sciences, Smith Hall, 101 Warren St., Newark, NJ 07102, USA*)

We isolated an unusual microsporidium from wild lubber grasshoppers, *Romalea microptera,* collected from Weeks Island, Louisiana, USA. We initially observed that the development of the isolate in the grasshopper host was not characteristic of other common microsporidia infecting terrestrial insects, including those found infecting grasshoppers. Large vesicles containing multiple developmental forms in different stages occurred in the midgut and gastric caecae cells. Transmission electron microscopy revealed that presporulation stages develop in association with the vesicle and dissociate from the membrane as they mature. The sporont plasma membrane is studded with electron dense exudates that persist until the resulting sporont is fully developed. The vesicles are remarkably varied, with no specific number of spores; some contain more than 60 spores and other developmental forms. Fresh uninucleate mature spores measure approximately 4 x 2 µm, and the spores average eight polar filament coils. The development is nearly identical to that of *Encephalitozoon cuniculi* but mature spores are significantly larger and the number of polar filament coils differs. The small subunit ribosomal DNA sequence places this microsporidium near *Encephalitozoon hellem* and well within the *Encephalitozoon* clade. With the exception of an *Encephalitozoon cuniculi* infection reported in mites that were fed on infected rabbits, no other arthropod microsporidia have been found to be congeneric with *Encephalitozoon* species. Our finding suggests the possibility of undiscovered invertebrate hosts for *Encephalitozoon* spp. that could serve as reservoirs for vertebrate pathogens. This novel invertebrate host-parasite system may offer some advantages over vertebrate-*Encephalitozoon* interactions for testing therapeutic systems.

8:15

The pathogenicity of Nosema apis, from Apis mellifera ligustica, to worker of Apis cerana cerana

Huang Shaokang, Ye Shengzhu, Dong Je

(*Bee Sciences College, Fujian Agriculture and Forestry University, 350002*)

To estimate the pathogenicity of *Nosema apis*,from Italian honeybee Apis mellifera ligustica, to Chinese honeybee, Chinese honeybee workers aged 2,5,8,11,14 days were individually fed with 5.12×10^4 spores of *N.apis* , then raised in cages in thermostat under 31±1℃,45～60% RH. The result showed, the average infection rate after 10 days post infection was 65.8%,range from 46.7%-72.6%.No age resistance was noted under experimental condition. The two-day old worker with lowest infection rate, seemed more resistant to N. apis compared with older groups.

8:30

Quantifying transmission of microsporidia in the gypsy moth, *Lymantria dispar*

Gernot Hoch[1], Vincent D'Amico[2], Dörte Goertz[1] and Leellen F. Solter[3]

(*[1]BOKU – University of Nat. Res. and Appl. Life Sci., Hasenauerstrasse 38, 1190 Vienna, Austria;*

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[3]*Illinois Natural History Survey, 1816 S. Oak St., Champaign, IL 61820, U.S.A.*)

The gypsy moth, *Lymantria dispar*, is regularly infected by different microsporida throughout its native European range. The pathogens belong to the genera *Nosema*, *Vairimorpha* and *Endoreticulatus*. Not very much knowledge exists on the transmission of these species in forest Lepidoptera. Frequently, only hypothetical pathways for transmission have been proposed based on the tissues that are infected. Quantitative data are particularly lacking. In a series of experiments, we have been trying to elucidate transmission pathways for selected microsporidia of the gypsy moth and to quantify them under both laboratory and semi field conditions. In the laboratory, important parameters of horizontal transmission were measured and compared between *Nosema lymantriae*, *Vairimorpha disparis* and *Endoreticulatus schubergi*. Spores of *N. lymantriae* and *E. schubergi* were released from the host in high numbers via feces after a latent period of 15 and 14 days, respectively. Although silk glands were heavily infected by *N. lymantriae*, spore release via silk was of minor importance. *V. disparis* appeared to depend predominantly on spore release after death of the host; low spore numbers were found irregularly in the feces of larvae. Horizontal transmission of *N. lymantriae*, was studied under semi field conditions. Inoculated larvae and uninfected test larvae were exposed together on caged, 2 m tall oak trees at different ratios for 21 days. Density of inoculated larvae affected prevalence of infections in test larvae (i.e., after horizontal transmission). Infection of test larvae increased with increasing density of initial infections. At higher densities, percent infection in test larvae appeared to level off.

8:45

Strategies and tactics for control of locust (*Locusta migratoria manilensis*) in China

Long Zhang, Yuhua Yan
(*Key Lab for Biocontrol of Pests, Ministry of Agriculture China Agricultural University, Beijing 100094, China*)

Locust plague had been recorded more than 800 times since B.C 707, is still a great potential threat to crop production in China. It has been developed a strategy for control locust since 1950s to emphasize on reforming breeding region of locust and using chemical pesticides. Some places of locust breeding regions have been reformed to crop field. But there are lot regions left as locust breeding region, recently estimated that locust occurs about 1,300,000ha controlled with chemical pesticides only. Therefore, it needs to develop alternative methods and strategies to control locust.

N. locustae has been used as biological control agent against grasshoppers in several countries as a long-term tactics (Henry 1981; Johnson 1997) . Based on achievements of mass produce of *N. locustae* using *L. m. manilensis* as host, researches on field epizootiology, and application techniques in different ecological conditions we have developed the strategies firstly to control locust with the pathogen to our knowledge. Strategy 1, when locust density is higher than 5 individuals/m^2, locust density should be reduced immediately to reduce the damage to crops and *Nosema* disease should be kept in locust population as long as possible. *Nosema* and insect growth regulator are integrated to use, separately interlaced spraying. The dosage of *Nosema* for use is $15X10^9$ spores/ha and insect growth regulator (Cascade®) is about 5-10 ml/ha. Application time is about at the 3rd instar stage. Strategy 2, when locust density is lower than 5 individuals/m^2, the solitary locust should be prevented to transferring into gregarious locust swarms. And outbreaks of higher gregarious locust swarms should be reduced. *Nosema* disease should be kept in locust population as long as possible. *Nosema* only is used, the dosage of *Nosema* for use is $15X10^9$ spores/ha, application time about at the 3rd instar stage. The results showed that the strategies are quite effective in locusts control after the demonstrations for 5 years in Tianjin, Hebei, Shandong, Henan, Anhui, and Hainan provinces.

9:00 STU

Infections experiments with different spore types and different microsporidian isolates of *Lymantria dispar*

Thomas Kolling and Andreas Linde
(*Fachhochschule Eberswalde, Dept. of Forestry, Applied Ecology, Alfred-Moeller-Str. 1, 16225 Eberswalde, Germany*)

The transmission of microsporidia in the genus *Vairimorpha* is, despite intensive field and laboratory studies, still an obstacle. *Vairimorpha* spp. infect, following a brief reproduction cycle in the midgut tissue, the larval fat body. As this tissue has no connection to the gut, the Malpighian Tubules, and the silk glands, the release of spores from infected hosts is difficult to explain. There is, however,

horizontal and vertical transmission. Furthermore, *Vairimorpha* spp. typically produce two spore types, the monocaryotic octospores (in packages of eight spores in a sporophorous vesicle) and the individual, diplocaryotic *Nosema*-type spores. The significance of these two spore types for the transmission of the infection had not been clarified, due to difficulties in the separation of the spores. In this study, we describe the separation of the two spores types of *Vairimorpha disparis* from *Lymantria dispar* L. and infection experiments with the two spore fractions. Furthermore, we investigated the minimum number of spores of different microsporidian isolates, needed for a successful infection of the host insect. The results of these studies are important to estimate the transmission rate of the microsporidia in the field after a release for biological control and the establishment of the pathogen in the host population. We used a micromanipulation device (Eppendorf combination of PatchMan and CellTram devices with Eppendorf CustomTips (Type IV), attached to a Leica inverse microscope) and adapted this technique for the separation of spores. Individual microsporidian spores can be located and imbibed into a micropipette through low pressure. By use of this technique we were able to separate and collect single spores from spore suspension of *Vairimorpha disparis*, *Nosema* sp. (Schweinfurt isolate), *Nosema* sp. (Veslec isolate), *Nosema* sp. (Levishte isolate), and *Endoreticulatus* sp., all isolated from infected tissues of *Lymantria dispar* L.. The pure samples of spores were used experimentally fed to larvae to investigate their role for the microsporidian infection. Octospores and *Nosema*-type spores of *Vairimorpha disparis* (dose of 50 – 200 spores/larva) were fed to third instar larvae of *Lymantria dispar.* The larvae were inspected for infection 14 days post infection (dpi). Only *Nosema*-type spores resulted in a typical *Vairimorpha* infection with both spores types present in high numbers in the fat body of the larva. No infections at all were found after feeding octospores to larvae. The biological significance of octospores of *Vairimorpha* remains unclear. The consequences of these results will be discussed. In a second set of experiments, we used a very low number of spores (1, 2, 5, 10, and 20 spores) for the oral infection of larvae. We wanted to prognosticate the total number of environmental spores after different time intervals post infection. As few as 2 spores are sufficient for the establishment of an infection in an individual larvae and resulted in spore numbers of $5x10^{09}$ to $1{,}1x\ 10^{10}$. The difference in spore production (quantitative) between the dosages will be calculated on the basis of the mass of the infected tissues. This is an important information for the evaluation of the chances of an released microsporidian to become established in a host population. Further results will be presented and discussed at the meeting.

9:15 STU

Effects of an anti-exospore monoclonal antibody on microsporidial (*Nosema bombycis*) germination in *vitro*

Fan Zhang[1], Meng Xing Lu[1], Shyam V Kumar[2], Jie Hong Zhu[1], Song Hong Chen[1], Xian Zheng Chen[1] and Jian Hong[1]

(*[1]Laboratory of Invertebrate Pathology, Zhejiang Unversity, Hangzhou 310029,People's Repubic of China; [2]P.G.Department of studies and Research in Sericulture Karnatak University, Dharwad-580 003,INDIA*)

Monoclonal antibody for the exospore of the anti-microsporidia (mAb) 3C2 which recognizes *Nosema bombycis* (*N.bombycis*) was prepared, and its effects on germination of the spores in *vitro* were studied. MAb 3C2 inhibited the germination of *N. bombycis* spores in *vitro* as visualized by significantly less number of germinated *N. bombycis* spores than in the control cultures with an irrelevant isotype mAb(P<0.01). The germination rate was not correlated with the mAb concentration. MAb 3C2 was recognized as a 34kDa antigen on the immunoblotting. It is considered that the surface proteins might be involved in the germination of spores. When the surface proteins are restrained or destroyed, the germination and invasion of the spores will be inhibited effectively. It is important to make sure the interaction between the spore surface proteins and the invasion. The present findings will be helpful to lead the development of novel strategies controlling the microsporidians.

9:30 STU

The comparison of rDNA of *Nosema ceranae* isolates

Wei-Fone Huang[1], Michel Bocquet[2], Ker-Chang Lee[1] and Chung-Hsiung Wang[1]

(*[1]Department of Entomology, National Taiwan University, 106, Taipei, Taiwan; [2]APIMEDIA, BP22, Pringy, France*)

Nosema ceranae is a pathogen of nosema disease that is a frequently encountered and important honeybee disease. *N. ceranae* is isolated from *Apis cerana* in 1996 and thought to be found

exclusively in *A. cerana*. Recently, *N. ceranae* has been reported in *A. mellifera* in many areas of the world, and it clarifies that the pathogen of nosema disease in *A. mellifera* may be *N. apis* or *N. ceranae*. The rDNA sequence is one of most available molecular markers for microsporidian classification. Here, we report the rDNA sequence comparison of the *N. ceranae* isolates that were collected from the honeybee colonies of Martinique Island, Spain, and Taiwan. It may have a contribution to elucidate the relationship between *N. ceranae* isolates of these areas.

Tuesday, August 29, 10:30-12:30, *Nanyuan Meeting Room*
Microbial Control Division Symposium: Novel approaches for dealing with difficult data
Convenor: Surendra Dara

10:30

Analysis, Interpretation, and Avoidance of Difficult Data in Bioassay

S. P. Wraight
(*USDA-ARS Plant Protection Research Unit,U.S. Plant, Soil and Nutrition Laboratory, Tower Road, Cornell University,Ithaca, New York 14853 USA*)

Problem data in biological assay of insect pathogens results from a number of difficult experimental design challenges, including: 1) screening of large numbers of pathogen isolates, 2) limited availability of inocula or test animals, 3) instability of pathogen preparations, 4) high variability in susceptibility of test animals, 5) censoring of data, and 6) high and variable levels of control mortality. Difficulties are exacerbated by inherent properties of pathogen dosage-mortality responses, especially low regression coefficients (slopes), imprecision of extreme lethal doses (e.g., LD_{95}), slow development of disease, and non-normal distribution of lethal dose estimates.

11:00

Top reasons why papers have been rejected for publication

Mark S. Goettel[1], Quirico Migheli[2] and Charles H. Pickett[3]
([1]*Lethbridge Research Centre, Agriculture & Agri-Food Canada, 5403 1st Avenue South, Lethbridge, AB, Canada T1J 4B1;* [2]*Dipartimento di Protezione delle Piante, Universita degli Studi di Sassari, Via E. De Nicola 9, I-07100 Sassari, Italy;* [3]*Biological Control Program, California Department of Food and Agriculture, 3288 Meadowview Road, Sacramento, CA, 95832, USA*)

Between 30 and 50% of manuscripts submitted to peer reviewed scientific journals are regularly rejected. This is a travesty as it demonstrates either very poor science or poor penmanship. The bottom line is that any scientific endeavor that is not in the end published, is essentially a loss of time, energy and resources. As Editors, members of Editorial Boards and as anonymous peer reviewers, we will present our experiences in an effort to alert scientists of the top reasons why papers are rejected. Most frequently, it is simply a lack of clarity. Often it is a lack of repetition of experiments, inadequate experimental design, or lack of novelty. More rarely it is pure plagiarism.

11:30

Lost to industrial secrecy, statistical insignificance and short attention span: dark, dead, and dated data

Jeff Lord
(*US Department of Agriculture, Agricultural Research Service, Manhattan, Kansas 66502, USA*)

There are many reasons for data failing to reach publication. Perhaps the most common is that the data are not statistically significant. Yet there can be great value in negative data. For example, there are many published reports of pathogen efficacy where unrealistic doses have been used. Negative results with realistic doses remain in notebooks while questionable positive results are cited. Including negative data in publications along with positive results should be encouraged. Industrial data is published at the pleasure of men who wear neckties. This means that unfavorable results are rarely reported. Because of the commercial and regulatory rather than academic purposes of industrial research, the data are sometimes not taken in a manner that is suitable for publication. Confidentiality agreements can prevent publication of data from collaborative research for up to ten years. Examples of data failing to reach publication for these reasons will be discussed.

12:00

Hard lessons and perspectives of laboratory bioassays and field trials with entomopathogenic fungi

Jarrod E. Leland[1] and Debbie Boykin[2]

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Three case studies will be examined that each presented challenges in overcoming practical constraints to obtain publishable bioassay data. First, experience from laboratory bioassays with *Metarhizium anisopliae* var. *acridum* against *Schistocerca gregaria* and *Schistocerca americana* involved limitations on insect production and coordinating experiments in two continents due to quarantine constraints. Second, development of formulations of *M. anisopliae* var. *acridum* and *Beauveria bassiana* involving coating conidia with lignin and application in oil or water carriers provided unique challenges in methodology of handling formulations and collaborating overseas experiments. Finally, screening of 25 isolates of entomopathogenic fungi against tarnished plant bug, *Lygus lineolaris*, and several beneficial insect species involved designing efficient bioassay methodology and working with fragile insects. Perspectives on strategies both in developing methodology and experimental design that in hind sight could have more efficiently provided quality bioassay data will be presented. In addition, laboratory bioassay data on *B. bassiana* isolates with *L. lineolaris* and several beneficial insect species will be considered as they relate to subsequent field trials comparing isolates and application rates.

Tuesday, August 29, 10:30-12:30, *Meeting Center*
Contributed papers: Bacteria 1
Moderator: Didier Lereclus

10:30

The *Bacillus thuringiensis* InhA metalloproteases*:* conclusive weapons for infection

Christina Nielsen-LeRoux[1, 2], Myriam Ellouze -Hajaij[1], Nalini Ramarao[1], Christophe Buisson[1], Elisabeth Guillemet[1], Michel Bréhelin[3], Michel Gohar[1] and Didier Lereclus[1]

(*[1] INRA, Unité Génétique Microbienne et Environnement, INRA, la Minière, 78285 Guyancourt; [2]Institut Pasteur, Departement of Micrbiology Institut Pasteur, 75724 Paris Cedex 15, France2; [3]INRA , Université de Montpellier II, 3Ecologie Microbienne des Insectes et Interactions Hôte-Pathogène Université Montpellier II, 34095 Montpellier Cedex 05, France*)

The main insecticidal activity of *Bacillus thuringiensis* (*Bt*) is due to the larval ingestion of the insect specific Cry toxins. However, strains of both crystal minus *Bt* and *B. cereus* are known to produce other factors contributing to the overall virulence of these bacteria toward insect. Such factors may interfere with immunity systems. The importance of the *Bt* PlcR regulator was demonstrated by reduced mortality in larvae of the greater wax moth *Galleria mellonella* (Gm) of spores from a *Bt* 407 Cry- *plcR* mutant (Salamitou *et al.,* 2000). PlcR governs many putative virulence factors among which the putative zinc protease InhA2 was discovered to be important for pathogenesis via the oral route (Fedhila *et al.* 2002). InhA2 is found as a major extra-cellular 85 kDa polypeptide in a *Bt* 407 Cry- culture in early stationary phase. InhA2 has 66% idendity with the InhA1 protein which is reported to inhibit antimicrobial peptides and found associated with the exosporium. InhA1 is regulated by Spo0A and present in the culture medium (from late exponential to late stationary growth).In order to better characterize the role of InhA1 and InhA2 during infection we have used several cellular and biochemical approaches. First, we estimated the implication of InhA1 and InhA2 in pathogenesis, individual interruption of both *inhA1* and *inhA2* resulted in reduced virulence towards Gm, both by oral and intrahemocoelic injection. Secondly, studies with a murin macrophage cell-line showed that InhA1 but apparently not inhA2 permits the *Bt* spores to escape from the macrophages (Ramarao & Lereclus,2005), then suggesting a role of InhA1 in cellular immunity as well. Similar studies related with insect hemocytes are ongoing. Purification of InhA2 was performed in order to characterize its enzymatic activity and specificity and to correlate this with the possible mode of action of InhA2 during the larval infection process. InhA2 was fully purified from a by anion-exchange chromatography in the presence of Ca^{2+} which is required for stability. Using Azo-casein as a substrate, InhA2 was found to be active in a large range of temperatures from, 25-55°C and in rather acid and neutral pH spectrum. The enzymatic activity, was inhibited by several

protease inhibitors and InhA2 degrades several substrates (albumine, collagene and actin). No direct cytotoxic effect was found towards mammalian Hela cells and no larvicidal activity was observed in *G. mellonella* following oral ingestion of pure InhA2. Meanwhile, when injected directly into the hemocoel, immune responses (melanization, encapsulation, nodule formation of hemocytes) are observed and the larvae die at 1μg. This result suggests that InhA2, like InhA1, interferes with insect immunity. In fact, InhA2 also degrades the antimicrobial peptide cecropine. Our results indicate that these two metalloproteases have complementary roles and may arm *B. thuringiensis* in the fight against its host.

10:45 STU

***Bacillus nematocida* kills nematodes with two coordinated pathogenic factors: Bae16 and Bace16**

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(*Laboratory for Conservation and Utilization of Bio-resources, Yunnan University,Kunming 650091,P.R.China*)

Bacillus nematocida, isolated from a soil sample, presented remarkable nematotoxic activity. The crude extracellular protein extract from culture supernatant of the bacteria killed about 80% of the tested nematodes within 24 h, suggesting the involvement of extracellular proteases. Two homogenous extracellular proteases, serine alkaline protease (designated Bace16) and neutral protease (designated Bae16), were purified by chromatography, and the hypothesis of proteinaceous pathogeny in the infection of the bacteria was confirmed by the experiments of killing living nematodes and by the degradation of purified nematode cuticle when treated with the homogenous proteases. Characterization of Bace16 revealed the molecular mass of 28 kDa and the optimum activity at pH 10, 50℃. The protease Bace16 was highly sensitive to PMSF (phenylmethyl sulfony fluoride) (5 mM) indicating it belonged to the serine protease family. The other protease of Bae16 showed a molecular mass of approximately 40 kDa and displayed optimal activity at pH 6.5, 55℃. The two proteases could both hydrolyze collagen and the cuticle of nematodes although Bae16 revealed relatively weaker activity compared with Bace16. But the addition of the Bae16 to bioassay experiments exposed to serine protease Bace16 increased significantly the nematicidal efficacy. These phenomena might illustrate that protease Bace16 and Bae16 synergize to kill nematodes. Thereafter, the genes for the two virulence proteases were both cloned, and the deduced amino acid sequences were determined individually. The nucleotide sequence of Bace16 showed significant similarity with subtilisin BPN' and *Bae16* had 94% sequence identity with neutral protease gene from *B. amyloliquefaciens*. However, both of the sequences showed low homology (13%-43%) with other cuticle-degrading proteases previously reported in fungi. Recombinant mature Bace16 (rm-Bace16) and Bae16 (rm-Bae16) were expressed in *Escherichia coli* BL21 using *p*ET30 vector system and a bace16 knockout mutant was constructed by homologous recombination. Bioassay experiments demonstrated that these two recombinant proteases could still destroy the nematode cuticle, besides, compared with the wild type, the bace16 mutant had considerably lower proteolytic activity and lost approximately 55% nematicidal activity. Our present study revealed the two proteases with different characteristics produced by the same strain co-ordinated efforts to kill nematodes. These data helped to understand the interaction between this bacterial pathogen and its host. *Correspondence author: kqzhang111@yahoo.com.cn

11:00 STU

Requirement of *spoIIIAE* gene and *spoIVF* operon for sporulation and producing δ-endotoxins in *Bacillus thuringiensis* G03

Changpo Sun[1], Fuping Song[2], Jie Zhang[2] and Dafang Huang[1]

([1]*Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100081,China;* [2]*State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100094,China*)

Bacillus thuringiensis are known to generate spore and insecticidal crystalline-inclusions. We had constructed and screened a *B. thuringiensis* G03 mini-Tn*10* insertion library for loss of spore and crystal. Two clones were isolated, corresponding to one mini-Tn*10* insertions mapping to the *spoIIIAE* locus. The *spoIIIAE*⁻ mutant strain was blocked at stage III of sporulation. The inactivation of *spoIIIAE* gene resulted in none of spore and faint virulence to some pests. The *spoIVF* operon of *Bt* G03 was knocked out by gene homologous recombination and relative mutant was obtained. This mutant strain was blocked at stage IV of sporulation. It had lost the ability of sporulation and production of crystal also. Besides,the growth velocity of mutant strains became slower than that of

wild strain at 30℃. The promoter of *cry1Aa* was cloned from *Bt* G03 and fused with the *LacZ* gene in the vector of pHT315. Then this plasmid containing the *cry1Aa'-'lacZ* was introduced into wild strain and mutant strains by electroporation. The curve of β-galactosidase activity showed two peaks in wild strain. But in mutant strains, the β-galactosidase activity was little and didn't show peak. These results suggested that the activation of sigmaE and sigmaK had been severely reduced in these two mutant strains. Overall, our findings suggest that the *spoIIIAE* gene and *spoIVF* operon encode adaptive factors that may act in formation of spore and crystalline -inclusions.

11:15

The organic composition of silk gland of silkworm, *Bombyx mori* L infected with *Bacillus thuringiensis*

Bharathi Depuru[1] and Yungen Miao[2]

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The organic composition of *Bombyx mori* L. was studied in a univoltine hybrid silkworm, Huahe (Chinese) × Dongfei (Japanese) infected with *Bacillus thuringiensis*. A significant decrease was noticed in the weight of posterior silk gland and the biochemical constituents causing pathological symptoms to silkworm. The decrease in the biochemical components of posterior silk gland indicates that *Bacillus thuringiensis* affects the synthesis of silk proteins which might influence on the quality of silk.

11:30 **STU**

Molecular characterization of the plasmid genome from *Bacillus thuringiensis* subsp. *tenebrionsis* YBT-1765

Junyan Huang, Suxia Guo, Dongmei Han, Li Wang, Ziniu Yu and Ming Sun

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A 20-kb DNA fragment containing a plasmid replicon was isolated from *Bacillus thuringiensis* subsp. *tenebrionis* YBT-1765 and characterized. By Southern blot analysis, this replicon region was determined to be located on pBMB165, the largest detected plasmid of strain YBT-1765. This plasmid was mapped partially for *Bam*HI and for *Hind*Ⅲ restriction sites as well, and then the total size of pBMB165 (82 kb) was identified. Deletion analysis revealed that a replication initiation protein (Rep165), an origin of replication (*ori165*) and an iteron region were required for replication. In addition, two overlapping ORFs (*orf6* and *orf10*) were found to be key elements for the stability control of plasmid replication. Sequence comparison showed that the replicon of pBMB165 was homologous to replicons of the pAMß1 family, indicating that pBMB165 replicon belongs to this family. The presence of five transposable elements or remnants thereof in close proximity to and within the replicon control region leads us to speculate that genetic exchange and recombination are responsible for the divergence among the replicons of this plasmid family. On the other hand, a new cryptic plasmid pBMB175 from *Bacillus thuringiensis* subsp. *tenebrionsis* YBT-1765 was isolated and characterized. Plasmid curing experiment revealed that the smallest plasmid pBMB175 in *B. thuringiensis* subsp. *tenebrionsis* YBT-1765 was genetically stable in its native host at high temperature (highest temperature is 46℃). Sequence analysis showed that pBMB175 (14841-bp and 31% GC content) contained at least eighteen putative open reading frames (ORFs), among which nine ORFs displayed the homology with the hypothetical proteins in rolling-circle replication plasmid pGI3. Deletion analysis revealed that the pBMB175 minireplicon located in a novel 1151-bp fragment. This fragment contains ORF7 coding sequence, which encodes an indispensable protein ((Rep175, 149 amino acids [aa]) for plasmid replication. Rep175 has not any significant homology with known function proteins. Furthermore, a putative double-strand origin (*dso*), having not any DNA similarity with characterized *dso* of other replicon so far, was identified in this minireplicon fragment. These features showed that pBMB175 could be grouped into a new plasmid family.

11:45 **STU**

Anthrax virulence plasmid pXO1 conservative fragments within *Bacillus cereus* group and their phylogenic relatedness with pathogenicity

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The presence of one of the anthrax virulence plasmid pXO1 conservative fragments was analyzed in *Bacillus cereus* group strains, representing 6 subspecies, by polymerase chain reactions. 12 out of 24 strains showed PCR-positive for an ORF101 homologous sequence. Two pXO1-ORF101-like fragments from a *B. cereus* B-4ac and a commercial *Bacillus thuringiensis kurstaki* HD1 were cloned, sequenced and expressed in *Escherichia coli*. Toxicity assays revealed that the product encoded by the pXO1-ORF101-like fragment had no impact to either Vero cells or Chinese Hamster Ovary cells, suggesting that this fragment probably not contribute to enterotoxic activity. Sequence alignment of the pXO1-ORF101 from three *B. anthracis* and ORF101-like fragments from other 12 *B. cereus* group isolates indicated high identity (more than 90%) and the presence of subgroup- and strain-specific SNPs among these fragments. The evolution affiliation also indicates that ubiquitous presence of pXO1-ORF101-like fragments among *B. cereus* group strains may correlate with phylogenetic relatedness with the pathogenicity within the *B. cereus* group.

12:00 STU

Toxicity Analysis of Truncated Insecticidal Crystal Protein Cry1Ah from *Bacillus thuringiensis*

Jing Xue[1], Fuping Song[1], Dafang Huang[2] and Jie Zhang[1*]

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Cry1Ah protoxin from *Bacillus thuringiensis* is a 134-kDa insecticidal protein. After ingestion by susceptible insects, it dissolve in the midgut and is activated to a 65 kDa active toxin core by midgut proteases. In order to reveal the minimal active fragment of Cry1Ah polypeptide, 7 pairs of primers were designed to generate different PCR products. Seven PCR products amplified by different primers using *cry1Ah1* gene as template were cloned into the pET-21b vector respectively. These positive clones were transformed into *E. coli* strain Rosatta2 (DE3) cell separately, and protein expression was induced by isopropyl-β-D-thiogalactopyranoside (IPTG) with 0.05 mM. Protein concentrations were determined using bovine serum albumin (BSA) as standard. Insecticidal activity against second-instar larvae of *Plutella xylotella* was measured by leaf dip bioassay. Bioassay result showed that the active fragment of Cry1Ah-truncated polypeptide was located between amino acid 20-640. As the insecticidal activity of the truncated protein containing amino acid 1-657 is similar to fragment 20-640, fragment 47-640 and 20-639 was declined significantly, so the C-terminal site of the minimal active fragment of Cry1Ah protein is located at amino acid 640. The N-terminal section of active fragment is between amino acid 20 to 47, and the precision site is still need to be studied.

12:15

Molecular dynamics simulations of the *Bt* toxin Cyt1A: the model and its validation by fluorescence resonance energy transfer

Xiaochuan Li[1], Kerrick Nevels[1], Dexuan Xie[2], Marianne P. Carey[3] and Peter Butko[1]

([1]*University of University of Southern Mississippi, Department of Chemistry and Biochemistry, Hattiesburg, MS 39406, USA;* [2]*University of Wisconsin, Department of Mathematical Sciences, Milwaukee, WI 53211, USA;* [3]*Case Western Reserve University, Department of Biochemistry, Cleveland, OH 44106, USA*)

Cyt1A is an insecticidal cytolytic toxin produced by the spore-forming bacterium *Bacillus thuringiensis* var. *israelensis*. Knowledge of molecular details of the toxin's conformation changes in the presence of lipid membranes is important for elucidating the toxin's mode of action, but the structure of the membrane-bound Cyt1A has not been experimentally determined. Therefore we initiated molecular dynamics simulations of the toxin in silico. A 3-D molecular structure of Cyt1A in solution was generated by homology modeling and potential energy minimization using CHARMM and NAMD. Short periods of molecular dynamics runs identified the regions in the molecule that manifest increased conformational flexibility and thus are likely to participate in the initial membrane binding and conformational changes. The simulated structure was used to study the relevance of the four conserved blocks in the amino-acid sequences of all Cyt toxins and the effect of a single amino-acid mutation, K225A, that is known to abrogate the Cyt1A toxicity in vivo. The results suggest that the mutant's loss of toxicity is due to an overall change in conformation and a diminished stability, rather than due to a localized alteration of the "binding site" or "active site", which is often observed in enzymes and binding proteins. Selective randomization of atoms in the computer model revealed that the four conserved blocks are important for proper folding and stability of the toxin

molecule. The computer model was validated experimentally by fluorescence resonance energy transfer (FRET): we determined the distance between the single cysteine 191 that was fluorescently labeled by 1,5-IAEDANS (5-((((2-iodoacetyl) amino)ethyl)amino)naphthalene- 1-sulfonic acid) and the distant pair of two neighboring tryptophans (158 and 161). The experimental and simulated values were 2.7 ± 0.3 nm and 2.3 ± 0.2 nm, respectively. The agreement between the two numbers gives credence to our computer model and at the same time confirms the predicted structure of Cyt1A in water. Simulations of Cyt1A and FRET measurements in the presence of lipid membranes are currently under way. It is hoped that this work will bring a long-anticipated insight into the structure of the Cyt1A bound to the lipid membrane.

Tuesday, August 29, 10:30-12:30, *Multifunctional Hall*
Contributed papers: Viruses 1
Moderators: Linda King and Xinwen Chen

10:30

***Helicoverpa armigera* nucleopolyhedrovirus *orf80* encodes a late, nonstructural protein**

Dun Wang[1, 2] and Chuan-xi Zhang[1]
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Homologues of *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV) *orf80* are found in all 23 completely sequenced members of the lepidopteran nucleopolyhedroviruses and granuloviruses, but so far their functions are unclear yet. In this article, the ORF80 (*ha80*) of *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV) was characterized. Northern blot and western blot analyses demonstrated that *ha80* was expressed as a late gene and encoded a nonstructural protein of HearNPV. *Ha80* gene was transcribed beginning at 12 h post-infection in infected *Helicoverpa zea* cells (HzAM1). Western blot analysis using a rabbit derived polyclonal antibody showed the product of *ha80* in infected cells was a 31 kDa protein, in tune with the theoretical size of 30.8 kDa. The protein was firstly detected in the cytoplasm of infected HzAM1 cells 12 h p.i., and was transported into nucleus after 36 12 h p.i. in infection.

10:45 STU

The *Helicoverpa armigera* nucleopolyhedrovirus FGF is essential for BV infection

Xiang Li, Changyong Liang, Jianhua Song, Xinwen Chen
(*State Key Laboratory of Virology, Wuhan Institute of Virology, the Chinese Academy of Sciences, Wuhan, 430071, the People's Republic of China*)

Fibroblast growth factor (FGF) is a key regulator of developmental processes affecting the growth, differentiation, and migration of many cell types. A FGF homology (vFGF) is found in all lepidopteran baculoviruses. It has been demonstrated that *Autographa californica* nucleopolyhedrovirus (AcMNPV) vFGF is chemotatci factor and might play key rule in viral oral infection. Here we analyzed the *vfgf* gene (open reading frame 113) of *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV). The HearNPV *vfgf* transcripts were detected from 18 to 96 h post-infection (p.i.) of HzAM1 cells with HearNPV. Polyclonal antiserum recognized a 35 kDa protein from 24 to 96 h p.i.. Western blot analysis showed that vFGF was secreted into the culture medium, suggesting that it acts as an extracellular ligand. HaSNPV vFGF had strong affinity to heparin, a property important for FGF signaling via an FGF receptor. Unlike its AcMNPV homology, which was able to stimulate migration of several different types of insect cells, HearNPV vFGF specially chemoattracted Hz-AM1, but not other insect cell lines such as Sf9, Se-UCR nor mammalian cells: 293 and HepG2. It is most notable that HearNPV vFGF was also a structural protein of budded virus but absent in occlusion-derived virus (ODV), which coordinated to the chemotatic activity analysis, implying that it my have important function for BV infection. Then a recombinant HearNVP bacmid lacking a` functional vFGF (HaΔFGF) was constructed. Transfection-infection analysis indicated that HaΔFGF could not set up the second infection. However, restoration of *vfgf* rescued the infectivity. Those results indicated that vFGF plays an essential role in HearNPV BV infection.

11:00 STU

Functional analysis of baculovirus DNA photolyase genes

Fang Xu[1], Margit Lampen[1, 2], Christina Van Houte[1], André P.M. Eker[2], Just M. Vlak[1] and Monique M. Van Oers[1]

([1]*Laboratory of Virology, Wageningen University, Binnenhaven 11, 6709 PD Wageningen, the Netherlands;* [2]*Department of Cell Biology and Genetic, Erasmus University Medical Center, Dr Molewaterplein 50, 3015 GE Rotterdam, the Netherlands*)

Baculoviruses in general are sensitive to UV radiation and have no mechanism to repair UV-induced DNA damage. Cyclobutane pyrimidine dimers (CPDs) are formed in the DNA upon UV irradiation. CPD DNA photolyases (PHRs) are able to repair such dimers using blue light as an energy source. Two open reading frames (ORFs) *phr*1 and *phr*2 coding for putative class II CPD DNA photolyases were discovered in the genome of *Chrysodeixis chalcites* nucleopolyhedrovirus (ChchNPV) (van Oers et al., 2004, Virology; ibid. J. Gen. Virol., 2005). A putative DNA photolyase gene was also identified from *Trichoplusia ni* single nucleopolyhedrovirus (TnSNPV) (Willis et al., 2005, Virology). The TnSNPV *phr* gene has the highest amino acid identity (78%) to the *phr*1 gene of ChchNPV. The expression and functional study of these *phr* genes may provide further information on the possible role of these DNA photolyases in DNA repair and in UV protection in these baculoviruses. In order to test whether the ChchNPV *phr1* and *phr2* genes encode functional enzymes, an assay was developed involving a photolyase-deficient *E. coli* strain (*E. coli* KY29). To this end, the viral *phr* genes were cloned in the expression vector pKK223-3 and the recombinant plasmids were introduced into *E. coli* KY29. The photolyase corresponding to *phr*2 was able to functionally complement the photolyase-deficient *E. coli* strain. On the contrary, phr1 could not rescue photolyase activity and may be an inactive enzyme or a pseudogene. A flag-tagged *phr*2 protein was localized in the cytoplasm of Sf21 cells in a transient expression study using anti-flag antibody, but a flag-tagged *phr*1 protein could not be detected. Similar experiments are also conducted with GFP- fusion proteins. The presence of DNA photolyase genes may be a general feature of NPVs belonging to group II, which infect lepidopteran insects of the *Plusiinae* subfamily. This hypothesis is tested by PCR, amplifying a conserved region of CPD photolyases. The ecological and evolutionary relevance of the observations will be discussed. (monique.vanoers@wur.nl)

11:15 STU

The Baculovirus P10 protein forms two distinct cytoskeletal-like structures with different cellular localisation properties

David CJ Carpentier, Caroline M Griffiths and Linda A King

(*Insect Virus Research Group, School of Biological and Molecular Sciences, Oxford Brookes University, Headington, Oxford, OX3 0BP, United Kingdom*)

The polyhedrin and p10 gene products are expressed at extremely high levels very late during infection by *Autographa californica* nucleopolyhedrovirus (AcNPV), the prototype member of the *Baculoviridae* virus family. The role of Polyhedrin as the main component of the paracrystaline occlusion bodies (OBs) that protect virions from environmental factors and mediate virus host-to-host spread is well understood. Although it has been studied for several decades the role of the P10 protein remains a mystery. Homologues of the AcNPV p10 gene have been found in all sequenced genomes of nucleopolyhedroviruses (NPVs) isolated from lepidopteran hosts, suggesting an important role in the infection process, but seem to be absent from the three non-lepidopteran NPVs that have been sequenced. Early electron microscopy studies identified P10 as a component of large fibrilar structures found in the cytoplasm and nucleus of cells late in the infection cycle. These structures appear to interact with structures thought to be involved in the maturation of the polyhedrin OBs suggesting P10 might play a role in this process. Other studies have suggested that P10 might play a role in mediating the lysis of the cell or the nucleus, facilitating the release of OBs into the environment. Neither of these roles has been studied in depth, however recent studies using co-immunoprecipitation and yeast-2-hybrid studies have shown an interaction between P10 and the host cell microtubule cytoskeletal network. Using confocal laser scanning microscopy we have identified two distinct intracellular structures formed by P10 during infection; a network of thin filament structures that appear to colocalise with and possibly reorganise the microtubule network in the host cell cytoplasm, and thicker perinuclear tubular structures that seem to form a cage or shell around the nuclear OBs. To investigate whether P10 acts as an adaptor linking the microtubule cytoskeleton to processes involve in viral replication we are using co-immunoprecipitation and protein characterisation studies to isolate and identify other viral and cellular protein components of

these intricate and extensive intracellular P10 filament and tubule structures. Confocal microscopy studies are underway to identify the effect of deleting p10 on other structures and processes involved in virus replication and Ob maturation. The aim of these studies is to gain a greater understanding of the role played by this evolutionarily conserved protein in the replication and spread of Lepidopteran nucleopolyhedroviruses.

11:30 **STU**

38K is Required for *Autographa californica* Multiple Nucleopolyhedrovirus Nucleocapsid Assembly

Wenbi Wu, Tiehao Lin, Lijing Pan, Mei Yu, Zhaofei Li, Yi Pang and Kai Yang

(*State Key Laboratory of Biocontrol, Sun Yat-sen University, Guangzhou 510275, People's Republic of China*)

38K (*Ac98*) of *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) is a highly conserved baculovirus gene which function is unknown. To determine the role of *38K* in the baculovirus life cycle, a *38K* knockout bacmid containing the AcMNPV genome was generated through homologous recombination in *Escherichia coli*. And then, a *38K* repair bacmid was constructed by transposing the *38K orf* with its native promoter region into the *polyhedrin* locus of the *38K* knockout bacmid. After transfecting these viruses into *Spodoptera frugiperda* cells, the *38K* knockout bacmid leads to a defect in infectious budded virus （BV） production while the *38K* repair bacmid can rescue this defect and the BV titers can reach the wild-type virus levels. Slot blot analysis indicated that *38K* deletion did not affect the levels of viral DNA replication. Subsequent immunoelectron microscopic analysis revealed that masses of electron lucent tubular structures containing the capsid protein vp39 were observed, suggesting that nucleocapsid assembly was interrupted in cells transfected with *38K* knockout bacmids. In contrast, the production of normal nucleocapsids was restored when the *38K* knockout bacmid was rescued with a copy of *38K*. Recombinant virus expressing 38K fused to green fluorescent protein as a visual marker was constructed to follow protein transport and localization within the nucleus during infection. Autoflourescence was first detected along the cytoplasmic periphery of the nucleus and subsequently localized to the center of nucleus. These results demonstrate that 38K plays a role in nucleocapsid assembly and is essential for viral replication in the AcMNPV life cycle.

11:45 **STU**

HA2 from the *Helicoverpa armigera* nucleopolyhedrovirus: A WASP-related protein that activates Arp2/3-induced actin filament formation

Qian Wang[1,2], Changyong Liang[1], Jianhua Song[1] and Xinwen Chen*[1]

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Filamentous actin is required for productive replication of lepidopteran nucleopolyhedroviruses. We have demonstrated that nucleocapsids of the *Helicoverpa armigera nucleopolyhedrovirus* (HearNPV) are capable of nucleating actin polymerization *in vitro* in a dose-dependent manner. Actin polymerization is the main factor for cell locomotion and has also been utilized by the bacteria, *Listeria*, and by vaccinia virus for intracellular and intercellular movements. The WASP family of proteins has been shown to stimulate the assembly of branched actin filaments by the Arp2/3 complex. The process is conserved in eukaryotic cells. Here, we found that the HA2 of HearNPV ORF2, a WASP homologue could nucleate branched actin filaments in the presence of Arp2/3 complex *in vitro*. We have further shown that the WCA domain is critical for HA2 function. We also demonstrated that HA2 co-localizes with Arp2/3 complex in the nucleus of infected cells, indicating that HA2 and Arp2/3 complex is involved in nuclear-actin polymerization. In summary, HA2 activates Arp2/3-induced actin filament network formation *in vitro* and *in vivo*. Moreover, nucleocapsids of HearNPV appear to induce actin polymerization into branched structure in absence of Arp2/3 complex. We postulate a possible model on how HA2 is used to activate nucleation.

12:00 **STU**

The cytoplasmic tail domain of baculovirus group II F proteins is essential for viral infectivity

Gang Long[1, 2], Xiaoyu Pan[1], Marcel Westenberg[2], Zhihong Hu[1] and Just M. Vlak[2]

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Netherlands)

F proteins from baculovirus nucleopolyhedrovirus (NPV) group II members are newly found viral envelope fusion proteins present on budded viruses (BV). In group I baculoviruses, such as AcMNPV, GP64-like proteins have the same function. Baculovirus F proteins undergo furin-like proteolysis processing prior to maturation and hence consist of two covalently linked subunits, a large (C-terminal) and small (N-terminal) subunits. Baculovirus F proteins have structural and functional homology with F proteins of vertebrate enveloped viruses, such as influenza. F proteins from different baculoviruses have a cytoplasmic tail domain (CTD) at the C-terminus, ranging from 48 (*Spodoptera litura* MNPV) to 78 amino acid residues (*Adoxophyes honmai* NPV). This is much longer than the CTD from GP64-like envelope fusion proteins, which is only 8 amino acids. Here we have investigated the functional role of the CTD of *Helicoverpa armigera* single capsid nucleopolyhedrovirus (HearNPV) as a representative of group II baculoviruses. We combined a newly constructed HearNPV *f*-null bacmid knockout-repair system and an AcMNPV *gp64*-null bacmid knockout-pseudotype system to study the functional role of the CTD in group II baculovirus F proteins. We show that the F protein in HearNPV is essential for spread of the virus from cell to cell. In addition, the CTD of the HearNPV F protein is also important for production of infectious BV. Its size is correlated with the production of BV in a length-dependent manner. However, when the CTD is entirely removed, the residual HearNPV F proteins still rescue infectivity in a *gp64*-null AcMNPV background. Thus, deletion of the CTD does not affect F protein synthesis, processing and fusogenicity. Altogether, our results indicate that the CTD of the F protein is essential for viral infectivity in its own genetic background and probably important for the incorporation F protein in BVs.

12:15 STU

The unique functions of a marvelous gene p13 from type II baculoviruses

Enqi Du[1], Feng Yan[1], Weixin Jin[2], Wenke Zhou[1], Yipeng Qi[1]

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P13 gene was firstly discovered in Leucania separata multiplenuclear polyhederosis virus (LsMNPV) by our laboratory as early as 1995. At present, about nineteen baculovirus p13 like genes have been reported, while its function hasn't been studied to today. In according with the first nomination, all the p13 homologies were designated p13 gene although their molecular weight was 31-kDa. P13 gene was contained specifically in Group II NPVs and some GVs except for Group I NPVs. which contains GTGTTATA box, CATT box, TTAAG box of early and late promoter core sequence, and an enhancer hr (n) upstream the promoter in 5'UTR.Our work have further confirmed p13 is an early and late gene by western-blot and luciferase assay. Interestingly, we also found that p13 promoter not only had no host specification, but also increased nearly 100 times activity in heterogenous cells. Although P13 has a leucine zipper-like motif in the middle, our results showed P13 protein could not form oligomeration by HPLC and yeast two-hybrid identification. When p13 was expressed in sf9 cells, P13 could down-regulate polyhedrin and eGFP expression drived by polyhedrin promoter, and the repression efficacy was increased with the advance of p13 expression phase and leucine zipper mutant could rescue the repression no matter p13 expression in early or late phase. However, P13 didn't repress the BV production by TEM detection. Although we still have no direct evidence to identify that leucine zipper motif of P13 could specifically bind with polyhedrin promoter, our results have confirmed that P13 could down-regulate the gene expression drived by polyhedrin promoter in leucine zipper motif. Surprisingly, we find P13 could accelerate insects death rate no matter injection AcMNPV expressing P13 or co-injection p13 protein with wt-AcMNPV,or DsRNA interference p13 gene.As to the exact mechanism,it is still on study.Confocal resuts showed P13 early located most in nuclus. With the phase of infection, P13 location diverted from nucleus to cytoplasm membrane, and P13 in nucleus were decreased dramatically. Once TMs delection, P13 was continually decreased in cytoplasm membrane and enriched in nucleus.P13 repression on AcMNPV was correspondingly increased, which showed P13 repression efficacy was directly related with its consentration in nucleus.

Wednesday, 8:00-10:00, *Nanyuan Meeting Room*
Fungi Division Symposium: Ecology of Entomophthorales
Convenor: Ming-Guang Feng

8:00

Host-pathogen interaction in Entomophthorales in agro-ecosystems: initiation of epizootics and relationship between host species and fungal genotype

Jørgen Eilenberg, Annette Bruun Jensen and Charlotte Nielsen
(*Department of Ecology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK 1871 Frb C, DENMARK*)

It is important to understand different key elements which determine the initiation and development of epizootics by Entomophthorales in pest insects. We have studied two systems: 1) Aphids in cereals and Entomophthorales (*Pandora neoaphidis* and others). 2) Flies (Anthomyiidae) and *Entomophthora muscae* and *Strongwellsea castrans*. Concerning system 1), our recent studies have elucidated the diversity of *P. neoaphidis* in relation to the distribution of clones of *Sitobion avenae* in a cereal field in Denmark. Also, we studied the initiation of epiziootics in spring. Concerning system 2), our studies have paid attention to the genetic profile of the pathogen in relation to host species. Our findings will be discussed in relation to possibilities for biological control.

8:30

Persistence and Spread of *Entomophaga maimaiga* Infecting *Lymantria dispar*

Ann E. Hajek[1], Charlotte Nielsen[2] and Patrick C. Tobin[3]
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Entomophaga maimaiga was first reported infecting gypsy moth, *Lymantria dispar*, larvae in northeastern North America in 1989, after which time this pathogen spread across the distribution of gypsy moth in North America. Activity by this pathogen has persisted in long-term plots in central New York since initial epizootics in 1991 and 1992 and gypsy moth populations have remained at low densities. Levels of infection are associated with the proportion of the days with rain throughout the period when larvae of this univoltine host are present. Gypsy moth is an invasive species and, although it was first released in North America in 1868, the distribution of this species is still expanding. *E. maimaiga* readily moves into newly colonizing host populations. Recent studies are focusing on dispersal of *E. maimaiga* into areas newly colonized by its host to create a model with the goal of being able to predict spread by *E. maimaiga*. Studies are being conducted at the edge of gypsy moth spread in Wisconsin by caging larvae on the forest floor and in the foliage, collecting cadavers, rearing field-collected larvae and exposing larvae to soil samples in the laboratory. During 2005, sites with extremely low densities of gypsy moth were sampled. Male moth catch was not a good indicator of presence of *E. maimaiga* and this pathogen was only found at sites where gypsy moth larvae were dense enough that larvae could be collected and not where we only caged larvae. Results will be presented from 2006 field studies concentrating on areas with slightly higher host densities.

9:00

Intraguild interactions involving Entomophthorales

Judith K. Pell[1], Jason Baverstock[1], Ariel W. Guzman Franco[1] and Helen E. Roy[2]
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Intraguild interactions occur between species that share a common resource. Such interactions can occur between species from unrelated phylogenetic groups, e.g. *Pandora neoaphidis* (entomophthoralean fungus), *Coccinella septempunctata* (predatory beetle) and *Aphidius ervi* (hymenopteran parasitoid) utilising aphid populations, or between species from taxonomically similar groups e.g. the two entomophthoralean fungi, *Pandora blunckii* and *Zoophthora radicans*, that both infect the diamondback moth, *Plutella xylostella*. Intraguild interactions directly affect the population size of all species involved and can lead to competition, co-existence and/ or exclusion. In the examples given, the species described are natural enemies of pests and so negative interactions between them could also influence their efficacy as biological control agents. Laboratory studies at a number of spatial scales indicate that the interactions between species within a guild are complex.

Foraging predators and parasitoids have positive effects on *P. neoaphidis* through enhanced transmission and dispersal but negative effects through competition for hosts and predation of infected hosts. The combined effect of all three natural enemies can be additive with respect to aphid population regulation. There is the potential for competitive exclusion of parasitoids by *P. neoaphidis* because of the shorter developmental period of the fungus. However, at the largest spatial scales evaluated, parasitoids did not appear to incur a fitness cost through foraging in patches containing *P. neoaphidis*, even in the presence of a large quantity of fungus and therefore fewer available aphid hosts. In the diamondback moth system, although *P. blunckii* was more virulent than *Z. radicans*, both species were able to co-exist in individual hosts. However, a reduction in fitness was observed for both pathogens; fewer conidia and resting spores were produced from dual infected hosts than from hosts infected with only one species of fungus. The complexity of these interactions and their relevance for agroecosystem biodiversity and biological control are discussed.

9:30

Transmission of obligate aphid pathogens (Entomophthorales) with host dispersal flight: from biological hypothesis to confirmation

Ming-Guang Feng

(*Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, 310058, China*)

The Entomophthorales includes over 20 species of obligate or non-obligate aphid pathogens that take important parts in natural control of aphids. Since most of the pathogens have resting spores to overwinter or survive adversity, primary infection at the early stage of an aphid epizootic in the field is considered to be initiated by a possible source of fungal inocula in soil. However, this plausible speculation never suits to *Pandora neoaphidis*, an obligate aphid pathogen most prevalent in global aphid populations but known to have no form of resting spores discovered. A hypothesis is hence proposed that an epizootic may start from inocula borne by hosts themselves. In other words, alates carry pathogens from place to place as they fly for suitable plants. This hypothesis would become evident only if the fungal pathogens were commonly present in migratory alates or if infected alates were able to fly for dispersal, colonize plants and transmit fungal infection to their progeny or neighbors. To testify the hypothesis, more than twenty thousands of migratory alates (including aphid species infesting vegetables, cereals and cotton) trapped from air in several provinces of China in the past five years were individually reared and examined; nearly 700 alates infected with *P. neoaphidis* were flown in a computer- monitoring flight mill system and then reared for observations. Summarized results from the field and laboratory studies are given and discussed with a brief review of related publications. Conclusively, the hypothesis is biologically supported either because that several fungal pathogens (including *Pandora*, *Entomophthora*, *Conidiobolus*, *Zoophthora* and *Neozygites*), obligate or non-obligate, were frequently found in the air-trapped alates or because the infected alates had all the concerned capabilities.

Wednesday, 8:00-10:00, *Xiyuan Meeting Room*

Nematode Division Symposium: Emerging pest targets for Entomopathogenic nematodes

Convenor: David Shapiro-Ilan and Richou Han

8:00

Emerging Pests Targets for Entomopathogenic Nematodes in China

Richou Han, Li Cao, Guohong Wang, Jinghua Chen and Xuehong Qiu

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In recent years emerging pest targets for entomopathogenic *Steinernema* and *Heterorhabditis* nematodes were evaluated in China. New invasive pests such as oriental fruit fly *Bactrocera dorsalis* (Hendel), asiatic palm weevil *Rhabdoscelus lineaticollis* (Heller) and Banana moth *Opogona sacchari* (Bojer) were introduced into China. Oriental fruit fly attacks many tropical fruits. Asiatic palm weevil is an important pest of palm plants and sugarcane. Banana moth damages various ornamentals and economic crops such as banana, sugarcane and maize. Four species or strains of entomopathogenic nematodes, *S. carpocapsae* All, *S. carpocapsae* A24, *S. feltiae* SN and *H. bacteriophora* H06 were used to control the oriental fruit fly in the laboratory and in the field. *S.*

carpocapsae All showed the best control of this fly and 86.3% larval mortality was obtained after 9 days with 300 infective juveniles (IJs) per cm^2 in the soil. The mixture of 48.0% chlorpyrifos EC （1000mg/l） and 70.0% imidacloprid （500mg/l）and *S. carpocapsae* All (4000 IJs/ml) was used for the control of *R. lineaticollis* on the palm and after 7days the mortality of the weevil larvae in the combined treatment （98.0％）was significant higher than those in chlorpyrifos （69.0％）, imidacloprid（0％）or *S. carpocapsae* All（68.4-78.6％）, respectively. *S. carpocapsae* A24 at a dose of 2857 IJs/ml caused 90.9% larval mortality of the banana moth on an ornamental *Dracaena fragrans*. *H. indica* LN2 also gave 88.2% mortality of the fourth instar larvae of the chive midge *Bradysia odoriphaga*, which destroys the Chinese chive, with 400 IJs per larva. The results would provide new target insects for the commercialization of entomopathogenic nematodes.

8:24

Entomopathogenic Nematodes and Emerging Pests in Latin America: The Quest for a Sustainable World

S.Patricia Stock[1], Jesus Alcazar[2], Juan Carlos Lopez-Nunez[3], Luis Leite[4] and Mayra Rodriguez Hernandez[5]

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Public awareness of the problems developed from the use of chemical pesticides (i.e. adverse impact on human health, wildlife, environmental pollution, pesticide resistance and pest resurgence) has dramatically increased over the past year, in most Latin American and the Caribbean countries. Environmentalists, scientists and labor groups from various countries not only demand for laws to regulate chemical pesticide use, but also to disseminate and sponsor the search and consideration of natural enemies and entomopathogens to control a wide array of invertebrate pests of agricultural and forestry significance. Entomopathogenic nematodes (EPN) (Steinernematidae and Heterorhabditidae) are one of the beneficial organisms that are currently considered in biological control and/or integrated pest management programs. Emphasis has been place on the discovery of native species and strains, as well as in the introduction of exotic species, particularly currently available EPN formulations for the control of diverse invertebrate pests of agricultural and forestry relevance. Major pest problems for which EPN have been considered are: coffee berry borer (Hypothenemus hampei), coffee root mealybug (Dysmicoccus texensis), potato borer (Tecia solanivora), cassava burrower bug (Cyrtomenus bergi) several root weevils (Cosmopolites spp., Graphognatus spp.), scarab beetle complex, many lepidopterans including fall armyworm (Spodoptera frugiperda), corn earworm (Heliothis zea), diamondback moth (Plutella xylostella), among many others. Interest has also been set at the production level, with formulation of entomopathogenic nematodes being restricted to plantation industries. However, collectivized farming systems also produce EPN and a variety of other biocontrol agents including predators, parasitoids and other entomopathogens, for augmentation. In this presentation, a review of the research being conducted in those Latin American and Caribbean nations where the use of entomopathogenic nematodes has most extensively been implemented will be presented. The potential of these nematodes for control of emerging pests will also be discussed.

8:48

New and upcoming target pests for entomopathogenic nematodes in North America.

David I. Shapiro-Ilan[1] and Parwinder Grewal[2]

(*[1]USDA-ARS, 21 Dunbar Rd. Byron, GA 31008, USA; [2]Department of Entomology, Ohio State University, Wooster, OH 44691, USA*)

Research and commercial application of entomopathogenic nematodes in North America has a long history. In the pursuit of commercial viability, there have been a number of success stories, but also quite a number of dead ends. In this presentation, we provide insight into new opportunities for entomopathogenic nematodes that are on the horizon. Some novel targets that are currently being researched include a variety of wood boring insects and weevil pests. Novel application methods and formulations that are being developed will also facilitate expanded use of nematodes as microbial control agents.

9:12

Current and Future Uses of Nematodes in Western Europe

Michael J Wilson[1], Cyrille Verdun[2], Ralf Udo Ehlers[3]

(*[1]University of Aberdeen, School of Biological Sciences, Cruickshank Building, Aberdeen, AB24 3UU, UK; [2]Becker Underwood Ltd, Harwood Industrial Estate, Littlehampton, BN17 7AU, UK; [3]Chrisitian Albrechts University, Klausdorfer, Str 28-36, 24223, Raisdofr, Germany*)

Nematode-based biological control agents are now sold throughout western Europe in a broad range of market segments. Several companies are producing Steinernematid or Heterorhabditid nematodes for both domestic use and for export. Traditional key target pests have been the black vine weevil and sciarid fly larvae in protected ornamental crops and mushrooms. There is increasing interest in the potential of nematodes to control foliar pests such as leaf miners. There is also interest in using nematodes to control both foliar and soil stages of thrips. In addition to entomopathogenic nematodes, the slug parasitic nematode *Phasmarhabditis hermaphrodita* is also sold. This nematode can control a range of slugs but is particularly effective at controlling the gray garden slug, *Deroceras reticulatum.* Until recently this has been primarily sold for use in home gardens, but now it is sold to control slugs in Brussels sprouts, iceburg lettuce and orchids, particularly in the Netherlands.

9:36

Emerging pest targets for entomopathogenic nematodes in Asia outside of China

Ho Yul Choo[1], Dong Woon Lee[2], Sang Myeong Lee[3], Satoshi Yamanaki[4], Sudershan Ganguly[5] and Vacheree Somsook[6]

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Entomopathogenic nematodes have received strong attention as biocontrol agents of insect pests in Asian countries. Emerging pest targets are the same or different depending on situation of each country. In Korea, many Korean isolates have been being used against important local and exotic insect pests of rice, vegetables, forest, fruit, turfgrass, and greenhouse crops. The important emerging targets are fungus gnats (*Bradysia difformis*, *Mycophila spreyeri*, and *Lycoriella mali*)), white grubs (*Exomala orientalis*, *Popilia quadriguttata*, and others), leaf-feeding caterpillars (*Palpita indica*, *Anomis* spp., and others), and fruit insects (*Curculio sikkimensis*, *Cydia kurokoi*, and others). In Japan, *S. carpocapsae* is commercially produced and being used against vegetable, turf, orchard, fig, ornamental insect pests. The emerging targets are cutworms (*Spodoptera litura* and *Agrotis* spp,), hunting billbugs (*Sphenophorus venatus*), black vine weevil (*Otiorhynchus sulcatus*), long-horned beetle (*Psacothea hilaris*), sweet potato weevils (*Cylas formicarius* and *Euscepes postfasciatus*), palm weevil (*Rhynchopherous ferrugineus*), and peach fruit moth (*Carposia niponensis*). In addition, emerging targets of *S. glaseri* are white grubs. In India, the emerging targets of *S. carpocapsae* are *Tryporyza incertulus* on rice and sugarcane, *T. incertulus*, *Chilo suppressalis*, *Cirphis compta*, *Pseudalentia separata*, *Cnaphalocrosis medinalis*, *S. litura* on rice, potato, and tobacco, and *Anomala* sp. and *S. litura* on potato. *Papilio* sp. on citrus and *Leucinodes orbonalis* on brinjal are targets of *S. carpocqapsae* and *Heterorhabditis indica*. In addition, *S. bicornutum* is being used for *A. ipsilon* on tomato, *H. indica* for *Helicoverpa armigera* on Pigeon pea and cotton, and *S. thermophilum* for *Plutella xylostella* on cabbage, *Pieris brassicae* on cauliflower, *S. litura* on cotton, and *C. partellus* on maize. In Thailand, *S. carpocapsae* is being used against caterpillars, *Cossus* sp. and *Microchlora* sp. on Longkong (*Lansium domesticum*), *S. exigua* on marigold, flea beetle (*Phyllotetra sinuate*) on Cruciferous, carpet beetle (*Ataenius nigricans*) on turfgrass, sweet potato weevil (*Cylas formicarius*) on sweet potato, and *Dipspyres areolata* on strawberry. Notable successes have been demonstrated on some of these emerging targets in each country.

Wednesday, August 30, 8:00-10:00, *Meeting Center*
Contributed papers: Bacteria 2
Moderator: Zhiming Yuan

8:00

What is the mechanism of resistance to *Bacillus thuringiensis* toxin Cry1Ac in a greenhouse population of cabbage looper, *Trichoplusia ni*?

Ping Wang[1], Jian-zhou Zhao[1], Ana Rodrigo-Simón[2], Wendy C. Kain[1], Alida F. Janmaat[3], Anthony M. Shelton[1], Juan Ferré[2] and Judith Myers[3]

([1]*Department of Entomology, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456, USA;* [2]*Department of Genetics, University of Valencia, Dr. Moliner 50, 46100 Burjassot* (*Valencia*)*, Spain;* [3]*Department of Zoology, University of British Columbia, Vancouver, British Columbia, V6T 1Z4, Canada*)

The cabbage looper, *Trichoplusia ni*, is one of only two insect species that have evolved resistance to *Bacillus thuringiensis* (*Bt*) in agricultural production situations. To study the mechanism of *Bt*-resistance in a *T. ni* population that evolved resistance in commercial greenhouses, the genetic trait of resistance to the *Bt* toxin Cry1Ac from a greenhouse population was introgressed into a highly inbred susceptible laboratory *T. ni* strain. The *T. ni* introgression strain, GLEN-Cry1Ac-BCS, and its near-isogenic susceptible strain were used in this study to identify the mechanism of Cry1Ac resistance evolved in the greenhouse population. The Cry1Ac-resistance in *T. ni* is a monogenic, autosomal and incompletely recessive trait. Biochemical analyses indicated that the activities and composition of midgut proteases, immune response, and the activity and composition of midgut esterase were not altered in the GLEN-Cry1Ac-BCS strain. The pattern of cross-resistance of the GLEN-Cry1Ac-BCS strain to 11 *Bt* Cry toxins suggested that the resistance is correlated with the Cry1Ab/Cry1Ac binding site in the larval midgut. Further analysis of specific binding of *Bt* toxins Cry1Ab and Cry1Ac to the midgut brush border membranes confirmed the loss of the binding site for Cry1Ab and Cry1Ac in the midgut of the resistant larvae. We conclude that the mechanism for the Cry1Ac resistance that evolved in the greenhouse population of *T. ni* is alteration of the midgut binding site shared by Cry1Ab and Cry1Ac.

8:15 STU

Production of *Bt* Cry1Ac resistance in cotton bollworm, *Helicoverpa zea* (Boddie)

Konasale J Anilkumar and William J Moar

(*Department of Entomology and Plant Pathology, 301 Funchess Hall, Auburn University, Auburn, Alabama-36849, USA*)

Insecticide resistance is the primary concern with *Bt* crops in the US. For Bt cotton (Bollgard®), target pests include tobacco budworm, *Heliothis virescens,* pink bollworm, *Pectinophora gossypiella*, and cotton bollworm (CBW), *Helicoverpa zea*. Although results from lab-selected Cry1Ac-resistant TBW and PBW have contributed to insect resistance management (IRM) policy making, this has not occurred with CBW; there is no stable highly *Bt*-resistant CBW strain. Reasons include high tolerance to Cry1Ac, difficulties in mass rearing, and access to Cry1Ac. Because each species has different *Bt* resistance characteristics, potentially impacting IRM strategies, characterizing *Bt* resistance in CBW is critical, especially because Bollgard® does not express a "high dose" against this pest. Additionally, selection experiments cited above have been conducted with MVP II, a commercial formulation containing Cry1Ac. Because Bollgard® expresses Cry1Ac protoxin that is at least partly activated to toxin, we hypothesize that selection using MVP II may not adequately reflect resistance selection occurring *in planta*. Therefore, we initiated selection experiments with MVP II and Cry1Ac toxin.

A susceptible laboratory strain was established from a Monsanto colony, September, 2004. The baseline susceptibility (LC_{50}) of this strain to MVP II and Cry1Ac toxin was 26µg/g and 9µg/g diet, respectively. Subsequently, we selected CBW populations resistant to MVP II or Cry1Ac toxin. Current resistance ratio for AR strain is over 100-fold after 11 generations of selection. The MR strain completely crashed after 9 generations after developing only 17-fold resistance after 7 generations due to higher fitness costs associated with fertility. Results on the rate of resistance development, observed fitness costs and cross resistance to other Cry proteins will also be discussed.

8:30

Lack of irreversible binding as a novel mechanism of resistance to *Bacillus thuringiensis* Cry1Ab toxin

M. Sales Ibiza-Palacios[1], Juan Ferré[1], Satoshi Higurashi[2], Kazuhisa Miyamoto[3], Ryoichi Sato[2] and Baltasar Escriche[1]

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Bacillus thuringiensis Cry toxins are widely used as bioinsecticides in crop pest management because of their environmentally friendly characteristics. A serious threat to the use of formulations or transgenic crops based on Cry toxins is the evolution of resistance in the target insect populations. The mode of action of Cry toxins involves binding to the brush border membrane (BBMV) of midgut epithelial cells, and lack of binding has been long shown to confer high levels of resistance. However, in some cases, high levels of resistance were not associated to lack of binding. Two kinds of protein (Aminopeptidases, APNs, and Cadherin-like proteins) are possible receptors for one class of *Bt* toxins (Cry1A). However, little is known about the involvement of these proteins in Cry1A binding to BBMV in solution. We have analysed for Cry1Ab the activation, the reversible and irreversible binding, and the levels of putative receptors in two susceptible strains (Kin and N65) and on one naturally resistant strain (C7) of *Bombyx mori*. The strains did not show apparent differences in proteolytic cleavage of the toxin. Specific binding of ^{125}I-labeled Cry1Ab was found in all strains. Binding in the two susceptible strains had two components, reversible and irreversible binding. However, the resistant strain lacked irreversible binding. Highly specific antibodies raised against APN1, APN3, and BtR175 (cadherin-like) proteins of *B. mori* were used to block Cry1Ab binding in N65 strain. A significant reduction was observed when anti-BtR175 and -APN1 were used in the association experiments. Anti-BtR175 produced a striking reduction in reversible and irreversible binding. In contrast, the anti-APN1 specifically affected the amount of irreversible binding, while reversible binding was not affected. The analysis of putative receptors indicated a 4-fold reduction of APN1 in the resistant strain, which might be related to the resistance to Cry1Ab, however other alterations in the protein are possible. Our data reports by the first time that insect resistance to Cry toxins may be due to lack of irreversible binding.

8:45

Cross-resistance between *Bt* and non-*Bt* insecticides in *Plutella xylostella*

Ali H Sayyed[1,2], Graham Moores[3], Denis J Wright[2] and Neil Crickmore[1]

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Plutella xylostella (the diamondback moth) readily acquires resistance to a wide variety of insecticides, both biological and synthetic. It was the first insect reported to have become resistant to *Bt* toxins in the field. In order to manage the risk of resistance development, rotations of different insecticides are commonly employed to control this pest. We have found that a *Bt*-resistant field population of *P. xylostella* was also relatively insensitive to the pyrethroid deltamethrin, and that upon laboratory selection of increased resistance to *Bt* toxin an increase in the resistance to deltamethrin was also observed. Furthermore selection with deltamethrin resulted in an increased cross-resistance to both *Bt* toxin and the insecticide acetamiprid to which the population had not previously been exposed. This simultaneous acquisition of resistance to two completely different insecticide types has significant implications for the control of this insect. In this paper we will present our data on this cross-resistance phenotype and consider the possible mechanisms that could account for it.

9:00 STU

Cellular mode of action of the *Bacillus sphaericus* binary toxin

Onya Opota[1], Nils Gauthier[2], Emmanuel Lemichez[2], Colin Berry [3], David Pauron[1]

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The binary toxin (Bin) produced by *Bacillus sphaericus* is one of the few available biopesticides to control *Culex* and *Anopheles* mosquito species by targeting their larval stage. Bin binds to Cpm1

(*Culex pipiens* maltase 1), its specific receptor located on the membrane of midgut epithelial cells. The emergence of resistant strains, and especially those who bind the toxin as well as susceptible strains has lead us to focus on the postbinding cellular events. We previously reported that when expressed in the mammalian epithelial MDCK cell line (MDCK-Cpm1), Cpm1 characteristics are fully conserved. Moreover Bin specifically induces the appearance of intracytoplasmic vacuoles related to cytopathological effects observed in intoxicated larvae. Cpm1 is located in lipid raft microdomains and that Bin induces the opening of pore in the cellular membrane. We are now investigating the cellular mode of action of Bin on MDCK-Cpm1, including endocytic and intracellular trafficking pathways and induced vacuolation. Using fluorescent derivatives of Bin subunits, BinA and BinB, we found that BinB specifically binds to MDCK-Cpm1 and allows BinA binding which does not bind by itself. These results confirm previous data from Bin binding to *Culex* brush membrane vesicles. Together they induce cell giant intracytoplasmic vacuoles within few hours. BinB alone but not BinA is able to induce the formation of vacuoles but slower than the BinA/BinB mix. After binding to membranes, BinB, BinA together with Cpm1 are internalized into cell peripheral early endocytic compartment. The BinB/BinA/Cpm1 complex accumulates in a region close to the nucleus. At this stage, the toxins partly colocalized with the early sorting endosomal marker Rab5 and the recycling endosome marker Rab4. A slight colocalization is also observed with the mitochondriae, the endoplasmic reticulum and the Golgi apparatus. Small vacuoles subsequently appear in this region and enlarge to occupy the whole cytoplasm. These vacuoles contain both the late endosomal marker Rab7 and the lysosomal marker Lamp1. In contrast, neither Cpm1 nor BinB or BinA are localised on the membrane or inside the vacuolating compartment. This pattern is also observed when BinB is used alone. The vacuolation induced by Bin is Cdc42 independent and is not prevented by inhibition of either clathrin-dependent endocytosis or actin-dependent internalisation. These results will be discussed in the light of a putative intracellular mode of action of Bin. **Experimental methods:** The clonal MDCK (Madin and Darby Canine Kidney) cell line expressing Cpm1 was previously obtained (1) by stable transfection of the *Cpm1* cDNA subcloned in the pcDNA3.1 mammalian expression vector. Bin was produced in the *Bacillus thuringiensis* 4Q2-81 strain and BinA and BinB were produced in fusion with glutathione-S-transferase in *E. coli* as reported earlier (2). All toxin subunits self-activated by incubation at 22°C for 48-60 hours. The activated forms were purified by affinity chromatography on FPLC using benzamidine-Sepharose columns (GE Healthcare, France) and stained with ALEXA 488 and ALEXA 546 (Molecular Probes). 50nM Bin or 25nM BinA/BinB induced maximal vacuolation of MDCK-Cpm1 within six hours of incubation at 37°C. These results served as a reference for the rest of the study, and all kinetic studies were done for six hours. We first examined the dynamics of BinA versus BinB internalisation process. We then investigated the fate of Cpm1 by immunodection versus one or the other component of Bin. The route of BinA, BinB or Cpm1 inside the cell was established by transitory transfection of MDCK-Cpm1 with plasmid vectors expressing specific markers of intracellular compartments fused with fluorescent proteins. This latter strategy was also used to determine the origine of the vacuolating compartment. We next studied the effects of factors known to modulate characterized endocytic pathways. Actin-dependent endocytosis was investigated by disruption of the F-actin cytoskeleton by cytochalasin D. Clathrin pathway was inhibited by expression of dominant negative mutant of proteins implicated in the formation of clathrin-coated vesicules. The same method was applied to test whether Bin endocytosis was dependent on the Cdc42 GTPase that control the endocytosis to GPI anchored proteins enriched early endosomal compartments (GEECs). Cells were analyzed by fluorescence and confocal microscopy and when necessary they were totally reconstructed to observe the entire population of vesicles.

9:15 STU

A second GTPase modifying toxin, named LopT2, is encoded by a remnant prophage in *Photorhabdus luminescens* and produced in insect specific organs

Sonia C. P. Costa[1, 2], Karine Brugirard-Ricaud[1], Michel Brehelin[1], Alain Givaudan[1] and Robert Zumbihl[1]

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The entomopathogenic Gram-negative bacteria *Photorhabdus luminescens* are release in the haemocoel from his nematode host. Once in heamolymph the bacteria depress insect immune response to protect the symbiotic complex from cellular and humoral defence reactions, among them the phagocytosis. Genomic analysis revealed the presence of a new effector homologous to LopT,

named LopT2, which is not encoded by the TTSS locus but by a remnant prophage locus. There is a high similarity between LopT and LopT2 sequence and the biggest difference between those two *Photorhabdus* toxins relies in the first 60 amino acids and in the fact that no chaperone was found to be associated with *lopT2* gene. The reorganization of actin cytoskeleton is essential for phagocytosis and is regulated by proteins of the Rho GTPases family. In the present work, we studied the effects of LopT2, in comparison with LopT1, and we performed HeLa culture infections experiments, using *Yersinia enterocolitica* transformed with constructions for LopT1 (YeO9 pLopT1SpcT) and LopT2 (YeO9 pLopT2). The immunodetection of RhoA, Rac and Cdc42 revealed that there was a distribution of these proteins from the cell membrane to the cytosol. We performed the LopT2 characterisation with *in vitro* studies and we analysed the effect of the purified recombinant toxin on membrane proteins extracts from *Spodoptera littoralis* haemocytes and HeLa cells. We observed that Rac, RhoA and Cdc42 are released from membranes to the soluble fraction after toxin treatment. Furthermore, we investigated lopT2 expression localisation in the orthoptera *Locusta migratoria* after injection of *P. luminescens* LopT2-GFP and we show that LopT2 is site-specific produced *in vivo* in phagocytic insect organs, as obtained with LopT. To study the LopT2 and LopT anti-phagocytic effect, we performed a gentamycin killing assay of *E. coli* in J774.A1 cells treated with YeO9 pLopT1SpcT and pLopT2 and we observed that there is a diminution of *E. coli* phagocytosis. Our results shows that *P. luminescens* presents two toxins, LopT and LopT2, with an anti-phagocytic effect as their target are the Rho GTPases, they are produced specifically in the insect's phagocytic organ and they effect the *E.coli* phagocytosis by J774.A1 cells.

9:30 STU

Mutations of Residues in Three Domains of *Bacillus thuringiensis* Cry1C δ-Endotoxin Affect Insecticidal Activity

Yu Ren[1], Fuping Song[1], Dafang Huang[2] and Jie Zhang[1*]

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Cry1Ca δ-Endotoxin is toxic to *Spodoptera exigua*. We found that several residues in the α6-α7 loop were involved in the formation of salt bridges and some in the loops of Domain Ⅱ were important epitopes related to receptor binding and those in β17 of Domain Ⅲ changed the permeability of brush border membrane vesicles and interacted with R^{254} in block 2 through sequence alignment with Cry1Aa. Site-directed mutagenesis was used to study the relation between structure and function. T^{221}D substitution in Domain Ⅰ resulted in complete loss of toxicity toward *Spodoptera exigua*. ^{276}PQ^{277}-GS in domain Ⅱ and G addition between G^{439} and T^{440} exhibited significantly reduced toxicity, while mutation of R^{522}E in DomainⅢ showed a slight reduction in toxicity. Overall we conclude that the residue in the α6-α7 loop and the residues in the loops of Domain Ⅱ and the residue in β17 are all crucial sites for toxicity.

9:45 STU

Cry1Ac N-terminal mutants with increased toxicity towards the diamondback moth

Mark Bruce and Neil Crickmore

(*University of Sussex, Department of Biochemistry, School of Life Science, University of Sussex, Falmer. Brighton. BN1 9QG. UK*)

A Cry1Ac mutant called TLL has been shown to be more toxic towards a resistant population of *Plutella xylostella* (SERD4) compared to wildtype toxin. TLL was created in an attempt to produce a variant of Cry1Ac resistant to N terminal cleavage. TLL has five mutations at known or putative trypsin and chymotrypsin cleavage sites in the N terminus of Cry1Ac, it is resistant to N terminal cleavage by trypsin, but not by gut extract. The gut extract activated TLL shows three bands when run on SDS-PAGE. The larger two bands appear to be the same size as gut extract activated wildtype (60-65KDa), but the third band is smaller (roughly 55KDa). As well as this difference in proteolytic processing we have been attempting to correlate the increased toxicity of TLL with other biochemical and physiological properties including solubility and ability to insert into planar lipid bilayers.

Mutants containing one or more of the amino acid changes present in TLL have been created. Studies on the toxicity and biochemical/physiological properties demonstrate that many of the differences in solubility and proteolytic processing seen with TLL and wildtype Cry1Ac do not correlate with the observed differences in toxicity.

Wednesday, August 30, 8:00-10:00, *Multifunctional Hall*
Contributed papers: Viruses 2
Moderators: Johannes A. Jehle and Chuanxi Zhang

8:00

Salivary gland hypertrophy virus (SGHV) as a threat to the success of SIT eradication programs for tsetse flies

Adly ABD-ALLA[1, 3], Hervé Bossin[1], François Cousserans[2], Andrew Parker[1], Max Bergoin[2] and Alan Robinson[1]

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Salivary gland hypertrophy virus (SGHV) is a linear double stranded DNA virus with rod shaped particles, 50 nm in diameter and 700 – 1000 nm in length. While the virus was reported more than thirty years ago, attention has focused on the virus recently for several reasons: i) the success of the tsetse SIT eradication programs on the island of Unguja, United Republic of Tanzania has encouraged similar programs to eradicate tsetse in other African countries; ii) during the mass rearing of tsetse in the FAO/ IAEA laboratories the collapse of one tsetse colony was observed and the virus was thought to be the probable cause due to the observation of a high percent of individuals with hypertrophied salivary glands. To study in detail the biology of the virus and to understand its mode of transmission, a PCR based detection method was developed, from a partial sequence of the viral DNA from which two pairs of primers were derived. The PCR detection showed a higher prevalence of the virus in tsetse colonies than the observed prevalence of SGH symptoms (while SGH prevalence was 4-5%, the virus prevalence based on PCR was higher than 90% in *Glossina pallidipes*). The detection of the virus based on the PCR showed that the virus is distributed in several tsetse species colonized in different countries, including France, Belgium, and Ethiopia. To assess the impact of virus infection on the biology of tsetse flies, a method to obtain a virus free colony was tested. To classify the virus, sequencing of the complete viral DNA was undertaken.

8:15

Nucleopolyhedrosis Virus Introduction in Australia

Patrick Buerger[1], Caroline Hauxwell[2] and David Murray[3]

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Nucleopolyhedrosis virus (NPV) has become an integral part of integrated pest management (IPM) in many Australian agricultural crops. This is a culmination of years of work conducted by researchers at the Queensland Department of Primary Industries(QDPI) and Ag Biotech Australia Pty Ltd.

In the early 1970's researchers at QDPI identified and isolated a virus in *Helicoverpa armigera* populations in the field. This NPV was extensively studied and shown to be highly specific to *Helicoverpa* and *Heliothis* species. Further work showed that when used appropriately the virus could be used effectively to manage these insects in crops such as sorghum, cotton and sweet corn. A similar virus was first commercially produced in the USA in the 1970's. This product, Elcar, was introduced into Australia in the 1970's by Shell Chemicals with limited success. The reason for poor adoption of Elcar could be attributed to the enormous success of the synthetic pyrethroids at that time. The importance of integrated pest management was probably also not widely accepted at that time. Gradual development of insect resistance to synthetic pyrethroids and other synthetic insecticides in Australia and the increased awareness of the importance of IPM meant that researchers once again turned their attentions to environmentally friendly pest management tools such NPV and beneficial insects. In the 1980's a company called, Rhone-Poulenc registered an NPV for use in Australian sorghum and cotton. This product, Gemstar®, was imported from the USA. In 2000 Ag Biotech Australia established an in-vivo production facility in Australia to produce commercial volumes of a product similar to the imported product for sale. This product was branded, Vivus®, and was first registered and sold commercially in Australia in 2003. The initial production of Vivus used a virus identical to the American product but replicating it in an Australian *Helicoverpa* species, *H.armigera*. Subsequent research collaboration between QDPI and Ag Biotech reinvigorated interest in the local

virus strain. This was purified and the production system adapted to produce it on a commercial scale. This new version of Vivus, which was branded Vivus Gold, was first registered and sold commercially in 2004. Widespread insect resistance to insecticides and a greater understanding of integrated pest management is leading increasing adoption of technologies such NPV in Australian agriculture.

8:30

Protection mechanism by lignin additives for baculoviruses against the negative effect of uv radiation

S. Elnagar[1], M.A.K. El- Sheikh[1], A. Amin[1], G. Fédière[1], A. A. Atwaand[1] and M. Khattab[1]

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The virus suspension containing 1% of any of the tested lignin additives had almost the same pH value of around 7.55. Therefore, it was difficult to state that the pH value of lignin additive played a key role in protecting the virus. The spectrophotometer, showed also that, all the lignin additives were similar in their high rate of UV-absorption (from 210-900 nm), especially in the most effective UV inactivation region (300-320 nm). Scaning Electronmicrograph (SEM) of polyhedra combined with the lignin additive indicated the possibility of physical protection of the polyhedra crystal by the tested additive. The latter appeared to surround the polyhedra with a continuous layer which was visible in the dark field of transmission electromicroscopic examination.

8:45

Field resistance of codling moth to Cydia pomonella granulovirus: Occurrence, genetics and breaking

J. A. Jehle[1], K. E. Eberle[1], S. Asser[1], S. M. Sayed[1] and M. R. Rezapanah[2]

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Cydia pomonella granulovirus products have been used for codling moth control for many years. Recently, local codling moth field populations with low susceptibility to CpGV have been observed in Germany and Franc. In order to evaluate further resistance management, information about the genetics and mechanism of resistance are essential. Experiments to gain information about the inheritance pattern of resistance have been performed. Bioassays with neonate larvae of a susceptible laboratory strain and a resistant strain originating from a field population were done. Dose-mortality response after 7 days indicated that the resistant strain (LC_{50}= 1.99×10^5 OB/ml) was 100 times less susceptible to infection with the virus strain CpGV-M as the susceptible one (LC_{50}= 1.90×10^3 OB/ml), even it was reared without any selection pressure for almost two years in the laboratory. Reciprocal crosses between the parental strains did not differ in their response to CpGV-M (LC_{50}= 1.90×10^4), hence the resistance was not sex linked but inherited autosomally. Estimation of the dominance values suggested that resistance was inherited incompletely dominant. Mortality data obtained from backcrosses between the F_1 and the susceptible strain suggested that inheritance of resistance was due to a non-additive, polygenic trait. In order to test alternatives to the conventional CpGV-M based products, the same experiments were performed using the Iranian virus isolate CpGV-I12 for bioassays. Regarding the susceptible strain, CpGV-I12 had the same effectiveness (8.97×10^2 OB/ml after 7 days) and besides worked 10 times better against the resistant strain (LC_{50}= 1.27×10^4 OB/ml). In 14-day bioassay, the resistance breaking isolate CpGV-I12 worked against the resistant strain (LC_{50}= 3.06×10^2 OB/ml) as well as the conventional CpGV-M against the susceptible strain. Resistance to CpGV is therefore not complete but could be overcome by application of an alternate virus isolates.

9:00

Virulence of a Nucleopolyhedrovirus to Balsam Fir Sawfly (Hymenoptera: Diprionidae)

Shiyou Li

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Lethal and sublethal effects of a nucleopolyhedrovirus, isolated from natural populations of balsam fir sawfly, *Neodiprion abietis* of a forest defoliator in North America, were studied in the laboratory. The

results showed that *N. abietis* responded to virus challenge in a concentration-dependent manner, i.e., the higher virus concentration, the higher larval mortality. LT_{50}s (the time at which 50% of the larvae died) varied from 4.9 to 16.3 days depending upon concentrations and instars tested. At a given concentration of the virus, significantly higher mortality was observed in younger larvae than older instars, indicating that young larvae were more susceptible to the virus than old ones. Although sublethal doses did not kill larvae directly, they greatly reduced feeding activity of survivors. Pupal weight and adult emergence were also significantly reduced by sublethal doses of the virus. Significantly more male adults were emerged from the virus-treated group than the control, suggesting that females were more susceptible to the virus than males. To produce large quantity of the virus for field operational use, laboratory mass production techniques were investigated. At present, only natural host, *N. abietis*, can be used to propagate the virus. To rear larvae for virus production from overwintering eggs collected from the field early in the season could prolong production duration in the laboratory. Based on virus produced by each dead larva and mortality in a given larval stage, 3rd instars were found to be optimal stage to inoculate for maximum virus production.

9:15 STU

Competition and transmission rate of wild type and recombinant HaSNPV in *Helicoverpa armigera* larvae

Liljana Georgievska[1, 3], Jenny Cory[2], Wopke van der Werf[3] and Just M. Vlak[1]

([1]*Laboratory of Virology, Wageningen University, Binnenhaven 11, 6709 RD Wageningen, The Netherlands;* [2]*Great Lakes Forestry Research Centre, Sault Ste. Marie, Ontario P6A 2E5, Canada;* [3]*Laboratory of Crop and Weed Ecology, Haarweg 333, 6709 RZ Wageningen, The Netherlands*)

HaSNPV virus infects the major insect pests on cotton, the bollworm *Helicoverpa armigera.* Baculoviruses are useful and effective biological control agents against insect pests. Naturally existing wild type viruses have some drawbacks, as they are relatively slow killers and not enough efficacious in controlling insects. The availability of new genetic technologies makes it possible to generate viruses with improved insecticidal characteristics. The recombinant HaSNPV-LM2 (egt-, AaIT), a derivative of the wild type *Helicoverpa armigera* single-nucleocapsid nucleopolyhedrovirus (HaSNPV-WT), has an enhanced speed of action and is marked by the absence of the ecdysteroid UDP-glycosyl transferase (*egt*) gene and the presence of the insect scorpion toxin gene (*AaIT*). The impact of this research is to obtain a better understanding of the competition between baculoviruses with differential biological properties, i.e. the HaSNPV-LM2 and HaSNPV-WT, since there is a good chance that insects will acquire both viruses during their life and transmit the virus horizontally or vertically. Since these two types of virus have different biological characteristics, it is important to quantify the horizontal transmission rate of such viruses. Therefore, a caged field trial was carried out in cotton in China to estimate the transmission rate of HaSNPV-WT and recombinant HaSNPV-LM2 in the *H. armigera* population. This field experiment was performed using six cotton plants per cage. The infected insects used were 2nd instar (infected with 5 x LD99) and were released, six per plant, in the following set-up: (1) control (no virus-infected infector larvae released); (2) infectors inoculated with HaSNPV-WT; (3) infectors inoculated with HaSNPV-LM2; (4) infectors inoculated with a 1:1 mixture of HaSNPV-WT and HaSNPV-LM2 (5) half of the infectors singly inoculated with HaSNPV-WT and the other half inoculated only with HaSNPV-LM2. After 3, 5 and 7 days post infection, respectively, six healthy 3rd instar larvae were released per each cotton plant to test the horizontal transmission over time. The test larvae were removed after 24 hours of their release on the plant and further reared on artificial diet and checked daily for virus mortality. The transmission rate was found to be higher for HaSNPV-WT, compared with HaSNPV-LM2. Greenhouse experiments in a similar set-up independently confirmed the findings from the field experiments. We have quantified the relative proportion of each baculovirus genotype in the mixed infected insects using quantitative PCR (QT-PCR). Our results indicated that there is higher transmission rate of HaSNPV-WT compared with HaSNPV-LM2. This finding contributes to the understanding of the long-term population dynamics of this particular insect pathogen system, but also has a major implication for evaluating the long-term biosafety of recombinant baculoviruses in the field in general. This project is supported by the Netherlands Foundation for the Advancement of Tropical Research (WOTRO). liljana.georgievska@wur.nl

9:30 STU

Quantitative relationship of two viruses (MrNV and XSV) in white tail disease of *Macrobrachium rosenbergii* de Man

Hua Jun Zhang[1], Jian Min Wang[1], Jun Fa Yuan[1], Li Juan Li[1], Jian Hong Zhang[1], Jean Robert Bonami[2] and Zheng Li Shi[1]

([1]*State Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, 430071 Wuhan, China;* [2]*Pathogens and Immunity, UMR5119, ECOLAG, CNRS/UM2, Université Montpellier 2, Montpellier, France*)

White tail disease (WTD) causes a high mortality rate in the freshwater prawn *Macrobrachium rosenbergii*. Two kinds of viral particles have been isolated from WTD prawns, *Macrobrachium rosenbergii* Nodavirus (MrNV), associated with extra small virus (XSV). We purified MrNV and XSV separately by sequential sucrose gradient and CsCl isopycnic centrifugation. Healthy post-larvae (PL) were infected with the purified via an immersion method. Three groups of PL were challenged with various combined doses of MrNV and XSV. Signs of WTD were observed in Groups 1 and 2 that had been challenged with combinations containing relatively high proportions of *Mr*NV and relatively low proportions of XSV. By contrast, there was little sign of WTD in Group 3 that had been challenged with a relatively higher proportion of XSV than *Mr*NV. A two-step Taqman real-time RT-PCR was developed and applied to quantify viral copy numbers in each challenged post-larva. Results showed that genomic copies of both viruses were much higher in Groups 1 and 2 than they were in Group 3 on the 8^{th} and 24^{th} day post infection, indicating that *Mr*NV plays a key role in white tail disease of M. *rosenbergii*. Statistic analysis with SPSS software resulted in a linear correlation between *Mr*NV and XSV genome copies in each prawns tested (n=80) ($P<0.01$), demonstrating that XSV is a satellite virus dependent on *Mr*NV, but its role in pathogenicity of WTD remains unclear.

9:45

Building up standard of cotton bollworm (Helicoverpa armigera (Hübner)) Nuclear Polyhedrosis Viruses wettable powder

Jiang Hui，Wang Xiaojun, Lin Ronghua, Han Xianguo, Chen Kun, Chen Hongying and Liu Qiong

（Institute for the Control of Agrochemicals, Ministry of Agriculture，Beijing 100026）

Applying of biopesticide of insect virus is one kind of important measures in control of agriculture pest. Many researches have been done to detection and identification of insect virus, especially in China. More than 20 kinds of insect virus have been assessed in field experiment, 13 kinds of them were registered in China. Nuclear Polyhedrosis Viruses of cotton bollworm (Helicoverpa armigera (Hübner))(HaNPV) are the most important kind of insect virus in control of cotton bollworm. And products of HaNPV wettable powder were applied widely in Hubei, He'nan and Xinjiang province in order to alternate the chemical insecticides. 19 families have got the temperature ID for industry product. But until now, there is no authoritative standard built up to detect and identify insect viruses. The method of detection and identification to insect viruses limited the development and industrialization. Until now, the detection and identification of insect viruses was not coincidental, which influence the quality of HaNPV product. In this study, some qualitative analysis, such as restriction endonucleases and serology in ELISA, were carried out, while bioassay method was compared to hemagglutination assay for the quantitative analysis. Some indexes of in physics and chemise including fineness, wetness, suspension and pH were established. The building of this standard can do help to the register of insect virus of HaNPV.

Wednesday, August 30, 10:30-12:30, *Xiyuan Meeting Room*
Microsporidia Division Symposium: Microsporidia in silk moth
Convenor: Gernot Hoch and Leellen Solter

10:30

***Nosema bombycis* and the Silkworm Industry**

James J. Becnel

(*U. S. Department of Agriculture, Agriculture Research Service, Center for Medical, Agricultural and Veterinary Entomology, 1600 S. W. 23rd Drive, Gainesville, Florida 32608*)

Sericulture was started in China nearly 5200 years ago and has had a great influence on international trade and cultural exchange for thousands of years. The commercial silkworm strain and rearing

techniques were introduced to the Korean peninsula in the 11th century BC, and to Japan during the 5th century BC. The "Silk Road" refers to the route from China to the Middle East over which silk was transported commercially and eventually the technique of sericulture was introduced there about 138 B.C. Sericulture was eventually introduced into Europe in the 6th century A.D. The most commonly used silkworm is *Bombyx mori.* The Chinese accumulated much knowledge on silkworm biology and records from Yong-jia in southern Zhejiang Province in the 4th century AD described a method to rear silkworms for 8 generations a year by keeping the eggs at low temperature to avoid diapause in the next generation. Over the long history of silk worm rearing, several diseases have been notable but paramount among these has been the microsporidian parasite *Nosema bombycis* Naegeli, 1857. *Nosema bombycis* is the first named species of microsporidia and therefore the type for the genus as well as the phylum Microsporidia. This disease has had a tremendous impact on sericulture in a number of important ways from the early selection of *B. mori* strains resistant to *N. bombycis* by Chinese scientists to the work of Louis Pasture in the nineteenth century that determined pebrine disease was caused by *N. bombycis*. An overview of the history of *N. bombycis* and sericulture will be presented as an introduction to this symposium on "Microsporidia of Silk Moth".

11:00

Diversity Among Microsporidian Parasites Causing Silkworm Pebrine Disease

Ji-Ping Liu[1], Judith E. Smith[2] and Ling Zeng[1]

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The microsporidian parasite *Nosema bombycis* is known to cause significant disease in the silkworm *Bombyx mori*. Control relies on screening of female moths to select disease free eggs for production. We have devised a PCR based assay to allow sensitive detection of microsporidia and to further enable discrimination of species via sequencing. In practice, when applied to standard sericulture screening protocols, the assay was able to detect *Nosema bombysis* (N.b.) spores at 10^3-10^4/ml. The PCR method was applied to nine parasite isolates obtained from China and amplified products were sequenced and aligned with SSUrDNA sequence from known lepidopteran infective microsporidia. The majority of isolates fell into the true *Nosema* clade and were closely related to *Nosema bombycis* reference isolates. However one isolate fell into the *Nosema*/*Varimorpha* clade and was closely related to species obtained from the pest species *Pieris rapae* and *Cerace stipatana*. Wider phylogenetic analysis of silkworm isolates from China, Japan and India reveals a similar pattern of diversity with isolates derived both clades of the genus *Nosema*. These data suggest that it is important to evaluate the host range of *Nosema* parasites and that pest species which attack Mulberry may represent an important reservoir of disease.

11:30

From *Nosema bombycis* rDNA organization to revise the *Nosema* isolates in Taiwan

Chih-Yuan Wang, Wei-Fone Huang and Chung-Hsiung Wang

(*Department of Entomology, National Taiwan University, 106, Taipei, Taiwan*)

Nosema bombycis is a type species of the genus *Nosema*. The organization of *N. bombycis* rDNA, 5'-LSU (large subunit)-ITS (internal transcribed spacer)-SSU (small subunit)-IGS (intergenic spacer)-5S-3', had been reported. This characteristic can be used to define the species that belong to true *Nosema* species. Nine *Nosema* isolates, 7 from lepidopteran species and 2 from hymenopteran species (honeybees), in Taiwan were revised based on the gene organization and identities of rDNA genes and spacers. The result showed that the isolates from lepidopteran species are true *Nosema* species, they are high identities in the sequences of genes (LSU, SSU and 5S), and low identities in the sequences of spacers (ITS and IGS). The isolates from honeybees, *Apis mellifera* and *A. cerana*, are the same species, *N. ceranae*. Both isolates showed a different organization in rDNA genes, 5'-SSU-ITS-LSU-3', and a high diversity in gene sequences compared with those of true *Nosema* species. We suggest that the *Nosema* isolates from lepidopteran species in Taiwan is a *Nosema* species complex that is related closely to *N. bombycis* but far from the *Nosema* species from honeybees.

12:00

Impact of *Nosema sp.* infection on nutritional physiology and growth of the tasar silkworm *Antheraea mylitta*

Sudhansu Sekhar Rath, Mrinal Kanti Singh and Suryanarayana N.

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Nosema sp. infection in the Indian tasar silkworm, *Antheraea mylitta* has multidirectional effect on its host. The instar duration was extended significantly (P<0.001) except in I instar. The infected larvae took about 48 days to reach the spinning stage against 40 days normally need by the uninfected ones. The final weight attained by the larva at the end of each instar of development declined significantly following infection, so also gain in weight and relative growth rate (RGR). The growth recorded/ day declined in infected larvae over uninfected ones from 8.2% during I instar to 43.3% during V instar. Food ingestion and digestion increased with advancement of the instar significantly irrespective of the status of the larvae but the relative consumption rate (RCR) declined. All these parameters register a significant decline in infected larvae (except food digested during II instar). The decline was more during III instar. In contrast, the approximate digestibility (AD %) remains at a significantly higher level in infected larvae than uninfected ones leaving the I instar larvae unaffected. The efficiency of conversion of ingested food (ECI) and efficiency of conversion of digested food (ECD) did not reveal a definite pattern of change following the microsporidia (*Nosema sp.*) infection. The values of ECI significantly change during II, III and V instar; while the change in ECD during II, IV and V were significant. Thus, during the entire larval life all the parameters tends to decline significantly following microsporidia infection but AD register a significant increase. *Nosema sp*. spore concentration has increased 270.7 times during larval development in the course of experimentation.
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Note: The author is an invited speaker of SIP.

Wednesday, August 30, 10:30-12:30, *Meeting Center*
Contributed papers: Bacteria 3
Moderator: Dafang Huang

10:30

Identification of *Bacillus cereus* internalin and other candidate virulence genes specifically induced during infection in insects

Nadine Daou, Sinda Fedhila, Christina Nielsen-LeRoux and Didier Lereclus
(*Unité Génétique Microbienne et Environnement, INRA, La Minière, 78285 Guyancourt cedex, France.*)

Bacillus thuringiensis and *B. cereus* are closely related gram-positive bacteria with a broad host spectrum. *B. thuringiensis* is well known as entomopathogen and *B. cereus* is frequently associated with food-borne infections causing gastroenteritis. We developed an *in vivo* expression technology (IVET), with an insect host, for identification of the *B. cereus* genes specifically expressed during infection. This IVET-based approach uses site-specific recombination to identify transient promoter activation. The activity of a promoter upstream from the recombinase gene *tnpI* results in a loss of resistance to spectinomycin and a gain of resistance to kanamycin. We constructed a genomic library of *B. cereus* ATCC14579 by cloning DNA fragments upstream from *tnpI*. The library was screened *in vivo* by oral infection of the insect *Galleria mellonella*. We selected 100 clones resistant to kanamycin and sensitive to spectinomycin from dead larvae. Sequencing of the inserts led to the identification of 37 potential *in vivo*-induced genes (*ivi* genes) belonging to several different functional classes: regulation, metabolism, DNA repair and replication, cell division, transport, virulence and adaptation. These presumed *ivi* genes were subjected to a second round of screening in insects and specific *in vivo* induction was confirmed for twenty of the 37 genes. We analysed gene *ivi29* which encodes an internalin-like protein with four distinct domains: an N-terminal signal peptide for export, a NEAT domain thought to be involved in iron transport, a leucine-rich repeat domain that may interact with host cells, and a C-terminal SLH domain presumably anchoring the protein in the bacterial membrane. As suggested by a Fur box in the promoter, transcriptional analysis showed *ivi29* expression to be repressed by iron, suggesting that expression was induced *in vivo* due to iron deprivation in the host. This iron-regulated, leucine-rich surface protein was designated IlsA. Disruption of *ilsA* reduced the virulence of the bacteria to the insect larvae indicating its role in the overall pathogenesis of *B. cereus*. The use of bacteria harboring a transcriptional fusion between the *ilsA* promoter and the *gfp* reporter gene indicates that *ilsA* expression is activated when the bacteria invade the hemocoel of the infected insect.

10:45 STU

Preliminary Characterization of a thermostable DNA polymerase I from a mesophilic *Bacillus sphaericus* strain C3-41

Han Bei, Hu Xiaomin, Liu Haizhou, Cai Ya jun, Yuan Zhiming

(*Wuhan Institute of Virology, Chinese Academy of Science, Wuhan 430071, China*)

A thermostable DNA polymerase I from a mesophilic *Bacillus sphaericus* strain C3-41 was characterized in this study. The *polI* was cloned, sequenced and over-expressed in *Escherichia coli*. The expressed 110 kDa fusion protein of PolI was stable at 70℃ for 1 hour. Compared with The DNA polymerase I of *E. coli* (TaKaRa®), the relative polymerase activity of this PolI was 3.33±0.1 RFU μl^{-1} at 37℃ by the fluorescent quantitative analysis. It showed higher polymerase activity than *E. coli* PolI at higher temperature, with a relative activity of 3.75±0.1 RFU μl^{-1} at 70℃. The *polI* sequence analysis and the protein structure prediction indicated that this protein had a high similarly to other PolI from thermophilic micro-organisms. This information is of importance for future study for evolution of the house-keeping gene *polI* in entomopathogenic bacterium *B. sphaericus*.

11:00 STU

Identification of Three Zwittermicin A Biosynthesis-Related Genes from *Bacillus thuringiensis* YBT-1520

Changming Zhao, Yi Luo, Chunxu Song, Hui Zeng, Ziniu Yu and Ming Sun*

(*College of life science & Technology, Huazhong Agricultural University, Wuhan 430070, PRC*)

Zwittermicin A (ZwA) is a novel linear aminopolyol antibiotic. A 16kb DNA fragment from Bacillus cereus UW85 (zwa16Bc) suggested the hypothesis that the premature ZwA is synthesized by a nonribosomal peptide synthetase (NRPS) and polyketide synthetase (PKS) hybrid pathway from five kinds of precursors, then a hypothetic carbamoyltransferase catalyses the premature ZwA into mature ZwA. However, neither the carbamoyltransferase coding gene nor the synthetase gene coding for 2,3-diaminopropionate, one of the five precursors, has been identified so far. Bacillus thuringiensis YBT-1520, a ZwA-producing strain, contains a counterpart of zwa16Bc. A bacterial artificial chromosome (BAC) library of YBT-1520 has been constructed. Three genes, zwa6, zwa5A and zwa5B related to the biosynthesis of ZwA were identified from BAC clones 1F8 and 5E2, whose inserts overlap zwa16Bc, by partial DNA sequencing. All these three genes were found to exist in the corresponding location of B. cereus UW85. The ZwA biosynthesis cluster is determined to be at least 38.6kb and is located on the chromosome but not on the plasmid. ZWA6 shares 56% amino acid identity with a typical carbamoyltransferase in Streptomyces avermitilis. ZWA5A and ZWA5B are homologs of cysteine synthetase and ornithine cyclodeaminase, respectively, which synthesize 2,3-diaminopropionate by their concerted actions in Viomycin biosynthesis. The identification of these three genes further supports the hypothesized ZwA biosynthesis pathway.

11:15

Amino acid substitutions in aA and aC of Cyt2Aa2 alter hemolytic activity and host specificity

Boonhiang Promdonkoy[1], Amporn Rungrod[1], Patcharee Promdonkoy[1], Wanwarang Pathaichindachote[1], Chartchai Krittanai[2] and Sakol Panyim[2]

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Cyt2Aa2 is a hemolytic and mosquito larvicidal toxin produced by *Bacillus thuringiensis* subsp. *darmstadiensis*. The toxin exhibits *in vitro* cytolytic activity against broad range of cells including erythrocytes but shows specific *in vivo* toxicity against larvae of Dipteran insects. We have substituted amino acids in aA (A61C) and aC (S108C and V109A). Expression in *E. coli* revealed that all 3 mutants were produced at high level as inclusion bodies similar to that of the wild type. All mutant proteins could be solubilized in 50 mM Na_2CO_3 pH 10.5. Activation of solubilized mutant toxins by proteinase K yielded similar product to that of the wild type. Hemolytic activity assays against sheep RBC showed that the mutants A61C and S108C significantly reduced the activity whereas the mutant V109A did not affect the hemolytic activity. Interestingly, the mutant A61C exhibited high larvicidal activity to both *Aedes aegypti* and *Culex quinquefasciatus* larvae. The mutants S108C and V109A showed low activity against *C. quinquefasciatus* larvae but relatively high toxicity to *A. aegypti* larvae. Our results suggest that amino acids in aA and aC act as specificity determinant for the Cyt toxin.

11:30 STU

Cloning and expression of *gabT* and *gabD* of *Bacillus thuringiensis* YBT1520

Li Zhu[1], FuPing Song[2], Jie Zhang[2] and DaFang Huang[1]

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The *gabT-gabD* pathway of γ-aminobutyrate (GABA) utilization is common to many bacteria. The *gab* cluster which specifies the synthesis of the enzymes of GABA degradation pathway contains three key genes: *gab*, encoding Glutamate decarboxylase (EC 4.1.1.15); *gabT*, encoding GABA transaminase (GSST; EC 2.6.1.19); *gabD*, encoding succinic semialdehyde dehydrogenase (SSDH; EC 1.2.1.16). We found that the organization and arrangement of *gab* cluster in *B. thuringiensis* is common in *B. cereus* groups, but different from that in *B. subtilis* by complete genome sequence comparison. So we have characterized two genes of *gab* cluster in *B. thuringiensis* strain YBT1520: the *gabT* gene consists of 1,440bps encoding a protein of 479 amino acids with a molecular mass of 52.6 kDa. The *gabD* gene consists of 1,449bps encoding a protein of 483 amino acids with a molecular mass of 52.2kDa. The nucleotide sequences of two genes show 62% and 52% respectively similarity to those homologous genes of *B. cereus* group by Blast analysis. The *gabT* gene of YBT1520 has been cloned into expression vector pET-21b, and expressed in *E coli* BL21(DE3) cell in soluble form. The expression product is a fusion protein of about 53KDa with 6 His-tags by SDS-PAGE analysis, and demonstrated its activity of GABA transaminase by enzyme assay. The other gene *gabD* of YBT1520 has been cloned into expression vector pGEX-4T-1, and expressed a fusion protein about 78 kDa with GST tag in *E coli* BL21(DE3) cell. We have identified both its crude protein extracts and purification protein all exhibit SSDH activity by enzyme assay. The further study will focus on the structure and function of *gab* cluster so as to clarify the relationship between the spore and crystal formation and GABA shunt in *B. thuringiensis*.

11:45 STU

Expression of *Vitreoscilla* hemoglobin gene in *Bacillus thuringiensis* improve the cell density and insecticidal crystal proteins yield

Feng Liang, Chen Shouwen, Sun Ming, Yu Ziniu

(*State Key Laboratory of Agricultural Microbiology, National Engineering Research Center for Microbial Pesticides, Huazhong Agricultural University, Wuhan, 430070, P.R.China*)

VHb gene (vgb) was integrated into the chromosome of Bucillus thuringiensis BMB171 using integrative vector pEG491 after changing the promoter of luxS. The expression of VHb was confirmed by CO-difference spectra analysis. Bioreactor experimental results showed that the critical oxygen concentration (COC) of the VHb+ strain was 12%, which was 40% lower than the VHb- strain. The cell density of the VHb+ strain in high, middle and low aeration/agitation cultures were 1.05, 1.11 and 1.44 fold respectively of the VHb- strain. Under same conditions, insecticidal crystal proteins (ICP) yields of the VHb+ strain were 1.18, 1.56 and 3.68 fold of the VHb- strain. Bioassay data on Helicoverpa armigera also showed the cultivation product of VHb+ strain improved insect potency. These results indicated that the expression of VHb in the Bucillus thuringiensis improved the cell density and ICP yield, especially under low aeration/agitation cultivation.

Key Words: Vitreoscilla hemoglobin, Bucillus thuringiensis, critical oxygen concentration, insecticidal crystal proteins

12:00 STU

The prepation of the HBF-1 polyclonal antibody and its application to detection of the protoxin in soil

Rui-hua Wu[1, 2], Shu-liang Feng[2], Guo-xun Li[3], Rong-yan Wang[2], Jin-yao Wang[2], Wei-ping Cao[2], Lin-xin Du[2] and Jian Song[2]

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The mixture of endospore and parasporal protoxin from *Bacillus thuringiensis* HBF-1 was treated with Na_2CO_3 solution to get the insecticidal protein. The extracted protein was purified by SDS-PAGE preparation electrophoresis. The highly purified protoxin with molecular weight of 130 KD was used as standard protein and antigen which was used to immune New Zealand white rabbits, and then high

immunity serum was got, and the titre of antibody reaches 1/128000 by using the indirect enzyme-linked immunosorbent assay (ELISA), at the same time IgG was extract from the serum by ammonium sulfate precipitation technique and DEAE cellulose chromatographic fractionation. With the method of $NaIO_4$, HBF-18 antibody was labelled to make enzyme-labelled antibody. After tested, in the combination of enzyme –labelled antibody, the concentration of HRP and Ab was 0.0696 mg/ml and 0.349 mg/ml, respectively; HRP/Ab was 1.856%; the combination rate of enzyme was 12.10%. The high quality polyclonal antibody was used to assess its specificity against several other *B. thuringiensis* strains which were also toxic to harmful scarabaeoid beetles larvae. Protoxins from *B. thuringiensis* strain HBF-18 and 185 reacted with the HBF-18 antibody. With the polyclonal antibody which had good specificity in the testing to monitor the fate of the toxins in soil, three kinds of ELISA reactions, direct ELISA, indirect ELISA and double antibody sandwich ELISA were developed. Result showed that the latter was a better method. And the conditions of the reaction were confirmed. When crystals from strain HBF-1 were placed in soil and then solubilized with alkali treatment or Polytron or a Vortex mixer, the concentration of the protoxins could be determined using the ELISA(s). Comparison of degradation the crystal in non-sterile soil and sterile soil indicated that biotic degradation is one of the main factors contributed to degradation. This agreed with the results of other studies that toxins could be biodegraded by soil microorganisms.

12:15 STU

Insecticidal Toxicology of HBF-1 Strain from *Bacillus thuringiensis* on *Anomala corpulenta* and *A. exoleta* larvae

Jian Song[1], Shu-liang Feng[1], Rong-yan Wang[1], Jin-yao Wang[1], Wei-ping Cao[1], Li-xin Du[1], Jie Zhang[2] and Fu-ping Song[2]

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This study is to get more information on the toxicology of HBF-1 strain to *Anomala corpulenta* and *A. exoleta* larvae and its interaction with chemical pesticides. After a series of experiments, the following results were got: The survival rate of *A corpulenta* larvae and *A.exoleta* larvae was decreased, the antifeeding and growth inhibition of *A. corpulenta* larvae were increased, the feeding selectitity to feedstuff of *A. corpulenta* larvae enhanced as the concentration of HBF-1 strain was increased and the time of infected with HBF-1 strain was prolonged. When the *A. corpulenta* larvae treated with HBF-1 strain at the concentration of 10 μg/g soil for seven days, the antifeeding rate and growth inhibition rate were 4.74% and 9.84%, 30.75% and 73.87% for fourteen days. When the *A. corpulenta* larvae treated with HBF-1 strain at the concentration of 160 μg/g soil for seven days, the antifeeding rate and growth inhibition rate were 39.01% and 83.61%, 86.10% and 97.75% for fourteen days. *A. corpulenta* larvae were more repellent to the feedstuff containing HBF-1 strain of high concentration. The sensitivity of *A.corpulenta* to the chemical insecticides increased gradually with increasing exposure time to HBF-1 strain and HBF-1 strain concentration. After the larvae were treated by a mixture of HBF-1 strain and chemical insecticides, its sensitivity were significantly increased by comparing to the larvae treated with chemical insecticides alone. When the larvae treated with a mixture of HBF-1 strain and chemical insecticiedes at the ratio of 1:50 and 1:200, its sensitivity to Phoxim increased 6.06 and 4.00 times while the sensitivity to Chlorpyrifos increased 16.05 and 12.78 times respectively. The activity of AchE in larvae was decreased slightly compared to the blank control and the activity of GsTs and CarE increased in some extent. The histopathology of *A corpulenta* larvae and *A.exoleta* larvae infected by HBF-1strain were studied by means of optical instrument The results showed that the primary symptom of *Anomala corpulenta* and *A. exoleta* larvae after HBF-1 strain infection is not visible, with the increasing time, the infected insect appears inactive, paralysis and lost its sensitivity to the environment, finally the insect become blackish, straight or shrinked until to dead. The histopathology of scarabaeid larvae infected by HBF-1 strain was studied, malformation and vacuolization of the mid-gut cell appeared three days after HBF-1 strain infection, the mid-gut cells were serious damaged and even destroyed completely after 7 days, while the epithelium disappeared after 10 days.

Wednesday, August 30, 10:30-12:30, *Nanyuan Meeting Room*
Contributed papers: Microbial Control 1
Moderators: Wendy Gelernter and Svetlana Gouli

10:30

Microbial insecticides: some thoughts on history, commercialization and the future

Wendy Gelernter
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In the long history of microbial insecticide use, the development of commercial products, which began in earnest only in the 1950s, represents a mere snippet of time. However, it is in this short period that years of effort have come to fruition in the form of products used around the world – from entomopathogenic fungi such as *Metarhizium* and *Beauveria*, which are widely used on field and greenhouse crops, to the planting of over one hundred million hectares of crops engineered to produce *Bacillus thuringiensis* toxins. Onchocerciasis has been eliminated from seven West African countries using an integrated program that includes *B. thuringiensis* var. *israelensis,* and the soybean looper is controlled by the *Anticarsia* nuclear polyhedrosis virus on over one million hectares in Brazil . For every wonderful success story though, there are always the many mistakes upon which all progress rests. It is in this spirit that I review the past fifty years of microbial insecticide development efforts in an attempt to understand what we have done that has worked best, what hasn't, and how we can apply this information to make future efforts more productive. To do this, it is useful to divide the past fifty years into three eras: 1) 1950 – 1980: Visionary entrepreneurs; 2)1980 – 1995: Irrational exuberance; 3) 1995 – present: Back to the future. These eras are defined by the technologies that were developed, the types of companies that developed them and the socioeconomic forces that shaped them.

10:45

Lessons Learned from LUBILOSA

Roy Bateman
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The normal method of controlling locusts and grasshoppers is with chemical pesticides, using ultra-low volume (ULV) application methods. However, many of these chemicals may cause environmental damage and some are hazardous to humans and domestic animals. In response to concerns over pesticide use, an internationally funded and executed Programme called LUBILOSA was set-up for biological control of locusts and grasshoppers in environmentally sensitive areas. The Programme very rapidly focused on the development of a fungus called *Metarhizium anisopliae* var. *acridum* as the most promising agent for biological control delivered in a way that was compatible with standard, existing ULV application techniques. Products such as 'Green Muscle' have now been adopted by commercial partners but reaching this stage was expensive: the LUBILOSA Programme eventually cost donors approximately 15 million (although this is more than an order of magnitude cheaper than developing a chemical pesticide). The purpose of this paper is to discuss briefly how some of the lessons learned might be used to develop the next generation of mycopesticide products, based on truly virulent yet specific, naturally-occurring, fungal isolates, for environmentally safe pest control. During the course of the Programme, it became clear that two of the key technical challenges in the development of mycoinsecticide product were mass production and delivery systems (including formulation and application). For example, these procedures are linked by a critical process: the separation of fungus spores from their substrate, and a purpose-built spore harvesting device was developed. Such "enabling technologies", can assist in the exploitation of a range of micro-organisms by overcoming the crucial laboratory to field transition. All this was made possible by the enthusiasm of collaborating scientists belonging to a number of organisations: not least those that carried out the crucial and extensive testing in the field. However, 'Green Muscle' is a technical solution to a real need, which was only delivered by also taking a rigorous approach to product development, registration and commercialisation.

11:00

Ecological mechanism of sustainable pest control in pine plantation ecosystem

Zengzhi Li, Meizhen Fan, Degui Ding, Bin Wang and Baoyu Han
(*Department of Forestry, Anhui Agricultural University, Hefei, Anhui 230036, P. R. China*)

Our long-term observation in Masson's pine plantation on use of *Beauveria bassiana* has demonstrated that the use of the fungus could suppress the pest populations sustainably at low level.

However, it was also demonstrated that the caterpillar population fluctuated slightly with long-term suppression where the fungus was inoculatively released, while the population fluctuated sharply with shorter suppression where the fungus was inundatively released, suggesting that the density-dependant mortality caused by the fungus overcompensated upon the rising pest population. Community diversity of invertebrates and entomogenous fungi for a whole year in 3 forest farms with similar climate and vegetation was studied in Anhui, East China, and the result showed that the diversity index was the highest where innoculative release of *B. bassiana* had been used. A primary component analysis indicated that such community had clear time order and was the most stable. Comparatively, the index was the lowest, with badly ranked time order and poor stability where inundative release had been used. Further esterase and RAPD analyses and other molecular markers showed a surprising result: in a Masson's pine stand of 350 ha, 118 *B. bassiana* strains of different genetic characters were isolated from 30 different insect species except the released strain in the whole year after releasing! They belonged to 30 different esterase types and 7 different RAPD genotypes. Therefore, the population of *B. bassiana* in the ecosystem of the pine displayed an unbelievable high heterogeneity which has not been recorded. The above esterase types and RAPD genotypes contained different host spectra forming respective host chain (Table 1), and forming an integrated timely host spectrum (Table 2). A complicated host web formed when all spectra were synthesized. In addition, the host chains combined other strains surviving in soil and litter and spreading by air flow, forming more complicated web of parasitism-saprophytism and becoming an indispensable component in food web in forests. It was also found that some strains attributed to different genotype host chains could transfer within the chain. Meanwhile, some common hosts appeared in different host chains. In another word, some link of the chains, i.e. niches, are shared by different host chains, causing niche overlap of strains of different genotypes. It was also confirmed in the present study that niche overlap also happened among different insect species. Among the abundant hosts, 2 weevils, *Brachyderes incanus* and *Sympiezomias veletus* accounted for small portion of species amount, but for 64.7% of all beetle cadavers, suggesting their important role in keeping *B. bassiana* population stable in the ecosystem. Due to host transfer and niche overlap occurring in the ecosystem, the released strain and predominant indigenous strain can find proper hosts to persist from abundant hosts when target host is at low level; the other strains can also transfer their hosts through niche overlap on some hosts to suppress the target host when these strains are at low level. From the highly heterogeneous population, abundant strains of *B. bassiana* can suppress complementarily various forest pests including the target pest, co-play the role of a density dependent factor and make up the single factor role of the released strain, and avoid the overcompensation which causes sharp population fluctuation, resulting in a sustainable control. Therefore, the length of the sustainable control period is correlated with genetic diversity of *B. bassiana*, because the adaptability of a highly heterogeneous population is very strong; the genetic diversity of *B. bassiana* acts upon species diversity in the pine ecosystem. Therefore, *B. bassiana* is a keystone species for maintenance of insect community stability in the pine ecosystem, which plays an important role in keeping biodiversity of the pine ecosystem and community stability of the pine plantation. An ecological disaster will happen if it disappears or it is weakened. Based on above results, the mechanism of the sustainable control is revealed through population heterogeneity of *B. bassiana* in forest, *B. bassiana* as a keystone species, Host transfer, and niche overlap. Therefore, an ecological mechanism of sustainable pest control by entomogenous fungi and a new application strategy for application of entomogenous fungi are proposed, advocating that the commonly used strategy of inundative release of fungal insecticides should be substituted by a strategy of long-term innoculative release.

11:15 STU

Production of biomass and shelf life screening of the lepidopteran specific entomopathogenic fungi *Nomureae rileyi*

Akbar Ali khan Pathan, Narasimha Reddy Parine and Uma Devi Koduri

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Nomureae rileyi (Farlow) Samson is a lepidopteran specific entomopathogenic fungus. The fungus was observed in populations of the *Spodoptera litura* and *Helicoverpa armigera*, in the humid Guntur region of South India. Regular surveys of peanut and chilly fields for *N.rileyi* induced epizootics in this area were conducted in a linear manner around villages in the district of Guntur. A preliminary laboratory bioassays at a concentration of 1 x 10^7 conidia ml ($^{-1}$) indicated that two of the *N. rileyi* isolates of *S. litura* origin were better in terms of time taken for mycosis and mortality in both the test larvae: *S.litura* (>80% mortality in 7 days) and *H.armigera* (>75% in 9 days).But the most important

consideration in the development of a production system for this fungus as a mycopesticide is the biology and the sensibility of this fungus on invitro growth conditions. In the present study we made an attempt to optimize an effective biphasic solid state fermentation process for the mass production of *N. rileyi* on broken rice with the two most virulent isolates PADP11 and PAKR. The isolates were then tested for optimized conidial yield in different polypropylene autoclavable bags for proper aeration against the SACO2 (Belgium) bags as control. A series of moisture conditions have been optimized for the maximum production of conidia. Best growth was observed in SACO2 bags with 40% moisture (6.9×10^{10} conidia/gm of substrate and 3.4×10^{13} conidia/gm of conidial powder), moderate growth was observed with 30% moisture content in 10/10 cms nylon filter patched hand made aeration bags (6.2×10^{9} conidia/gm of substrate and 3.2×10^{11} conidia/gm of conidial powder) while optimal growth at 30% moisture content was recorded in the two sided cotton plugged hand made bags (9.7×10^{8} conidia/gm of substrate and 2.1×10^{9} conidia/gm of conidial powder). Shelf life of the conidia during storage is a major problem with *N. rileyi.* Thus, the determination of the correct shelf life method for each fungal species and periodical monitoring to check their morphology, pathogenecity and genetic stability has become a requirement, and studies are necessary to understand the problems related to storage conditions in order to select the best preservation method for an effective formulation and to have an effective shelf life. The objective of the present study was to evaluate the viability of fungal strains preserved under 13 different combinations of additives. Of the 13 different combinations tested, 9 showed > 90% viability at room temperature even after six months.

11:30 STU

Expression and Characterization of a novel vegetative insecticidal protein gene of *Bacillus thuringiensis*

Liang Xiao, Yuehua Chen and Jun Cai

(*Tianjin Key Laboratory of Microbial Functional Genomics, Department of Microbiology, College of Life Sciences, Nankai University, Tianjin 300071, China*)

Bacillus thuringiensis strain 9816C is a wild *Bt* strain with high potency against *Spodoptera exigua* and *Helicoverpa armigera* and its culture supernatant also exhibits high toxicity to this two pests. The full length Vip3Aa gene from Bt9816C was amplified and cloned to pUC18-T vector. This gene sequence has been submitted to GenBank (Accession No. AY945939) and named Vip3A18 by the *Bacillus thuringiensis* delta-endotoxin nomenclature committee. BLAST result revealed that sequence of *vip3Aa18* is 99% identical to other published *vip3Aa* genes and SMART program revealed there was a carbohydrate binding domain (CBM_4_9) existed in the C-terminus of its amino acid sequence. By using Conserved Domain Architecture Retrieval Tool (CDART), two crystal insecticidal proteins, Cry9Ed1 (AAS68357) and Cry9Ec (AAS68357) were found sharing similar domain architecture. The full length fragment of Vip3A18 was inserted into pET 28(a+) vector and expressed in *Escherichia coli* BL21. Bioassay demonstrated that the recombinant *E. coli* had high toxicity against *S.exigua* and *H.armigera*, while it lacked toxicity to nematode *Caenorhabditis elegans.* Moreover, bioassay results showed that Vip3Aa18 synergized the activity of Cry1Ac towards *H. armigera.*

11:45 STU

A structured model for the entire fermentation of *Bacillus thuringiensis* var. *kurstaki*

Ana Karin Navarro and Fermín Pérez-Guevara

(*CINVESTAV, Department of Biotechnology, Av. IPN 2508, San Pedro Zacatenco, Mexico City, México. PC 07360*)

The structural model proposed here describes the entire *Bacillus thuringiensis* fermentation at different glucose concentration, from 25.1 to 54.1 g/L. The model included the evolution of the three stages of *B. thuringiensis* fermentation; vegetative growth, transition and sporulation, through the key compounds; cell count, poli-b-hydroxybutyrate, Cry protein and dipicolinic acid DPA (endospores). A Gompertz model was used to follow the production of the key compounds. The disappearance, or dead, of vegetative cells was modeled taking into account that endospore (DPA) are formed from biomass. The PHB consumption was modeled considering that PHB serves as an endogenous reserve of carbon and energy for DPA and Cry production. Correlated for several model parameters were successfully with the total solid concentration. The Gompertz model allowed to estimate the inflection point for each key compound curve. The inflection point of each key compound indicated critical times during fermentation. Like people, cells usually deliberate very carefully before committing dramatic changes in their lifestyle, and weighs its options before committing to a developmental

pathway. *B. thuringiensis* culture showed two dramatic changes when signals induce cells to change between one stages to another, that can be related with the inflection points obtained. The inflexion point of growth corresponds with mid-exponential growth and sugar half consumption when TCA enzymes formation was observed and PHB production by g-aminobutiric acid cycle started. The inflexion point of PHB production corresponds with DPA and Cry production start. The timing of the decision to commit to spore formation is extremely critical because once committed, there is no turning back. At the inflexion point of PHB production the cell seems to have taken the decision to sporulate based upon having sensed that enough resources will still be available for completion of sporulation.

12:00 STU

Screening and breeding of *Bacillus thuringiensis* subsp. *kurstaki* with high toxicity against *Spodoptera exigua*

Zhang Xiao-peng, Gong yu-hua, Chen Shou-wen*, Yu Zi-niu

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Aims: The present work aimed to screen and breed B. thuringiensis subsp. kurstaki strain with high toxicity against Spodoptera exigua. **Methods and Results:** A high toxicity strain BMB005 was screened via investigating toxicity of 200 B. thuringiensis subsp. kurstaki strains against S. exigua. It was exposed to UV ray and then appropriate mutants were treated by Diethyl sulfate. The Mutants were primarily screened to obtain over-producing Zwittermicin A by agar plug plate diffusion method, and then rescreened to obtain over-producing Zwittermicin A and high producing delta-endotoxins mutants which were finally bioassayed to validate the toxicity against S. exigua. Mutant BtD1-23 with highest toxicity against S. exigua was obtained. In 3-l fermentor level, its toxicity against S. exigua and Heliothis armigera increased by 115.4% and 25.9% respectively as compared to BMB005. **Conclusions:** The good result showed that the novel model was efficient for screening and breeding of B. thuringiensis subsp. kurstaki strain with high toxicity against S. exigua. **Significance and impact of the study:** This study provided a new strategy for screening and breeding of B. thuringiensis subsp. kurstaki strain with high toxicity against S. exigua.

Wednesday, August 30, 10:30-12:30, *Multifunctional Hall*
Contributed papers: Viruses 3
Moderators: David A Theilmann and Linda Guarino

10:30

Baculovirus immediate early 1 protein is a broad-spectrum bridge for enhancer function *in trans*

Yin Chen, Xu'ai Lin, Yongzhu Yi and Zhifang Zhang

(*Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing,100081, China*)

Enhancers are classically defined as cis-acting DNA regulatory elements that stimulate transcription, independent of their position and orientation with respect to the transcriptional initiation site. In some cases, via a protein bridge, enhancers can function in *trans* on a separate DNA molecule to the promoter. A plasmid containg *homologous region 3* (*hr3*) enhancer from *Bombyx mori* nucleopolyhedrovirus (BmNPV) failed to stimulate the expression of another plasmid containing *luciferase* gene (*luc*) under control of a promoter in co-transfection assays, but strong stimulation occurred when cells were infected by BmNPV. Co-transfections of *hr3* enhancer, *luciferase* gene driven by BmNPV helicase promoter and a wildtype BmNPV genomic library presented in pUC19 revealed that IE-1, the essential viral factor, contributed to the *hr3* enhancer function *in trans*. Further investigations proved that IE-1 could mediate *hr3* enhancer function *in trans* to stimulate promoters from various origins. Among the tested promoters were homologous promoters from BmNPV *helicase* and *gp64* gene and heterogeneous promoters of host derived *larva serum protein* (*lsp*) and mammalian virus derived SV40 or CMV promoters. The stimulating effects ranged from 40 to more than 100 folds. These results suggested that IE-1 was a broad-spectrum bridge for enhancer function *in trans*. Functional dissections of the *hr3* enhancer clarified that the 30-bp imperfect palindrome was

essential for the enhancer function *in trans*.

10:45

Baculovirus genes involved in bmnpv *ubiquitin* gene expression in transient expression assays

Xu'ai Lin, Yin Chen, Yongzhu Yi and Zhifang Zhang

(*Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing, 100081, China*)

We developed a method to identify baculovirus genes required for late gene expression that is based on accession of clones from a *Bombyx mori* nucleopolyhedrovirus genomic DNA library. A plasmid containing the *luciferase* gene under the control of *Bombyx mori* nucleopolyhedrovirus late *ubiquitin* gene promoter was constructed (pBmubiluc). A low level of luciferase activity was observed in cells when pBmubi595luc co-transfected with pIE-1 DNA only, that is to say, IE-1 was necessary but not sufficient for expression from *ubiquitin* promoter, other genes in BmNPV were required for expression from the *ubiquitin* promoter. Transient expression assay experiments indicated that the late promoter was active in *Bombyx mori* cells when co-transfected pBmubiluc with some BmNPV genomic library DNA and *ie-1* gene, but not when pBmubiluc was transfected alone. The nucleotide sequence of these plasmids was determinated, based on the results, several repeated gene in these fragments were subcloned to pGEM3Z, co-transfected with pBmubiluc and pIE-1, transient expression assay analysis indicated that these can stimulate ubiquitin promoter activity. Subsequent subcloning and luciferase assays indicated that, in the present of IE-1, at least HE65, P35 and late expression factors LEF-6, LEF-11 were required for the activation of Bm NPV *ubiquitin* promoter.

11:00 STU

Functional analysis of the AcMNPV budded virus regulatory protein EXON0

Minggang Fang[1], Xiaojiang Dai[2] and David A Theilmann[1,2]

([1]*Department of Plant Science, Faculty of Land and Food System, University of British Columbia, Vancouver, B.C., Canada V6T 1Z4, Vancouver, B.C., Canada V6T 1Z4;* [2]*Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Summerland, B.C., Canada V0H 1Z0*)

The *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) late gene *exon0* (*orf*141) is required for the efficient production of budded virus. Deletion of *exon0* reduces the levels of budded virus (BV) up to 99% (Dai *et al*., 2004, *J. Virol.* Vol. 78, 9633). AcMNPV *exon0* encodes a protein of 261 residues that forms dimers and domain mapping has shown that the N-terminal acidic region and the coiled-coil leucine zipper domain have the greatest impact on BV production (Fang *et al*, manuscript in preparation). In this study, the cellular localization of EXON0 was analyzed by cellular fractionation, GFP fusion and immunofluorecence. Western blot analysis showed that EXON0 co-purified with both BV and occlusion-derived virions. Fractionation of virion components also showed that EXON0 appears to be a structural component of nucleocapsids. The fate of nucleocapsids in cells infected with EXON0 deletion viruses was further investigated by transmission electron microscopy to aid in determining how this protein is required for efficient BV production. Tandem affinity purification, immunoprecipitation and yeast two hybrid assays were used to try and identify proteins that potentially interact with EXON0.

11:15 STU

Functional comparison of the *Autographa californica* multiple nucleopolyhedrovirus transcription factors IE0 and IE1

Yingchao Nie[1] and David A Theilmann[1,2]

([1]*Department of Plant Science, Faculty of Land and Food System, University of British Columbia, Vancouver, B.C., Canada V6T 1Z4;* [2]*Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Summerland, B.C., Canada V0H 1Z0*)

The *ie0-ie1* immediate early gene complex of *Autographa californica* Multiple Nucleopolyhedrovirus (AcMNPV) produces the primary early transcription factors IE0 and IE1. It is the only AcMNPV gene that is known to produce a protein as a result of splicing. Peak expression of IE0 is at early time post-infection whereas IE1 peaks at late time p.i. A recent study on AcMNPV *ie0-ie1* using viruses that express only IE0 or IE1 showed that either protein can support virus growth, however both are required to obtain a wild type infection (Stewart et al., 2005, *J.Virol.*, 79, 4619). This result shows that IE0 and IE1 play critical but different regulatory roles in the viral infection cycle. In support of this, IE0 and IE1 have also been shown to be mutually antagonistic in the activation of late gene expression (Huijskens *et al*., 2004, *Virology*, 323, 120). *Orgyia pseudotsugata* multiple

nucleopolyhedrovirus (OpMNPV) IE0 and IE1 have also been shown to differentially activate specific promoters. AcMNPV IE0 and IE1 only differ by 54 amino acids at the N-terminus and it is unknown how this domain alters the function of the protein during infection. The objective of this study was to further investigate the functional differences of AcMNPV IE0 and IE1. Using an *ie0-ie1* deletion virus, recombinant viruses were constructed that expressed IE0 or IE1 fused with fluorescent proteins to compare the subcelluar localization via laser confocal microscopy. Using CAT assays we have also compared the ability of AcMNPV IE0 and IE1 to activate various AcMNPV early promoters to identify different transactivation targets. Tandem affinity purification has also been used to determine if the extra N-terminal domain of IE0 results in proteins specifically interacting with IE0 compared to IE1.

11:30 **STU**

***Lef*-2 dual role in DNA replication and late gene expression during baculovirus- infection.**

Clare Allen[1, 2], Linda King[2] and Robert Possee[1]

([1]*NERC Centre for Ecology & Hydrology, Mansfield Road, Oxford, OX1 3SR, UK;* [2]*School of Biological and Molecular Sciences, Oxford Brookes University, Headington, Oxford, OX3 0BP UK*)

Late gene expression factors (*Lef*s) are a class of early genes that regulate late gene expression and DNA replication during baculovirus infection. Individual *Lef*s can be subdivided based on their specific role during infection, either in replication, late gene expression or host determination. Earlier research identified six *Lef*s essential (IE1, *Lef* 1, *Lef*-2, *p143*, *DNA pol*, and *Lef*-3) and three stimulatory (*p35*, *IE2, Lef*-7) to plasmid DNA replication through the use of transient based assays. The isolation of a mutant virus that was deficient in late gene expression (VLD1) led to the identification of a point mutation within the *Lef*-2 gene by marker rescue experiments and sequence analysis. The cellular consequences of the VLD1 phenotype was delayed synthesis of late proteins such as chitinase, lower viral titres and temporal delay of DNA replication. To ensure that the observed late gene deficiency exhibited was the result of the *Lef*-2 mutation, an additional virus (AcORF6^{3260} (1)) was constructed to solely mimic this alteration. The role of the *Lef*-2 gene in baculovirus-infection is thought to be involved and has been shown to be essential for DNA replication. In contrast, the AcORF6^{3260} (1) virus experimentally showed that there was no significant difference in plasmid replication compared to wild-type, but appeared to express very late genes such as polyhedrin at a lower level. This led to the suggestion of a dual role for the *Lef*-2 gene. To further explore the potential of the dual role of *Lef*-2 gene in DNA replication and late gene expression a variety of modern techniques were employed. Using real-time PCR (Q-PCR) and standard calibration curves, the DNA replication levels over 24hpi were calculated for each individual virus. Results confirmed previous research that the VLD1 phenotype did exhibit delayed DNA replication levels, but the AcORF6^{3260} (1) virus levels were comparable to AcMNPV. Further characterization of the *Lef*-2 gene involved the use of q-PCR to examine expression levels of early, late and very late genes between AcMNPV and AcORF6^{3260} (1) viruses over 48hpi. The C-terminal of the *Lef*-2 gene is highly conserved and rich with cysteine residues. Serine mutational analysis of five highly conserved cysteines using PCR and marker rescue into an AcΔ*Lef*-2.neomycin virus construct are being carried out to examine the subsequent consequences on gene expression.

11:45

Role of the RNA triphosphatase domain of LEF-4 in late gene expression and viral replication

Yi Li and Linda Guarino

(*Texas A&M University, Department of Entomology, 2475 TAMU, Texas A&M University, College Station, TX 77840*)

Baculovirus encode an RNA polymerase that transcribes viral late genes. This 4 subunit complex also has promoter recognition, mRNA capping, and polyadenylation activities. The capping activity has been mapped to the LEF4 subunit, which has guanyltransferase (GTase) and RNA triphosphatase activities (RTPase). Transient expression assays, as well as the isolation of a temperature sensitive virus, indicate that the LEF4 subunit is required for late gene expression and virus replication. In addition, construction of LEF-4 knockout virus indicates that the GTase function is required for viral replication. Here we report on the requirement for RTPase activity. To address the role of baculovirus RTPase activity, site specific mutations were constructed that destroyed either RTPase activity in LEF4 without affecting GTase activity. The mutant constructs were tested for the ability to support late gene expression by transient assay, and also reconstructed into whole virus to determine levels of virus replication in the absence of RTPase activity. Transient expression assays revealed that RTPase

activity was dispensable as reporter levels were only slightly decreased as compared to wildtype protein. Results with mutant virus were similar. No viable virus was recovered in the absence of LEF4 or with a LEF4 mutant containing a critical substitution within the GTase domain. RTPase mutants, however, were viable and, furthermore, the yields of infectious virus were indistinguishable from wildtype. The lack of a requirement for RTPase function was surprising as this enzyme activity is required for the formation of an authentic cap structure which is required for efficient translation. This suggests that either the host enzyme or the viral protein PTP, which also has RNA triphosphatase activity, can substitute for LEF4.

12:00 **STU**

Characterizing the region of the polyhedrin promoter affected by a few polyhedra mutant baculovirus

Carolyn Pritchard[1, 2], Barbara Kelly[1], Linda King[2], Rosie Hails[1] and Robert Possee[1]

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Amplifying baculoviruses in cell culture frequently results in the selection of spontaneous mutants termed the few polyhedra (FP) virus. The mutants are characterized by producing fewer polyhedra per cell, have occlusion bodies which lack virions or contain a few virus particles with altered morphology. The virus-infected cells yield increased numbers of infectious budded virus particles (BV). The mutations within FP viruses map to the late gene 25K. These comprise insertions or deletions within the coding sequence. It has been demonstrated that 25K is essential for the optimal expression of polyhedrin, but expression of p10 remains unaffected. An *Autographa californica* nucleopolyhedrovirus (AcMNPV) *FP-25K* mutant (AcdefrT) that also had a mutation within the anti-apoptotic *p35* was isolated from virus-infected *Trichoplusia ni* (TN-368) cells. In addition to inducing apoptosis in *Spodoptera frugiperda* cells, it also caused plasma membrane blebbing late in infection of TN-368 cells accompanied by down regulation of polyhedrin protein synthesis. To determine why polyhedrin gene expression appears to be reduced in cells infected with *FP-25K* mutants but p10 expression is unaffected, we designed and constructed hybrid very late gene promoters to direct expression of a reporter chloramphenicol acetyl transferase (CAT). Baculovirus very late gene promoters comprise transcriptional control regions either side of a TAAG motif. The upstream and downstream regions were exchanged between polyhedrin and p10 gene promoters (*pol*TAAG*p10*.CAT; *p10TAAGpol*.CAT) as well as constructing copies of the authentic wild type promoters. These promoters were inserted into the *egt* locus of wild-type and *FP-25K* mutant viruses. In AcdefrT, *pol*TAAG*p10*.CAT and *p10TAAGpol*.CAT hybrid promoters give identical CAT expression to that from native *p10* up to 15 h p.i. However, by 48 h p.i., although the *pol*TAAG*p10*.CAT construct gave similar expression to the *p10* native promoter, the *p10TAAGpol*.CAT results resembled that of the native polyhedrin promoter. This suggests that the downstream region of the polyhedrin promoter may be affected by the *FP-25K* mutation.

12:15

Unique expression strategy of cricket densonucleosis (AdDNV) genome

Peter Tijssen[1], Yi Li[2], Zoltan Zadori[1], Francoise-Xaviere Jousset[3], Jozsef Szelei[1], Mohamed El-Far[1], Joseph Woodring[4], Regina G. Kleespies[5] and Max Bergoin[3]

(*[1]INRS-Institut Armand-Frappier, Laval QC, Canada H7V 1B7; [2]Huazhong Normal University, Wuhan, 430079 P.R. China; [3]Université Montpellier II, Montpellier, 34095 France; [4]Universität Bayreuth, Bayreuth, 95440 Germany; [5]Federal Biological Research Centre for Agriculture and Forestry, 64287 Darmstadt, Germany*)

The Acheta domesticus densonucleosis virus (AdDNV) has been isolated from crickets in France about 30 years ago. Commercial growers in Europe lost almost all of their cricket stocks about 5 years ago, apparently via AdDNV. Although European growers believed the virus came from imported American Acheta, these crickets have not yet been affected. Indeed, we observed that American crickets are initially resistant, although they can be infected by feeding them infected European cricket carcassess. With each generation more characteristics of infected animals occur in the American Acheta. Infected crickets lie on their backs and do not move although their heart and malpighian tubules are still functional. The most obvious pathological change is seen in the gut. Infected females always have less food in the gut, and especially striking is the completely empty digestive caecae. The genome of the original French isolate as well a German isolate before and after passing through American crickets was cloned and sequenced. Sequence differences between the

original and new isolates were about 1%, whereas initial passing through American crickets that was expected to undergo mutations to adapt to the new host, did not show any differences yet. The transcription map was determined and showed that, in contrast to other ambisense densoviruses, splicing occurred in both the nonstructural (NS) and the capsid protein (VP) gene cassettes. Interestingly, the splicing observed when VP was expressed by the baculovirus system demonstrated an additional alternative splicing about the phospholipase A2 motif as compared to splicing patterns in crickets. The splicing pattern in crickets predicts 3 NS proteins (NS1, 566 codons; NS2, 276 codons: NS3, 169 codons) and 2 VP ORFs of 597 (ORF-A) and 268 (ORF-B) codons that are linked by splicing. The largest VP of ORF-A was shown by N-terminal protein sequencing to code for VP2. The larger VP1 is, therefore, generated by splicing ORF-A and B and requires a frameshift. This is unique among the parvoviruses. The small ORF-B contains the coding sequence for the phospholipase A2 motif that we have shown to be critical for the entry of the virus and the entry of the viral genome into the nucleus.

Wednesday, August 30, 14:00-16:00, *Meeting Center*
Bacteria Division Symposium: Bacteria in Bio-control in Asia: Natural and Bio-tech strains
Organizer: Ray Akhurst, Moderator: D.H. Dean

14:00

Application of mosquitocidal *Bacillus sphaericus* and the resistance management in China

Yuan Zhiming
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Due to the high specific larvicidal toxicity, long-term effect in field and the safety to mankind and environment, *B. sphaericus* has been widely used in mosquito control management in China. *B. sphaericus* strain C3-41, a high toxic strain isolated from a mosquito breeding-site in China, has a high toxicity against *Culex* sp., followed by *Anopheles* sp. at laboratory and field conditions. *B. sphaericus* C3-41 liquid formulation (Jianbao®) is produced in Wuhan and more than 100 tons was used each year for mosquito breeding-site control, including sewage ditches, ponds, puddles, cesspits, marshes, small tanks and a large number of temporary habitats in construction areas after rainfall. There has been 80 cities and towns all over China using the C3-41 as a main agent in mosquito integrated control management and about 10,000 hectares of mosquito breeding-sites were treated each year. In certain southern cities , such as Shengzhen, Dongguan and Fushan, C3-41 formulation has been continually used for more ten years and the mosquito population has been controlled at the tolerable level.

For many years, it was considered that larvicides based on *B. sphaericus* would not lead to resistance in mosquitoes and this was one of the main advantages of microbial insecticides over synthetic chemical insecticides. However, the target *C. quinquefasciatus* have the ability to acquire resistance to *B. sphaericus* binary toxin under long-continuous selection pressure, with a resistance ratio 100,000-fold resistance to *B. sphaericus* in field. The appearance of high-level of resistance in mosquito is the threat to the future application of *B. sphaericus* as mosquito control agents. Because there is no cross-resistance to *Bti* and some other *B. sphaericus* strains within the *Bs*-resistant populations, and it is suggest using *B. sphaericus* and *Bti* alternatively for control of C. *quinquefasciatus* in suburb of cities or towns for avoiding appearance of resistance.

Key Words: *Bacillus sphaericus, Culex quinquefasciatus*, mosquito control, resistance, mosquitocidal activity, Toxin

14:30

Transgenic bacteria expressing combinations of genes from *Bacillus thuringiensis*

Arieh Zaritsky and Eitan Ben-Dov
(*Ben-Gurion University of the Negev, Department of Life Sciences, Ben-Gurion University of the Negev, POB 653, Be'er-Sheva 84105, Israel*)

To avoid resistance in insect pests against *Bacillus thuringiensis* (*Bt*), combinations of several toxin genes are cloned for expression in microorganisms that protect the toxins from sunlight inactivation. All 15 combinations of *cry4Aa*, *cry11Aa*, *cyt1Aa* and *p20* from subsp. *israelensis* (*Bti*) were cloned in *Escherichia coli*, and the most mosquito larvicidal (with all 4) was transferred into the nitrogen-fixing,

filamentous cyanobacterium *Anabaena* PCC 7120. It displays the highest toxicity against larvae of *Aedes aegypti* and *Anopheles* sps. ever reached in transgenic cyanobacteria. This gene combination in *E. coli* is highly toxic against larvae of *Culex quinquefasciatus* as well; larval mortality of lines that had been selected for resistance against various combinations of the toxins was only 2.5-3-fold lower. The combination without *cyt1Aa* is 1000-fold less toxic, except the line selected with Cyt1Aa. The results confirm our notion that recombinant cyanobacteria producing all *Bt*i's mosquito larvicidal toxins would be as effective as Bti itself, but with additional advantages, and may replace Bti for field delivery. Being of a laboratory origin, it will eventually disappear from the natural habitat by competition with endogeneous species, thus furnish temporal refugia. It is cheaply mass-produced and its activity persists longer than Bti thus anticipated to be highly cost-effective. The transgenic *Anabaena* endured exposure to sunlight and silt conditions longer than Bti powder (Bactimos) did. *cry1Ac* and *cry1Ca* from subsps *kurstaki* HD-73 and *aizawai* 4J4 respectively, were similarly added to *E. coli* with *cyt1Aa* and *p20*. Toxicity of the clone expressing all 4 genes was very toxic against susceptible larvae of Cotton Bollworm (*Helicoverpa armigera*), displaying substantial synergy between *cyt1Aa* and both *cry* genes. Cyt1Aa may thus be an important asset for management of resistant insects pest. The Lepidopteran and Coleopteran species that are not responding to Cyt1Aa may differ in the phospholipids composition of their epithelial midgut cell membranes.

15:00

Microbial control of scarabs in Japan

Shin-ichiro Asano[1], Hisanori Bando[1], Noriko Shisa[2], Katsuyoshi Takeuchi [2] and Toshihiko Iizuka [3]
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In Japan, scarabs are important insect pests which do causing big damage to roots of turf and dry field farming thing. Chemical insecticides such as organophosphates, neonicotinoides have been used to control these insect pests. Microbial insect control agents such as *Bacillus thuringiensis*, and *Paenibacillus popilliae* (milky disease) are also used but the usage is limited due to a number of problems including low efficacy. Recently, new potent strains of *B. thuringiensis* and *P. popilliae* were discovered in Japan. The strain of *B. thuringiensis* is called SDS-502 and its insecticidal protein gene, *cry8Da*, has been cloned and characterized. The strain of *P. popilliae* is called Hime and its insecticidal protein gene, *cry43Aa*, has been cloned and characterized. Laboratory tests showed the Cry8Da insecticidal protein had substantially higher specific activity against Japanese beetles than insecticidal proteins (Cry9Da and Cry43Aa proteins) isolated from other *B. thuringiensis* and *P. popilliae* strains. The Cry8Da protein showed the activity against alder leaf beetle, cloverleaf weevils and some others. SDS-Biotech in Japan have been testing sprayable formulations of this Bt SDS-502 strain for 4 years and have found high potential on the turf grass and the peanut field, the sweet potato field as a commercially competent biopesticide. In addition, the transgenic turf containing the *cry8Da* gene was created, and it showed strong resistance against the scrabs.

15:30

Toxicity of *Bacillus thuringiensis* crystal proteins against plant root-knot nematode

Ziquan Yu, Suxia Guo, Ziniu Yu, Ming Sun
(*State Key Laboratory of Agricultural Microbiology, College of Life Science and Technology, Huazhong Agricultural University, Wuhan 430070, PR China*)

Plant-parasitic nematode devastate a wide range of crop plants world-wide and cause great losses every year. Control phytoparasitic nematode with *Bacillus thuringiensis* is an effective and environmental safety way. A few of work on B. thuringiensis against nematode mainly focused on Caenorhabditis elegans, a free-living nematode. While those against plant root-knot nematode (RKN), the economically most important plant-parasitic nematode, is very few. RKN's mouthpart is piercing-sucking (stylet) which is different from C. elegans. The diameter of RKN stylet is so narrow that the parasporal crystal protein could not pass through, bringing the difficulty on bioassay. A bioassay was developed to assess the toxicity of *B. thuringiensis* crystal protein against Meloidogyne sp., one of the most important RKN, under laboratory conditions. Based the bioassay, several nematicial activity B. thutingiensis strains, like YBT-1518, YBT-1532, BMB020, BMB021 and BMB027 were isolated. Pot test showed the YBT-1532 could significantly reduce the infection of Meloidogyne sp. to tomato. A few of nematicidal crystal protein genes, cry1Ea6, cry6Aa2, cry5B-like

were isolated and were demonstrated toxicity to Meloidogyne sp. The action mode of crystal protein against plant-parasitic nematode is not clear. How toxin enter into root-knot nematode is unknown. Western blotting showed Cry6Aa2 crystal protein could be detected in infected Meloidogyne sp. and the molecular weight of Cry6Aa2 crystal protein was founded increased. This confirmed the toxin entered into the RKN body through some way, and bound to a specific receptor. Infection of Meloidogyne sp. with Cry6Aa2 labeled with FITC showed the crystal protein may entry into the body of Meloidogyne sp. through cuticle but not the stylet. A novel negative regulation factor which suppressed the expression of cry6Aa2 was identified in strain YBT-1518 (containing cry6Aa2 and cry5B-like gene as well as a gene encoding a 45kDa crystal protein with novel N-terminal amino acid sequence). cry5B-like gene was not expressed in wild type strain YBT-1518, but it was expressed a 140 kD-protein in a plasmid cured stain BMB171. This may be associated with the negative regulation factor in cry6Aa2 operon. This finding might give a new idea to find novel crystal protein gene and enhance the yield of crystal protein in *B. thuringiensis*.

Wednesday, August 30, 14:00-16:00, *Nanyuan Mmeeting Room*
Contributed papers: Fungi 3
Moderators: Tariq M Butt and Mingguang Feng

14:00

Laboratory bioassays of entomopathogenic fungi for control of western flower thrips *Franklinella occidentalis* in horticultural growing media

Minshad Ali Ansari[1], Michael Brownbridge[2], Farooq Ali Shah[1], Mark Whittaker[3], Munoo Prasad[4] and Tariq M Butt[1]

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Using a new bioassay method, the efficacy of 6 isolates of *Metarhizium anisopliae* (i.e. V275, ARSEF 3297 and 4456, ERL 700, CA1 and F 10), and one isolate of *M. flavoviride* (F 62), *Beauveria bassiana* (CLO 61), and *Paecilomyces fumosoroseus* (CLO 55) was compared to the standard insecticide, fipronil, against soil-dwelling stages of western flower thrips (WFT), *Franklinella occidentalis* under controlled conditions. WFT enter the soil as late second instars, and go through pre-pupal and pupal stages before adult emergence. Given the amount of time spent in the soil, these stages make excellent targets for entomopathogenic fungi applied to growing media used in plant production. Horticultural growing media (peat- and bark-based mixes) were premixed with dry fungal conidia to obtain a concentration of 1×10^{10} conidia/L of compost, or the recommended dose of fipronil. After correction for control mortality, emergence of WFT adults in the fungal treatments was reduced by 70-90% 11 days post-treatment. However, adult emergence from the fipronil treatment was only reduced by 40-45%. This study clearly demonstrates that fungi can effectively control WFT in potting media and could become useful components of a holistic control strategy. In addition, application of fungi may also impact other greenhouse pests with a soil stage in their life cycle.

14:15

Field evaluation of *Beauveria bassiana* isolates from *Lygus* spp. for control of *Lygus* spp. (Hemiptera: Miridae)

Jarrod E. Leland[1], Michael R. McGuire[2], Tina G. Teague[3], Jennifer Lund[3], Steinkraus C. Donald[4] and Gore Jeff[1]

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Isolates of the entomopathogenic fungus *Beauveria bassiana* from *Lygus* spp. populations in the Mississippi Delta and San Joaquin Valley of California were evaluated for characteristics relevant to field efficacy and mycoinsecticide development. Evaluation criteria included; pathogenicity to the tarnished plant bug *L. li*neolaris and the Western tarnished plant bug *L. hesperus*, impact on beneficial insects, conidial production, mycotoxin production, survival under solar radiation, and temperature

tolerance. Based on these criteria one isolate from each collection was selected for field trials and comparison to the commercial *B. bassiana* isolate (GHA) in field trials against *L. lineolaris* in pigweed and *L. hesperus* in alfalfa. Improvements in mass production provided for additional field trials with the Mississippi *B. bassiana* isolate against *L. lineolaris* in cotton, radish, mustard and canola to evaluate application rates, formulation strategies and potential for using *B. bassiana* in combination with insect growth regulators in trap crops. Prevalence of infection and population change following application were determined for *L. lineolaris* and beneficial insect predators. Caged *L. lineolaris* adults were used to evaluate the persistence of *B. bassiana* conidia on plant surfaces.

14:30

Grain-Based Production of the Entomopathogenic fungus Nomuraea rileyi

David Holdom and Hauxwell Caroline

(*Department of Primary Industries and Fisheries, 80 Meiers Road, Indooroopilly, Queensland, Australia 4068*)

Nomuraea rileyi is a fungus pathogenic to *Helicoverpa armigera* and other Noctuidae. Its use as a biopesticide has been limited by the difficulty of producing condia on commercially viable media such as rice. Addition of the salt mixture from Czapek's medium to rice substantially increased yields of conidia of *N. rileyi*. Doubling the magnesium concentration, quadrupling the other salts and adding zinc and manganese further increased yields. Bioassays of conidia of *N. rileyi* against *Helicoverpa armigera* showed that they were equivalent to conidia produced on a standard agar medium, Sabouraud's maltose agar supplemented with yeast extract. The supplement also increased the yield of conidia on other grains, and increased yields of another entomopathogenic fungus, M*etarhizium anisopliae*.

14:45

Evaluation of the potential of native fungal isolates and *Metrahizium anisopliae* var. *acridum* for the greater wax moth, *Galleria mellonella* (L)

Namusana Hellen, Emiru Seyoum and Bekele Jembere

(*Department of Biology, Addis Ababa University, P.O.Box 1176, Addis Ababa, Ethiopia*)

The potential of six fungal isolates including, DLCO-AA5, DLCO-AA14, DLCO-AA83, DLCO-AA 109, IITA 18 and IMI 330189 was evaluated in the laboratory for their pathogenicity to adult greater wax moths (GWM). Adult GWM were found to be susceptible to all isolates and concentrations ranging from $4x10^4$ to $c4x10^7$ conidia/ml. Comparison of post treatment mortality of adult, greater wax moths showed that there was no significant difference in pathogenivcity between the different isolates used and the control groups until day 3 after treatments. Post treatment mortality due to mycosis by the different isolates however increased gradually reaching up to 100 % by day 14 with isolates DLCO-AA 5, IMI330189, DLCO-AA-83 and DLCO-AA-109. It was further noted in the present work that all isolates even at the lowest concentration ($4x10^4$ conidia/ml) were able to cause target infection of > 90% by day 14. Death was considered to be due to mycosis only when external mycellial growth over the body of surface sterilized cadavers and the characteristics red coloration were apparent following incubation (Prior *et al*, 1993; Bateman *et al*, 1993; Odino, 1994; Inglis *et al.*; 1996). Further laboratory based experiments were also carried out to investigate host specificity of two isolates of Metarhizium (DLCO-AA-83 and IMI330189) and one isolate of Beauveria (IITA 180) by inoculating Ethiopia races of honey bees (*Apis melifera*). Each isolate was applied topically by spraying 10 ml of $2x10^7$ conidia /ml into the bee hives. The standard isolate, IMI330189 was found to non pathogenic to the native honey bees. Post treatment mortality of the non-target honey bees was subject to examination similar to the target GWM.

15:00

Assessment toxicity of *Beauveria bassiana* blastospores against colding moth *Cydia pomonella* (Lepidoptera: Tortricidae) in laboratory

García-Gutiérrez Cipriano[1], Solis-Soto Aquilés[2], Galán-Wong Luis J.[3], González-Maldonado Ma. Berenice[1] and Medrano-Roldán Hiram[2]

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The colding moth (CM) *Cydia pomonella* L. is the major pest of apples orchards in Durango, Mexico. In this place the commercial strains Bea-Sin™ (*Beauveria bassiana* spores) and Meta-Sin™

(*Metarhizium anisopliae* spores) were used at dose of $1.2x10^{12}$ spores/ha, also the native strain *B. bassiana* code BbPM without formulate had toxic effects at dose of $1.2x10^{9}$ blastospores/ml; Bea-Sin™ presented the lowest percentage of damage fruits 2.04% following BbPM 2.38% and Meta-Sin™ 4%; so both infective bodies were effectives for CM control at field level trials. On other hand, many studies concerning to key fermentation parameters (agitation, flow rate, cultivate medium, pH) have been realized to improve the fermentation processes; the mixing and aeration conditions are the most important variables to arise the nutrients distribution, oxygen supply to the organisms, the carbon dioxide remove, and provide cooling and other properties that have a strong influence on the productivity to obtain an efficiency of entire fermentation process, in such away the aim of this study was to determinate the optimal mixing and aeration conditions using single and a combination of Rushton-Maxflo impellers, and the assessment of blastospores toxicity on CM larvae. BbPM with the better yield was used to calculate LC_{50} using an artificial diet contamination bioassay at dosage of $1.2x10^{9}$ to $3.8x10^{5}$ blastospores/ml on five days CM larvae. The larvae mortality was determined at 24, 48 and 72h after fungi-insect exposure, as well as the mycosed insect percentage during 8 days. The strain gotten the better yield with a Rushton-Maxflo impellers combination ($1.2x10^{9}$) to 400 rpm and 1.0 vvm, followed by Rushton ($3.4x10^{8}$) and Maxflo impeller ($1.7x10^{8}$ blastospores/ml) at same conditions. The LC_{50} for BbPM strain produced under impellers combination was $8.82x10^{6}$ blastospores/ml. The strain caused 96% of larvae mortality at 72h and 9% at dose of $3.8x10^{5}$ at 24h. There are statistical effects between dose and mortality (F = 9.784, p £ 0.0.01), but this phenomenon was not found between infection time and mortality (F = 0.180, p£ 0.835). Eight days after fungi-insect exposure 82% larvae were mycosed. This work is an important effort for transfer this knowledge from laboratory to pilot plant scale in the near future for blastospores production.

15:15 STU

Enhanced efficiency of *Beauveria bassiana* blastospore-based transformation system by restriction enzyme-mediated integration

Qiong Jiang, Sheng-Hua Ying and Ming-Guang Feng

(*Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, 310058, China*)

Common transformation methods for genetic manipulation of *Beauveria bassiana* and other fungal biocontrol agents are based the use of fungal protoplasts, conidia or *Agrobacterium* mediated by glycol (PEG), electroporation or particle bombmardment. Recently, a blastospore- based, more convenient method has been developed for integration of foreign genes into *B. bassiana* at a mean frequency of 24 transformants per microgram of DNA. In this study, restriction enzyme-mediated integration (REMI) technique was applied to improve the blasotospore method for higher transformation frequency. As a result, the frequency for integrating phosphinothricin-resistant gene *Bar* into the blastospores of a wild *B. bassiana* strain (ARSEF2860) was doubled via the prepared plasmid pAn52-1N-Bar containing fungal *GPD* promoter. Factors recognized to affect the frequency included blastospore age or status, enzymes, and buffer solution. Colony growth of the wild stain was entirely inhibited on Czapek's agar medium containing 150 µg/ml phosphinothricin whereas the resultant transformants grew well on the medium containing 400 µg/ml phosphinothricin. This resistance was retained after consecutive subcultures under selective or nonselective pressure. Both PCR (with sense primer BarF and antisense primer BarR) and Southern Blotting analyses confirmed successful integration of the *Bar* gene into the genomes of 10 *B. bassiana* transformants randomly taken and presence of only a single copy of the inserted gene in all. Conclusively, the blastospore method can be improved with the REMI technique and perhaps more with other advanced techniques for efficient genetic manipulation of *B. bassiana* and other entomopathogenic hyphomyctes.

15:30 STU

Pathogenicity of *Beauveria bassiana* towards Fuller's rose weevil larvae in soil

Carolyn Mander[1], Trevor Jackson[2] and Bruce Chapman[1]

([1]*Bio-Protection and Ecology Division, PO Box 84, Lincoln University, Canterbury, New Zealand;* [2]*AgResearch, PO Box 60, Lincoln, Canterbury, New Zealand*)

Testing of candidate strains for pathogenicity towards the target insect is a critical step in strain selection for microbial control. However, traditional bioassay methods such as maximum-challenge assays do not equate with infection rates in complex environments such as soil. Therefore, assessments of the pathogenicity of *Beauveria bassiana* towards Fuller's rose weevil (FRW) (*Naupactus cervinus*) larvae were conducted in kiwifruit orchard soil to provide a closer

approximation to field conditions. This also allowed soil effects to be investigated. Initially, thirteen strains of *Beauveria* spp. and *Metarhizium anisopliae* were screened for pathogenicity towards FRW larvae in soil. All strains tested were pathogenic but varied in virulence. Three strains of *B. bassiana* and one strain of *B. brongniartii* inducing high rates of mortality were further investigated to assess the effects of soil-type, larval age and treatment time on larval mortality. Mortality was significantly reduced in native orchard soil in comparison with oven-dried soil, suggesting that the effective dose is less in more fungistatic soils. Fifth instar larvae were more susceptible to mycosis than 3rd and 4th instar larvae. Mortality was significantly reduced for larvae placed in soil pre-inoculated with conidia than larvae placed in soil immediately post-inoculation, suggesting the effective dose was reduced after one week. Conidial germination on the larval cuticle was also examined using fluorescence microscopy. A positive relationship between cuticle germination and virulence was found.

15:45 STU

Variation in carbendazim resistance and ovicidal activity of *Paecilomyces fumosoroseus* strains against *Tetranychus cinnabarinus*

Wei-Bing Shi[1] and Ming-Guang Feng[1, 2]

([1]*Institute of Insect Science, College of Agriculture and Biotechnology, Hangzhou, Zhejiang, 310029, China;* [2]*Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, 310058, China*)

The sensitivities of six *Paecilomyces fumosoroseus* (Pfr) strains to gradient concentrations of the fungicide carbendazim were assayed at 25°C based on the sizes of their colonies and the counts of colony forming units (CFU). The inhibitory effect of carbendazim concentration on CFU counts fit well to a logistic equation for all the strains ($r^2 \geq 0.90$). The fitted minimum inhibitory concentrations (MIC) classified four strains to medium carbendazim resistance ($20 \leq MIC \leq 100$ mg/ml) and two strains to low resistance ($5 \leq MIC \leq 20$ mg/ml). The strain Pfr6206 had an MIC of 93.5 mg/ml, approaching to the high resistance level of MIC>100 mg/ml. All the fungal strains were also bioassayed for their lethal effects on the eggs of *Tetranychus cinnabarinus* at 25°C under a photophase of 12:12 (L: D) by spraying conidial suspensions onto fava bean leaves with the mite eggs. Egg mortalities at three gradient concentrations (no. conidia per mm^2) of each fungal strain differed significantly. The fungal strains caused egg mortalities of 37-85% at the high concentration of >1200 conidia/mm^2. The LC_{50} estimates determined by the fitted concentration-mortality relationships were 243, 357, 853 and 913 conidia/mm^2 for the strains Pfr6206, Pfr2175, Pfr116 and Pfr153, respectively. However, ovicidal activity was very poor for the strains Pfr612 and Pfr4205. Thus, the strain Pfr6206 with greater carbendazim resustance and better ovicidal activity would be potential for developing a fungal formulation against the mite pest in crop systems where the fungicide is often used.

Wednesday, August 30, 14:00-16:00, *Multifunctional Hall*
Contributed papers: Viruses 4
Moderators: Robert R Granados and Yi Pang

14:00 STU

Identification and analysis of a viral-like chitinase gene isolated from *Spodoptera exigua*

William Ian Tyne, Xiahong Yu and Robert D Possee

(*Centre for Ecology and Hydrology, Oxford, CEH Oxford, Mansfield Road, Oxford, United Kingdom, OX1 3SR*)

Chitinases are responsible for breaking down the polysaccharide chitin. Insects utilize chitinase during ecdysis. The enzyme is secreted into the apolysial space where it is partially responsible for the lysis of the old endocuticle. Baculovirus-encoded chitinases have endo- and exolytic activities and are involved in the liquefaction of the host in the terminal stages of infection. This appears to result in more efficient release of virus from the insect cadaver. Laboratory-based experiments also suggest that this process aids transmission of virus to a new host. The origin of baculovirus chitinases is uncertain. They are closely related to bacterial chitinase A genes, particularly those from *Serratia marcescens*, but similar genes have also been discovered in lepidopteran species. A viral-like chitinase gene (chi-h) was cloned and characterised from *Spodoptera exigua*. This species was chosen as we have been unable to detect a persistent baculovirus infection in this host, unlike many other Lepidoptera. The sequence of *Se*chi-h showed a high degree of homology to other viral and viral-like

chitinases. The activity of the protein was assessed by expressing the *Se*chi-h using a chitinase-negative baculovirus vector. To confirm the expression of the chitinase protein a culture of *S. frugiperda* cells was infected with the recombinant virus and the protein content analyzed by mass spectrometry 2 days post infection. The results of the mass spectrometry analysis indicate the expression of a chitinase protein that is in the region of 64 kiloDaltons, there were no indications of glycosylation of the protein. The recombinant budded virus was injected into late third instar larvae of *S. exigua*. These were subsequently monitored for signs of viral death and liquefaction. A number of the larvae were completely liquefied, indicating that the viral-like chitinase is functional when produced by the baculovirus within the insect host. This suggests that acquisition of the chitinase gene from a host would have represented an immediate advantage to the ancestral baculovirus recipient.

14:15

Insect cell culture as protein factories: progress and challenges

Robert R Granados[1] and Guoxun Li[2]

([1]*Boyce Thompson Institute, Tower Road, Cornell Univ., Ithaca, NY 14853-1801;* [2]*Liayang Agricultural College, Qingdao, Shandong Province, 266109, P.R. China*)

For over two decades the baculovirus expression system has been used successfully to produce a wide variety of foreign proteins in insect cell cultures. The commercial market and the use of cell lines for academic research is currently dominated by two cell lines; the *Spodoptera frugiperda* line, SF21 (and its clonal isolate SF9), and the *Trichoplusia ni* line, BTI 5B1-4, commercially known as High Five cells. Few novel cell lines with improved properties have been established by insect cell culturists in recent years unlike the development of many innovative baculovirus vectors and formulations of insect culture media. Current research efforts to develop improved insect cell liens have involved the cloning of existing SF21 or High Five cells. Furthermore, stable and transient transfection of insect cell lines with select genes have been successfully accomplished to improve protein production and biological activity. These advances have launched a major effort by industry for the commercialization of research reagents, human therapeutics and vaccines. Cell culture production of viral insecticides for control of agriculture and forestry pests is currently in development.

14:30

Advanced baculovirus expression vectors enabling easy and fast purification of recombinant proteins

Jae Young Choi[1], Yang-Su Kim[1], Heekyu Choi[1], Jong Yul Roh[1], Joong Nam Kang[1], Yong Wang[1], Soo Dong Woo[2], Byung Rae Jin[3] and Yeon Ho Je[1]

([1]*School of Agricultural Biotechnology, Seoul National University, Seoul 151-742, Korea;* [2]*College of Agriculture, Life Sciences, Chungbuk National University, Cheongju 361-763, Korea;* [3]*College of Natural Resources and Life Science, Dong-A University, Busan 604-714, Korea*)

The major drawback of baculovirus expression system is the tedious efforts required to purity recombinant virus from non-recombinant backgrounds. In addition, it has been very difficult and complex to purify proteins expressed using baculovirus vectors from infected insect cells. For the easy generation of pure recombinant baculovirus, extracellular RNase gene from *Bacillus amyloliquefaciens*, barnase gene, was introduced into *Autographa californica* nucleopolyhedrovirus virus (AcNPV) genome under the control of *Cotesia plutellae* Barcovirus (CpBV) promoters.For the investigation of the detrimental effect of barnase on insect cells, recombinant bacmids harboring barnase gene under the control of CpBV promoters on their genome, bAcFast-3004ProBarnase and bAcFast-3005ProBarnase, were constructed. While no viral replication were observed when the recombinant bacmids were transfected solely, recombinant viruses were generated when the bacmids were co-transfected with the transfer vector, pAcUWPolh, through the substitution of the barnase gene with the native polyhedrin gene by homologous recombination. Moreover, no non-recombinant backgrounds were detected from unpurified recombinant stocks in PCR analysis. To improve the complexity of existing protein purification process, we constructed a novel baculovirus transfer vector, pBac9-Polh-EKA, by which target genes were expressed as fusion protein of polyhedrin-adaptor-enterokinase site-target protein. When the EGFP was expressed using this novel vector system, the expressed polyhedrin-EGFP fusion protein formed fluorescent granules in the nucleus of infected cells through aggregation between polyhedrin part of the fusion proteins. These granules could be simply purified by sonication and centrifugation of infected cells. By means of enterokinase treatment to the purified granular form of the fusion protein, native EGFP could be obtained as intact form without any extra amino acid residues in N-terminus.

14:45

The establishment of a controllable expression vector system in baculovirus

Jui-Ching Wang[1] and Yu-Chan Chao[1, 2]

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Previously, we established a tetracycline-responsive expression system (TRES) in insect cells. In this system, the TRE-CMVm promoter, a minimal promoter which should be silent without stimulation, can be strongly activated by the tetracycline controllable transactivator (tTA). This is a controllable regulation by the addition or removal of doxcycline. However, the TRES is so far not successfully reported to function in the genome of the baculovirus. Due to the strong activation of the TRE-CMVm promoter by a newly identified activator, the polyhedrin upstream (*pu*) sequence in the genome of the baculovirus, the basal levels of gene expression under TRE-CMVm promoter is high. Thus, tTA can not further activate this promoter for regulatory gene expression in the baculovirus. In this study, by the use of the *pag* promoter, a promoter derived from a baculovirus-unrelated Hz-1 virus, a TRES was established in the genome of this virus. High level tTA activations were found in the transient transfection experiments for this promoters. The folds of activation could be further enhanced by the addition of three TRE elements. The transfer vector which contains the entire TRES was then inserted into the genome of the baculovirus. Strong expression of the luciferase reporter gene was detected, and this expression could be well-controlled by the addition of doxcycline. The successful establishment of the TRES in baculovirus would be very useful for the functional study of many baculovirus or cellular genes, especially for the study of lethal genes. It will also be useful for the expression of poisonous or particular foreign genes, which may interfere with the propagation of the virus or the growth of the cells.

15:00

Antiviral effect of extracts of Spondias mombin and Newbouladi laevis on the infectivity of cowpea aphid borne mosaic virus (CABMV) genus potyvirus

Chinwe C. Ukoha[1], Godfrey E. Ezeifeka[2] and Chinyere N. Umeaku[1]

(*[1]Dept. of Microbiology, Anambra State, University of Science and Technology, Uli, Nigeria; [2] Dept. of Applied Microbiology & Brewing,, Nnamdi Azikiwe University, Awka, Nigeria.*)

Cowpea aphid borne mosaic virus (CABMV) genus *potyvirus* is a widespread most Virus occurring virtually everywhere cowpea is cultivated. In Africa, it causes the most important virus disease in cowpeas and is a major constraint to its production.The use of resistance is currently the most practicable means of controlling CABMV and resistance has been found in germplasm collections and breeding lines. However, obtaining a widely adaptable resistance is difficult because of a high degree of pathogenic variability. In this study, ethanol and water extracts of two Nigerian indigenous plants were tested for antiviral activities against the infectivity of *cowpea aphid-borne mosaic virus* (CABMV) genus *potyvirus.* The mean percentage inhibition of the infectivity of the virus by ethanol and water extracts of *Sondias mombin* were 75.5% and 65% respectively, while that of *Newboulodia leavis* were 65.5% and 65.25% respectively. The effects of dilution on the activity of the two plants were also assayed. Dilution generally reduced the inhibitory activity of both extracts. The dilution of the test extracts resulted in a reduction in their inhibitory activity. The greater the dilution, the lower the percentage of inhibition. However extracts of *Sondias mombin* were still able to inhibit CABMV at a dilution of 1: 32. Generally, a medium level of that can be inhibition was observed for the extracts, therefore, there is need to investigate additional plants for greater antiviral activity as the advantages derived from the use of botanicals in plant virus control include lack of phytotoxicity, environmental friendliness, acceptability and cost effectiveness.

15:15 STU

A Cell Line (NTU-MV) established from *Maruca vitrata* (Lepidoptera: Pyralidae): characterization viral susceptibility, and polyhedra production

Shih Chia Yeh[1], Song Tay Lee[2], Chih Yu Wu[1] and Chung Hsiung Wang[1]

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NTU-MV cell line was established from pupal tissues of *Maruca vitrata*, an important pest of the beans. This cell line contained four major cell types: polymorphic cells, round cells, spindle-shape

cells, and comma cells. The doubling time of MV cells in TNM-FH medium supplemented with 8% FBS at 28℃ was 27 hours. The chromosome numbers of MV cells varied widely from 16 to 268. The patterns of esterase, malate dehydrogenase (MDH), and lactate dehydrogenase (LDH) of MV cell line were different from other cell lines that are routinely maintained in our laboratory. The sequence of internal transcribed spacer regions (ITS) of MV cells was 98% identical to that of *M. vitrata* larvae. These results confirmed that this new cell line was indeed derived from *M. vitrata*. NTU-MV cells showed a high susceptibility to MaviNPV and an extreme yield of polyhedra after two weeks virus infection. In conclusion, NTU-MV cell line will be an available tool for studying MaviNPV and mass production of MaviNPV polyhedra.

15:30 STU

Study the infectivity of budded viruses of wild type and recombinant HearNPVs by quantitative PCR

Huiyuan Wang, Manli Wang, Wentao Dai, Fei Deng, Zhihong Hu and Hualin Wang
(*State Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, Hubei, 430071, P. R. China*)

A previously constructed recombinant *Helicoverpa armigera* single nucleocapsid nucleopolyhedrovirus (HearNPV) $vHa^{+gp64+egfp}$, which expressed GP64 from AcMNPV, was found with significantly increased infectivity than its parental virus vHa^{+egfp}. In this study, quantitative PCR with TaqMan probe specific to *lef-8* gene was used to determine genome DNA copies of the budded virus and compared to viral titers to investigate the infectivity of progeny viruses in these viruses. The results showed that for vHa^{+egfp}, one $TCID_{50}$ unit tested by end point dilution assay was equivalent to 10^3 copies of viral genome DNA. The same ratio was also found for wild type HearNPV. For $vHa^{+gp64+egfp}$, however, one $TCID_{50}$ unit was equivalent to 10^2 copies of viral genome DNA. Based on the titer determined by quantitative PCR, we compared the growth curve of $vHa^{+gp64+egfp}$ with vHa^{+egfp} and the result showed that the supernatant of $vHa^{+gp64+egfp}$ was ten times more infectious than vHa^{+egfp} when propagated in the HzAM1 cells. To determining the quantity of recombinant virus entered into cells, we detected the genome DNA copies of recombinant virus per infected cell at the early stage of the infections. The genome DNA copies in one HzAM1 cell infected with $vHa^{+gp64+egfp}$ were ten times higher than that of vHa^{+egfp} when the same DNA copies were performed in the initial infection. In summary, we proposed that the expression of GP64 enhanced the ability of $vHa^{+gp64+egfp}$ to enter the HzAm1 cells, which means that less recombinant $vHa^{+gp64+egfp}$ virions is sufficient to trigger an effective infection in the host cells.

15:45

A cytoplasmic polyhedrosis virus isolated from the pine processionary caterpillar, *Thaumetopoea pityocampa*

Ikbal Agah Ince, Remziye Nalcacioglu, Ismail Demir and Zihni Demirbag
(*Karadeniz Technical University, Department of Biology, Faculty of Arts and Sciences, Karadeniz Technical University, 61080, Trabzon, Turkey*)

A Cytoplasmic polyhedrosis virus (CPV) was isolated from the larvae of *Thaumetopoea pityocampa* and shown to cause an infection of midgut cells. This viral infection revealed several important diagnostic symptoms, including discoloration of the posterior midgut, reduced feeding, and extended development time of the larvae. The virus infection is lethal to *Thaumetopoea pityocampa*, and with the increasing doses kills the larvae within 4-5 days post infection. Electron microscopy studies showed typical cytoplasmic polyhedral inclusion bodies that are icosahedral, and ranged from 2.4 to 5.3μm in diameter. Electrophoretic analysis of the RNA genome showed that The virus has a genome composed of 10 RNA segments. Based on morphology and nucleic acid analysis, this virus was named *Thaumetopoea pityocampa* cytoplasmic polyhedrosis virus (TpCPV), belongs to the genus cypovirus, family Reoviridae.

Wednesday, August 30, 16:30-18:30, *Meeting Center*
Poster Session II

Bacteria II

BP18

Cloning and characterization of the STAT gene from *Hyphantria cunea*

Hong Ja Kim[1], Yong Min Kwon[1], Yong Il Kim[1], Yeon Soo Han[2], In Hee Lee[3], Beong Rae Jin[4], Young Jin Kang[5] and Sook Jae Seo[1]

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STAT proteins mediate the signaling of cytokines and a number of growth factors from the receptors of these extracellular signaling molecules to the cell nucleus. A new insect member of the STAT family of transcription factors (Hc-STAT) has been cloned from the fall webworm, *Hyphantria cunea*. The *H. cunea* STAT cDNA contains an ORF of 2301 nucleotides capable of encoding 767 amino acid polypeptide with a predicted molecular mass of 88 kDa. The domain involved in DNA interaction and the SH2 domain are well conserved. The Hc-STAT mRNA is expressed at all developmental stages, and the protein is present in hemocytes, fat body, midgut, epidermis, and Malphigian tubule. Also bacterial challenge results in nuclear translocation of Hc-STAT in infected tissues and induction of its DNA-binding activity. *In vitro* and *in vivo* treatment with pervanadate induced translocation of Hc-STAT into the nucleus.

BP19

Comparative analysis of two attacin genes of *Hyphantria cunea*

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Insects express a battery of potent antimicrobial proteins in response to injury and infection. Here we report the cloning of two attacin genes from *Hyphantria cunea.* The two cDNAs encoded the different length of precursor proteins with 233 and 248 amino acid residues, respectively. The two attacins show 45.9% identity at the amino acid level, while 35.2% identity at the necleotide level. Attacin A and B were highly homologous to *Bombyx mori* attacin with identity of 52.8% and 59%,respectively. Attacin B is typical glycine-rich protein (13.8%), while attacin A is leucin-rich protein (13.4%). The attacin B expressed at all developmental stages while attacin A showed stage-specific expression during prepupal and pupal stages in *H.cunea.* Attacin A and B showed similar hydrophilicity pattern, whereas both proteins showed different secondary structure in which attacin A has no tendency to form helices but attacin B contains substantial amount of helices.

BP20

Migration of *Bacillus thuringiensis* towards bean leaves

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Bacillus thuringiensis, a world wide used biopesticide, has been proposed as a plant mutualist offering protection from phytophagous insects. For this, it should colonize leaves from soil, where it has been mostly found, and it should be able to grow on leaves. In this study we have attempted to assess both ideas. Two different *B. thuringiensis* strains were inoculated to soils, seeds or to bottom leaves of bean plants to check whether they were able to migrate to top leaves, under controlled conditions. In all treatments, *B. thuringiensis* isolates were recovered from leaves but populations were very low (less than 10 cfu/cm^2 of leaf). In addition, the number of *B. thuringiensis* bacteria diminished progressively as leaves were sampled more distant from the soil or from the inoculated leaves. This indicates that *B. thuringiensis* migrates with difficulty from the soil or the seed to the leaves or between leaves of the same plant. Moreover, *B. thuringiensis* colonies did not grow well on BDM, a medium designed to

simulate the nutrient composition of the phylloplane. It seems that *B. thuringiensis* has greater nutrient requirements than other bacterial species clearly natural inhabitants of the bean phylloplane.

BP21

A Mini-Replicon from pBtoxis of *Bacillus thuringiensis* subsp. *israelensis*

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A 2.2-kb fragment containing a replicon from pBtoxis, the large plasmid that encodes the insecticidal endotoxins of *Bacillus thuringiensis* subsp. *israelensis*, was identified, cloned and sequenced. This fragment contains *cis* elements, including iterons, found in replication origins of other large plasmids and suggests that pBtoxis replicates by a type A theta mechanism. Two genes, *pBt156* and *pBt157*, encoding proteins of, respectively, 54.4 kDa and 11.8 kDa, were present in an operon within this mini-replicon, and each was shown by deletion analysis to be essential for replication. The deduced amino acid sequences of the 54.4-kDa and 11.8-kDa proteins showed no substantial homology with known replication (Rep) proteins. However, the 54.4-kDa protein contained a conserved FtsZ domain, and the 11.8 kDa protein contained a helix-turn-helix motif. As FtsZ proteins have known functions in bacterial cell division and the helix-turn-helix motif is present in Rep proteins, it is likely that these proteins function in plasmid replication and partitioning. The mini-replicon had a copy number of 2-3 per chromosome equivalent. A plasmid constructed to synthesize large quantities of the Cry11A and Cry1A endotoxins demonstrated that this mini-replicon can be used to engineer vectors for *cry* and *cyt* gene expression.

BP22

Trisodium citrate influenced productions of thuringiensin, PHB and heat in cultivation of *Bacillus thuringiensis* YBT-032 cells

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Influence of 1.00 g l^{-1} trisodium citrate addition in *Bacillus thuringiensis* YBT-032 batch cultivation on productions of thuringiensin, PHB and heat was investigated. Relative to the control, the addition of trisodium citrate (as a potential source of NADH and/or ATP) significantly stimulated thuringiensin production rate (from 3.68 to 5.13 mg g^{-1} h^{-1}) and transformation of glucose to thuringiensin (from 100.30 to 141.12 mg g^{-1}), but substantially decreased that of PHB production (from 5.93 to 4.07 mg g^{-1} h^{-1}). Precursor supplies of thuringiensin increased from 17.30 to 23.60 mg l^{-1} and that of PHB decreased from 0.076 to 0.029 g l^{-1}. The heat production in thuringiensin synthesis phase increased from 9.60 to 11.56 J. Meanwhile, pyruvate kinase activity decreased from 0.42 to 0.27 U mg^{-1} of protein. The results indicate that trisodium citrate can act as a modulator to adjust the proportion of glucose metabolism in different pathways by energy system of cells and increased the amount of carbon skeletons that can be utilized for adenine compounds biosyntheses. The information can be utilized for fed-batch fermentation with continuing glucose-citrate feed to achieve a high thuringiensin yield.

BP23

Cloning a Novel Crystal Protein Gene from a "non-insectidical" *Bacillus thuringiensis* strain YBT978

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As the implication of finding certain insecticidal or other biological activities from Bacillus thuringiensis strains which were described "non-insecticidal" previously, a novel cry gene, designated cry7Ba1, was cloned and characterized from a "non-insecticidal" Bacillus thuringiensis subsp. huazhongensis strain YBT978. The deduced amino acid sequence of Cry7Ba1 is best homologous with Cry7Ab2, Cry7Ab1 and Cry7Aa1 with the respective identities of 58.2%, 57.9% and 57.1%, especially in the carboxy-terminal half with the identities of 87.9%, 86.9% and 87.9%, respectively.

However, its amino-terminal half, which is best similar with Cry7Ab2, Cry7Ab1 and Cry7Aa1 with the respective identities of 37.1%, 37.0% and 36.4%, showed much lower homology with other known Cry or Cyt proteins. The cry7Ba1 gene was transferred to an acrystalliferous B. thuringiensis CryB. The resulting combinant BMB0502 produced typical bipyramidal crystals with the similar shape and size to that in strain YBT978. Although strain YBT978 was considered to be non-insecticidal by using spore-crystal mixture, the solutions of crystal proteins from YBT978 and BMB0502 showed very high toxicity to Plutella xylostella. These may imply the large possibility to identify and isolate genes encoding novel parasporal inclusion proteins with insecticidal or other valuable biological activities from "non-insecticidal" B. thuringiensis strains.

BP24

Synergism between Thuringiensin and Cry1Aa, Cry1Ac, Cry1B and Cry1C against *Helicoverpa armigera* and *Spodoptera exigua*

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Four kinds of purified Insecticidal Crystal Proteins (Cry1Aa, Cry1Ac, Cry1B and Cry1C) were respectively mixed with thuringiensin at a certain ratio to assess the synergism between them. Our results shown that thuringiensin could widen the insecticidal spectrum of ICPs and shorten the lethal time of target pests. The synergism factor was 2.13 for *H. armigera* when first-instar larvae were fed with the combination of Cry1Aa and thuringiensin at a rate of 8:2 (v/v) and the LT_{50} decrease of 12.8 hours for Cry1Aa; and the counterpart factor was 3.41 when fed with the combination of Cry1Ac and thuringiensin at a rate of 5:5 (v/v) and the LT_{50} decrease of 9.2 hours for Cry1Ac. While, The synergism factor was 2.44 for *S. exigua* when first-instar larvae were fed with the combination of Cry1C and thuringiensin at a rate of 5:5 (v/v) and the LT_{50} were decreased by 11.5 hours for Cry1C. An improved method was used to determine the optimal ratios between ICPs and thuringiensin in this study. All the results could provide a new strategy to improve the application of *Bacillus thuringiensis* preparations in the bio-control of pest.

BP25

Characterization of the replication of plasmid pBMB2062 from the YBT1520 strain of *Bacillus thuringiensis*

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Strain YBT-1520 of *B. thuringiensis* subsp. *kurstaki*, isolated by our laboratory from Chinese soil samples in 1990, displays high toxicity towards lepidopteran pests. The strain contains at least seven indigenous plasmids (Sun et al., 2000) and two plasmids (pBMB67 and pBMB9741) among them have been sequenced previously. In this study, the smallest of these resident plasmids, named pBMB2062, was sequenced and characterized. The plasmid is steady and widespread in *B.thuringiensis* and this 2-kb element carries two ORFs. We proved that ORF1 is necessary for replication, while ORF2 is a putative antitoxin gene. To explore the pBMB2062 potential function as antitoxin of TA module, we collected 500 sequences from TA module belonging to the seven known TA gene families and present an exhaustive search for TA module locate on pBMB67, pBMB9741 and other nine RCR plasmids from *B. thuringiensis* sequenced previously. Based on sequences similarities, we found the small plasmids only contain antitoxin gene. It could confer that the function of pBMB2062 is playing role in reducing the activity of another system toxin in YBT1520 strain.

BP26

Physiological Characterization of Accumulated Poly-β-hydroxybutyrate (PHB) in *Bacillus thuringiensis*

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Poly-β-hydroxybutyrates (PHBs) are accumulated during exponential growth and then utilized very fast during the early stationary phase in *Bacullus thuringiensis* which produce spore and synthesize insecticide proteins in the late life. PHB is deposited as carbon storage and energy in cell. To address

those questions, we inserted an against erythromycin gene within the gene *pha*C, encoded the key gene of PHB biosynthetic enzyme in *Bacillus thuringiensis,* obtained PHB-negative mutant in this study. PHB-negative mutant of *Bacillus thuringiensis* was unable to synthesize PHB and its generation time was longer than the parent strain on Luria-Bertani medium. The ability of vegetative cell and spore of parent strain against UV irradiation and heat was much greater than the mutant strain. Physiological studies showed that the PHB-negative mutant strain excreted more formate, lactate, acetate, pyruvate, β-hydroxybutyrate, fumarate, malate citric acid and glutamine than the parent strain. The NAD^+/NADH and $NADP^+$/NDDPH ratio in the PHB-negative mutant strain was lower than that in the parent strain. When we fermented the parent strain and the mutant strain, the latter produce much less spore and can synthesize insecticide proteins but not form crystal. From those results, we can conclude that the accumulated PHB is important to *Bacillus thuringiensis* significantly when it forms spore and synthesizes crystal insecticide protein.

BP27

Protective Effect of Poly (γ-glutamic acid) on *Bacillus thuringiensis* Active Components Against High Temperature and UV Irradiation

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Poly (γ-glutamic acid)(γ-PGA) could effectively reduce high temperature damage to insecticidal active components of *Bacillus thuringiensis* (*Bt*) preparation during spray drying, and the best protective effect could be obtained by adding 0.3%(w/v) γ-PGA. γ-PGA also could remarkably increase the resistance of *Bt* preparation against ultraviolet (UV) irradiation, after 5h-irradiation, the residual insecticidal activity of *Bt* preparation not containing γ-PGA decreased 77.55%, while the value of the *Bt* preparation containing 0.3%γ-PGA decreased 35.87%. The potted plant experiment suggested that *Bt* preparation containing γ-PGA had longer residual insecticidal time, for its resistance against sunlight irradiating and rainwater scouring was effectively improved through adding γ-PGA.

Key words: poly (γ-glutamic acid), Bacillus thuringiensis, protection, ultraviolet, high temperature

BP28

Study on reconstruction of flexible polypeptide linker about scFv

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ScFv (single-chain F_V antibody fragment) is a small molecule antibody in which the V_H and V_L domains of the antibody are joined by a polypeptide linker. The lengths and sequences of the linker peptide can significantly affect the affinity of scFv. When the linker is > 12 amino acids in length, the scFv usually shows a monovalent antigen binding affinity similar to the Fab fragment of the parent antibody. As the linker shortened to 3-12 amino acids in lengths, the linker of the domains which are too short to allow pairing of the domains on the same chain favor pairing between domains in two adjacent chain. In this way the diabody have produced. When the linker is < 3 amino acids the triabody have produced. The linker we commonly used was $(GGGGS)_3$ peptide. In this study we construct the linker consisting 0, 3, 5, 8, 12, 15, 19 resides to study the effect of different length linkers on scFv affinity, hoping it can be used in medicine and detection. ★Corresponding author. E-mail: wshyyl@sina.com.

BP29 STU

***Spodoptera exigua* selection using a marginally toxic Cry protein provided a wide range of toxin resistance.**

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Spodoptera exigua (Hübner), the beet armyworm, is an important insect pest that is world wide distributed. The beet armyworm has a wide host range, occurring as a serious pest of vegetable, field and flower crops. Products based on *Bacillus thuringiensis* (Bt), which contains more than one Cry toxin, are used for controlling this insect pest. The long-term efficacy of Bt depends on the understanding and managing pest resistance. We have selected a strain of *S. exigua* to assess the

ability to generate resistance to a single Cry toxin. Two laboratory strains of this insect species with little differences on Bt susceptibility were used. The most tolerant one was used to perform the selection experiments and the most susceptible one was used as control. Laboratory continuous selection experiments were carried out with activated Cry1Ab toxin. Regarding to LC_{50} values of the control strain, 25-fold increase in tolerance was achieved after five generations and, after 18 generations, an increase of more than 100-fold was observed. Analysis of genetic and biochemical basis of resistance showed that the resistance was inherited as a recessive and autosomic trait, and it is not based in a midgut degradation. Selected insects exhibited similar resistance levels (more than 100-fold) of cross-resistance to other *S. exigua* highly toxic Cry toxins (Cry1C, Cry1F and Cry1Da). The high Cry1Ab resistance obtained has limited relevance for *S. exigua* control, because of its low toxicity to this insect species. However, the bases of such resistance can be useful as a model for other insect species highly susceptible to Cry1Ab. The unusual cross-resistance to toxins without common receptors with Cry1Ab (Cry1C and Cry1D) indicates the occurrence of a wide resistance mechanism not based in the presently well-described ones (reduced specific binding and alteration of proteolytic process). The cross-resistance detected could be a threat for the Bt control of *S. exigua* pest. The main part of formulated products contains an important amount of Cry1Ab, which can spoil the future use of more optimal toxic Bt toxins.

BP30 STU

Characterization of Mn superoxide dismutase cDNA from *Hyphantria cunea*

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Reactive oxygen species (ROS) is toxic to living organisms, because its high reactivity causes oxidative damage to proteins, nucleic acids, and lipids. Superoxide dismutase (SOD) is an enzyme facilitating the removal of superoxide anions from living organisms. This study focused on the cloning of SOD cDNA from *Hyphantria cunea*. cDNA encoding MnSOD was amplified by RT-PCR from *Hyphantria cunea*. The deduced amino acid sequence of MnSOD from *Hyphantria cunea* indicated that the residues forming Mn binding site are conserved and the sequence showed high homology to that of *Bombyx mori*.

BP31 STU

Transferrin inhibits stress-induced apoptosis in a beetle*

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Transferrin in insects is known as an iron transporter, an antibiotic agent, a vitellogenin, and a juvenile hormone-regulated protein. We show here a novel possible function/role of the insect transferrin. Stresses, such as iron overload, bacterial or fungal challenge, cold or heat shock, wounding, and H_2O_2 or paraquat exposure, cause up-regulation of beetle *Apriona germari* transferrin (*AgTf*) gene in the fat body and epidermis and increase in AgTf protein level. To understand whether *AgTf* is involved in stress response in beetle larvae, we performed the reduction of endogenous *AgTf* mRNA levels in fat body via RNA interference (RNAi). The RNAi-mediated *AgTf* reduction resulted in rapid induction of apoptotic cell death of fat body during exposure to heat stress. The observed effect of *AgTf* RNAi indicates that AgTf inhibits heat stress-induced apoptotic cell death, suggesting a function/role(s) for AgTf in defense/stress responses in a beetle. *This work was supported by the Brain Korea 21 project.

BP32 STU

Plasmid transfer among *Bacillus cereus* group strains within lepidopteran larvae

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The plasmid pHT73 was transferred from *Bacillus thuringiensis* subspecies *kurstaki* KTO to several *B. cereus* group strains in three lepidopteran larvae by conjugation. The results demonstrated that the donor and recipient spores of the *B. cereu*s group strains could germinate and multiply in the infected larvae of *Spodoptera exigua, Plutella xyllostella* and *Helicoverpa armigera*, and that gene transfer took place between *B. thuringiensis* strain KTO and other *B. cereus* strains *in vivo*. The highest transfer ratio reached 5.83×10^{-6} CFU /donor, and the transconjugants gained the ability to produce the insecticidal crystal. These results indicated that horizontal gene transfer among *B. cereus* group strains in susceptible insect larvae might play a key role in the acquisition of extra plasmids and evolution of these strains.

Study on the isolation of endogenous fungus producing anti-termite compounds from *Juniperus virginiana L.* and *Chamaecyparis lawsoniana*（*A.Murr.*）*Parl*

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Termite is one of the five pests worldwide, which caused the economic loss up to several billions each year. Traditionally, chemical agents have been used to kill termites, with such shortcomings as inexact effect, environmental pollution and damages to human and animal health. Other methods such as burrowing or finding pesticide also have different shortcomings. This study aims to develop a kind of low-toxic, non-damaging anti-termite reagent producing from endogenous fungi, thus finding a new method to kill termite. Isolation and identification of the indicating microbes Odontotermies formosanus was sampled from Luojiashan, Wuhan, China. The microbes, which used cellulose or lignin as the only sources of carbon, were isolated from the disinfected *Odontotermies formosanus* abdomen. Through morphological observation of the mycelium, consolidation and morphology of sporum, 18srDNA (or 16srDNA) sequence analysis, these strains were identified as *Penicillum, ssp*, *Fusarium oxysporum*, *Bacillus cereus* and *Paenibacillus polymyxa*. Isolation and identification of the function microbes Branches of *Juniperus virginiana L.* and *Chamaecyparis lawsoniana*（*A.Murr.*）*Parl.* from America were cut into small segments, which were disinfected and washed. Bacteria and fungi were isolated by streak culture. The ferment liquid of the function microbes was collected and concentrated. Bacteriostatic test was carried out on the indicating microbes above by Oxford cup methods using the condensate as bacteriostatics. Through morphological, physiological and biochemical identifications and 16srDNA （or 18srDNA） sequence analysis, the study screened several functional strains with good bacteriostatic effects to the indicating microbes. These strains include bacteria（identified as *Paenibacillus alginolyticus* and *Bacillus cereus*）; fungus（identified as *Aspergillus spp.* and *Penicillium spp.*）. *Anti-termite tests of the functional microbes* The dried products from fermentation liquid containing functional microbes were fed to termites in the test groups. Control groups 1 and 2 were fed with filter paper wetted with 1mL sterilized water and 1mL sterilized liquid culture used for fermenting the functional microbes, respectively. Mortality of the termites was recorded daily. At 10 days, significant differences were found by F test ($P<0.01$, $n=5$). In summary, both kinds of tree endogenic functional fungus fermentation liquids can efficiently kill *Odontotermies formosanus*, suggesting that anti-termite compounds existed in the fermentation liquids. However, more work need to be done on the structure of anti-termite compounds and anti-termite mechanisms. These studies may undoubtedly provide a new way to develop an anti-termite drug with such characteristics as natural, low-toxic, and may be produced industrially.

Location of the *thu* gene responsible for synthesis of extrotoxin (thuringiensin) in *Bacillus thuringiensis* CT43

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Thuringiensin, produced by some strains of *B. thuringiensis*, is a kind of insecticidal activity material belonging to nucleotide analogue with small molecular weight of 701kDa, and it has the characters of wide toxicity spectrum, low-toxicity, heat-stability, slow-degradation and so on. In this research the *thu* gene, which is responsible for synthesis of extrotoxin (thuringiensin), is located in a 150 kb plasmid of *Bacillus thuringiensis* CT43 with high thuringiensin production. A series of mutants of *B. thuringiensis* CT43 were obtained by plasmid elimination experiment. The native plasmid patterns of the mutants mentioned above were detected by pulsed field gel electrophoresis(PFGE), and the HPLC

experiment was conduct to determine whether the mutants produce thuringinsin or not. Taken together, a 150 kb plasmid is predominantly described to be involved in production of thuringiensin. Moreover, we have proved that the *CryIB* gene is also located in this same 150kb plasmid by PCR technique.

Fungi II

FP11

Identification of insect pathogenic fungus, *Cordyceps sphecocephala*, and its cultivation on host insect, drone of honey bee (*Apis mellifera*)

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Cordyceps sphecocephala is a fungal pathogen growing on a dead wasp, having anti-cancer effect according to a recent research result. There are very few investigations on the specie of *C. sphecocephala* and it is difficult to commercialize due to its fastidious characterization. A drone of *Apis mellifera* needs 24 days to develop from an egg to a fully grown male and may live as an adult for several months. Its only function in the bee's community is as a potential mate for a virgin queen and then it usually completes its span or is expelled from the hive in the autumn. Therefore, the apicultural industry has been seeking for the way to utilize the drones economically. The purpose of this study is to produce *C. sphecocephala* by using the drones as host insects and consequently promote their industrial utilization. Perithecia, asci, ascospores, etc. of *C. sphecocephala* were examined to find out its telemorphic characteristic. Colonies of isolates from ascospores grew quite slowly. It was 32 ㎜ diameter when cultured at 25℃ on the potato dextrose agar for 30 days. To confirm the success of isolation, ITS 1, ITS 2 and 5.8 rDNA regions amplified from colony isolated from ascospores. *C. sphecocephala* was able to be proliferated by using honey bee larva and adult bee as host. The fungi can grow on drone larva and adult. They grow best on drone larva under the culture condition of 25℃, 50 days. Under that condition, 30 drones can produce maximal 67mg dry weight. By using the drone of honey bee as host insect, the comparative study results with the conventional cultivation are as follows: the production period of the mycelium is shortened by 8 days, the total cultivation period is shortened by 16 days, the stroma-like stalks is produced, but organ showing maturity like ascus or perithecium is not developed.

FP12

Isolation and PCR-based detection of Entomopathogenic Fungus, *Ascosphaera apis* from honey bee (*Apis mellifera*) larva and their breeding environment

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Chalkbrood of honeybee, *Apis mellifera* is highly infectious disease that is caused by *Ascosphaera apis*, entomopathogenic fungi occurring only honeybee larval stage. The environment of disease occurrence is humid and high temperature in the hives. Dead brood was founded in the combs and on the ground around hives. A rapid and sensitive detection of *Ascosphaera apis* in honeybee larva, hive and their breeding environment were carried by polymerase chain reaction. In the early stage of chalkbrood diseases, the white/grey mass was formed on the surface of larvae. The larvae were soon shrunken to mummy, getting hard, and forming a hard. In the late stage of chalkbrood diseases, the mycelium grew densely, covering the larvae to the extent that it filled the whole cell. When the larvae were infected with one sexual type of *A. apis,* they became white mummies while the grey-black mummies were arisen from invasion by both sexual types. *A. apis* (Aaj623) was heterothallic, mostly grown 57.6 ㎜ in diameter in 7 days on potato dextrose agar. Spore cysts were globose, nearly smooth on outer surface, evenly verrucate on inner surface, 45～95 ㎛ in diameter(average 74 ㎛ diameter). Spore balls were globose, 9～17 ㎛ in diameter (average 12.9 ㎛ in diameter), lacking a conspicuous granular coating. Ascospores were hyaline. The combination of primers of CBP1 and CBP3 was superior in PCR to the other 3 primers applied. Each primer about 20 bases in size has reported as specific primers. PCR products appeared 500bp in size. In case of the detection of *A. apis* from the infected larva, soil, debris and pollen, sensitivity by PCR were higher than that of

microscopic observation.

FP13

Fungi associated with Hemlock Woolly Adelgid, *Adelges tsugae*, and development of the most active isolates for pest control

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The northeastern US forests with a predominance of hemlock trees are in alarming decline due to two exotic Homopteran insect pests, the hemlock woolly adelgid (HWA), *Adelges tsugae* Annand, and the elongate hemlock scale (EHS), *Fiorina externa* Ferris. The insects are rapidly spreading across the eastern seaboard. The woolly cover of HWA provides exceptional defense against insecticides, natural enemies and adverse weather conditions, while the high reproductive rate of insects allow populations to recover quickly even when high annual mortality rates occur. HWA has been present in the USA for around 80 years as an adventitious species remaining in contact with local entomopathogenic and entomophilous microorganisms including fungi. It was interesting to study the activity of local fungi associated with HWA. Since 1996, we collected pathological material from different geographical locations among the HWA infestation and among the numerous isolates recovered, three entomopathogenic species were found; *Beauveria bassiana*, *Lecanicillium lecanii*, and *Paecilomyces farinosus*. Different local and geographically distant strains of four entomopathogenic fungi including *B. bassiana*, *L. lecanii*, *P. farinosus*, and *Metarhizium anisopliae* were tested in laboratory conditions for infection activity to HWA, fungal growth rate, productivity, and mass-production potential. Three of them, including two strains of *L. lecanii* and one strain of *B. bassiana* were selected for field experiments. These strains were used for preparation of special formulations based on oil, whey proteins, and a mixture of oil and whey proteins. The principal goal of the field experiments included establishing the possibility of using ULV equipment for different types of fungal formulations, to study all spray parameters, the conidial viability after application and the level of HWA mortality. The ULV sprayer (Ultrafan MK2, Micron Sprayers Ltd.) provided cover on all sides of the tree branches with the fungal suspensions. Conidia showed a high level of viability in droplets throughout a five week observation period. The level of HWA mortality fluctuated from 42 to 87% depending on the formulation type, with oil formulations being the most effective yielding an insect mortality of 87%. Formulations based on whey proteins and whey and oil had the same level of efficacy, ranging from 42 to 72%.

FP14

Understanding and assessing the complex of fungi impacting the Elongate Hemlock Scale, *Fiorinia externa*, in New England

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During 2003-2006, a study of the dynamics of mycoses manifested among elongate hemlock scale (EHS) populations was conducted throughout regions where strong epizootics were found and associated fungi isolated. Epizootic is accompanied by specific sclerotic formations on the scale surface. Initially several fungal species were isolated from insects with specific sings of the disease and from sclerotic formations. One of these isolations formed the sclerotic masses on media and was able to infect elongate scale. Initially, the fungus was identified as *Hypocrella* sp., however, it was atypical because this genus is the ascigerous, or perfect stage, of the subtropical fungi of genus *Aschersonia*. The fungal cultures and insects with signs of the disease were sent to several specialized scientific institutions in the USA, China, Czech Republic, and Thailand. As a result, four different identifications including *Aschersonia marginata, Fusarium meristomoides, Tubercularia sp., and Myriangium* sp. were received. Finally in our Entomology Research Laboratory this fungus was identified based on DNA analysis as *Colletotrichum acutatum*. This is a very well-known phytopathogen, and there is information about infection activity close species *C. gloeosporioides* to scale in Brazil. Numerous additional fungal isolates receiving from insects with signs of mycoses were identified based on DNA analysis as *Cordiceps* sp., *Nectria vilior, Myriangium duriae, Lecanicillium lecanii, C. acutatum, Phialophora oxyspora*, *Botrytis* sp., *Fusarium* sp., *Rhinocladiella*

sp., and *Cladosporium* sp. All species can have a productive relation with insects, but only some of them are forming the sclerotic formations. It is *C. acutatum, M. duriae, Botrytis* sp. and *Fusarium* sp. Fungus *C. acutatum* is showing the highest level of infection activity but usually inoculated insects and insect cadavers had contained only mycelia without any reproductive morphological structures. This circumstance is not gave possibility to establish action of fungus as real pathogen, or as stress factor serving for activation of other fungal species. Reisolation of fungus from cadavers has given several fungal species including *C. acutatum*, *Rhinocladiella* sp., *Cordiceps* sp., *Botrytis* sp., and sometimes *Myriangium duriae*. The result of mycological analyses, experimental insect inoculations, and pathological examination of insects it is possible to draw a conclusion that strong epizootic in EHS populations were provoked the complex of entomopathogenic and entomophylous fungi.

FP15

Genetic diversity of Japanese isolates of *Verticillium lecanii* (*Lecanicillium* spp.)

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Recently, it was suggested that a major part of the species formerly classified in *Verticillium* sect. *Prostrata*, especially *V. lecanii* and *V. psalliotae*, be transferred to *Lecanicillium* and that *V. lecanii* was subdivided into three species and renamed *L. lecanii*, *L. muscarium*, and *L. longisporum* according to morphological observations and PCR-RFLPs of ITS, mtDNA and β-tubulin (Zare and Gams, 2001). In order to clarify whether *V. lecanii* of mainly Japanese isolates were identified with *Lecanicillium* spp., DNA polymorphism in Bt-1 region of β-tubulin gene of *V. lecanii* isolates from insects, phytopathogenic fungi and other substrates were analyzed by PCR-RFLP. The size of the PCR product from this region was 540bp in all isolates used in this study except for one isolate (560bp). Enzymatic digestions of this region with *Alu* I, *Cfo* I, *Hinf* I, *Hae* III were detected several fragment pattern, reflecting intraspecific variation in this region. All isolates amplified 540bp were recognized same fragment pattern as digested with *Alu* I, *Cfo* I, *Hinf* I, whereas consisted of two pattern as digested with *Hae* III. On the other hand, one isolate of 560bp product were detected unique fragment pattern. These results suggested that the isolates used in this study were belonging to *L. muscarium* and *L. longisporum* except for one isolate which alone could not be belonged to both *Lecanicillium* spp. This result was associated with the previous study that was divided into three groups according to DNA polymorphisms of mitochondrial large subunit rDNA and Bt-2 region of β-tubulin gene, but not ITS, IGS, mitochondrial small subunit of rDNA and histone 4 region (Sugimoto *et al*, 2003).

FP16

Host range of a fungus associated with epizootic in elongate hemlock scale.

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The northeastern US hemlock forests [*Tsuga canadensis* (L.) Carrière] are in alarming decline due to two exotic Homopteran insect pests, the hemlock woolly adelgid, *Adelges tsugae* Annand (Homoptera: Adelgidae) (HWA) and the elongate hemlock scale, *Fiorinia externa* Ferris (Homoptera: Diaspididae) (EHS). In 2002 an epizootic was reported in an EHS population found in the Mianus River Gorge Preserve in Bedford, NY, USA. We investigated this epizootic as it promised to be an opportunity to identify a natural control agent of EHS. Up to 85% of the insects sampled in this area were partially or completely covered with sclerotic formations. We molecularly characterized this fungus, and determined its biology and host range. Phylogenetic analysis showed that the epizootic-causing fungi had 100% similarity with phytopathogenic strains of the widely known genus, *Colletotrichum*. Using five different genes, no genetic variation was seen in several isolates tested from various localities within the region of the epizootic. This lack of variation across the epizootic range, suggests that the epizootic spread from a single focus of dispersion. Fungal host range trials reveal a propensity of the fungus to infect and cause massive mycosis in Homopteran insects. However, endophytic fungal growth, morphologically identical to *Colletotrichum,* was observed in Rosaceae (Morus sp.) and Lauraceae (Sassafras) bushes, and Magnoliaceae (Magnolia and Liriodendron) and Rosaceae trees (apple) present in several areas of the epizootic. In addition, we have also inoculated and endophytically infected a Gramineae species (barley) and a Legumisosae species (bush beans) and biotrophically infected a Rosaceae species (strawberries). Given that this fungal strain is capable of infecting insects and establish mainly endophytic growth in plants, we propose two possible pathways

that could have led to its present day distribution: a) outbreak of a cryptic, previously unreported entomopathogenic strain either already in the environment or introduced with the insect, or b) a new recombinant strain, with a selective advantage towards arthropods rather than plant infection. Currently, this entomopathogenic fungus and the epizootic that it causes appear to be of critical importance in the reduction of EHS on hemlock and its rapid spread.

FP17

Studies on the infective characters of *Nomuraea viridulus*

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The infective characters of a local entomopathogenic fungus, *Nomuraea viridulus* was studied. *Nomuraea viridulus* was able to grow at the temperature ranging from 15 to 35^{o}C in yeast-peptone-dextrose medium. The greatest mycelial growth and sporulation were found at 30^{o}C. The present data was quiet fit the phenomenon as the cadaver was collected in the hot summer. *N. viridulus* was able to grow at the range of nine tested culture media, e.g. Sabouraud's dextrose agar (SDA), Peptone glucose agar (PGAP, Potato dextrose agar (PDA), Yeast extract glucose agar (YEGA), Czapek's-Dox agar (CDA), V8 juice agar, Corn meal agar (CMA), and Beef extract agar (BEA). The greatest mycelial growth was found at MEA, and the highest sporulation was found at PGA. It was demonstrated that conidia of N. *viridulus* produced extracellular enzymes when they grew on selective media, such as gelatin, chitin or Tween 20 media that could be the enzymes responsible for killing the insect. Very low mortalities were shown when this fungus applied to control flea beetle (*Phyllotreta striolata*) and aphis (*Lipaphis erysimi*).

FP18 STU

Thermal adaptation of *Metarhizium anisopliae* strains in association with components of their cell wall hydrophobin-like proteins

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Aerial conidia of *Metarhizium* spp. are active ingredients of fungal formulations against insect pests. Conidia more tolerable to outdoor thermal stress may enhance adaptation of the formulations to more variable insect habitats for better efficacy but this thermotolerance to large degree depends on fungal candidates for conidial production. To search for more thermotolerable candidates, 18 strains of *M. anisopliae*, *M. anisopliae* var. *anisopliae* and *M. anisopliae* var. *acridum* from different host and geographic origins were compared for their conidial tolerances to the thermal stress of 48°C and colony growth rates at 10-35°C. As a consequence, both indices varied greatly among the tested strains. Hydrophobin-like or formic-acid-extractable proteins associated with conidial walls of the strains were found highly diverse in both composition and quantity. Up to nine proteins ranging from 11.7 to 18.4 kDa were distinguished from conidial extracts but only a few were recognized to be possibly involved in thermal adaptation of the fungal strains based on stepwise multivariate correlation of the two indices to the contents of the distinguished proteins. The conidial thermotolerance was associated mainly with three of the proteins (r^2=0.95), including two stimulators (11.7 and 17.5 kDa) and one suppressor (12.6 kDa). However, the 12.6-kDa protein tended to facilitate the colony growth rates at 10-20°C whereas the 11.7-kDa component displayed inverse effects on the growths of the fungal strains at the lower temperatures. The facilitating effect of the 11.7-kDa component on fungal growth diminished as the temperature increased to the optimal 25 or 30°C. Three different proteins (13.4, 17.5 and 18.4 kDa) present in fewer fungal strains were associated significantly with better colony growths at the high temperature of 35°C. The results indicate a complexity in thermal adaptation of the fungal strains but suggest some useful indices for recognizing more thermotolerable strains for microbial control.

FP19 STU

Variable benzimidazole resistance and thermotolerance of *Beauveria bassiana* are associated with mutations of its beta-tubulin sequence

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Fungicide resistance and thermotolerance of fungal biocontrol agents such as *Beauveria bassiana* are of merits for enhancing fungal formulations against insect pests in the field. Among 20 wild *B. bassiana* strains tested in this study, 19 were sensitive or highly sensitive to carbendazim (methyl 2-benzimidazole carbamate), a typical benzimidazole fungicide, despite low resistance associated with one strain. Sequential mutagenesis of a carbendazim-sensitive wild strain (MIC=1.32 mg/ml) under artificial selection pressure generated 11 mutants sharing a common MIC of >1000 mg/ml with no visible variation either in colony growth or in conidiation capacity. This indicates at least 758-fold increase of the resistance among the mutant strains. However, accompanied with the enhanced resistance, all the mutants became less thermotolerable. Stressed at 48°C, conidial LT_{50}s of the mutants varied from 1.8 to 9.6 min and were significantly lower than the parental LT_{50} (36 min). Moreover, the contents of hydrophobin-like proteins in conidial walls declined significantly among the mutants compared to that of the wild parent. Mutations commonly relating to benzimidazole resistance in fungi were located at Q134, F167 and/or E198 around the taxol-binding site of beta-tubulin by sequencing the beta-tubulin of the mutants. However, other 37 amino acid residues in the sequences were also mutated with 1-5 mutated residues associated with each of the sequences. All mutations restricted to the half of beta-tubulin close to alpha-tubulin were diverse in spatial structure and associated with variation of both fungal thermotolerance and fungicide resistance but their interactions were complicated.

FP20 STU

Isolation of ESTs expressed by *Metarhizium anisopliae* in the invasion process of *Plutella xylostella* using RDA

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In this era with pollution, caused by human activity, drastically affects the ecosystems in different ways, the interest to explore new alternatives that permit an integral pest management to diminish the use of chemical pesticides to a minimal level, is increasing. One attractive alternative to chemical pesticides is the use of microorganisms as natural pest enemies. *Metarhizium anisopliae* is a well-known entomopathogenic fungus, used commercially to control several agricultural insect pests, including vectors for human diseases. *Metarhizium anisopliae* is a broad-range insect pathogen but different isolates have different host specificity. In order to discover potential genes involved in this recognition process, a Representational Difference Analysis (RDA) was performed, using cDNA obtained from conidia of *M. anisopliae* strain CARO19, grown in cuticles from *Plutella xylostella* (as specific host) and *Phyllophaga ravida*. 450 clones were obtained, 16 sequences (including sequences with homology to ribosomal proteins, elongations factors, GTP binding proteins, metabolic proteins and putative cell wall proteins) were evaluated by RT-PCR and Northern blot from RNA obtained when conidia from *M. anisopliae* CARO19 were grown in minimal medium with glucose, chitin or cuticle of *P. ravida* or *P. xylostella*. The higher expression is observed when *M. anisopliae* grows in cuticle from the specific host *P. xylostella*. These sequences represent potential genes involved in the recognition process and current experiments are under way to delete some of them, in order to determinate their participation in this complex process.

FP21 STU

Pathogenicity of hybrid strains of *Verticillium lecanii* (*Lecanicillium spp.*) to eggs of the soybean cyst nematode

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It is known that the entomopathogenic fungi *Verticillium lecanii* colonizes eggs, cysts, and/or females of the soybean cyst nematode (*Heterodera glycines*) (Meyer et al., 1990). Two strains of *V. lecanii* (Vertalec and Mycotal) are exploited commercially as useful biological control agents against pest insects. A third strain, B-2, has a high colonizing ability on plant leaf surfaces. These three strains were fused together to obtain 174 useful new hybrid strains (Aiuchi et al., 2004). Some of these hybrid strains reduced *H. glycines* populations in greenhouse studies (Watanabe et al., 2006). In the

present study, nine hybrid strains plus three parental strains were tested on water agar for their pathogenicity to eggs of *H.glycines.* Two hybrid strains AaF17, AaF80 and Mycotal showed >20% parasitism to eggs. AaF23 and BbF17 showed a low degree of parasitism to eggs but these two strains showed high inhibition (85.3% and 84.4%, respectively) of egg hatching. The effect of all fungi on egg hatching wasn't correlated with parasitism to eggs in this study, indicating that other factors may be involved in reducing egg hatching. Hybrid strains among two entomopathogenic strains (Vertalec and Mycotal) showed various pathogenicities to the soybean cyst nematode. They also showed that protoplast fusion could be an effective method for extending the fungal spectrum for biological control. Next, we also tested for activity of fungal culture filtrates against *H.glycines*. Our result showed that the culture filtrates of hybrid strains also have various effects on *H.glycines*. We will discuss a correlation between direct fungal effect and the effect of fungal culture filtrates.

Histopathological studies of spruce budworm infected by *Hirsutella longicolla* and *Toypocladim niveum*

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Determining the causes of the death of the spruce budworm (*Choristoneura fumiferana*) and evaluating their impact on the population dynamic are major objectives of a long-term ecological study at the Canadian forest service. We report here histopathological studies of spruce budworm infected by two entomopathogenic fungi, *Hirsutella lomgicolla* and *Tolypocladim niveum.* Sequential observations of infections of two pathogenic fungi (*H. longicolla* and *T. niveum*) to the spruce budworm were made under the light microscopes. Both pathogenic fungi entered midgut of the larvae by penetrating the cuticle of the larvae, generating spherical hyphal bodies in the hemocoel of the larvae after 3d infection, causing the fat body tissue lyses. The fat body tissues were gone after 10d infection of *H. longicolla.* The difference of is that the fat body tissue lyses more quickly by *H. longicolla* than by *T. niveum.*

Survival of *Beauveria bassiana* on cadavers of *Monochamus alternatus* adults

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Survival of *Beauveria bassiana* on cadavers of *Monochamus alternatus* adults was studied in the field. Adults of *Monochamus alternatus* were inoculated with *B. bassiana* conidia, and the obtainedcadavers with abundant conidia production (average 1.6×10^9/individual) were placed on the ground of a mixed stand of *Pinus densiflora* and *Quercus serrata*. Number of conidia on 5 cadavers were counted every 30 days, and germination rates of the conidia on 5 cadavers were counted every 7 days. Number of conidia on the cadavers declined to 1/10 with average 1.6×10^8 conidia/individual 30 d after setting up of cadavers, and declined to 3.7×10^7 conidia/individual after 60 d. After 90 d, conidial number on the cadavers declined to 1.5×10^6 /individual, which was 0.98% of the beginning and was nearly equal to the number at the time while inoculated. Germination rate of the conidia on cadavers in the stand was more than 97% within 21 days with uniform germination, and its variation coefficient was less than 3%. After 77 d, it kept more than 90%, and its variation coefficient was less than 5%. After 84 d, variation extent of germination rate became higher, and its variation coefficient remained at 7.39-26.31%. After 119 d, the germination rate of survival conidia on cadavers remained 67.2%. No significant variation in conidial germination rate was detected between in soil and on cadavers, and its *t*-test was nearly the same. The result suggested that the cadavers with *Beauveria bassiana* played an important role in maintaining fungus quantity in the stands.

Microbial Control II

MCP14

Characterisation of a *Bacillus thuringiensis* isolate that is highly toxic to *Eldana saccharina* (Lepidoptera: Pyralidae)

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New isolates of *Bacillus thuringiensis* were isolated from the cadavers of *Eldana saccharina* Walker larvae. Spore-crystal and crystals suspensions of isolate SB-4 were highly toxic to *E. saccharina* larvae. Scanning electron microscopy demonstrated that SB-4 produced compound crystals consisting of interlocking bipyramidal and cuboidal crystals. Southern blot analysis showed that the *cry1A* gene was located on two SB-4 plasmids. PCR, using *cry1A*-specific primers designed to amplify the complete *cry1A* gene and the 170 bp preceding the start codon, amplified a fragment with an open reading frame that differed from the *cry1Ac* holotype by four bases. The study showed that SB-4 has potential as a bioinsecticide and that *cry1Ac* genes are promising genes for use in the development of Cry-expressing transgenic sugarcane or recombinant sugarcane-associated bacteria for the control of *E. saccharina.*

MCP15

Experimental use of *Epinotia aporema* granulovirus (EpapGV) in Argentina

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Epinotia aporema (Lep. Tortricidae) is a major pest of legume crops in South America. An indigenous granulovirus (EpapGV) was characterized and the results indicated its potential as a candidate bioinsecticide for the management of *E. aporema*. Towards this end, a formulation of this baculovirus was developed and tested under laboratory conditions, and the registration process was initiated in order to evaluate its performance under field conditions. In addition, to increase the prospective utilization of EpapGV and to design optimized strategies in the management of lepidopteran pests in the main Argentinean soybean area, we tested its interaction with *Anticarsia gemmatalis* nucleopolyhedrovirus (AgMNPV). The ability of granuloviruses to enhance NPVs infection is well documented, and important from a practical point of view. Bioassays were conducted on third instars of *A. gemmatalis*, using the synchronous per oral technique. The LD_{50} value of AgMNPV was estimated at 168 OBs/larvae. Based on this result, three doses of AgMNPV were selected (300, 150 and 50 OBs/larva) and tested alone, in mixture with EpapGV (6000 OBs/larva), and also in combination with 0.1% boric acid. Mortality was recorded daily, and viral identity in dead larvae was determined by PCR using specific primers. An apparently synergistic action was observed in the viral mixtures, in which a decrease of the LT_{50} was verified (*i.e.*, 6.3 days with a dose of 300 AgMNPV OBs/larva *vs.* 5.4 days with 300 AgMNPV + 6000 EpapGV OBs/larva). Similar results were obtained with the combination of AgMNPV and 0,1% boric acid. However, the addition of this component to the mixed viral suspension did not produce any additional effect. These results suggest that EpapGV increases the virulence of AgMNPV, and the use of formulations that include both viruses, might be a valuable tool for pest management in areas with temperate climate, where AgMNPV has a slow speed of action, and also taking into account that *A. gemmatalis* and *E. aporema* are frequently present simultaneously on legume crops.

MCP16

Viability studies for a Brazilian isolate of SfMNPV production in suspension cultures

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In vivo production of viral biopesticides is the major source of viral insecticides currently in the marketplace. However, this system presents limitations during production scale-up. For the *Spodoptera frugiperda nucleopolyhedrovirus* (SfMNPV), the insect used for replication has cannibalistic characteristics, thus production is even more difficult. Besides, liquefaction of the larvae cuticle is very intense and collection of the virus particle becomes a limitation. Insect cells are commonly used for *in vitro* baculovirus production. Most of these cell lines are derived from Lepidoptera species. The Sf21 cell line is derived from *Spodoptera frugiperda* caterpillar ovarian tissue, and its clonal isolate Sf9, have been used for biopesticide production due to their ease of growth in suspension cultures. In this study, the *in vitro* production capabilities of a Brazilian

SfMNPV isolate obtained from cornfields was evaluated. Comparison of polyhedra production was carried out using both Sf21 and Sf9 cells, based on volumetric and specific yields. Both cell lines were cultivated in Hyclone medium supplemented with different fetal bovine serum concentrations (2.5 and 5%). The best results were obtained using Sf9 cells supplemented with 5% serum. These results were further confirmed quantitively through kinetic parameter estimation for both cells lines and different serum concentrations. After seven successive passages, this system still presented high specific polyhedra production.
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MCP17

Kaolinite effect on spouted bed drying process and baculovirus biopesticide hygroscopicity

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Entomopatogenic viruses used as insecticides, especially the ones of the baculovirus group (*Baculoviridae* family, genus Nucleopolyhedrovirus and Granulovirus), have been mentioned as having a high potential for becoming safe and useful pest control agents. Baculoviruses possess characteristics that ideally suit then for use in integrated pest management programs. Commercially, dried biopesticide formulations have an advantage over liquid products for storage and handling because of reduced weight, package size, and storage stability. Viral biopesticides were available in the marked as wettable powders for application as sprays. Spray drying has proved to be the most successful approach for producing stable baculovirus formulations with fine particles. However, high costs are preventing Brazilians industries to use this method for *Anticarsia gemmatalis* nucleopolyhedrovirus formulations. In a previous study it was indicated that spouted bed could be considered a viable alternative for baculovirus biopesticide formulation production. No damage was observed in the dried biopesticide and no significant difference was observed between viral biological activities of dried samples compared with a standard virus. Kaolinite is usually used as carrier in baculovirus biopesticides formulations due its low cost and to improve the produced powder stability. In this work, the influence of kaolinite on the drying process and final product hygroscopic stability was studied. Spouted beds in continuous feed experiments were carried out with different kaolinite proportions. The results indicate that kaolinite presence and higher temperatures produced dryer powders. Efficient formulation of a biological control agent requires small particle sizes. The biopesticide powder obtained after spouted bed drying process presented a high percentage of particles around 29 mm. This condition ensures that the formulation will remain uniformly suspended and free of nozzle-plugging particles in the mixing tank throughout the application period. Equilibrium hygroscopic isotherms were obtained using dynamic method for different kaolinite-baculovirus formulations. Higher kaolinite proportions decrease the powder hygroscopicity showing a Type I curve from BET classification, which is characteristic of microporous solid materials. The best-fitted model was the modified Halsey with a R^2 = 98.62% and relative mean error of 1.78%. On the other hand, low kaolinite formulation presented a higher hycroscopic characteristic and a Type II curve from BET classification that is typical for biological products. Modified Henderson model showed the best fit for this case, with R^2 = 96.97% and relative mean error of 7.18%.

MCP18

The use of a nucleopolyhedrovirus for the suppression of its natural host, the balsam fir sawfly (*Neodiprion abietis* Harris)

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Balsam fir sawfly (BFS) has become a major forest insect pest of balsam fir (*Abies balsamea* (L.) Mill.) in Newfoundland. Western Newfoundland, in particular, is currently experiencing the worst outbreaks of BFS on record. Defoliation caused by BFS can lead to severe reductions in wood volume increment with negative impacts on future supplies of this most important natural resource in the region. The primary natural regulator of BFS populations is a nucleopolyhedrovirus (NeabNPV). NeabNPV has recently been registered with the Pest Management Regulatory Agency (Health Canada)

for the biological suppression of BFS populations under the tradename, Abietiv. An understanding of BFS ecology and NeabNPV-BFS interactions was needed for registration and the efficient use of NeabNPV in aerial spray programs. Laboratory and field studies were conducted to elucidate patterns of NeabNPV dispersal within groups of larvae and at the population level. Field experiments included large-scale aerial applications of NeabNPV. Our results showed that NeabNPV is rapidly communicated between individuals within a larval cohort. NeabNPV can spread hundreds of meters from the point of aerial application within a single season. Significant suppression of balsam fir sawfly populations, with subsequent decreases in defoliation, are most evident in the year following NeabNPV applications. These results are being integrated into the development of NeabNPV aerial application strategies for use against BFS populations.

MCP19

Susceptibility of *Pyrausta sticticalis* to *Bacillus thuringiensis*-based formulations depending on host plant

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The beet web worm, *Pyrausta sticticalis* L., is a serious pest insect damaged to various crops in Russia. Susceptibility of *P. sticticalis* larvae to *Bacillus thuringiensis* (*Bt*)-based formulations registered in Russia was shown in our earlier research. However, some recent data have discovered the significance of tritrophic interactions (plant – phytophagous insect -- biocontrol agent) for microbial insect control. Therefore, the aim of this study was to evaluate the susceptibility of *P.sticticalis* larvae to two *Bt*-based formulations on two species of host plants. Lucerne and carrot were chosen as common plants strongly damaged by the beet web worm. After emerging from eggs, larvae were reared on these plants under laboratory conditions. Larval development was rather quick both on carrot and lucerne (12-14 days). Third instar larvae were exposed to plant treated with suspension of *Bt*-formulation of different concentrations. Lepidocid® containing spores and crystals of *Bt* subsp. *kurstaki* and Bitiplex® based on hydrolysed crystals of the same subspecies were used for insect treatment. Data for corrected mortality showed greater susceptibility of the insect to the Lepidocid® when beet web worm larvae were fed on lucerne compared to larvae fed on carrot. More than 90% larvae fed on lucerne plant died whereas the most part of larvae fed on carrot plant was survived at 3 day after treatment with Lepidocid® (0.5%). LC_{50} for this preparation was 10 times greater when larvae were fed on carrot compared with LC_{50} obtained for larvae fed on lucerne. The influence of host plant on Bitiplex® activity toward beet web worm was almost the same despite this preparation caused the less rate of larval mortality both on carrot and lucerne compared with mortality caused by Lepidocid®. Further research on the effect of host plant on mortality of *Pyrausta sticticalis* larvae induced by *Bacillus thuringiensis*-based formulations is required.

MCP20

Development of PCR-RFLP approach using three chitinase genes for the genetic characterization and identification of *Metarhizium* strains

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Metarhizium anisopliae is an entomopathogenic fungus that infects its hosts by actively penetrating the cuticle using mechanical pressure and enzymic degradation with synergistic action of hydrolases. Hydrolases such as proteases and chitinases are implicated in breaching the host cuticle which is composed of a complex of proteins and chitin. Chitinases from glycohydrolase family 18 belong to Class III and Class V. Class III chitinases possess serine/threonine-rich domain and chitin-binding domain that help in attachment to the fungal cell wall and maybe involved in growth and morphogenesis. Class V chitinases lack the chitin-binding domain and therefore may contribute to the utilization of exogenous chitin as a source of nutrients. Our goal was to clone and sequence chitinase genes of Class III and Class V from *M. anisopliae* to design specific primers and develop a PCR-RFLP approach for characterization and identification of *M. anisopliae* strains. We have isolated and sequenced one Class III (*ChiIII*) and two ClassV (*ChiVa* and *ChiVb*) chitinase genes from two defined *M.anisopliae* strains from Switzerland and India. Specific primers were designed by aligning the obtained sequences to those of different *Metarhizium* species previously isolated. The DNA sequences of *ChiIII*, *ChiVa* and *ChiVb* for the two type strains showed 91%, 98% and 98% identity.

We report for the first time the *ChiVb* chitinase gene in *M.anisopliae* with 70% similarity to *B.bassiana*. Specific primer pairs were designed for each chitinase based on obtained sequences and a PCR-RFLP approach was developed. Based on these chitinases the two strains could be distinguished. The PCR-RFLP method for chitinases may provide a tool for detection and identification of *Metarhizium* strains and to monitor the *M.anisopliae* strains released for biological control purposes.

MCP21

Potential of *Lecanicillium* species for dual microbial control of aphids and the cucumber powdery mildew fungus, *Sphaerotheca fuliginea*

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In vitro detached leaf and leaf disc bioassays were conducted against four species of aphid and the cucumber powdery mildew respectively with three isolates of *Lecanicillium*: *L. longisporum*, *L. attenuatum*, and an unidentified isolate, to determine the possibility of dual microbial control of both aphid and powdery mildew. The *Lecanicillium* species had high virulence against the aphids *Aphis gossypii*, *Aulacorthum solani*, *Macrosiphum euphorbiae* and *Myzus persicae*. The susceptibility of *A. gossypii* and *A. solani* differed according to isolate; LT_{50} on *A. gossypii* by *L. longisporum* (Petch) Vertalec , *L. attenuatum* CS625 and *Lecanicillium* sp. DAOM 198499 was 2.1, 5.5 and 5.4 days, respectively and on *A. solani* 1.9, 3.1 and 6.4 days, respectively. The isolate from Vertalec required only 3 days to incur 100% mortality in *M. euphorbiae* and its LT_{50} on *M. persicae* was 2.4 days. LT_{50} by isolate CS625 was 2.6 days on *M. euphorbiae* and 3.9 days on *M. persicae*. For studies against powdery mildew (*Sphaerotheca fuliginea*), suspensions of conidia and blastospores of the *Lecanicillium* species were applied onto 1.5 cm leaf discs dissected from cucumber plants previously inoculated with *Sphaerotheca* spores. Powdery mildew did not develop when the applications were made 1 and 7 days after *Sphaerotheca* inoculations. When *Lecanicillium* was applied to highly infected leaf discs on days 10 and 14, the application suppressed subsequent production of powdery mildew spores as compared to the controls. These results suggest the potential of a dual role for *Lecanicillium spp.* as microbial control agents against aphids and powdery mildew.

MCP22

Impact of SDS in baculovirus occlusion body purification buffer on biological activity

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Efficacy of a baculovirus as a biological control agent is based on its biological parameters, LD_{50}, LC_{50} or ST_{50}, LT_{50}. Sodium dodecyl sulfate (SDS) is commonly used in concentrations ranging from 0.1-1% to purify the occlusion bodies from baculoviruses-infected dead larvae. Several studies have shown that SDS can disrupt occlusion bodies structural integrity; however, the possible effects of SDS concentrations were not evaluated in terms of viral efficacy. In our study, effects of different SDS concentrations (between 0.1-2%) in occlusion body purification buffer were examined in terms of their effects on baculoviral biological activity parameters. Higher concentrations of SDS resulted in higher LD_{50} and ST_{50} values, indicating that biological parameters of baculoviruses could be negatively affected by higher concentrations of SDS.

MCP23 **STU**

The distribution and expression of chitinolytic enzymes from *Bacillus thuringiensis*

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The combination of chitinase and Bt ICPs has proven to increase the larvicide activity. The purpose of this study was to detect the *Bacillus thuringiensis* strains produced chitinolylic enzymes. 1,001 Bt strains were tested in a medium containing colloidal chitin as the sole carbon source and all of the selected strains could grow at 30℃ over 72 hours. Among them, 542 strains were found to form the clear haloes surrounding the colonies. Moreover，106 strains could form rather big haloes. Chitinase genes of 930 Bt strains could be detected by PCR using specific primers that were designed based on homology to *Bacillus thuringiensis* chitiase. 54 *B.thuringiensis* strains known their serotypes were

analyzed and it suggested that the serotypes and chitinase did not have definite relationships in *B.thuringiensis*. It is well established that chitinolytic enzymes synthesis in most of the chitinase producing bacteria is inducible by chitin or chito-oligosacharidens. In this study, among 43 chitinase producing strains, about 45% Bt strains could constitutively expressed the chitiase at high levels when they were grown in the absence of inducer. Only 14% strains must be induced to express the chitinase.

MCP24 STU

Effects of sublethal nucleopolyhedrovirus infection on the metabolic rate of *Helicoverpa armigera* (Lepidoptera: Noctuidae)

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Nucleopolyhedrovirus (NPV) research has focused on lethal infections in insects and the immediate reduction of pest populations. In recent years, interest in sublethal infections as a means toward long-term suppression of pest populations has increased. Previous studies have shown that individuals surviving NPV infections are often impaired relative to controls. Typical sublethal effects include changes in development time, reduced fecundity, reduced egg viability, and changes in sex ratio. These observations suggest that the mechanisms by which insects overcome sublethal infections are associated with a fitness cost. To determine whether or not fitness costs are reflected in the metabolic rate of infected larvae, the respiration rates of third instar *Helicoverpa armigera* larvae infected with sublethal doses (LD_{25}, LD_{50} or LD_{75}) of *H. armigera* single nucleocapsid NPV were monitored. Respiration rates, measured as the amount of CO_2 produced ($ml.mg^{-1}.h^{-1}$), were recorded daily for 6 days post infection (d.p.i.) using closed-system respirometry. Consistent trends in daily respiration rates in controls, infection survivors and larvae that eventually died were observed throughout the study period. The post-infection respiration rate of LD_{25} or LD_{50} infection survivors did not differ significantly from that of uninfected controls, however the 4 d.p.i. respiration rate of LD_{75} infection survivors was significantly higher than that of uninfected controls. The respiration rate of LD_{50} and LD_{75} infection survivors was significantly higher than that of LD_{25} infection survivors 4 and 5 d.p.i. respectively. For all doses tested, the respiration rates of larvae that eventually died from NPV infection were similar to infection survivors, but significantly lower 4 d.p.i. The results suggest that the metabolic cost associated with the initial period following NPV infection is low, but that a significant metabolic cost is incurred as the infection progresses.

MCP25 STU

Isolation and Characterization of Novel Insecticidal *cry1*-Type Genes from *Bacillus thuringiensis* K1 Strains

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To identify novel *cry1*-type insecticidal genes, 56 lepidopteran-specific *Bacillus thuringiensis* (Bt) strains were isolated from Korea. Universal oligonucleotide primers, K5un2 and K3un2, were designed and used to amplify all known *cry1*-type genes with PCR from 56 Bt isolates. The restriction fragment length polymorphism (RFLP) patterns of the PCR-amplified fragments revealed 7 distinct patterns, and a standard isolate was selected from each one of the RFLP groups. Through cloning and sequence analysis of the PCR-amplified fragments showing unique RFLP pattern, 7 novel *cry1*-type genes were identified. Bt K1 strain was selected to isolate and characterize the active regions of the novel genes, and then 5 genes were cloned from Bt K1. Furthermore, structural and flank regions of these genes were obtained by inverse PCR and their complete open reading frames were detected. In the alignment of the nucleotide sequence with the known *cry* genes, 3 novel genes (*cry1-1, cry1-7* and *cry1-44*) were identified from Bt K1. To verify the activity against lepidopteran larvae, the 5 genes were expressed using baculovirus expression vector system and the bioassay was performed to *Plutella xylostella* (Px) and *Spodoptera exigua* (Se). All novel *cry1*-type genes had high toxicities against Px larvae and *cry1- 1*had dual toxicities against Px and Se larvae. Finally, each novel gene must be a very useful resource for development of microbial insecticide and insect resistant plants.

MCP26 STU

Cloning and heterogeneous expression of a *mel* gene from a wild-type melanin-yielding *Bacillus cereus* strain Bt799

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Tyrosinase is the key enzyme in melanin bio-synthesis process, and the *mel* gene encoding tyrosinase may be used as a biomarker in transcriptional/translational signal screening. *Bacillus cereus* Bt799 is a wild-type melanin-yielding strain that could produce 36 mg/ml melanin on LB medium. Based on the sequence of tyrosinase gene from Bc10987, a 744 bp DNA fragment was amplified by PCR and then sequenced from strain Bt799. Sequence analysis shown that this DNA fragment was identical to that had of tyrosinase gene from Bc10987. The amplified fragment was inserted into an expression vector pET-28(a) and a *Bt-E.coli* shuttle vector pBU4 under a Cry3A promoter, resulting recombinant plasmids pETyr799 and pTD799 respectively. The recombinant plasmids were transferred into *E.coli* BL21 and crystal-minus B. thuringiensis subsp. israelensis (Bti) 4Q7 by electroporation. Induced by IPTG, The recombinant *E.coli* could produce melanin at the presence of L-tyrosine, which verified the *mel* gene function. The recombinant Bti 4Q-7 strain exhibited melanin positive phenotype on both casein medium and LB medium. SDS-PAGE and native-PAGE revealed that the 28kD tyrosinase enzyme proteins were expressed in high level and its catalyst melanin could be synthesized up to 16 mg/ml. The establishment of heterogeneous expression system of tyrosinase in G- and G+ bacterial enabled industrial enzyme production and opened further applications area for melanin and tyrosinase.

Key words: Tyrosinase, Melanin, *Bacillus cereus*, Cloning, Heterogeneous expression.

MCP27

Study on the Bioactivities of Plant Extracts against *Lasioderma serricorne*

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The bioactivities of 4 solvent (acetone, ethanol, ethyl acetate and petroleum ether) extracts from 21 kinds of plants against the adult of Lasioderma serricorne were determined. The result showed that the extracts from AGS、CTM、CMI、SJA、ACS、AKI、ACI and TFG among 21 kinds of plants had better contact toxicity with mean corrected mortality between 80% and 100%. The 4 solvent (acetone, ethanol, Ethyl acetate and Petroleum ether) extracts from AGS had best contact toxicity with LC50 1.0926mg/ml、0.3131mg/ml、0.3366mg/ml and 0.1935mg/ml. The active ingredient of the AGS extract was determined as α-asaron by column chromatography、thin layer chromatography and GC-MS, respectively. * Corresponding Author: Laboratory of Insect Microbiology, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, P. R. China. Tel: +86-27-87286968; E-mail: jianhl@mail.hzau.edu.cn

Identification of an Insect Intestinal Mucin from the Lepidopteran Peritrophic Membrane of *Helicoverpa armigera*

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In insects, the gut epithelium is generally lined by a unique protein-chitin structure, the peritrophic membrane (also known as peritrophic matrix)(PM).Functions of the PM include facilitation of effient food digestion, protection of the gut epithelialsurface, prevention of microbial infections, and sequestering dietary toxins and chemical pesticides.The importance of the PM in interactions of insects with plant insecticidal compounds, synthetic chemical pesticids and micribial pathogens has been broadly documented. Helicoverpa amigera, cotton boll worm, is a worldwide pest, therefore a better understanding of the molecular basis for the structure and physiological function of the PM will be expectation to improve the efficiency of bio-insecticides.

A cDNA library was consructed from H. amigera midgut mRNA using a Smart cDNA library Construction Kit following the manufacture's instructions. An anti-serum, against Trichoplusia ni insect intestinal mucin(IIM), was used to screen a cDNA encoding PM protiens. A cDNA of 1256 nts in length deducing a protein of 363 amino acids was obtained. The putative protien, Ha IIM, had high

identity with T. ni IIM and contained cysteine-rich regions similar to chitin-binding domains. Furthermore, putative polyadenylation signal sequence, AATAAA, was present 1193 nt downstream of the translational stop codon. Also, other nucleotide sequences of Ha IIM gene will be obtained.

Nematodes

NP1

Gene clone of insecticidal protein from *Xenorhabdus nematophila* HB310

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The genome cosmid library was constructed in order to clone insecticidal genes from *X. nematophila* HB310 strain. The two clones with oral activity against neonate *H. armigera* larvae were obtained after screening 562 *E. coli* clones by the bioassay. The two cosmid clones were given the numbers pWEB216 and pWEB338. pWEB216 had higher toxicity than pWEB338. LC_{50} level of pWEB216 clone to *H. armigera* neonates was $9.5x10^{10}$ cells/mL. In order to identify the toxin genes presented in pWEB216 and pWEB338, 9 pairs of primers were designed according to the insecticidal toxin genes of *X. nematophila* PMFI296 in GenBank to PCR amplification of toxin gene fragments from these clones. Nine fragments and six fragments were obtained from pWEB216 and pWEB338 respectively. Online BLAST analysis showed that the sequences of pWEB216 PCR products had 95%~100% homology to those toxin genes of *X. nematophila* PMFI296, BP and TccC1 strains.

NP2

Insecticidal activity and midgut histopathological effects of *Xenorhabdus nematophila* on *Pontia daplidice*

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Xenorhabdus nematophilus HB310 is the insect pathogen that is mutualistic with entomopathogenic nematode *Steinernema carpocapsae*. *X. nematophilus* HB310 showed high oral insecticidal activity against *Pontia daplidice*. The bacteria broth (5.994×10^{8}cells/mL) killed 100% *P. daplidice* first-instar larvae within 72 h. LC_{50} level against *P. daplidice* first-instar larvae was determined to be 2.7418×10^{6} cells/mL. The larvae also showed strongly repellent activity.The antifeeding rate of 3th instar larvae was 81. 02 % in selective test and 93.16 % in non- selective test at 48h. The symptoms showed that the histopathology of the *P. daplidice* midgut was similar to that of other novel midgut-active toxins such as the δ-endotoxins from *Bacillus thuringiensis*, as well as Tca from *Photorhabdus luminescens* W14. The mid-gut cells were serious damaged at 60h after exposed the toxin-treated food and at least destroyed completely after 72h .

NP3

Insecticidal activity of the toxins from entomopathogenic nematode symbiotic bacteria

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Bacteria of the genera *Xenorhabdus* and *Photorhabdus* have a mutual symbiotic relationship with entomopathogenic nematodes (EPN) of the families Steinernematidae and Heterorhabditidae, respectively. The bacteria play an important role in the pathogenicity of the nematode-bacteria complex to their insect hosts by producing insecticidal toxins and other bioactive compounds. It was found that some toxins of EPN symbiotic bacteria could effectively kill a wide range of insects. 152 strains of symbiotic bacteria were isolated from 28 strains of entomopathogenic nematodes. Bioassays showed the insecticidal activities vary according species and isolates. The highly virulent strains is selected through a large number of bioassays. The insecticidal toxins of the 28 highly virulent strains were isolated primarily. Bioassay results showed that all crude extract had highly virulence to *Galleria mellonella*，*Ostrinia furnacalis*，*Plutella xylostella*，*Mythimna separata*， *Laphygma exigua* and *Tenebrio molitor*. Bioassays showed that the oral insecticidal activity of Ln5 strain was higher than others. Partial 16S rDNA genes sequences of 23 isolates and 5 reference strains of the genera

Xenorhabdus and *Photorhabdus* were determined by direct sequencing of PCR products. Aligned sequences against those from described species were subjected to phylogenetic analysis by DNAstar software. The homology of 16S rDNA between Ln 5 and *X. pionarii* was 99.7%. This bacteria may be new weapon for insect pest control.

NP4 **STU**

The application of *Ovomermis sinensis* in cooperation with Bt (*Bacillus thurigiensis*) to the control of *Helicoverpa armigera*

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This thesis analyses the application of *Ovomermis sinensis* in cooperation with Bt to the control of *Helicoverpa armigera* The results revealed that when the concentration of Bt was 1 mg/ml, in the third day of the experiment the death rates of *Helicoverpa armigera* were both zero by using *Ovomermis sinensis* or Bt respectively, but when using them both, the death rate of *Helicoverpa armigera* came up to 35%. And when the concentration of Bt was 3 mg/ml , the death rates of *Helicoverpa armigera* came up to 75% by using Bt respectively compared to 40% by using them both. As it known, if the concentration of Bt is too much, the insects will have resistance to Bt. The results indicated that the control effect of *Helicoverpa armigera* by using *Ovomermis sinensis* and Bt together was apparently better than by using them respectively when the concentration of Bt was right.

Viruses II

VP18

Genome sequence and genome organization analyses of *Trichoplusia ni ascovirus 2c* (*Ascoviridae*)

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The complete *Trichoplusia ni* ascovirus 2c (TnAv-2c) genome was sequenced. The TnAV-2c has a circular genome containing 174,059 base pairs (bp) with 165 open reading frames (ORFs) of greater than 180 bp and two major homologous regions (*hrs*). The base composition of the genomic DNA is 64.6% A+T and 35.4% G+C. Fifty-four ORFs of TnAV-2c had homologues in other insect viruses, such as ascovirus, iridovirus, baculovirus, and entomopoxvirus; thirty ORFs showed low homology with that from different parasitic protozoa and twelve ORFs were unique to TnAV-2c. One of the most important features is that TnAv-2c has 15 ORFs which could be grouped into six gene families. Repetitive sequences are also a major feature of the TnAV-2c genome. Three major conserved repeat fragments were identified and those fragments were interspersed in two regions. One repeat contained a perfect palindrome (TTTTGTCGCGACAAAA), and this palindrome repeated ten times in the *hr1* and *hr2*. BLAST analysis revealed that there were 16 enzymes involved in gene transcription, DNA replication, and nucleotide metabolism in the TnAV-2c genome, and most of them had high amino acid sequence identities with their homologues in other insect viruses. In addition to the 12 ORFs which showed significant homologies to *Spodoptera frugiperda* ascovirus 1a (SfAV-1a), twenty five ORFs shared the highest homologies with iridovirus homologues. Sequence analysis showed that the codon usage bias appears to be similar to SfAV-1a other than to iridovirus.

VP19

Presence of nuclear polyhedrosis virus in *Neodiprion sertifer* populations in Latvia

Liga Jankevica

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The European pine sawfly *Neodiprion sertifer* (Geoffr.) is often present in the pine stands in western part of Latvia. Since 1908 outbreaks of *N. sertifer* are recorded regularly after 10 - 12 years in the same region of Kurland. Nuclear polyhedrosis viruses (NPV) were isolated from diseased *N. sertifer* larvae in 1970. Ns NPV Latvian isolate was used as a source of the virus preparation applied to control the populations of the European pine sawfly in 1991 and 1992. Laboratory of Experimental Entomology either has been studying the NPVs persistence and possibilities for prolonged regulation

of insect populations. We have studied the occurrence of viral infections in *N. sertifer* populations. The aim of our studies was to observe *N. sertifer* populations, to search for Ns NPV and to increase knowledge concerning their occurrence and environmental effects. In 2005 we observed territories covered by outbreak of *N. sertifer.* We recorded high level of defoliation in inspected pine stands. Presence of NPV was observed in primary regions the outbreak. Approximately 20% of all colonies were destroyed by NPV. This work has been financially supported by the grants from the Latvian Council of Sciences.

VP20

Choristoneura fumiferana defective nucleopolyhedrovirus spindlin is a superior model for studying baculovirus GP37-type proteins

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The spindlin protein (GP50) of *Choristoneura fumiferana* defective nucleopolyhedrovirus (CfDEFNPV) is encoded by the *gp50* gene. The protein, GP50, exhibits high identity with GP37 proteins from the other baculoviruses and a distant similarity to entomopoxvirus fusolin and bacterial chitin-binding proteins. The gene encoding spindlin is present in most baculoviruses and is rarely expressed as heavily as in CfDEFNPV. The propagation of CfDEFNPV in various cell lines indicated that Cf70, Sf21 and High-5 cell lines were permissive for CfDEFNPV replication. Virus growth kinetics and spindlin production demonstrated that vDEF.GFP (green fluorescent protein GFP gene replacing of CfDEFNPV *polh*) grew faster and produced more spindlin in High-5 cell line than in Cf70 and Sf21 cell lines although the titers in the three cell lines were similar 6 days postinfection. CfDEFNPV spindles were over produced exclusively in the cytoplasm of infected cells as bipyramidal inclusion bodies. The size of the mature spindles was variable in different cell lines. CfDEFNPV spindle did not occlude virions and consisted of a pure crystal. It accumulated in the endoplasmic reticulum near the nuclear envelope and appeared surrounded by a membrane of smooth endoplasmic reticulum. At least two peptides of Ca.39 kDa and Ca.31 kDa were produced when spindles were solublized in an alkaline carbonate buffer. *In vitro* chitin binding assay analysis results showed that solublized spindlin with its two degradations was efficiently bound to chitin. Gut juices treatment of CfDEFNPV spindlin revealed that CfDEFNPV spindlin was cleaved into at least 2 fragments of 39 kDa and 31 kDa by neat *Bombyx mori* gut juice and no cleavage by *C. fumiferana*, *C. occidentalis* and *Trichoplusia ni* gut juices. *B. mori* gut juice cleavage patterns were similar to those treated with carbonate buffer. The interaction of CfDEFNPV spindlin and peritrophic membranes (PMs) from various insect hosts was tested. There was no altered protein profile between spindlin treated PMs and control PMs.

VP21

Sequence analysis of the genome of _Maruca vitrata_ multicapsid nucleopolyhedrovirus (MaviNPV)

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The complete genome sequence of a multicapsid nucleopolyhedrovirus isolated from *Maruca vitrata* (MaviNPV) was determined and analyzed. The viral genome was 111,953 bp long with an overall G+C content of 39% and contained 126 open reading frames (ORFs) encoding predicted proteins of over 50 amino acids. The genome organization of MaviNPV was closely related with that of *Autographa californica* MNPV (AcMNPV) or *Bombyx mori* NPV (BmNPV). Except the ORF (mavi74) was not found in AcMNPV and the two ORFs (mavi74 and mavi108) were missing in BmNPV, all of the other MaviNPV ORFs have homologues both in the AcMNPV and BmNPV genome. The MaviNPV genome lacks *bro* (baculovirus repeat ORF) genes that are found in AcMNPV and BmNPV. Five homologous regions (*hrs*) were located within the MaviNPV genome, each containing small imperfect palindromes embedded within direct repeats. Based on genomic structure, MaviNPV is a new species of the genus NPV.

VP22

Replication of _Bombyx mori_ nucleopolyhedrovirus in nonpermissive insect cell lines

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The susceptibility of cells from *Spodoptera frugiperda* (Sf9 and Sf21), *Trichoplusia ni* (Hi5) and *S. exigua* (Se301) to *Bombyx mori* nucleopolyhedrovirus (BmNPV) was examined. Even though these cells were usually considered nonpermissive to BmNPV, we could observe cytopathic effects, increase in virus yield and viral DNA synthesis by BmNPV in Sf9, Sf21 and Hi5 cells. The very late gene expression of BmNPV in these cell lines was also detected through the expression of b-galactosidase gene under the control of the polyhedrin promoter. Sf9 cells were most susceptible to BmNPV in all aspects, followed by Sf21 and Hi5 cells in decreasing order, while Se301 cells failed to show a distinct virus replication. This particular difference of virus susceptibility in each cell line may be utilized in future studies for understanding the mechanism of host specificity of NPVs.

VP23

Sequence analysis on the genome of the *Chroristoneura biennis* entomopoxvirus

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The two-year-cycle spruce budworm, *Choristoneura biennis*, harbours a number of viruses including an entomopoxvirus (CbEPV) that has been previously isolated and characterized with respect to a number of its genes and proteins. The viral genome is approximately 280 kb in size and, typical of EPVs, contains approximately 80% A+T residues. The genome was nebulized, cloned and partially sequenced. To date, contigs that cover in excess of 60% of the total genome were generated. Almost all the genes related to functions such as DNA replication, repair, nucleotide metabolism, transcription, RNA modification and protein modification that have been previously found in the *Amsacta moorei* entomopoxvirus (AmEPV) have homologues in the CbEPV genome. Indeed, the sequence so far clearly indicates that the CbEPV genome is closely resembles that of AmEPV in terms of sequence identity and gene organization. A homologue of the baculovirus inhibitor of apoptosis, p35, was also found in the CbEPV genome. Homologues of certain open reading frames, such as MSV127 of the *Melanoplus sanguinipes* entomopoxvirus were found in the genome of CbEPV but not in AmEPV. All of the 6 gene families reported AmEPV had homologues in CbEPV. The 17K ORF family, which contains the KilA-N domain (conserved DNA-binding domain) has 6 members in CbEPV (5 in AmEPV); MTG (methionine-threonine-glycine) motif gene family has 6 members in CbEPV (3 in AmEPV); ALI-like (alanine-leucine-isoleucine) gene family has 5 members in CbEPV (5 in AmEPV). One copy of each of the AMV176 gene family (unknown function), the tryptophan repeat gene family and the LRR (leucine-rich repeat) gene family has been located in the CbEPV genome.

VP24

Characterization of a new baculovirus isolated from *Iragoides fasciata* and its infection of TN-5B1-4 cell line

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The tea slug moth *Iragoides ragoides* is one of the main pests attacking tea bushes. A nucleopolyhedrovirus (NPV) newly isolated from the diseased larva of *Iragoides ragoides* was characterized. Electron microscopic observations showed that *Iragoides fasciata* NPV (IrfaNPV) was a single nucleocapsid type virus. An *Eco*R I/*Xho* I 11.6kb insertion clone containing the polyhedrin gene was sequenced. Using the amino acid sequences of 58 baculovirus polyhedrin genes deposited in the GenBank , the phylogenetic relationship of IrfaNPV polyhedrin, lef2, lef11, odv-e56, ORF1629, pk-1 and ptp with other baculoviruses was analyzed. The phylogenetic tree showed that IrfaNPV was a member of the GroupI NPVs and was most closely related to AcMNPV, BmNPV and RaouMNPV. The proliferation of IrfaNPV in the non-host cell line Tn-5B1-4 was investigated, using a fluorescence Quantitative PCR assay. The results showed that IrfaNPV could infect the TN-5B1-4 cells and form BVs, which could normally release to the medium. But the IrfaNPV polyhedra could not be normally formed in the nucleus and the number of BVs in the medium was very low, only as 1/1140 high as that of AcMNPV BVs. (Author for correspondence: Chuan-Xi Zhang, chxzhang@zju.edu.cn

VP25

Salivary Gland Hyperplasia Virus of the House Fly, *Musca domestica* (Diptera: Muscidae)

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A virus causing salivary gland hyperplasia (SGH) has been detected in house fly populations in northern Florida. This non-occluded, rod-shaped, double-stranded DNA virus had a genome size of approx. 137 kbp. A field survey conducted in 2005 and 2006 examined the incidence of the SGH virus in feral house fly populations at three dairy farms in Florida. Infection rates were highest (maximum, 34%) during periods of peak fly abundance. Transmission tests demonstrated that healthy flies became infected when presented with food and water dishes from cages housing infected flies; flies also became infected after being placed in cages formerly occupied by infected flies. Laboratory experiments have investigated the impact of viral infection on house fly fitness and reproduction. Compared with healthy females, infected females laid significantly fewer eggs, and dissections revealed a suppression of ovarian development in these flies. Female flies that became infected as young flies did not mate or develop their ovaries. Ongoing studies have incorporated the SGH virus into bait systems in an attempt to deliver the SGH virus into healthy house fly populations.

VP26

Ascertaining the efficiency of granulovirus based bio-pesticides in *Cydia pomonella* and *Adoxophyes orana* control, using PCR based techniques

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Polymerase chain reaction (PCR) based techniques were developed for the detection of *Adoxophyes orana granulovirus* (AdorGV) and *Cydia pomonella granulovirus* (CpGV) based on granulin gene. Specific primers were used to detection both AdorGV and CpGV. Specificity of the virus detection methods were confirmed by PCR with generic primers flowed by cleavage PCR amplicon with *EcoRI*. The reliability of PCR based detection was again confer with sequencing of PCR amplicons and their analysis. The biological efficiency of AdorGV (Capex® -II, Andermatt Biocontrol, Switzerland) and CpGV (formulated in ZD Chelčice, CR) based microbial agents were evaluated in *A. orana* and *C. pomonella* control, respectively. The virus based bio-pesticides were directly tested in apple orchards around the Czech Republic. The mortality of the larvae and fruit damage were evaluated in treated orchards, in the year of virus treatment and the years following. The reduction of population density of *A. orana* ranged from 40% to 80% after the direct treatment with Capex® -II. Within the two subsequent years, the population density of *A. orana* was considerably reduced in all tested apple orchards. The efficiency of CpGV based preparation in the CpGV-treated orchards were varied according to locality. The fruit injury (at harvest) in CpGV-treated orchards ranged from 1.6% to 28.5% and number of larvae per tree ranged from 1.8 to 17.1. The viruses were tested by PCR (maintain above) on the survival larvae of *A. orana* (tested AdorGV) and *C. pomonella* (tested CpGV) after the virus treatment and as well as the subsequent years. The high level of AdroGV persistence was recorded in surviving larvae after direct treatment by AdroGV causing high mortality of larvae even in next generations. The population density was reduced by AdroGV under damage threshold during two years after the virus treatment. In contrast to AdorGV, the persistence of CpGV among the *C. pomonella* individuals surviving CpGV treatment was low in CpGV-treated orchards in first year of treatment. CpGV was not detected in *C. pomonella* larvae in subsequent years after virus treatment. From our results, it could be concluded that both garanulovirus based bio-agents are an effective means of pest control, and the virus persistence in survival larvae in particular has a supplementary benefit in this approach.

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VP27

Promoter Analysis of *Bombyx mori* Nucleopolyhedrovirus Ubiquitin Gene

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Ubiquitin is a small eukaryotic protein involved in a number of basic cellular processes. In this study, transcription and promoter analysis of BmNPV *ubiquitin* gene were carried out to understand more

fully the regulation of the gene expression. Multiple transcripts encompassing ubiquitin were detected by Northern blot analysis. To define the nucleotide sequences that regulate transcription of the gene, a series of mutations was generated by progressive deletion or single mutation in the nucleotide sequence upstream ATG. In the presence of viral factors, the remarkable reduction in the promoter activity was observed when deleted –382 to –124 bp upstream of ATG, the result showed that – 382 to – 124 bp upstream of ATG was required and sufficient for the promoter activity. Mutations of TATA box could reduce the promoter activity distinctly. While CAAT mutation could increase the promoter activity. The TAAG mutation has different effect on the promoter activity, the promoter activity were weakened when mutation of TAAG near to the ATG occurred, but the distal TAAG mutation can increase the promoter activity and make the promoter transcription independent of viral factors. The reporter gene can express in both *Bombyx mori* cells and *Spodoptera frugiperda* cells, which drove by the distal TAAG mutated promoter. Although ubiquitin from AcMNPV or BmNPV has very high identity, in the long evolution process, they formed different transcriptional regulation mechanism to escape the regulation of host ubiquitin transcription signal.

VP28 STU

Identification of baculovirus genes for activation of the *hhi1* promoter of Hz-1 virus

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Hz-1 virus is a so far unclassified virus mainly infects Lepidopteran insects. Genome analysis suggested that it is evolutionarily distant from the *Baculoviridae* (Cheng et al., J. Virol. 2002. 76: 9024-9034). A 6.2kb early gene, *hhi-1*, located in *Hind* III-1 fragment, reaches the highest expression at 2 hpi of Hz-1 virus infection. Our first experiments showed that the infection of AcMNPV could activate the expression of *hhi-1* promoter. To identify potential transactivators located on AcMNPV genome for such activation, we have cloned five reporter plasmids each containing progressively increasing upstream regulatory region of *hhi-1* promoter and performed temporal expression analysis with AcMNPV complete genome. Our result demonstrated that the highest activated area was located from the -62 to +277 nucleotide of *hhi-1* promoter. We also searched the entire 130kb AcMNPV genome and identified that there are two baculovirus genes *orf 147*(ie1) and *orf 135* (p35), which are responsible for the activation the *hhi-1* promoter. By using a DNA chip, we have also identified two, and only two, baculoviral genes which could be similarly co-activated by *orf 147* and *orf 135*. These results indicated that there is a unique controlling mechanism, cooperated by *orf 147* and *orf 135 in baculovirus, for the activation of specific down-stream genes*.

VP29 STU

Cloning of a gene encoding *Lymantria xylina* nucleopolyhedrovirus fusion protein and its expression in LD cells

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IPLB LD-652Y-5d cells infected with *Lymantria xylina* multiple nucleopolyhedrovirus (LyxyMNPV) were found that the infection cells were fused together and formed syncytial giant cells. This phenomenon is probably caused by the viral envelope fusion protein in the cell membrane of the infected cells. The viral proteins, GP64 and LD130, of NPVs mediate the cell fusion at low pH value had been reported. The envelope fusion protein of NPV Group I is GP64 while Group II is LD130. The LyxyNPV *ld130* had been cloned and sequenced, this gene consists of 2,025 bp. Comparison of the nucleotide and amino acid sequences of LyxyMNPV *ld130* with those of LdMNPV *ld130* showed 93 and 96% identities, respectively. Therefore, LyxyMNPV belongs to Group II. The LD130 of budded virus detected by Western blot revealed that LD130 is a viral structural protein. The predicted peptide sequence of LyxyMNPV LD130 contains signal and transmembrane domain. Analysis of LyxyNPV *ld130* transcript revealed both early and late transcriptional initiation sites located at upstream of the 5' ATG initiation codon. In addition, a poly A sequence located at 17 nt downstream of the 3' polyadenylation signal (ATTAAA). Based on the sequences of *ld130* gene, The LD130 were constructed with the promoter of immediately early gene and expressed by baculovirus transient expression vector in uninfected insect cells showed that LD130 could mediate the cell-to-cell fusion at

low pH. These results suggest that a functional homolog of LD130 envelope fusion protein in group II NPVs was found in LyxyMNPV.

VP30 STU

Influence of fetal bovine serum on the growth of insect cell cultures and baculovirus

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To determine the optimal condition of fetal bovine serum (FBS) on the growth of insect cells and the multiplicity of baculovirus, we tested it using three cell lines and two baculoviruses on the various concentrations of FBS. The growth of tested all cell cultures was higher in proportion to the concentration of FBS. In view of the time of subculture and the amount of FBS, we concluded that the most proper concentration of FBS is 7% for *Bombyx mori* (Bm5) and *Spodoptera frugiperda* (Sf21) and 10% for *S. exigua* (Se301) cells. The multiplicity of baculovirus, recombinant *Autographa californica* nucleopolyhedrovirus (AcNPV) in Sf21 cells and recombinant *B. mori* NPV (BmNPV) in Bm5 cells, were similar in both viruses after 3 days post-infection (p.i.). However, the multiplicity of both viruses showed the significant difference by the concentrations of FBS at 2 and 3 days p.i. The proper concentration of FBS for the multiplicity were 10% and 3% for recombinant AcNPV (BacPAK6) at 2 and 3 days, respectively, and 5% for recombinant BmNPV (BmK1-LacZ) at both 2 and 3 days. These results suggested that the optimal concentration of FBS should be determined by the used cell lines and viruses for their optimum production.

VP31

***Spodoptera litura* multicapsid nucleopolyhedrovirus inhibits *Microplitis bicoloratus* polydnavirus-induced host granulocytes apoptosis**

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Baculoviruses and parasitoids are critically important biological control agents in integrated pest management (IPM). They have been simultaneously and sequentially used to target insect pests. In this study, we examined the impacts of both baculovirus and polydnavirus (PDV) infection on the host cellular immune response. Larvae of the lepidopteran *Spodoptera litura* were infected by *S. litura* multicapsid nucleopolyhedrovirus (SpltMNPV) and then the animals were parasitized by the braconid wasp *Microplitis bicoloratus*. The fate of the parasitoids in the dually infected hosts was followed and encapsulation of *M. bicoloratus* first instar larvae was observed. Hemocytes of *S. litura* larvae underwent apoptosis in naturally parasitized hosts and in nonparasitized larvae after injection of *M. bicoloratus* ovarian calyx fluid (containing MbPDV) plus venom (CFPV). However, assessments of the percentages of cells undergoing apoptosis under different treatments indicated that SpltMNPV could inhibit MbPDV-induced apoptosis in hemocytes when hosts were first injected with SpltMNPV budded virus (BV) followed by injection with *M. bicoloratus* CFPV. As the time of injection with SpltMNPV BV increased, the percentages of apoptosis in hemocytes population declined. Furthermore, *in vitro*, the percentages of apoptosis showed that SpltMNPV BV could inhibit MbPDV-induced granulocytes apoptosis. The occurrence of MbPDV-induced host granulocytes apoptosis was inhibited in the dually infected hosts. As hemocytes apoptosis causes host immunosuppression, the parasitoids are normally protected from the host immune system. However, in larvae infected with both baculovirus and PDV, the parasitoids underwent encapsulation in the host hemocoel. This might have implications for concurrent application of the virus and the parasitoid in the field. Although our findings demonstrated that SpltMNPV could inhibit MbPDV-induced apoptosis in *S. litura* granulocytes, what MbPDV gene(s) are responsible for inducing apoptosis in host hemocytes remains to be discovered. This gene and the one caspase inhibitor encoded by SpltMNPV, *p49*, will requires further research using Hi5 cells, a hemocyte-like cell line, and an *in vitro* approach to understand the mechanisms of anti-apoptosis and apoptosis in both baculovirus and polydnavirus.

VP32 STU

Expression of a *Microplitis bicoloratus* polydnavirus-encoded protein causes disruption of actin cytoskeleton in lepidopteran insect cells

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Microplitis bicoloratus is a braconid endoparasitic wasp associated with the polydnavirus of the bracovirus family named MbBV. Parasitism of *Spodoptera litura* larvae leads to an impaired cellular immune response and to absence of 42kDa actin in host hemocytes. In this work, we investigated if the absence of actin in blood cells was related to MbBV infection. A MbBV gene similar to *egf-like* genes identified in another bracovirus was partially cloned. This gene, named *Mbcrp*, is transcribed throughout the course of parasitism in host hemocytes and the 30kDa MbCRP protein was detected in hemocytes 6-7days after parasitization. The *Mbcrp* gene contains a cysteine-rich trypsin inhibitor-like (TIL) domain and expression of recombinant TIL domain inhibited expression of the 42kDa actin in *Trichoplusia ni* Hi5 cells. The 34.1kDa TIL-GFP fusion protein was located specifically in the cytoplasm. These results suggest that expression of MbCRP in lepidoptera insect cells is related to disruption of actin cytoskeleton .Further studies would be necessary to investigate whether MbCRP protein is an actin-binding protein or not through protein-protein interactions.

VP33 STU

Genome Analysis of *Cotesia plutellae* bracovirus

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Cotesia plutellae polydnavirus (CpBV) have a segmented genome consisting of multiple circular double stranded DNAs. Recently, we have developed an easy, simple and convenient system based on Tn7 transposition in order to clone genomic segments of CpBV in *Escherichia coli* cell and designated plasmid capture system (PCS). The PCS donor-S transferred a pUC19 origin of replication and an ampicillin resistance marker into CpBV genomic DNA by *in vitro* transposition. Through PCS system, we were able to clone 53 genomic clones ranging from 0.1 to 25.5 kb and further they were classified 29 segments by their sizes and restriction endonuclease patterns. Among them, a complete nucleotide sequences of 26 segments were determined and 125 putative genes were predicted from these segment. Whereas about 52.5% of predicted genes were hypothetical, 36 genes encoding protein tyrosine phosphatase were revealed to comprise the largest gene family. Also, 8 and 7 genes encoding *ank* and EP1-like protein consisted large gene families occupying about 6.4 and 5.6 % of total gene content of CpBV, respectively.

VP34 STU

The evolutionary analysis of *baculoviruses* based on variety evolution rates and function constraint

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Phylogenetic analysis of conserved genes, gene contents and the genomic organization are previously applied for evolutionary analysis of *baculoviruses*. The phylogeny-based studies alone, however, are not able to reveal the differences in the evolution speed and history. In this study, we estimated evolution rates to reveal function constraint and evolution traces of *lepidopteran baculoviruses*, especially the major envelop fusion proteins (F protein, F-like protein and GP64) which are the functional constraint and evolutionary selection pressure sensitive, are selected as subjects for evolutionary analysis. 30 core genes of NPVs and GV were evaluated and compared with the values of accumulation of synonymous mutations per synonymous site (dS) and nonsynonymous /synonymous substitution ratio (ω ratio, dN/dS) based on maximum likelihood (ML). The average values of dS of Group II was found significantly higher than of Group I, but the values of ω (dN/dS) was reverse. The high level accumulated of dS and lower value of omega in Group II indicated that Group II likely has a longer evolutionary history than Group I. Specially, F-like proteins of Group I have a much higher ω than F proteins of Group II (about 4.6 times), which indicated that F-like proteins evolve faster than F proteins. Considering function overlapping of GP64 with F, it is suggested that the function constraint of F-like proteins was relaxed after obtaining GP64. The highly

conservative GP64s was found having lowest value of ω than others of unique genes in group I NPVs, indicating that GP64 could be acquired in the Group I ancestor virus recently. Analysis the conservative inserted sites, orders and directions of GP64 in Group I shows its single origin. These results supported the hypothesis that Group I is a 'new' group of *Baculoviridae* and origin from an ancestral group II virus. Due to consequence of acquiring GP64, F protein lost part of its functions and the ancestral group II virus rapidly diverged into a new lineage-Group I NPVs.

VP35

Putative promoters isolated from infectious hypodermal and hematopoietic necrosis virus (IHHNV) of shrimp direct expression of a reporter gene in bacteria, insects and fish cells, and shrimp

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The lack of an immortal cell line and well-characterized promoters for the expression of heterologous genes are major limitations in studies involving molecular virology and functional genomics in shrimp. We have characterized two putative promoters of a shrimp virus, the infectious hypodermal and hematopoietic necrosis virus (IHHNV). IHHNV contains a linear single stranded DNA genome of ~4.1 kb in size and there are two putative promoters in the viral genome (Shike *et al.*, 2000). These two promoters, p2 and p61, are located upstream of the left (non-structural gene) and the right ORFs (capsid gene), respectively. In this study, the p2 and p61 promoter activity was evaluated via luciferase reporter vectors in recombinant bacteria, transfected insect cells (Sf9), fish cells (EPC) and shrimp tail muscle. The luciferase reporter vectors contained the luciferase coding region without or with the SV40 transcriptional enhancer. In bacteria luciferase expression by the p61 promoter was higher than the p2 promoter. However, in Sf9 and EPC cells p2 promoter-directed luciferase expression was higher than that of the p61 promoter. In shrimp, there was no significant difference in the luciferase expression driven by these two promoters. The presence of the SV40 enhancer element seems to have either a suppressive or no effect on activity of both promoters in bacteria, insect cells and shrimp. However, in EPC cells SV 40 enhancer element dramatically increased the activity of both promoters. Our data indicate that p2 and p61 are constitutive promoters, and these promoters can drive transient gene expression in both prokaryotes and eukaryotes.

Thursday, August 31, 8:00-10:00, *Meeting Center*
Bacteria Division Symposium: *Bt*- performance enhancement
Convenor: Yu Cheng Zhu

8:00

Synergistic Effect of Inorganic salts to Improve the Biological activity of *Bacillus thuringiensis* subsp. *aizawai* NT0423 against *Plutella xylostella*

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This study was carried out to find out some synergistic adjuvants for improving biological activity in *Bacillus thuringiensis* subsp. *aizawai* NT0423 formulation. *Bt. aizawai* NT0423 was initially isolated for using a potential biological agent by Seoul National University in Korea. About 20 materials, mainly salts, were tested on and compared against the 2nd instars of *Plutella xylostella* larvae using leaf dipping method in laboratory conditions bioassays. Among these tested materials, the possible candidates, $CaCO_3$, Na_2CO_3, MgO, $Ca(OH)_2$ and lactose showed some synergistic effect and were selected. The candidates were inserted into the wettable powder (WP) formulation of Bt., respectively. The physical properties such as wettability and dispersability were determined. Two formulations with $CaCO_3$ and Na_2CO_3 showing good physical properties were tested on *Plutella xylostella* at greenhouse and field condition. The two recipes showed around 30% and 20% higher activity and faster efficacy than a control recipe without synergists in the greenhouse and field test, respectively. These formulation receipes of the NT0423 were patented in 2002 and the product was registered as a bio-pesticide in 2004 in Korea.

8:30

A novel fonction of *Bacillus thuringiensis* Cry1C toxin on insect peritrophique matrix

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Bacillus thuringiensis Cry1 toxins are currently known for their strong host specificity; which is mainly due the presence of toxin receptors on midgut brush border membrane vesicles of susceptible insect larvae. Indeed, for most insects the toxin alone is sufficient to induce mortality but for others the association of spores or vegetative bacteria are necessary for mortality. *Galleria mellonella* is one of these so-called Type II insect, which is only weakly susceptible to Cry1C toxin (LD10>3µg/5th instar larvae (200 mg)). However, the association of 10^6 bacteria and toxin increases mortality to 80%. We have used this synergistic behavior between toxin and bacteria/spores to identify chromosomal virulence factors involved in the general pathogenesis of the infection process of *B. thuringiensis* in orally infected *G. mellonella*. However the precise role of the Cry1C toxin in this "synergy" oral infection process was not known. Our aim was to study the kinetics of bacterial development from the early infection to death and septicemia, with particular interest toward factors involved with intestinal barriers (peritrophic matrix (PM) and midgut). The PM is a cellular gel-like structure lining the epithelium which then protects the midgut from direct contact to food and pathogens. Histo-pathological observations on *G. mellonella* infected with toxin alone showed a structural modification of the PM but none on the midgut. Indeed, the gel-like and cohesive structure of PM collapsed 1-2 hours post ingestion, suggesting a direct effect of Cry1C. In order to show the degradation of MP, Cry1C toxin was mixed with Blue-Dextran beads (100.000 kDa size) which resulted in leakage of the large beads into the intestinal lumen. Thus, the role of Cry1C is to weaken the PM, which then reduces the protection of the epithelial cells and permits the bacteria to interact directly with the midgut cells and to pursue the infection process leading to septicemia and death. Similar studies are undertaking with other toxins and insects and we are attempting to determine the molecular role of Cry1C on PM of *G. mellonella*. Our observations, give further insight into the understanding of differences in Cry toxin susceptibility among various insects (Type I and Type II) but also suggest that Cry toxins may have a general and less specific effect on mucus-like structures, which could also include mucus from digestive tracts of vertebrates.

9:00

Potential use of proteinase inhibitors for insect control and Bt resistance management

Yu Cheng Zhu

(*USDA-ARS-JWDSRC, PO Box 346, 141 Experiment Station Road, Stoneville, Mississippi 38776, USA*)

Potential resistance development to Bt cotton in certain lepidopterans has prompted research to develop strategies that will preserve this environmental-friendly biotechnology. Proteinase inhibitors are potential candidates for enhancing Bt toxicity against lepidopteran pests and for expanding the spectrum of control for other insects. Interactions of Bt toxin from *Bacillus thuringiensis* and proteinase inhibitors were investigated by monitoring growth, development, and gut proteinase activities of the bollworm, *Helicoverpa zea*. Several proteinase inhibitors were combined with Bt protoxin Cry1Ac in artificial diet and fed to newly molted 3rd-instar bollworm larvae to determine effects on larval body weight and length, pupation progress, and mortality rate. Major midgut proteinase activities, including caseinase, tryptic, and chymotrypsin activities, were examined after treatment. A concentration of Bt at a level causing minimal mortality (<10%), was mixed with the following proteinase inhibitors: benzamidine, phenylmethylsulfonyl fluoride (PMSF), and N-α-tosyl-L-lysine chloromethyl ketone (TLCK). When compared with controls, the synergistic effect of Bt toxin and proteinase inhibitors caused significant decreases in mean larval weight and length over time. Midgut samples tested against the substrates azocasein, α-benzoyl-DL-arginine-*p*-nitroanilide (BApNA), and N-succinyl-alanine-alanine-proline-phenylalanine-*p*-nitroanilide (SAAPFpNA) showed significant decreases in the protease activity of larvae fed Bt plus inhibitor versus control. Interaction of Bt and proteinase inhibitors significantly retarded larval growth and resulted in developmental delay and up to 20% mortality.

9:30

Improved genetically engineered bacteria for controlling mosquito larvae

B. Federici, D. Bideshi, H. W. Park, J. Johnson, M. Tang, M. Wirth, Y. Sakano

(*Department of Entomology and Interdepartmental Graduate Programs in Genetics and Microbiology, University of California, Riverside, California 92521*)

New agents and strategies are needed to control mosquito vectors of diseases such as malaria, filariasis, and virus encephalitides. The larvicidal bacteria *Bacillus sphaericus* (Bs) and *B. thuringiensis* subsp. *israelensis* (Bti) hold promise, but use is limited by their high cost. Moreover, mosquito resistance has evolved rapidly to Bs where used intensively. Toxicity of these bacteria is due, respectively, to a single binary protein (BsB) in Bs, and four proteins in Bti, Cry4A, Cry4B, Cry11A, and Cyt1A. Cyt1A is of particular importance because it synergizes Cry toxicity and delays resistance to these. Additionally, Cyt1A suppresses high levels of resistance to Bs and expands its target spectrum. Recently, we used *cyt1A* promoters and a 5' mRNA stabilizing (STAB-SD) sequence to synthesize high levels of the Bs2362 binary toxin in acrystalliferous (4Q7) and crystalliferous (IPS-82) strains of Bti. The BtiIPS-82/BsB recombinant (LC_{50} = 0.37 ng/ml) was the most toxic of these against fourth instars of *Culex quinquefasciatus*, a vector of West Nile virus, making it 21 times as toxic as BtiIPS-82 (LC_{50}= 8.1 ng/ml), and 32 times as toxic as Bs2362 (LC_{50} =11.9 ng/ml). Moreover, BtiIPS-82/BsB completely suppressed extremely high levels of resistance (>100,000-fold) to Bs2362 in *Cx. quinquefasciatus*. Against *Aedes aegypti* and *Anopheles albimanus*, BtiIPS-82/BsB was approximately twice as toxic as BtiIPS-82. These new recombinant bacterial insecticides should be highly effective against *Culex* vectors and much less prone to resistance due to their high toxicity and endotoxin complexity combined with Cyt1A's synergistic and resistance-delaying properties.

Thursday, August 31, 8:00-10:00, *Nanyuan Meeting Room*
Contributed papers: Microbial Control 2
Moderators: Carlos A. Blanco and Bo Liu

8:00

Association of the components of the binary toxin from *Bacillus sphaericus* in solution and with model lipid bilayers

Panadda Boonserm[1], Seangduen Moonsom[1], Chanikarn Boonchoy[2], Boonhiang Promdonkoy[3], Krupakar Parthasarat[4] and Jaume Torres[4]

(*[1]Institute of Molecular Biology and Genetics, Mahidol University, Salaya, Phuttamonthol, Nakornpathom 73170, Thailand; [2]Institute of Science and Technology for Research and Development, Mahidol University, Salaya, Phuttamonthol, Nakornpathom 73170, Thailand; [3]National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, 113 Paholyothin Road, Klong 1, Klong Luang, Pathumthani 12120, Thailand; [4]School of Biological Sciences, Nanyang Technological University, 60 Nanyang Drive 637551, Singapore*)

We show herein that interaction in aqueous solution of the two components of binary toxin from *Bacillus sphaericus*, BinA and BinB, leads to a dramatic conformational change, from b turns or random coil, to b structure. Also, either BinA or BinB separately or their equimolar mixture, interact with lipid bilayers resulting in further conformational changes. Upon membrane association, the change in conformation observed for BinA or BinB separately is different from that observed when the proteins are combined, indicating that proper folding depends on the presence of the complementary subunit. We also show, in contrast to previous reports, that BinB, but not BinA, is able to insert in model neutral lipid monolayers.

8:15

Mechanism of *Bacillus brevis* against *Fusarium oxysporum* Schl. and Microcapsule approach for its formulated product

Lan Jiang-lin, Huang Su-fang, Che jian-mei and Liu Bo*

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Plant bacterial wilt disease, causing by *Fusarium oxysporum* Schl. is a worldwide soil-borne disease, which damages seriously the plants and leads to 20-80% economic lose. In order to reducing the pesticide residue in the soil, antagonistic bacteria have been studied and proved with potential as biological control agents against this disease. In 2000, a *Bacillus brevis* strain JK-2 was screened with high inhibition to the growth of *F. oxysporum* on Petri dish and good efficiency against the disease in potted plant bioassay. By using GFP technique, the JK-2 was induced with a green florescence protein

to investigate the biocontrol mechanism. Further studies showed that JK-2-GFP could enter the plant within 24h and propagate continually inside the plant tissue, without damage to the host. The possible mechanism for JK-2 was presumed that the bacterium might occupied rapidly the living spaces on plant rhizosphere and inside the plant body as well as competed the ecological niche and the nutritive with the pathogens, which prevented or delayed the invasion of *F. oxysporum* and then control the disease. The mechanism was similar as the "immunity protection" in zoology. Moreover, a microcapsule technique was applied to improve the stability of JK-2 and prolong the persistence in the field. A coagulating method by using glutin and arabic gum as shell materials. The effects of shell-material concentration, bacterium concentration，stirring speed and formaldehyde solidification on formation of microcapsule were studied．Results showed that using 2.5% glutin, 2.5% arabic gum and 10-30% bacteria fermentation liquid under conditions of 400 r/min stirring speed and 1% formaldehyde for solidification could result in round microcapsule with diameter of 30.8～57.3 μm and 70% retention ratio of the bacteria. This technique will provide a new approach for biocide formulated product.

8:30

Single Nucleotide Polymorphisms (SNPs) in Bt Toxin Binding Genes in Natural Populations of *Heliothis virescens*.

Omaththage P. Perera and Carlos A. Blanco

(*Southern Insect Management Research Unit, USDA-ARS, 141 Experiment Station Road, Stoneville, MS 38776, USA*)

Several Cry1A toxin receptors have been identified from the brush border membrane vesicles of tobacco budworm, *Heliothis virescens*. We selected three proteins included in the binding site group A, a Cadherin-like protein (HevCaLP) and two aminopeptidases, namely 170-kDa and 110-kDa *N*-aminopeptidases, to scan for single nucleotide polymorphisms (SNPs). Primers were designed to produce amplicons spanning putative Bt toxin binding domains of these three protein coding sequences. Heterpduplexes formed by annealing amplicons of field and control insects were subjected to CEL-I cleavage to identify insects with SNPs. Precise locations of polymorphisms were identified by analyzing the DNA sequences of SNP containing amplicons. SNP frequency as well as the effects of each SNP on the protein sequence and the Bt toxin binding were evaluated.

8:45 STU

Secondary structure Analysis of a highly mosquitocidal mutant strain of *Bacillus thuringiensis LDC-9* from Madurai, South India

Poornima K Kani, Mahalakshmi Ayyasamy, Sujatha Kabilan and Shenbagarathai Rajaiah

(*Lady Doak College, PG Department of Zoology & Research Centre, Lady Doak College, Chinnachokikulam, Madurai, Tamilnadu, India-625002*)

Mutant toxins, raised from *Bacillus thuringiensis LDC-9*, through UV exposure and chemical mutagenesis showed significantly improved toxicity against *Culex quinquefasciatus and Aedes aegypti* than its wild type. Bioassay result revealed 5 fold higher toxicity for the mutants raised chemically. UV exposed mutants produced pigmentation and it also produced a higher insecticidal effect against the mosquito larvae. The mutant LDC-M1 raised by chemical mutagenesis gave amplicons for all the cry toxins present in the wild strain in the Polymerase Chain Reaction. The sequence analysis of the cry genes from wild type and mutants revealed some random changes in the amino acid sequence in Cry 4 genes. Bioinformatic analysis of the sequence data shows changes in receptor sites, which might have increased the binding affinity of the toxin to the brush bordered membrane of the host. In conclusion toxicity assay on mosquito larvae *Culex quinquefasciatus* and *Aedes aegypti* suggested that the insecticidal activity of the *B.thuringiensis* LDC-M1 was significantly higher. Designing the secondary structure of the mutant cry gene could help significantly in predicting the functional aspect of the replaced amino acid in the sequence data.

9:00 STU

Location and identification of *cry* genes in *Bacillus thuringiensis* strain 4.0718

Zujiao Fu[1], Yunjun Sun[2], Xuezhi Ding[3], Shengbiao Hu[4], Xiaohong He[5] and Liqiu Xia[correspondence]

(*College of life science, College of Life Science, Hunan Normal University, Changsha 410081, P R China*)

Bacillus thuringiensis strain 4.0718 (CCTCC No.200016) which was isolated from soil can produce both bipyramidal and cuboidal crystals, and shows higher toxicity to insects in comparison with *B.*

thuringiensis subsp. *kurstaki* HD-1. In this study, characterization of this strain in regards to its fingerprints of genomic DNA, *cry*-type genes localization and identification was carried out by pulse field gel electrophoresis (PFGE), Southern blotting and the restriction fragment length polymorphism (RFLP), respectively. The findings showed that *B. thuringiensis* strain 4.0718 has an almost identical *Not* I digestion pattern of genome DNA and a slightly different extrachromosome pattern when compared to HD-1. PFGE and RFLP also showed that its *cry* genes are present on both genome and a plasmid and are consisted of at least *cry1Aa*, *cry1Ac*, *cry2Aa* and *cry2Ab* genes. Taken together, *B. thuringiensis* strain 4.0718 seems to be unique in its *cry*-type genes distribution.

9:15 STU

Diversity of *B. thuringiensis* strains from Madurai with insecticidal activity against different mosquito species

Mahalakshmi Ayyasamy, Poornima K Kani, Sujatha Kabilan and Shenbagarathai Rajaiah
(*Lady Doak College, PG Department of Zoology & Research Centre, Lady Doak College, Chinnachokikulam, Madurai, Tamilnadu, India, 625002*)

The characterisation of selected *B. thuringiensis* strains isolated from Madurai is presented. Characterisation was based on their insecticidal activity against *Aedes aegypti*, *Culex quinquefasciatus* to identify the active strain, scanning electron microscopy, sodium dodecyl sulfate polyacrylamide gel electrophoresis to determine the number and size of Cry proteins, plasmid profiles and PCR analysis using novel general and specific primers for *cry* and *cyt* genes encoding proteins active against mosquitoes (*cyt1, cyt2, cry2, cry4A, cry4B, cry10, cry11*). A potential strain *B.t* LDC-9 has activity against *Culex quinquefasciatus, Aedes aegypti* but has different protein and plasmid profiles. This strain harboring *cry4A, cry4B cry10, cry11*, cyt1 and cyt2 genes could be relevant for biological control of mosquitoes and future resistance management interventions. The PCR product of these genes in this strain was subjected to direct sequencing. BLAST Analysis of these genes indicates that it corresponds to Cry4A (DQ078744) (100% identity), Cry4B (DQ078743) (96% identity), Cry10A (DQ167578) (100% identity), Cry11A (DQ166531) (95% identity), cyt1A (DQ200984) (99% identity) and cyt2 (DQ171939) (92% identity). Protein sequence alignments revealed a high degree of conservation of the structural domains. It also helps in the identification of known *cry* genes encoding Cry proteins covering a wide phylogenetic distance and the detection and characterization of cry related sequences from novel *B. thuringiensis* isolates. The cloning and expression of the whole gene and characterization of its potential insecticidal activity against different mosquito species remain to be characterized.

9:30 STU

Production of thuringiensin by fed-batch culture of *Bacillus thuringiensis* subsp. *darmstadiensis* 032 with an improved pH-control glucose feeding strategy

Zhou Jing-Wen, Chang Ya-Fei, Yu Zi-Niu, Chen Shou-Wen
(*State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070, P.R. China*)

A improved pH-control fed-batch strategy for Bacillus thuringiensis subsp. darmstadiensis 032 producing thuringiensin was developed based on the analysis of the batch culture, constant rate fed-batch cultures and the original pH-control fed-batch. Having considered the pH variation and the glucose consumption status, the pH was adjusted from 6.5 to 7.0 by adding base in the late cultivation period of batch culture, and then the pH was kept at 7.0 by glucose feeding. The feeding was terminated when the pH couldn't be controlled by glucose feeding anymore. The proposed fed-batch strategy effectively avoided underfeeding or overfeeding, and it increased the thuringiensin yield and YP/X by 89.51% and 103.2% compared to that of the batch culture, respectively.

Thursday, August 31, 8:00-10:00, *Multifunctional Hall*
Contributed papers: Viruses 5
Moderators: Jim Maruniak and Peter J. Krell

8:00

Identification of Structural Proteins of *Culex nigripalpus* Nucleopolyhedrovirus (CuniNPV)

Omaththage P. Perera[1], Terry B. Green[2], Stanley M. Stevens, Jr.[3], Susan E. White[2] and James J.

Becnel[2]
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Occlusion derived virions (ODVs) of the nucleopolyhedrovirus of *Culex nigripalpus* (*CuniNPV*) were purified through ultracentrifugation and the resulting proteins were separated on SDS-PAGE. Proteins were identified using Edman sequencing, matrix assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, nano-electrospray quadrupole time-of-flight mass spectrometry of gel separated protein fragments (GeLC-MS/MS), or a combination of the above methods. Half of the 44 polypeptide sequences identified in this analysis were unique to CuniNPV and did not show any similarity to any sequence available in various protein databases. Of the 22 polypeptides that showed similarities to other baculovirus genes, only 17 sequences have previously been identified as structural proteins. Homologues of five newly identified CuniNPV structural genes were identified in the nucleopolyhedrovirus from *Autographa californica* (*AcMNPV*). This is the first report identifying these five genes as structural proteins of baculoviruses. The products of four genes, namely, lef-1 (cun045), alkaline exonuclease (cun054), helicase (cun089), and DNA polymerase (cun091) were not detected in the *CuniNPV* ODV preparations. These four genes are conserved among all annotated baculovirus genomes and their homologues have been detected in the ODV of AcMNPV.

8:15

The partial genome sequence of *Oryctes rhinoceros* virus

Yongjie Wang[1], Monique M. Van Oers[2], Allan M. Crawford[3], Just M. Vlak[2] and Johannes A. Jehle[1]
([1]*Laboratory for Biotechnological Crop Protection, Department of Phytopathology, Agricultural Service Centre Palatinate* (*DLR*)*, 67435 Neustadt an der Weinstrasse, Germany;* [2]*Laboratory of Virology, Wageningen University, Binnenhaven 11, 6709 PD Wageningen, The Netherlands;* [3]*AgResearch Invermay Agricultural Centre, Mosgiel, New Zealand*)

Oryctes rhinoceros virus (OrV) is an unassigned dsDNA virus with enveloped and rod-shaped virions. Two cloned PstI fragments C and D of OrV DNA have been sequenced and analysed. The PstI-C and -D sequences consist of 19,805 and 17,146 bp, respectively, comprising about 30% of the genome of OrV. Their AT content is of 58.6% and 58.0%, respectively. For each of the two fragments, 20 open reading frames (ORFs) of 150 nucleotides or greater with no or minimal overlap were predicted. Seven of the predicted 40 ORFs revealed the highest similarities to *Heliothis zea* virus 1 (HzV-1) ORFs, of which four, *lef-4*, *pif-2*, *dnapol* and *ac81*, are homologues to the conserved core genes in the family *Baculoviridae*. ORFD7 is similar to the baculovirus core gene *lef-5*. Two other genes were also homologous to *rr1* and *odv-e66* of baculoviruses. Five ORFs encode proteins homologous to cellular thymidylate synthase (TS), patatin-like phospholipase, mitochondrial carrier protein, ser/thr protein phosphatase, and serine protease, respectively. A *ts* and a *rr1* homologue are also present in HzV-1. The TS is phylogenetically related to that in eukarya and nucleo-cytoplasmic large dsDNA viruses. However, the remaining 25 ORFs bear poor or no sequence similarity to match with the current databases. Both the gene content of the sequenced fragments and the phylogenetic analyses of virus DNA polymerase suggest that OrV is most closely related to HzV-1, but distantly related to the *Baculoviridae*.

8:30

The genomic sequence of the *Gryllus bimaculatus* virus

Yongjie Wang[1], Regina G. Kleespies[2], Alois Huger[2] and Johannes A. Jehle[1]
([1]*Laboratory for Biotechnological Crop Protection, Department of Phytopathology, Agricultural Service Centre Palatinate* (*DLR Rheinpfalz*)*, 67435 Neustadt an der Weinstrasse, Germany;* [2]*Federal Biological Research Center for Agriculture and Forestry, Institute for Biological Control, Heinrichstr. 243, 64287 Darmstadt, Germany*)

The *Gryllus bimaculatus* virus (GbV) was found in dead and dying nymphs of the cricket, *Gryllus bimaculatus* (Orthoptera: Gryllidae) more than twenty years ago. It also infects other species of crickets, such as, *G. campestris*, *Teleogryllus oceanicus*, and *T. commodus*, and gives rise to considerable mortalities. The GbV is an unassigned dsDNA virus with enveloped and rod-shaped virions. The virions contain a closed circular dsDNA genome. The virus had been claimed to be a non-occluded baculovirus for some time, as it shared a similar virion structure and replication aspects. Now, it is taxonomically orphaned and deserves a new classification on the basis of its DNA sequence.

However, very little is known about its molecular genetics. In this study, the virus DNA was cloned and sequenced. The entire genome sequence of the GbV contains 96,944 bp and potentially encodes 98 predicted open reading frames (ORFs), 19 of which showed the highest similarity to these of *Heliothis zea* virus 1 (HzV-1). Fifteen ORFs are homologous to the baculovirus conserved core genes, which are also present in the HzV-1. Sequence and phylogenetic analyses suggest that the GbV is closely related to *Oryctes rhinoceros* virus (OrV) and the HzV-1, but distantly related to the baculoviruses.

8:45 STU

Identification of the Structural Proteins of the Occlusion-derived Virus of HearNPV

Fei Deng, Ranran Wang, Minggang Fang, Hualin Wang, Xushi Xu, Hanzhong Wang, Xinwen Chen and Zhihong Hu

(*State Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, P. R. China*)

Occlusion-derived virus (ODV) of baculovirus is responsible for initiating the primary infection of insect midgut epithelium. Therefore, identification of ODV structural components is fundamental for functional investigation of virulence and host specificity. The structural component of AcMNPV ODV has been reported before (Braunagel et al., 2003. PNAS 100: 9797-802). In this report, we describe SDS-PAGE and mass spectrum (MS) analysis of the ODV proteins of *Helicoverpa armigera* single nucleocapsid nucleopolyhedrovirus (HearNPV), a group II NPV. SDS-PAGE revealed forty-four bands and some proteins showed multiple bands such as GP41, ODV-E66, Polyhedrin, P49, ODV-E56, VP39, ODV-E25, P33, ODV-E18 and so forth. So far, twenty-three structural proteins of HearNPV ODV were identified by MS, nineteen of which have previously been reported as ODV structural proteins in AcMNPV: GP41, Ha66 (Ac66), VP80, P74, ODV-E66, P78/83, P49, ODV/BV-C42, Helicase, ODV-EC43, ODV-E56, ODV-EC27, VP39, Polyhedrin, ODV-E25, P33, DNA polymerase, P6.9 and ODV-E18; PIF-1 and Ha26 (Ac26) were also found in ODV. The other two proteins, HA44 and HA100, were identified as novel structural proteins. Data base search showed that HA44 is conserved in group II NPVs and granuloviruses, while HA100 is conserved in group II NPVs and appears to be a homologue of the poly(ADP-ribose) glycohydrolase (parg).

9:00

An investigation on biochemical variation of two isolates of potato tuber moth granulovirus, *Phthorimaea operculella* granulovirus

Mohammadreza Rezapanah[1], Amir Amiri Yekta[1] and Ahmad Dezianian[2]

([1]*Biological Control Research Department, Plant Pests and Diseases Research Institute, Tehran 19395, Iran;* [2]*Plant Pests & Diseases Research Dept, Semnan Agricultural & Resources Research Center, Semnan, Iran*)

Phthorimaea operculella granulovirus (PoGV) is a specific virus of Baculoviridae for controlling of potato tuber moth, *Phthorimaea operculella* (Zeller), one of the most damaging pests of potato in temparate, subtropical climates and also in Iran. A dead larva of potato tuber moth was collected from Chahardeh,Dibaj, Damghan in 2001. The propagated virus from the dead larva was compared with the isolate of CIP by Restriction Enzyme Analysis (REN) after their DNA isolation. The primary REN patterns revealed especial similarities and differences, therefore for detecting source of the virus, further studies are recommended.

9:15

Transcriptional analysis of *Choristoneura fumiferana* nucleopolyhedrovirus (CfMNPV) genes using an oligonucleotide-based DNA microarray

Dan-Hui Yang[1], Basil M Arif[2] and Peter John Krell[1]

([1]*Department of Molecular and Cellular Biology, University of Guelph, 488 Gordon Street, Guelph, Ontario Canada N1G 2W1;* [2]*Great Lakes Forestry Centre, 1219 Queen Street East, Sault Ste Marie, Ontario, Canada, P6A 2E5*)

An oligonucleotide-based two-channel DNA microarray was developed for characterization of temporal expression profiles of select *Choristoneura fumiferana* nucleopolyhedrovirus (CfMNPV) ORFs including its 7 unique genes. We explored a novel normalization protocol using CfMNPV viral genomic DNA (vgDNA) as equimolar reference standards for each probe in order to overcome the inherent variability problem of the traditional microarray normalization procedures. DNA microarray chips were constructed containing 70-mer oligonucleotide probes for 23 selected CfMNPV ORFs and

their complements. Total RNA was isolated at different times post infection and cDNA was synthesized. It was fluorescently labelled with Cy3, and co-hybridized to the microarray chips along with Cy5-labelled vgDNA. The temporal expression of the 23 CfMNPV genes representing all four temporal classes was monitored and discriminated using two-channel DNA microarray analysis. Host genes were unsuitable for normalization between arrays as their expression varied throughout viral infection. The DNA microarray results were selectively validated by quantitative RT-PCR. Transcription of the non-coding (anti-sense) strands of some of the CfMNPV selected genes including the polyhedrin gene was detected by array analysis emphasizing the added value of using single-stranded oligonucleotides as probes. The nature of the *polyhedrin* antisense transcription was further investigated using long-range PCR analysis and the possible roles of antisense transcripts are discussed.

9:30

Identification of baculovirus transactivator for early promoters using viral genomic library

Yin Chen[1, 2], Xu'ai Lin[2], Yiyu Lu[1], Yongzhu Yi[2] and Zhifang Zhang[2]

(*[1]Virus Reaserch Institute, Zhejiang Provincial Center for Disease Prevention and Control, Hangzhou, Zhejiang, 310009, China; [2]Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing, 100081, China*)

The baculovirus *Bombyx mori* nucleopolyhedrovirus (BmNPV) is an insect virus containing approximately 130 kb of double-stranded circular DNA. The BmNPV genome contains open reading frames potentially encoding more than 100 proteins. In this report, a genomic library of BmNPV was constructed by "partial filling-in" method. The genomic DNA of wildtype BmNPV was partially digested by *Sau*3AⅠ, and the fragments ranging from 3 Kb to 5 Kb were recovered from gel. The cohesive ends were filled in partially by incubating with dATP and dGTP in the presence of Klenow fragment of DNA polymeraseⅠ. The plasmid vector pUC19 was fully digested by *Sal*Ⅰ, and subsequently filled in by addition of dTTP, dCTP and the Klenow fragment. The vector and genomic fragments were ligated. The chemically transformed *E.coli* cells were cultured on IPTG/X-Gal plates. A total number of 238 white colonies were picked up and the plasmid DNA was extracted to co-transfection with the plasmid in which *luciferase* (*luc*) gene was driven by baculovirus *helicase* promoter. Eight of them exhibited strong activation of more than 10000 folds of luciferase activity. Sequence analyses revealed that all of them contained intact *ie-1* coding region. The *ie-1* gene was cloned by PCR to further confirm that only IE-1 but not other factors encoded by flanking sequences functioned as transactivator. Genome-wide screen also revealed that IE-1 was the only early factor of baculovirus stimulating the *helicase* promoter. The "partial filling-in" method was proved to be a feasible approach in construction of viral genomic library and subsequently function analyses suggested that the library was a powerful mean to investigate the transcriptional regulation of all DNA viruses.

9:45 STU

Characterization of a bacmid-derived defective baculovirus with a large deletion in the genome

Yi Huang[1, 3], Minggang Fang[1], Xinwen Chen[1], Ting Li[1], Just M Valk[2], Zhihong Hu[1] and Hanzhong Wang[1]

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More than twenty different bacmid genotypes were obtained during the construction of HaSNPV Bac-to-Bac system. One of the genotypes was HaBacHZ9 the genome size of which was approximately 30kb smaller than the wildtype HaSNPV-G4 which complete genome sequence had been reported. This study reported the complete sequence and the gene organization of HaBacHZ9. The genome size of HzBacHZ9 is 108,097b including 8.6 kb Mini-F cassette. In comparison with HaSNPV-G4, it revealed that about 50 open reading frames (ORFs) were missing in HaBacHZ9. Among the missing ORFs, 9 belonged to the conserved genes in baculovirus, 7 were replication and transcription related genes, 12 encoded for possible structure protein, and 26 ORFs were function unknown genes. In order to test the oral infectivity and easy observation, the polyhedrin gene and eGFP gene were introduced into HaBacHZ9, however, the transfection and infection results showed

that the virus of vHaBacHZ9-PH-eGFP can replicate in the nucleus as same as HaSNPV-G4, but there was no occlusion body formation in the nucleus though the polyhedrin gene promoted its promoter was reintroduced into the genome. The report gene, eGFP, also can't be observed though the cytopathic effects were apparent in infected cells. In addition, we found that the titer of vHaBacHZ9-PH-eGFP was nearly 100 times lower than HaSNPV-G4 from the one-step growth curve of the budded viruses of vHaBacHZ9-PH-eGFP. This may indicated that the budded virus of vHaBacHZ9-PH-eGFP has a lower infectivity in HzAm1 cells. When we injected the BV of vHaBacHZ9-PH-eGFP into larva because of no occlusion body formation, almost all of the larvae ware dead 7 days post infection, this results showed its infectivity in vivo. Investigation of the biological characters of this defective genome will shed light on the functions of these deleted genes or potential ORFs.

Thursday, August 31,10:15-10:30, *Meeting Center*
Lecture: Edward A. Steinhaus, Instigator, Catalyst, and Founder

Edward A. Steinhaus, Instigator, Catalyst, and Founder

Elizabeth W. Davidson
(*School of Life Sciences, Arizona State University, Tempe, AZ 85287-4501*)

Edward Steinhaus fused his unusual interests in microbiology and entomology when he was a graduate student at Ohio State University. But it was his acceptance of a position at the University of California at Berkeley, and the encouragement of Harry S. Smith, that led him to set up the first laboratory of insect pathology and to teach the first course on the subject in 1945. Out of this laboratory came many of the pioneers of our field; we will track the careers of those students, their students, and the many scientific descendents of Steinhaus which now number in the hundreds and are spread around the world. Among his many accomplishments, Steinhaus traveled to conferences in many nations in the 1950's and 1960's, including the first International Conference of Insect Pathology and Biological Control in Prague in 1958, as well as visits with colleagues in Europe and Asia. These contacts led to the international nature of our Society which we continue to celebrate. He was responsible for bringing together the group of scientists who founded our Society, and was the founding editor of the Journal of Invertebrate Pathology. He wrote or edited four seminal books on the topic, and left us with a treasure of his many memories in the form of *Disease in a Minor Chord*. In 1963 Steinhaus became founding Dean of the College of Biological Sciences at the University of California, Irvine. This talk will remind us of the amazing accomplishments of our founding President.

Thursday, August 31, 14:00-16:00, *Meeting Center*
Cross- Divisional Symposium: Nematodes and Bacteria: from Pathogenicity to Mutualism
Convenor: Raffi Aroian and Parwinder Grewal

14:00

Bacterial toxin - nematode interactions: using Bt toxins to control parasitic nematodes

Anderson Tan, Raffi Aroian, Xiang-Qian Li
(*University of California, San Diego, 9500 Gilman Drive, Mail Code 0349 La Jolla, CA 92093-0349 USA*)

Plant-parasitic nematodes (PPNs) cause significant economic losses worldwide. The current control strategies of PPNs include crop rotation, resistant varieties, chemical nematicides, and Integrated Pest Management. Under the Montreal Protocol, the main chemical nematicide, methyl bromide, is supposed to be phased out in developed countries in 2005, although the US government has asked for an extension. Transgenic plants expressing the environment-friendly and vertebrate non-toxic insecticidal crystal proteins of Bacillus thuringiensis are one of the modern breakthroughs in crop sciences. It has resulted in significant reductions in toxic chemical pesticide use and in significant gains in crop yields. Our laboratory has demonstrated that E. coli expressing Cry proteins present in the Cry5 and Cry6 subclades are toxic to phylogenetically diverse free-living nematodes. Among

these tested nematodes, Acrobeloides sp. belongs to the subclade of free-living nematodes closely related to the Tylenchida. These data suggest for the first time that Bt Cry proteins might have utility in controlling parasitic nematodes. Since to our knowledge it was not being tested but felt that it should be, we thus set out to test the hypothesis that Cry proteins expressed in transgenic plants might provide protection against plant-parasitic nematodes. To begin with, we are testing this hypothesis with Cry6A protein. In order to increase the expression level in transgenic plants, we synthesized "plant-friendly" versions of cry6A by assembling the entire genes de novo from 70-90-mer oligonucleotides and high fidelity PCR. The synthesized cry6A gene driven by enhanced 35S promoter was introduced into tomato hairy root via Agrobacterium rhizogenes-mediated transformation. It took us four years to maximize expression level to 0.1% of total soluble protein with further extensive modification of the original synthesized cry6A gene. Although Northern blot and RT-PCR sequencing results showed the correct cry6A transcripts, several truncated protein bands are observed. We are currently testing the transgenic resistance of tomato hairy root lines to Meloidogyne incognita. Our progress in testing these lines for control of nematode infections will be reported.

14:30

Elucidating the molecular mechanisms of bacteria-host interactions using the *C. elegans* pathogenesis model

Man-Wah Tan

(*Departments of Genetics, and of Microbiology and Immunology, Stanford University School of Medicine, Stanford CA 94305-5120, USA*)

Significant human sufferings could be attributed to pathogenic organisms. Annually, they account for over one quarter of human deaths worldwide and inflict significant economic losses when they infect livestock and crops. A thorough understanding of the molecular nature underlying the interactions between pathogens and their hosts would provide an important foundation for devising effective strategies to combat infectious diseases. Host-pathogen interactions are complex: they involve virulence determinants produced by pathogens and varied defense mechanisms deployed by the hosts. Thus, determining the genetic factors involved in each side of the interaction is crucial to understanding pathogenesis. Several *in vitro* and animal models have been used over the years to elucidate the molecular mechanisms underlying host-pathogen interactions. An important theme arising from these studies is that many of the offensive strategies utilized by pathogens to overcome host defenses are universal, irrespective of the evolutionary lineage of their host. Similarly, key features of the innate immune responses of the host are highly conserved across phylogeny. We use the *C. elegans* pathogenesis model to elucidate the molecular mechanisms of bacterial virulence and host defenses. A central innate immunity mechanism in most studied organisms involves the axis formed by PAMP-recognition Toll-like receptors and nuclear factor (NF)-kB transcription factors. However, *C. elegans* lacks the Toll-NF-kB axis, and yet effectively protects itself from diverse pathogens. This underscores the importance of other mechanisms in innate immunity. *C. elegans* protects itself from localized bacterial infections through the function and integration of several conserved signal transduction pathways. These include the Sma/Transforming Growth Factor beta, insulin, and p38 mitogen activated protein kinase pathways. However, to date, no transcription factor has been directly shown to mediate the function of any of these pathways in regulating transcriptional immune responses. Using a combination of genome-wide gene expression analyses, bioinformatics, and functional RNAi-based to study *C. elegans* responses to infection with a human bacterial pathogen *Pseudomonas aeruginosa*, we identified the tissue-specific GATA transcription factor ELT-2 as a major regulator of an early intestinal protective response to infection. This describes a mechanism specific to innate immunity, as both the response and the contribution of *elt-2* to this response were largely distinct from general stress responses. We further demonstrate that this function is conserved in evolution, as the human endodermal transcription factor GATA6 has a similar protective function in lung epithelial cells. These findings expand the repertoire of innate immunity mechanisms and illuminate a yet unknown function of endodermal GATA proteins. Finally, we showed that the ability of *Salmonella typhimurium* to establish a persistent infection in the *C. elegans* intestine requires the *Salmonella* pathogenicity island (SPI) 1 & 2, and the virulence plasmid (pSLT). The SPI2 and pSLT persistence defects *in vivo* could be rescued by the abrogation of host antimicrobial gene expression. This illustrates the power of analyzing mutants from the host and the pathogen to elucidate the specific *in vivo* function of host- and pathogen-derived factors in a pathogenic interaction.

15:00

Virulence of *Moraxella osloensis,* a bacterium associated with the slug-parasitic nematode *Phasmarhabditis hermaphrodita*, to the slug *Deroceras reticulatum*

P. S. Grewal

(*Department of Entomology, Ohio State University, Wooster, OH 44691, USA*)

Phasmarhabditis hermaphrodita (Rhabditida: Rhabditidae) is a lethal parasite of slugs in the families Arionidae, Limacidae, Milacidae, and Vaginulidae. The infective juvenile nematodes of *P. hermaphrodita* enter slug's shell cavity, beneath the mantle and release their associated bacteria, killing the host within 4-6 days. Although several bacterial species were identified from the nematode-infected slugs and from xenic nematode cultures, *Moraxella osloensis* was chosen to mass-produce the nematodes as it provided maximum nematode progeny. We found that *M. osloensis* plays a major role in the pathogenicity of the nematodes to the slugs. Axenic nematodes do not kill slugs and the number of bacteria carried by the infective juveniles directly correlates with the nematode-induced slug mortality. Investigation into the molluscicidal toxins produced by *M. osloensis* revealed that the bacteria produce an endotoxin consisting of a lipopolysacharide (LPS). The purified LPS is lethal to slugs when administered into the hemocoel or shell cavity. The LPS is a rough-type LPS with an estimated molecular weight of 5,300. Toxicity of the LPS resides in the lipid A moiety and was quantified to contain about 6 x 10^7 endotoxin units per mg. Coinjection of galactosamine with the LPS increased LPS toxicity to the slug by 2-4-fold. The galactosamine-induced sensitization of the slug to the LPS was reversed completely by uridine, thus indicating that slug hepatopancreas may be the potential site of action of the LPS. Gene expression profile of *M. osloensis* in the slug was analyzed and differentially expressed genes were identified using a selective capture of transcribed sequences (SCOTS) technique. Most identified sequences were homologous to those found in other pathogenic bacteria, and function as putative cell structure, energy metabolism, degradation, translocation, and proteins with unknown function. Several novel sequences that did not exhibit similarity to any genes or gene products in current databases, were also identified. We further evaluated the role of two SCOTS identified genes, *M5* (protein-disulfide isomerase) and *M8* (protein kinase) in the virulence of *M. osloensis* to the slug using mutation and complementation strategy. Mutants were constructed using insertion-deletion strategy by inverse PCR. The full length of *M5* and *M8* fragments were obtained by inverse PCR to complement mutants. Compared to the wild type, the virulence of *M. osloensiss* mutants to the slug was significantly reduced and the function could be complemented. Therefore, we conclude that the *in vivo* expressed genes, *M5* and *M8*, contribute to *M. osloensis* virulence to the slug. This study provides the first glimpse into the virulence genes of a pathogen in a primitive host system.

Thursday, August 31, 14:00-16:00, *Nanyuan Meeting Room*
Contributed papers: Microbial Control 3
Moderators: Roy Bateman and Ping Cheng

14:00

Design and evaluation of the 'MycoHarvester' for separation of powdery fungal conidia from substrates

Roy Bateman, Sylvia Mermelstein, Belinda Luke, Emma Thompson and Adrian Arnold

(*IPARC, Imperial College London, Silwood Park Campus, Ascot, Berks, SL5 7PY, UK*)

The development of a successful biopesticide depends on an efficient mass production system using cost-effective substrates. Working current mass production techniques for anamorphic entomopathogenic fungi such as *Beauveria* and *Metarhizium* spp. include 2-stage techniques involving aerial conidiation on solid substrates such as broken grain. For most application systems, the fungal product must be formulated in a way that is easy for operators to handle and does not cause blockages in filters, restrictors and other spray nozzle parts. This problem is especially acute with ultra-low volume (ULV) spraying techniques, where liquid flow is metered though very fine orifices, sometimes by gravity alone. High quality spore separation enables the development of other stable, suspension formulations, and by concentrating conidia into a pure product, facilitates drying to low moisture contents (in order to maintain shelf life). In addition, the process must involve minimal handling of substrate and operator contamination - especially inhalation of dust. This paper describes 10 years of development of a number of spore separation devices culminating in the MycoHarvester

version 5. Depending on the scale of the equipment, initial separation of the conidia from the rice is achieved either by tumbling (in large scale) or by creating a fluid-bed effect on the inoculated substrate (laboratory scale). Originally cyclones modified from commercial vacuum cleaners were used to classify and collect the fungal spores from the air stream created, but now proprietary stainless steel cyclones are used. Spore products can now meet the size specification required for ULV and other spraying techniques, and methods of particle size analysis are discussed.

14:15

The effect of water quantity, added during mass production, on *Beauveria bassiana* conidia yield and pathogenicity against *Oryzaephilus surinamensis*

Belinda Luke[1] and Maureen Wakefield[2]

(*[1] CABI, Silwood Park, Buckhurst Road, Ascot, Berkshire, SL5 7TA UK; [2]Central Science Laboratory, Sand Hutton, York, UK YO41 1LZ*)

Small to medium scale mass production of fungal conidia can be produced using a two-phase mass production system with a liquid phase followed by a solid substrate phase. This study examined the effect on conidial yield of varying the quantity of water added to the solid substrate (rice) phase. There were four treatments no water, washed rice, 300 ml and 600 ml water/kg rice. Two *Beauveria bassiana* isolates were tested, IMI 386243 and IMI 389521. For isolate IMI 386243 water quantity had no effect on conidial yield. For isolate IMI 389521 the general trend was for an increase in conidial yield with an increase in water quantity. Conidia pathogenicity against *Oryzaephilus surinamensis* did not appear to be effected by water addition to the solid phase during mass production.

14:30

Development of a mycoinsecticide for the control of *Helicoverpa armigera* infestation on pulses: Significance of back-up strains in the commercial production

S. Chavan[1], V. Ghormade[1], G. Kulkarni[2], A. Gondhalekar[1], A. Rajendran[1], M. Taranekar[1], S. Kulkarni[1], Y. Shauche[2] and M. V. Deshpande[1]

(*[1]Biochemical Sciences Division, National Chemical Laboratory, Pune –411008, India; [2]Molecular Biology Unit, National Centre for Cell Science, Pune-411007, India*)

Metarhizium anisopliae, an insect pathogenic fungus has been identified as a commercially viable strain for the control of *Helicoverpa armigera* infestation on pulses. It has been reported that number of commercial strains lose virulence towards the target pest due to their maintenance on artificial media, spontaneous mutation, low viability during preservation or even non-availability of specific raw material for spore production. In view of this fact, it is necessary to have potential back up strains which can maintain the quality and regular supply of the effective preparation. The desired properties to identify the back up strains are: virulence, pathogenesis related factors such as cuticle degrading enzyme activities (chitinase, protease, lipase and chitin deacetylase), and viability measured as conidial germination. Around 61 Indian strains of *M. anisopliae* isolated from soil under crops like pigeon pea, tomato, custard apple, etc. and from infected grubs and beetles have been isolated. From these, 3 back up strains have been identified which showed > 90% mortality of *H. armigera*, comparable cuticle degrading enzyme activities (> 3 x 10^{-3} U/ml measured as chitinase) and conidial germination within 12-16 h similar to the commercial strain. The morphological, cultural, biochemical and molecular (ITS and RAPD analysis) suggested that the back up strains are *M. anisopliae*. The solid state fermetation for conidia production and bioefficacy data will also be discussed.

14:45

Comparison of two different methods for quality of spray deposits after application of fungal formulations

Vladimir V. Gouli, Svetlana Y. Gouli, Carolina Provost, Bruce L. Parker and Margaret Skinner

(*Entomological Research Laboratory, University of Vermont, 661 Spear Street, Burlingtonm Vermont 05405-0105, USA*)

Spray application of microbial formulations is a very important part of the complex research connected with biological pest control. The evaluation of spray deposit quality demands simple and authentic methods. For this point, two different methods, including a method based on water and oil sensitive paper cards, and the Scotch tape method were estimated. New experimental formulations of the fungi *Beauveria bassiana* and *Lecanicillium lecanii* prepared in oil, special whey protein concentrate, and oil plus whey protein were tested. Hemlock plants were sprayed using ultra low

volume equipment (Ultrafan MK2, Micron Sprayers Ltd.) with suspensions of formulations containing $5x10^9$ conidia per ml. Spray deposits were sampled using the sensitive cards (TeeJet Epraing System Co) by placing the cards in different parts of the plants. The cards were examined using a reflected-light microscope objective. Hemlock twigs (5cm long) were utilized for estimation of the Scotch tape method. The technique of working with Scotch tape has been described (Gouli et al., 2005. J. of Mycology and Phytopathology, vol. 39, #3, Moscow). Several parameters were determined using these two methods, including the number of droplets per unit square, distribution of droplets according to the following four ranges; first - <25 mm, second - 25 – 50 mm, third – 50 – 100 mm, and fourth - >100 mm, and the number of fungal conidia on the plant surface. All of these parameters were made for the upper and under sides of the twigs. The sensitive cards and Scotch tape methods shoved the same total droplet numbers. In the case of formulation based on oil, there was statistically authentic tendency of the majority of droplets to be > 25mm using the sensitive card method. The calculation of conidia was possible only using the Scotch tape method. The microstructure of the sensitive paper card masked conidia, and furthermore, it was impossible to use any stain to contrast fungal material.

15:00

A phenologically based programme for season-long control of false codling moth on citrus, with particular use of a granulovirus and entomopathogenic nematodes

Sean Douglas Moore[1, 2], Antoinette P Malan[3] and Wayne Kirkman[2]

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False codling moth, *Cryptophlebia leucotreta* (Meyrick) (Lepidoptera: Olethreutidae), is a fruit pest of citrus, macadamias, stone fruits, avocadoes, grapes, peppers and litchis, in southern Africa. It can be a pest throughout the year, remaining active even in winter. It is capable of attacking newly set pea-sized fruit in spring, to ripe fruit in autumn and unharvested fruit in winter. It is therefore necessary to employ control measures throughout the year. The *Cryptophlebia leucotreta* granulovirus (CrleGV-SA) has been used successfully for two years now for the commercial control of *C. leucotreta* on citrus. Studies are currently being conducted to test the potential of entomopathogenic nematodes (EPNs), in the families Heterorhabditidae and Steinernematidae, as biological control agents for *C. leucotreta*. A control programme incorporating a granulovirus and EPNs is proposed. Cryptogran has been found to reduce *C. leucotreta* infestation more effectively, when applied early in the season (early summer) and when timed against a flight peak. In a trial where the efficacy of sprays applied at different phenological times were compared, a treatment on 7 December, applied against a flight peak, reduced infestation by an average of 62% over a 14 week period. A treatment applied on 10 January, between two flight peaks, only reduced infestation by 42%, over a 7 week period. Sprays applied during the last six weeks before harvest induce control for an even shorter period. It is proposed that during autumn and winter, EPNs could be applied to control pre-pupae and pupae in the soil. A survey has been undertaken in citrus orchards to obtain endemic, locally adapted EPNs. Twelve isolates of EPNs have been recovered and maintained for further study. All isolates were identified as *Heterorhabditis*. Laboratory bioassays conducted with EPNs and sentinel larvae and pupae of *C. leucotreta*, demonstrated that mortality and penetration of pupae was much lower than for larvae. Highest mortality and penetration rate of both larvae and pupae was obtained for *Heterorhabditis zealandica*. We therefore propose a phenologically discerning control programme for *C. leucotreta* on citrus, based around the use of Cryptogran and ENs.

15:15

Preparation of scFv and monoclonal antibody against HrpA

Shi Hua Wang and Zong Hua Wang

(*Key Laboratory of Biopesticide and Chemical Biology, Ministry of Education, China, College of Life Science, Fujian Agriculture and Forestry University, Fuzhou, Fujian 350002*)

Hrp gene is a gene family that regulates the bacteria triggering hypersensitive reaction and pathogenicity (*hrp*) in plant, and HrpA protein is major component of the filamentous surface appendage, playing a very important role in the interaction between bacteria and their host plant. The previously results indicated that the monoclonal antibody we achieved was of high specificity and affinity, which can be used as material in related experiments for better understanding Type III system (TTS) in bacteria. We further used phage display to obtain scFv against hrpA. The scFv DNA was

ligated into a phagemid vector and the ligated product was then transformed into *E. coli* to yield recombinant phages after infection with helper phage. After of panning with HrpA, the phage clones displaying scFv fragments of the antibody were selected by ELISA. High correlation was observed when identified HrpA with scFv and monoclonal antibody. Therefore, in this paper, monoclonal antibody and scFv antibody against bacterial HrpA protein were prepared to study the possibility of plant resistance improvement.

Thursday, August 31, 14:00-16:00, *Multifunctional Hall*
Contributed papers: Viruses 6
Moderators: Rollie Clem and Monique M. Van Oers

14:00

Characterization of early events during infection of TN368 cells with AcMNPV lacking *p35*

Bart Bryant and Rollie J. Clem
(*Molecular, Cellular, and Developmental Biology Program, Division of Biology, Kansas State University, Manhattan, KS 66506 USA*)

In addition to its well known role in inhibiting apoptosis, the AcMNPV *p35* gene is also required for melting of infected larvae. This includes *Trichoplusia ni* larvae, where apoptosis is not thought to occur following infection with *p35* mutant AcMNPV. Since larval liquefaction has been shown to be dependent on expression of the AcMNPV chitinase and cathepsin genes, we initially hypothesized that AcMNPV lacking *p35* may not express these genes as efficiently as wild type. However, infection of TN-368 cells with AcMNPV lacking *p35* or control revertant virus resulted in equivalent levels of both expression and enzyme activity of chitinase and cathepsin. Both of these viruses express GFP from the hsp70 promoter and we consistently observed lower GFP expression with the *p35* deletion virus when compared to the revertant virus at early times post infection. In addition, cell cycle analysis of infected TN-368 cells revealed a delay in cell cycle arrest with the *p35* deletion virus, further indicating a delay in early events after infection. This led us to examine the timing of entry for both viruses. A significant delay was observed in exit from the endosome for the *p35* deletion virus as compared to the revertant virus. Thus, mutant viruses lacking P35 enter TN-368 cells less efficiently and early events in the infection cycle are delayed, but by 48 hrs the infections are similar. We hypothesize that without P35, the virus may spread more slowly throughout *T.ni* larvae and this might contribute to the lack of larval melting.

14:15

Activation pathways and signal-mediated upregulation of the insect *Spodoptera frugiperda* caspase-1

Qingzhen Liu[1, 2] and Nor Chejanovsky[1]
([1] *Entomology Department, Institute of Plant Protection, Agricultural Research Organization, The Volcani Center, POB 6, Bet Dagan, 50250 Israel.;* [2] *State Key laboratory of Virology and Modern Virology Research Center, College of Life Sciences, Wuhan University, Wuhan 430072, P.R. China.*)

Sf-caspase-1 is the most studied effector caspase of Lepidoptera. Its activation is believed to follow a two-step mechanism: The first step requires cleavage by an initiator caspase at D^{195} (between the large and small subunits) releasing the C-terminal small subunit. This is blocked by the baculovirus caspase inhibitor P49. The second step removes the N-terminal prodomain by cleavage at D^{28} to generate the large subunit that is blocked by the baculovirus caspase inhibitor P35. Here we identified an alternative mechanism of Sf-caspase-1 activation and showed that both mechanisms are triggered by apoptotic stimuli following a distinct pattern. We also proved that expression of Sf-caspase-1 was upregulated upon reception of apoptotic stimuli. Moreover, we demonstrated that the stronger the stimuli, the higher the upregulation.

14:30

Functional analysis of *Helicoverpa armigera* single nucleopolyhedrovirus *inhibitor of apoptosis* genes

Marcel Westenberg[1], Job De Lange[1], Fei Deng[2], Hualin Wang[2], Zhihong Hu[2] and Just M. Vlak[1]
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Apoptosis is a defense mechanism of all living organisms, among others, to combat viral infections. As a response, many viruses have evolved mechanisms to prevent the host cell from entering apoptosis. Baculoviruses contain two classes of genes whose translational products can interfere with apoptotic pathways: *p35* and *iap* (*inhibitor of apoptosis*). The family of *iaps* is currently classified into five clusters (*iap1 – iap5*) based on sequence homology. All baculoviruses contains two or more *iap* genes, but so far at most one possesses an anti-apoptotic function. The baculovirus *Helicoverpa armigera* single nucleopolyhedrovirus (HearNPV) contains a homolog of *iap-2* and *iap-3*. To investigate at which time point and to what extent these *iap* genes are transcribed in HearNPV-infected HzAm1 cells, transcriptional profiles of these genes were made by RT-qPCR. Transcript of *iap-2* and *iap-3* could be found as early as 12 h and 4 h post infection (p.i.) respectively, which is compatible with the presence of a late (*iap-2*) or an early (*iap-3*) promoter motif. The transcription level of both genes reached a maximum at 72 h p.i, but surprisingly the amount of *iap-2* transcripts was at least 50 times higher than that of *iap-3* and comparable with that of *polyhedrin.* These IAPs were also transiently expressed in Sf21 cells to investigate if they could inhibit actinomycin-D induced apoptosis. Transfected cells were monitored by the simultaneously expression of GFP and apoptotic cells with condensed DNA were visualized by Hoechst-staining. Inhibition of apoptosis was only observed in cells expressing IAP-3 or the positive control AcMNPV P35. At the moment the host specificity of the HearNPV IAPs and their ability to rescue an AcMNPV *p35* deletion mutant are tested. The late and high level of transcription of *iap-2* however, together with its inability to inhibit apoptosis in an actinomycin-D assay, indicates that IAP-2 might have a different function.

14:45

Indirect and direct evidence for the role of trypsin in baculovirus infection.

Jeffrey M Slack[1], Susan D Lawrence[2], Peter J Krell[3] and Basil M Arif[1]

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Baculoviruses are a family of insect viruses that have been used for decades in biocontrol. Insects become vulnerable to infection when they ingest foliage contaminated with viral occlusion bodies (OBs). Liberated occlusion-derived virions (ODVs) initiate infection in epithelial cells when OBs dissolve in the alkaline environment of the larval midgut. It has been shown that P74, present on the surfaces of ODVs, was essential for infection. We have observed that P74 of the *Autographa californica* (*Ac*)MNPV is cleaved when a soluble form of the protein was incubated with insect midgut tissues under alkaline conditions and that cleavage was prevented by soybean trypsin inhibitor (SBTI). Biological assays were carried out and suggested that SBTI inhibited baculovirus infection and that trypsin enhanced infectivity. This may be due to trypsin cleavage and activation of P74. Analysis of the peptide sequences of P74 homologues identified a highly conserved trypsin cleavage site that could generate the observed cleavage product. Further more, mutagenesis of the trypsin cleavage sites in P74 had an effect on the oral infectivity. In this study we link molecular biology with practical biocontrol improvements and present evidence that plant products may affect baculovirus efficacy.

This work was initially supported by the United States Department of Agriculture. Continuing work is supported by the Canadian Insect Biocontrol Network, the Canadian Biotechnology Strategy Fund and Genome Canada through the Ontario Genomics Institute.

15:00

The 5' nontranslated region of *Varroa destructor virus* 1 (*Iflavirus*): Structure prediction and IRES activity in insect cells

Juliette R. Ongus[1], Els C. Roode[1], Cornelis W.A. Pleij[2], Just M. Vlak[1] and Monique M. Van Oers[1]

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Varroa destructor virus 1 (VDV-1) was originally isolated from the bee parasitic mite *Varroa destructor*, a serious pest of the honey bee *Apis mellifera*. The RNA genome of this picorna-like virus

belonging to the genus *Iflavirus* has been completely sequenced and is most closely related to *Deformed wing virus* (DWV) of bees. VDV-1 and DWV are both able to replicate in mites and honey bees as shown by a recently developed discriminative PCR method, specific for detection of the negative strand of either virus (J. Gen. Virol. 85: 3747-55). Functional studies on VDV-1 and DWV are hampered by the lack of suitable cell lines. In the current study, computer-assisted analysis was performed to predict the secondary structures of four *Iflavirus* 5' non translated regions (NTRs) and to identify potential internal ribosome entry sites (IRES). The relatively long 5'NTR sequences of VDV-1 and DWV (81% identity) were compared to the much shorter ones of the mutually related *Perina nuda picorna-like virus* (PnPV) and *Ectropis obliqua picorna-like virus* (EoPV). The existence of co-variation in two related sequences was regarded as a confirmation of the predicted structures. Intraspecies variations in VDV-1 isolates were also included in the analysis. Two iflaviruses, *Infectious flacherie virus* (IFV) and *Sacbrood bee virus* (SBV) have very short 5¢ NTR sequences and were not investigated here. Two types of putative IRES elements were identified, of which the VDV-1/DWV type showed much more complexity. The predicted 5' IRES structures of these iflaviruses do not have pseudoknots, in contrast to the intergenic IRES elements of Dicistroviruses, another group of insect picorna-like viruses. To test the activity of the putative VDV-1 IRES, a bicistronic construct was made in which the 5' NTR was cloned in between the reporter genes enhanced green fluorescent protein (EGFP, upstream) and firefly luciferase (Fluc, downstream). The expression of the reporter genes was tested in a transient expression assay. The presence of the 5' NTR of VDV-1 greatly enhanced the expression level of the downstream reporter gene (Fluc) in *Lymantria dispar* Ld652Y cells, showing that the 5' NTR of VDV-1 contains a functional IRES element. Less IRES activity was observed in *Spodoptera frugiperda* Sf21 cells, while the IRES was not active in *Drosophila melanogaster* S2 cells. VDV-1 is the first *Iflavirus* for which IRES activity has been experimentally confirmed. The potential of *L. dispar* Ld652Y cells to be a susceptible host for VDV-1 and DWV is discussed. e-mail: monique.vanoers@wur.nl

15:15

siRNA injection induce sequence-independent protection in *Penaeus monodon* against White Spot Syndrome Virus

Marcel Westenberg, Bas Heinhuis and Just M. Vlak

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White spot syndrome virus (WSSV) is a major disease in crustaceans, particularly shrimp, due to the current intensity of aquaculture practices. Novel strategies including vaccination to control this virus would be highly desirable. However, invertebrates lack a true adaptive immune response system and seem to rely on various innate immune responses. Nevertheless, preliminary experiments to vaccinate shrimps based on the WSSV major envelope protein VP28 resulted in enhanced tolerance to WSSV infection (Witteveldt *et al.*, J Virol 78: 2057-2061, 2004). An alternative and very specific approach to counteract WSSV infections in shrimp could be by the exploitation of RNA interference. As long dsRNA molecules induce a general, sequence-independent anti-viral immunity in shrimp (Robalino *et al*., J Virol 78: 10442-10448, 2004), it was investigated whether shorter 21 nucleotide-long small interfering RNAs (siRNAs) with homology to the WSSV *vp15* and *vp28* genes would give a sequence-specific interference response in the shrimp *Penaeus monodon*. *Vp28* siRNAs as well as nonspecific green fluorescent protein (*gfp*) siRNAs as control were able to specifically and efficiently silence their homologous genes in a heterologous baculovirus insect cell expression system showing proof of principle of this technology. However, in shrimps such a specific effect was not observed. Shrimp injected with *vp15* or *vp28* siRNAs before WSSV challenge gave a significantly lower mortality rate, but not significantly different from shrimp injected with the heterologous *gfp* siRNA. Thus, large dsRNA molecules as well as siRNAs induce a sequence-independent anti-viral reaction when injected in shrimp and hence this casts doubts on the feasibility of this approach to combat specific virus diseases in shrimp. (Westenberg *et al*., Virus Res 114: 133-139, 2005)

15:30

The *Aedes albopictus* Inhibitor of Apoptosis protects vertebrate cells from Bluetongue virus-induced apoptosis

Qianjun Li

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We sequenced and performed a functional analysis of the *i*nhibitor of *ap*optosis (*iap*) 1 gene from *Aedes albopictus* mosquitoes, designated Aa*iap*1. The Aa*iap*1 gene rescued cells from apoptosis when co-expressed with the *Drosophila* pro-apoptotic gene *hid* in *Spodoptera frugiperda* (*Sf*9) cells. The role of Aa*iap*1 gene during arbovirus infection was evaluated in the Bluetongue virus (BTV)-induced apoptosis system. BTV infection induced apoptosis in vertebrate cells via the intrinsic apoptotic pathway, including the translocation of *cytochrome C* and Smac/DIABLO, and the subsequent activation of caspase-9 and -3. Stable expression of Aa*iap*1 gene delayed BTV-induced apoptosis by 24 h, and reduced BTV progeny yield by 10-fold. These results suggest that Aa*iap*1 gene has an important role in regulating BTV-induced apoptosis.

Thursday, August 31, 16:30-18:30, *Meeting Center*
Contributed papers: Bacteria 4
Moderator: Brian Federici

16:30

Diversity of toxin gene from *Bacillus thuringiensis* against scarab larvae

Shu-liang Feng[1], Da-fang Huang[2], Rong-yan Wang[1], Jin-yao Wang[1], Wei-ping Cao[1], Li-xin Du[1], Jian Song[1], Rui-hua Wu[1], Fu-ping Song[2] and Jie Zhang[2]
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Researches on genetics of *Bacillus thuringiensis* (Bt) have made important progress recent years. Insecticidal proteins, coded for by genes located in plasmids, form typical parasporal crystalline inclusions during sporulation. Many strains contain more than one pesticide toxin protein gene. *Cry1Ac10* from strain YBT-1520 was first cloned insecticidal crystal protein gene from *B. thuringiensis* in China, subsequently, about 30 genes, such as *cry3Aa7, cry8Ca2* and *cry8E,* were cloned. Diversity of cry8-type gene encoding insecticidal crystal proteins by against scarab larvae is reviewed in this article. Ohba *et al.* first isolated a *B. thuringiensis* serovar. *japonensis* strain which was specific to only scarabaeid larvae. A new *cry* gene from this strain, *cry8Ca1* encoding protein exhibited larvicidal activity against *Anomala cuprea*, *A. rufocuprea* and *Popillia japonica* (Scarabaeidae) was cloned in 1992. Asano *et al.* have cloned *cry8Da1, cry8Da2* and *cry8Da3* genes in Japan subsequently. Strain HBF-1, isolated from Baoding soil sample, has high larvicidal activity to *A. exoleta* and *A. corpulenta* that are currently endangering large areas of forest and peanut. A *cry* gene named *cry8Ca2* cloned from HBF-1 have been registered in Gene Bank and proved by International Nomenclature Committee of Bt genes as a novel gene. It had been found that parasporal crystal from strain HBF-1 play a crucial role in killing target pest. *B. thuringiensis* 185 has high toxicity to larvae of *Holotrichia parallela* with LC_{50} of 0.9464(10^8cfu/ml).The HBF-18 strain has high toxicity to larvae of *H. oblita* with LC_{50} of 9.7356 (10^8cfu/ml) and also showed some extent of toxicity to *H. parallela*.But both of two strains have non insecticidal activity to larvae of Rutelinae. *Cry8Ea1* and *cry8Ga1* were cloned from strain 185 and HBF-18 respectively. There is no doubt that *cry8Ga1* and *cry8Ea1* which had high insecticidal activity to *H. oblita* and *H. parallela* that are two dominant species of underground pests respectively broaden the activity spectrum of Bt. There are about 9000 species of Melolonthinae, in which 500 kinds distributed in China. It is necessary to screen novel, broad spectrum and high activity insecticidal proteins genes against larvae of Melolonthinae.

16:45

A novel insecticidal factor from *Bacillus sphaericus* with no mosquitocidal activity

Hisashi Nishiwaki, Kenta Nakashima, Tadayuki Kawamura and Kazuhiko Matsuda
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Insects attacked by antlions *Myrmeleon bore* become limp with melanization. Since this symptom resembles that seen in microbial infection, a variety of bacterial species associating with the crop of the antlions were isolated to test their insecticidal activity against the larvae of common cutworms *Spodoptera litura* by injection. Several bacterial species isolated including a novel species were found to kill the cutworms and make the color of the caterpillar darkened. The 16S rRNA gene sequence

combined with morphological and biochemical properties of one of the insecticidal isolates A3-2 showed a close relationship of this isolate with mosquitocidal *B. sphaericus* subgroup IIA, which was further supported by DNA-DNA hybridization test. However, the genes encoding the mosquitocidal toxins of *B. sphaericus* subgroup IIA such as mtx 1, mtx 2 and binary toxin were not detected from A3-2, consistent with no insecticidal activity of A3-2 against mosquitoes *Culex pipiens*. The result suggests that this isolate is likely to kill caterpillars using novel toxins other than known mosquitocidal proteins. As a consequence of purification of an insecticidal principle from the culture broth of A3-2, the isolate was found to secrete a novel pore-forming toxin with a unique N terminal amino acid sequence. The pore-forming toxin was immunologically detected from caterpillars injected with A3-2, suggesting that the toxin probably contributes to the death of insects following the bacterial injection.

17:00

Molecular studies on Iranian native dipteran active *Bacillus thuringiensis* isolates

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This study was carried out to identify and characterize the Iranian native dipteran active *Bacillus thuringiensis* strains. For this purpose, about 100 strains were isolated from different ecological regions of Iran (cereal, sugar beet, legumes and vegetables fields). Characterization of isolates was based on morphological characteristics of crystals, plasmid profiles and protein band patterns as well as PCR analysis using novel general and specific primers for *cry* and *cyt* genes encoding proteins active against mosquitoes. Morphological characteristics of crystals were analyzed by gram staining and observation with common and phase contrast microscopes. The majority of isolates produced bipyramidal crystals and many of them showed other types of crystals (cuboidal, spherical, and heterogeneous forms). The protein pattern of spore-crystal suspensions was analyzed in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). These experiments showed that isolates had different protein bands from 65 to 130 kDa. Furthermore, a number of isolates had protein band of 25-28 kDa (Cyt proteins). For identification of dipteran active isolates, two types of primers (general and specific primers) for *cyt1*, *cyt2*, *cry2*, *cry4A*, *cry4B*, *cry10*, *cry11*, *cry17*, *cry19*, *cry21*, *cry24*, *cry25*, *cry27*, *cry29*, *cry30*, *cry32*, *cry39*, and *cry40* genes encoding proteins active against mosquitoes and also specific primers for 16SRDNA sequence were designed and synthesized. These primers were used for PCR analysis and screening of all isolates. The results of PCR analysis and plasmid profiles will be submitted.

17:15

Studying of natural strains of *Bacillus cereus-B.thuringiensis* from Siberia and Far East

Galina Kalmykova[1], Ljudmila Burtseva[1], Ivan Dybovskiy[1], Victor Glupov[1], Irina Andreeva[2], Anna Mokeeva[2], Svetlana Oreshkova[2] and Vladimir Repin[2]

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Bt strains have been found in samples of insect cadavers, soils from West Siberian plain, high-altitude sample of atmospheric aerosols where bioinsecticides are widely used and from bottom sediments of Baikal Lake, soils of Kunashir Island and Valley of Geysers that have never been treated with *Bt* formulations. We identified isolates of *Bt* ssp.*thuringiensis, kurstaki, sotto*, *galleriae, entomocidus, morrisoni, israelensis, novosibirsk, toguchini*. More than 50% of the natural samples contained *Bt* ssp.*kurstaki* isolates. It was found that most of *Bt* ssp.*kurstaki* strains belonged to K-73 crystovar. It was surprisingly because the base of bacterial insecticides is strains of K-1 crystovar. Strains of crystovar K-73 were only found in samples from Kunashir Island. *Bt* strains isolated from sediments of Baikal Lake were classified as *Bt* ssp.*galleriae. Bt* strains isolated from soil of the Valley of Geysers possess a number of morphological and biochemical peculiarities. These strains form mucous colonies. Under selection on mobility into U-shaped tubes vegetative cell of these strains were weakly mobile in contrast to motionless *Bt* ssp.*wuhanensis*. Their cells form parasporal inclusion bound to endospores like *Bt* ssp.*finitimus*, *pakistani, tochigiensis, silo*. To identify natural isolates we use information about typical strains of *Bt* that has been systematized in the computer program. This program allows to save and represent various information both textual and graphic one. Besides

cultural biochemical and serological methods that traditional for this group of microorganisms we also apply molecular methods such as SDS-PAGE and PCR-analysis. To select strains suitable for biocontrol we use characterization of parasporal crystal proteins with following confirmation in insect biotests.

17:30

Symbiosis in mosquitoes and its potential application in vector control

Akinkurolere Rotimi Oluwafemi and Hongyu Zhang

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Many insects have established an (extracellular/intracellular) symbiotic relationship with microorganisms they harbour within their bodies. Insects such as termites, Scarabs, mosquitoes and aphids feed on sub-optimal diets due to their restriction to a particular niche and the symbionts they harbour helps them to synthesize and make up for other nutritional requirements lacking in their food. Mosquito also have an association with vast array of symbiotic bacteria in its gut. Adult mosquito is attracted to pool of water with high bacteria load for oviposition while the bacteria later serve as source of food for the mosquito larvae. With bacteria being indispensable for normal larval growth in *Aedes* and *Culex* species, mosquitoes and other insects either fail to develop to full maturity or are unable to vector diseases that are lethal to human or livestock when they are raised under sterile conditions. In this paper, we are reviewing symbiosis in mosquitoes and its application under the following sub topics (1) symbiotic bacteria in mosquitoes; (2) acquisition and transmission of symbionts; (3) localization of symbionts; And discussing potential application of genetically transformed symbionts in control of mosquitoes and insect borne diseases like malaria etc. that are lethal to human or livestock.

17:45

Susceptibility of Legume Pod Borer (LPB), *Maruca vitrata* to the δ-endotoxins of *Bacillus thuringiensis* (Bt)

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Yard-long bean (YLB), *Vigna unguiculata* ssp. *sesquipedalis* is one of the most important vegetable crops in Southeast Asia. Legume pod borer (LPB), *Maruca vitrata* is the major insect pest that attacks floral buds and pods of YLB and thus drastically reduces the yield. Frequent insecticides spraying and short harvest intervals could adversely affect the human and environmental health. Safe and efficient pest control strategies are warranted to reduce the pesticide misuse in YLB. Hence, the objectives of this study are to evaluate the toxicity of insecticidal crystal proteins (ICPs) from *Bacillus thuringiensis* against LPB. Five tested Bt δ-endotoxins, *viz*., Cry1Aa, Cry1Ab, Cry1Ac, Cry1Ca and Cry2Aa were obtained from Jeroen Van Rie (Bayer BioScience, Belgium). Insect bioassays were done by incorporating the δ-endotoxins into the LPB artificial diet. The relative efficacy of δ-endotoxins against second instar larvae of LPB showed that the toxin Cry1Ab was the most potent toxin (LD_{50} 0.207 µg/g), followed by Cry1Ca, Cry1Aa, Cry2Aa and Cry1Ac in the descending order, with LD_{50}s 0.477 µg/g, 0.812 µg/g, 1.058 µg/g and 1.666 µg/g, respectively. Hence, commercial Bt formulations containing Cry1Ab and Cry1Ca toxins may immediately be used to control early larval stages of LPB in YLB. As continuous and large scale use of Bt formulations with single Bt toxin may pose the problem of insect resistance, it is recommended that the Bt formulations with two Bt toxins which would have different target sites in the midgut of LPB be used. As Cry1Ab is a major toxin in *Bacillus thuringiensis* subsp. *kurstaki* (Btk) and Cry1Ca is a major toxin in *Bacillus thuringiensis* subsp. *aizawai* (Bta), the resistance evolution to these toxins by LPB would significantly be delayed. Hence, combined use of Cry1Ab and Cry1Ca would be a most effective component in the integrated pest management of LPB. Besides, combined expression of Cry1Ab and Cry1Ca in transgenic YLB would also possibly provide better protection against LPB.

18:00

A 1.1 kb fragment downstream from the *bin* operon in *Bacillus sphaericus* 2362 affects Bin yield and crystal size

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Highly mosquitocidal strains of *Bacillus sphaericus* (Bs) such as 2297 and 2362 produce binary (Bin) toxins during sporulation. These toxins are composed of a 42-kDa toxic domain (BinA) and a 51-kDa binding domain (BinB) encoded by a two-gene operon. Based on slight differences in their amino acid sequences, four types of Bin toxins are known. Strain Bs2362 (Type 2), which produces a small parasporal crystal, has the highest toxicity against *Culex* species and therefore serves as the active ingredient of the microbial larvicide, VectoLex. Alternatively, strain Bs2297 (Type 3) shows relatively low toxicity even though it produces a much larger Bin crystal than Bs2362. While amino acid sequence differences may explain differences in toxicity, factors governing Bin crystal size and yield differences between these two strains are not known. When upstream and downstream nucleotide sequences of the *bin* operon from Bs2297 and Bs2362 were compared, a 1.6-kb fragment containing four open reading frames (*orfs*) was found in the latter strain downstream from the *bin* operon. Blast searches demonstrated that one of these *orf*s (*orf2*) shared significant identity with the multicopy suppressor gene (*SUR7*) of *Saccharomyces cerevisiae*. To determine whether this gene affected Bin synthesis, a 1.1-kb fragment containing this *orf* was cloned from the Bs2362 and inserted downstream from the Bs2297 *bin* operon using homologous recombination. Bin toxin yield by the recombinant Bs2297 containing this fragment was reduced by approximately 50 to 70%. Concomitantly, mosquitocidal activity of the recombinant Bs2297 against 4th instars of *Cx. quinquefasciatus* decreased by 68% compared to that of the wild type strain. When spore yields were compared, there was no significant difference between wild type and recombinant Bs2297 strains, whereas Bs2362 produced significantly more spores than the other two. These results suggest the additional sequence downstream from the *bin* operon in Bs2362 is responsible for the lower Bin yield and smaller crystal size characteristic of this strain.

18:15

Effect of Ciprofloxacin on Pathogenicity of Resistant *Escherichia coli*

Liancheng Lei and Wenyu Han

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Colibacillosis caused by strains which have multi-resistance and high level of MIC has already become an important and tough problem made tremendous lose s4in poultry farms. So we isolated prevalence avian pathogenic Escherchia coli (APEC) to disclose effect of ciprofloxacin (CIP) on pathogenicity of resistant strains. pathogenicity to mice of drug resistant wild strains did not decline. Most wild resistant strains still have full virulence, only a few of resistant strains maintain partly virulence, but can restore completely after transfer of culture several time in vivo. This finding also suggested that the pathogenicity of strains resistant to CIP could be decreased when the strains growed in media containing CIP in vitro (group3). However, the pathogenicity of these strains increased again when the mice injected with these strains were treated with CIP in vivo (group4).With the treatment with CIP in vivo,no matter which cultivation condition: drug or without drug, these mice (group2 and group4) injected with E.coli culture were to die easily,the living rate are low remarkabley. Especially, pathogenicity detection indicated that these mice suffered from more severe lesions to organs, such as heart, liver, spleen, lung, kidney. Pathogenicity of resistant E.coli was associated with CIP both in vivo and vitro.

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