The 4th Asia-Pacific Congress of Sericulture and Insect Biotechnology

April 23-25, 2015
Haeundae Grand Hotel, Busan, Korea

Program & Abstracts

Organized by

The Korean Society of Sericultural Science
Department of Life Science and Environmental Biochemistry, Pusan National University, Korea
National Academy of Agricultural Science, RDA, Korea

Supported by

Insect Biotech Co., Ltd., Korea
Institute for Research and Industry Cooperation, Pusan National University, Korea
Life and Industry Convergence Research Institute, Pusan National University, Korea
Korean Sericultural Association

The 4th Asia-Pacific Congress of Sericulture and Insect Biotechnology

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The 4th Asia-Pacific Congress of Sericulture and Insect Biotechnology

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The 4th Asia-Pacific Congress of Sericulture and Insect Biotechnology

Friday, 24 April

Insect Biotechnology (Room 3, 2F)

Insect Biotechnology (Room 3, 2F)

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Plenary Session

The 4th Asia-Pacific Congress of Sericulture and Insect Biotechnology

Thursday, 23 April (Room 1, 2F)

Novel approach on industrial insects in Korea

Kyu-seong Lee, Jong-Gill Kim, Pil-Don Kang, HaeYong Kweon

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Traditional usage of silkworm and honeybee is to harvest cocoon for dressing and honey for eating. Recently Korean scientists have investigated some novel function of silkworm, honeybee, and some other insects. These findings widen the usages and create market for industrial insects in Korea; functional food ingredient, biomedical materials, and natural medicine for animal.

The first innovation insect is silkworm. According to the Donguibogam, principles and practice of eastern medicine, silkworm is good for health. And silkworm pupa is a high-protein food as snack. So our group studied and evaluated the anti-diabetic effect of silkworm powder thorough animal experiment. Silkworm powder inhibits α-glucosidase to hamper the absorption of glucose in small intestine. Silkworm with 5^{th} instar 3 days shows the optimum anti-diabetic effect on human. Other memorial one was to develop artificial method of vegetable worms cultivation, called Nuedongchunghacho through silkworm lived. *Paecillomyces tenuipes* was sprayed on the 5^{th} instar silkworm, cultured to make cocoon, and then conditioned to grow synnemate formation. Vegetable wroms Nuedongchunghacho showed anti-cancer, anti-fatigue, and reinforcement of immunization. The latest innovative topic among sericultural product is silkworm cocoon. Cocoon made by silkworm has been used as textile fiber and surgical suture. It means that silk polymer is biocompatible and can be used as biomedical materials. Silk membrane for eardrum recover was developed. Eardrum attached with silk membrane showed rapid regeneration and good architecture.

Related in apiculture research, a new superior hybrid with high honey production was bred last year, and are mass-producing and delivering to beekeepers. Among bee products, bee venom has been used as a medicine but it is difficult to collect and isolate. We developed bee venom collector to harvest bee venom effectively with low damage on honeybee, and then developed the usability of bee venom for livestock and human as natural antibiotics. The cow and pig with bee venom show weight gain and resistance to disease. Cosmetics with bee

venom was developed and commercialized.

Among applied insects, bumblebee, mealworm beetle and white-spotted flower chafer have been studied. Bumblebee was imported as pollinating insect. Artificial mass-production and oviposition induction system was developed for commercialization of bumblebee stable. Pollinating activity of bumblebee has been studied and used applied to 17 horticultural plants including tomato. Some insects including *Tenebrio molitor*, *Protaetia brevitarsis* have been evaluated as new food resources. These insects have nutritional values and non-toxic.

In Korea, industrial insects, silkworm and honeybee, have been widen the application fields to functional food and biomedical resources. Some insects become industrial insects through values discovery like food resources and widen their applicable economical usage.

Key words: Silkworm, Honeybee, Bumblebee, *Tenobrio molitor*, *Protaetia brevitasis*

Opportunity and challenge of silkworm and insects in genome editing era

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Sericulture and insect biotechnology are developing for understanding the mechanisms at the physiological and molecular level. The further exploitation offers the profound knowledge to this Subject. Drosophila, the model insect, has been applied for this purpose and does provide valuable knowledge. But the diversity of insect species needs more targets and tools. Thanks to the progresses of genome research and the genome editing tools, the mechanism exploitation could be extended to many other insects.

Armed with genome information, transgenesis, and genome editing techniques, silkworm is considered as an ideal model for various researches of lepidopteran insects. In addition, with increasing demand for environmentally-friendly pest control, species specific gene targeting and genetic regulation are widely considered to be the future choice. Recently, the new technique of genome editing, Talents and CRISPR/CAS9, offers us convenient alternatives for gene knock out and knock in. Using *BmBlos2* gene as a target, both gene knock out and knock in strains were successfully created in silkworm. We have successed knocked out PBAN gene and some odor receptor genes, the further functional confirmation is being done. These approaches are valuable as the screened target gene could be deleted, or a transgene sequence inserted in a target locus. These methods are also applied to other lepidopteran pests, such as diamondback moth, spodoptera and caterpillars.

The genome editing is developing. Currently, its maturation and scope is limited to only a few species, but it opens the window for non-model insects. The opportunity and challenge are equal to everyone in this field.

Insect factory: Recombinant protein production using silkworm-baculovirus expression system

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Recent progress in the field of genome sciences enables us to obtain the information of genes or amino acid sequences easily. Also the progress in the analytical methods and bioinformatics reveal or predict the novel physiological or pharmaceutical functions of proteins. Based on these information, several researchers designed the new proteins with unique and multiple functions. Therefore, there is a strong possibility in medical or industrial application of recombinant proteins. All these basic and applied research, however, require the purified proteins retained their functions to continue and develop the researches.

For example, many of human cytokines and growth factors are excellent targets for drug discovery, but not establish the methods to produce recombinant proteins at low cost. Although effective utilization of potentially useful “seeds of industrial proteins” arouses us a great expectation, many of these are not commercialized mainly due to the difficulties in expressing recombinant proteins.

The baculovirus expression system is widely used for mass production of recombinant eukaryotic proteins because of the occurrence of post-translational modification similar to mammalian systems. Recently, screening of silkworm strains maintained in Kyushu University for high-expression of recombinant proteins and efficient recombinant baculovirus construction systems were reported. These make it one of the most efficient methods for mass production of recombinant eukaryotic proteins.

In this presentation, we would like to introduce several examples of recombinant proteins expression using silkworm-baculovirus system.

Key words: Baculovirus, Insect factory, Recombinant protein

Silk-based biocomposite materials: A high potential application in tissue engineering scaffolds

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Silk and silk-based materials have recently attracted renewed interest because of their unique structure and properties for biomedical applications. Easy processing, remarkable mechanical properties, superior functionalities, tailorable biodegradability and excellent biocompatibility of silk fibroin (SF) have been specially explored for a high potential application in tissue engineering scaffolds. Above all, silk biocomposite (biohybrid) scaffolds have focused on the development of biomedical devices for use in various tissue engineering scaffolds as forms of film, membrane, sponge, hydrogel, nonwoven mat, nanofiber, nanoparticle, etc. In this presentation, a current review on SF biocomposite scaffolds for tissue regeneration will be extensively covered in addition to our own research results.

Many different types and application fields of silk biocomposite scaffolds have been investigated and one specific example is involved in improving functionality and performance of SF scaffold with hydroxyapatite (HAp) for the bone regeneration. The SF is often hybridized with bioactive additives (e.g., VEGF, BMP-2, HAp, etc) or other biopolymers (e.g., collagen, gelatin, chitosan, hyaluronic acid, PCL, etc), and silk protein is known to have a great cytocompatibility (osteo-induction and differentiation). Therefore, in our study for example, SF scaffolds was hybridized with HAp in various morphological structural features and the fabricated SF/HAp biocomposites were characterized and evaluated for bone tissue regeneration.

Uniformly surface-coated SF nanofibers with HAp crystals could be fabricated by biomineralization the nanofibers in ten times concentrated simulated body fluid with a short immersion time (60-90mins). HAp nanocrystals were well deposited onto the surface of SF nanofibers and the SF biocomposite scaffolds were evaluated *in vitro* and *in vivo*. Another type of SF hybrid scaffold is the SF nanofiber matrix containing HAp nanoparticles inside the

fiber. After surface modification of HAps with hyaluronic acid (HA)-dopamine (DA) conjugate by enhancing dispersibility of HAps in SF dope solution for electrospinning, well dispersed HAps contained SF nanofibrous scaffolds were fabricated and evaluated. Also, injectable SF/HAp composite hydrogels were fabricated by ultrasonification method using aqueous SF solution and surface modified HAp nanoparticles conjugating with HA-DA. Processing conditions and fabrication methods for preparing various SF/HAp biocomposite scaffolds having different morphological structure were established and biological evaluation confirmed that the SF/HAp biocomposites can be applied as tissue engineering scaffold for bone regeneration.

Key words: Silk, Biocomposite material, Scaffold, Tissue regeneration

Branch Session I

The 4th Asia-Pacific Congress of Sericulture and Insect Biotechnology

Thursday, 23 April

Sericulture I (Room 1, 2F)

Production of silk degumming proteases

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Proteases were selected for silk degumming process. It was found that two proteases had a perfect degumming ability in silk yarn and did not affect fibroin fiber. In previous report, protease production was performed in a 500 mL shake-flask system. One of the protease was purified from *Bacillus subtilis* C4 (C4). An another one of the protease was cloned and expressed from *Bombyx mori* which was called cocoonase. The recombinant cocoonase (Bmcoc) was expressed in yeast cell, *Pichia pastorsis*. The later one not only had a degumming ability but also had a sericin degrading activity together with the color bleaching property. In this research, production of the alkaline proteases from C4 and Bmcoc were explored using fermentation technology. Mathematic models were applied to evaluate the enzyme production level. These proteases could provide excellent potential in silk degumming industry and could be applied with other proteins.

Key words: Silk, Degumming, Proteases, *Bacillus subtilis*, Cocoonase

Effect of degumming on the wet spinning and electrospinning performance of regenerated silk

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Recently, owing to excellent properties including biocompatibility and cytocompatibility, silk has attracted attention in biomedical applications. For the application, silk is regenerated to various fiber forms using wet spinning and electrospinning. Degumming and dissolution processes were necessary for the regeneration of silk. In the study, the effect of degumming method and condition on the wet spinning and electrospinning performance of regenerated silk was reviewed using the recent results of related studies. Different residual sericin content determines solution properties of regenerated silk including turbidity and viscosity resulting in different electrospinning performance and the diameter of resultant electrospun silk fiber. Also, the residual sericin affect wet spinning performance of silk and the maximum draw ratio of silk. Degumming method influences the molecular weight and solution viscosity of regenerated silk resulting in different electrospinning performance and the diameter of resultant electrospun silk fiber.

Key words: Regenerated silk, Degumming, Wet spinning, Electrospinning

How does silk provide delightful and attractive offers to physicists?

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Silk fibroin, extracted from the *Bombyx mori* cocoons, has been used as a luxury fabric for five millennia and is now a widely used and studied protein polymer for biomaterial applications due to several desirable features such as its biocompatibility, the ease for chemical modifications, its slow rate of degradation, and reasonable cost. A variety of processing methods to fabricate hydrogels, tubes, sponges, composites, fibers, microspheres and thin films makes it possible using silk fibroin as a biomaterial for implants, scaffolding in tissue engineering and *in vitro* disease models, as well as for drug delivery.

Recently physicists and physics-based engineers who are pursuing biomedical and biological applications based on their knowledge have been keeping observation upon the use of silk. For the electronics, silk could be applied as a substrate material to integrate the electrical circuits. Water-environment induces the time setting degradation, thereby removing the silk devices after the use. For the photonics, Biocompatibility, mechanical properties, and optical transparency of silk make it a unique biomaterial in the nano- and bio-photonics. Additionally silk can be nanopatterned and tunable. This allows manufacturing of structures such as photonic crystals and plasmonic resonators out of a pure protein film. The properties of silk allow these devices to be “biological activated” offering new opportunities for photo-induced diagnosis and therapy.

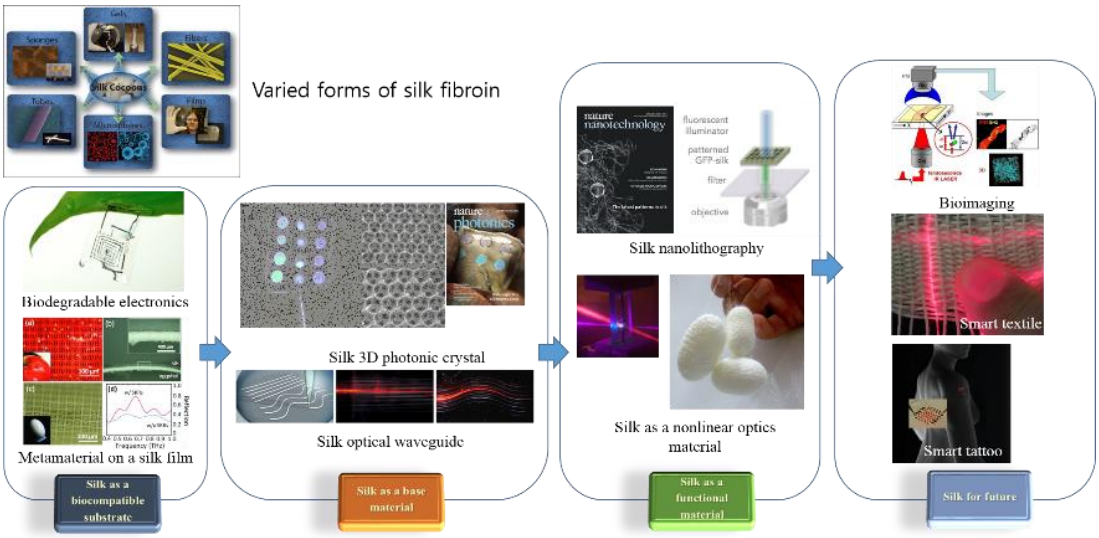

Fig. 1 Roadmap of silk nanophotonics

Here the author presents recent progresses for high-technological reinvents of silk fibroin, especially in the photonics, along with research background about nanophotonics. And remarkable suggestions for future research direction will be also presented as shown in Fig. 1.

Silk membranes for the guided bone regeneration

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Many kinds of membrane have been used for the guided bone regeneration (GBR) technique. However, most membranes do not fulfill all requirements for the ideal membrane for GBR technique. Among them, collagen membrane has been most widely used. However, its high price and weak tensile strength in wet condition are limitations for wide clinical application. Synthetic polymers also have been used for GBR technique.

Recently, silk based membrane has been considered as a membrane for GBR technique. Silk fibroin usually induces foreign body reaction when it is implanted into the bone defect. If the silk fibroin is degraded by the acid treatment, its molecular weight can be decreased below 1 kDa. This low molecular silk protein can increase the alkaline phosphatase activity and collagen synthesis in MG63 cells. When these low molecular silk protein is used with platelet-rich-fibrin, they can increase bone regeneration in the rabbit calvarial defect model and peri-implant bone defect model. Silk membrane still has not been commercialized for the GBR procedures. However, recent several studies reported its potential application as a membrane for the GBR procedures.

Though there have been many promising preclinical data for a silk membrane, the clinical data for the silk membrane has been few. Electrospun silk membrane had been applied to patients successfully. Adverse effect has not been reported related to the silk membrane. Considering that silk membrane can be provided cheap price to the patients, its clinical application should be encouraged.

Key words: Silk, Guided bone regeneration, Membrane

The nano silk fibroin scaffolds for bone tissue regeneration

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Bone tissue has the remarkable ability to regenerate itself provided a proper support is given to the injured or fractured bone in order to hold it in place. Many synthetic and metallic materials have been in use for this purpose. However, they cause immunogenic reactions and other associated long term medical complications. In the present study, a technology has been developed to synthesize composite silk scaffolds for bone tissue regeneration which has a range of advantages including biocompatibility, biodegradability, bioactivity for cell growth and the high mechanical strength. The 3D porous architecture and nano silk fibroin protein provide several sites for cell attachment, proliferation and differentiation into the specialized bone tissue.

Silk secreted by the silkworm consists of two main proteins, sericin and fibroin. Nano silk fibroin (NSF) material was prepared by first removing the sericin, the glue protein from split cocoons using sodium carbonate (degumming) followed by dissolving the fibroin in ternary solution ($CaCl_2$-ethanol-water mixture).The solution was then dialyzed to remove salts and subjected to Ultrasonication to disperse the NSF particle which have tendency to agglomerate. The prepared NSF solution was analyzed for particle size using particle size analyzer which confirmed the nano size of fibroin. The obtained NSF solution was also characterized by UV Spectroscopy, SDS-polyacrylamide gel Electrophoresis and Fourier transform infrared spectroscopy. The solution was lyophilized to get nano silk fibroin powder and studied the morphology using scanning electron microscope which confirmed the rod shaped fibrous structure. The composite scaffold of NSF was prepared by mixing with chitosan/ Poly

Ethylene Glycol (PEG) and/or Poly vinyl Alcohol (PVA) in various combinations and concentrations and analyzed for various properties.

Silk biomaterials are inherently very stable to changes in temperature and moisture, along with being mechanically robust; due to the extensive network of physical cross links formed during the assembly process the scaffolds of nano silk fibroin showing high cross linking seen in SEM images (Fig.3). The enzyme could be immobilized on scaffold of NSF (Fig 2) help in retaining the activity of the enzyme at room temperature. The tensile strength of fibroin based scaffold was found to be 5 MPa and peak load was 248 N (25kg). The average ultimate strength in tension of bovine trabecular bone was 7.6 ± 2.2 MPa (Fig 4)The ultimate tensile strength and fragility test of fibroin based scaffold have values nearing bovine trabecular bone hence can be used as bone implants in case of bone damege/fracture.

Key words: *Bombyx mori*, Nano silk fibroin, Scaffold

Structure and application of Korean *Bombyx mori* silkworm cocoon

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Silkworm cocoon spun by silkworm, *Bombyx mori*, has been used mainly as textile fiber. Recently, silk polymer extracted from cocoon has been considered as a promising biomedical material. The authors examined the general characteristics of natural *Bombyx mori* cocoon, the structural characteristics using infrared spectrometer, mechanical properties using universial testerm, and the feasibility for dental membrane. The morphology of silkworm cocoon was shown like a nonwoven type. Tensile strength and extention rate of silk layer was different with the sample. Cell test using MG63 cell showed that some silk layer exhibited good cell comparability and alkaline phosphotase activity. Animal experiment showed that silk layer was good for the regeneration of new bone formation with minimal inflammation. Therefore, silk layer might be used as a novel dental membane for bone regeneration.

Key words: Silkworm, Cocoon layer, Biomedical application, Structure

Sericin stabilizes fibroin in a phase separated system

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Despite the long history of silk fiber, the spinning mechanism of silk fiber is still not fully solved. The biggest secret is how the concentrated fibroin is stable in the silkgland. In 2003, Jin and Kaplan reported an important result in Nature. Based on the fact that the fibroin is a type of block copolymer consist of hydrophilic and hydrophobic blocks, they found that fibroin exist in a self-assembled micelle structure which enables stabilization of fibroin even at high concentration. However, the micelle structure was observed in a blend film of fibroin and PEG, where PEG is not the component in the silkgland. Instead, in the silkgland, another protein exists with fibroin, which is sericin. Therefore, we blended sericin instead of PEG with fibroin in order to verify the formation of micelle structure of fibroin. As expected, we also could find the fibroin micelle in the presence of sericin. However, more interesting results were found during the study. We found that sericin can retard the crystallization rate of fibroin. It means that sericin can prevent the premature crystallization of fibroin in the silkgland, but do not perfectly inhibit the crystallization of fibroin that will occur during the spinning. We suggested that there might be interaction between fibroin and sericin at the interface through hydrogen bonding. However, these results do not reflect the real situation in the silkgland. In the former study, fibroin and sericin are separated in microscale, but these proteins are separated in macroscale in the silkgland. In this study, we report that sericin can still retard the crystallization of fibroin even in macroscale separated system. We poured the sericin solution carefully on the top of fibroin solution while not disturbing the interface between two protein solutions. We observed the gelation time of fibroin solution beneath the sericin solution. The interface was maintained throughout the experiment unless the concentrations of the two solutions were equal. Surprisingly, the gelation time of fibroin was delayed in the presence of sericin even though they are phase separated. In general, the gelation of fibroin is induced by the conformational transition from Silk I (random-coil predominant) to Silk II (β-sheet predominant) structure, which is the same transition that

occurred during the silk spinning. Therefore, the delay of gelation time of fibroin indicates that the sericin can contribute on the stabilization of fibroin in the silkgland. Conventionally, the role of sericin during the spinning of silk fiber was underestimated and not studied in details. It was suggested the sericin might acts as a lubricant during the spinning. However, our results here suggest that sericin might have more important role during the spinning that stabilize the concentrated fibroin in the silkgland and prevent premature crystallization.

Key words: Sericin, Fibroin, Spinning mechanism, Phase separation

Branch Session I

The 4th Asia-Pacific Congress of Sericulture and Insect Biotechnology

Thursday, 23 April

Insect Biotechnology I (Room 2, 2F)

Transformation of *Bacillus megaterium* for producing insecticidal proteins

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A soil bacterium, *Bacillus thuringiensis* produces crystal proteins toxic to a range of insects and nematodes during sporulation. As a result, it has been used as a control agent for insect pests and disease vectors for many decades. Since the first crystal protein gene (*cry*) was cloned in 1980, more than 300 *cry* and *cyt* (for cytolytic) have been identified from different subspecies and strains of *B. thuringiensis*. Many recombinant strains producing different combinations of Cry and Cyt proteins have been constructed using electroporation technique. *Bacillus megaterium* is known as "big beast" due to its large size with a volume approximately 100 times that of *Escherichia coli*. It is a Gram-positive, aerobic spore-forming bacterium found in widely diverse habitats from soil to seawater, rice paddies, honey, fish and dried food. It can grow in simple media on more than 62 carbon sources out of 95 tested, which has made it an ideal industrial organism for more than 50 years. However, it has not been studied much whether this bacterium can be used to produce insecticidal proteins such as Cry and Cyt from *B. thuringiensis*. Therefore, in this study, several plasmids containing different copy numbers and antibiotic-resistant genes were transformed into *B. megaterium* to determine the optimal plasmid as a vector and the selected plasmid was used to produce insecticidal proteins.

Tribolium castaneum RR-1 cuticular protein TcCPR4 is required for formation of pore canals in rigid cuticle

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Insect cuticle is composed mainly of structural proteins and the polysaccharide chitin. Despite a rather limited composition, insects produce diverse cuticles with the proper combination of mechanical properties (strength, hardness and flexibility). Adult beetles are covered mostly in a hard cuticle, but they can fly because the cuticle is lightweight. The rigid cuticle is comprised of three major functional layers, namely the outermost envelope, the protein-rich epicuticle and the innermost chitin-protein rich procuticle. In addition, there is a large number of vertically oriented columnar structures denoted as pore canals that contain chitin fibers (pore canal fibers, PCFs) that are absent in soft and flexible cuticles.

The CPR family is the largest family of cuticle proteins (CPs), which can be further divided into three subgroups based on the presence of one of the three presumptive chitin-binding sequence motifs denoted as Rebers-Riddiford (R&R) consensus sequence motifs RR-1, RR-2 and RR-3. The TcCPR27 protein containing the RR-2 motif is one of the most abundant CPs present both in the horizontal laminae and in vertical pore canals in the procuticle of rigid cuticle found in the elytron of the red flour beetle, *Tribolium castaneum*. Depletion of TcCPR27 by RNA interference (RNAi) causes both unorganized laminae and pore canals, resulting in malformation and weakening of the elytron. In this study, we investigated the function(s) of another CP, TcCPR4, which contains the RR-1 motif and is easily extractable from elytra after RNAi to deplete the level of TcCPR27. Transcript levels of the *TcCPR4* gene are dramatically increased in 3 d-old pupae when adult cuticle synthesis begins. Immunohistochemical studies revealed that TcCPR4 protein is present in the rigid cuticles of the dorsal elytron, ventral abdomen and leg but not in the flexible cuticles of the hindwing and dorsal abdomen of adult *T. castaneum*. Immunogold labeling and transmission electron microscopic analyses revealed that TcCPR4 is predominantly localized in pore canals and regions around the apical plasma membrane protrusions into the procuticle of rigid adult cuticles. RNAi for *TcCPR4* resulted in an abnormal shape of the pore canals with amorphous

PCFs in their lumen. These results support the hypothesis that TcCPR4 is required for achieving proper morphology of the vertical pore canals and PCFs that contribute to the assembly of a cuticle that is both lightweight and rigid.

This work was supported by NRF (NRF-2012R1A2A1A01006467).

Key words: *Tribolium castaneum*, Cuticle protein, Cuticle/Exoskeleton, Pore canal fiber

Development of new technologies for utilizing baculoviruses and insect cell lines

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Baculovirus expression vector system (BEVS) has been expanding its capability in biotechnology from valuable protein production to challenging applications such as gene therapy. In our laboratory, aiming at utilization of insect-related properties, noble applications of BEVS including a long-term storable protein production system using diapausing pupa of giant wild silkworms and artificial diets containing stable isotopes for labeling whole insect body as well as recombinant protein have been successfully developed. In addition, genetic engineering of insect cell line using *piggyBac* transposon, which have enabled us to elucidate the mechanism of piRNA-mediated transgene-specific gene silencing, and genome editing of insect cell line for altering posttranslational modification properties are in progress.

Key words: Baculovirus, Insect cell line, Posttranslational modification, Diapause, Genome editing

Comparative transcriptome analysis of midgut-expressed genes between mulberry feeders and nonmulberry feeders

Takashi Kiuchi[1], Huabing Wang[1], Munetaka Kawamoto[1], Takashi Yamano[1], Yusuke Hiramatsu[1], Koji Kadota[1], Yutaka Suzuki[2], Sumio Sugano[3], Susumu Katsuma[1], Toru Shimada[1*]

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The mulberry is the most suitable diet for the silkworm, *Bombyx mori*. However, almost plant-eating insects cannot grow by the mulberry leaves. It is supposed that large amounts of sugar-mimic alkaloids, such as 1-deoxynojirimycin and 1,4-dideoxy-1,4-imino-D-arabinitol, in the mulberry latex inhibit some disaccharidase activities and prevent the growth. Most bombycoid silkworms also cannot grow by the mulberry leaves, and only the species of *Bombyx* and *Rondotia* conquer the toxic sugar-mimic alkaloids in the mulberry latex. These mulberry-feeding silkworms and other mulberry-feeding worms, such as *Hyphantria cunea* and *Glyphodes pyloalis*, have some defense system against the sugar-mimic alkaloids. However, the molecular mechanism is unknown. The goal of our study is to identify the evolutionary key genes involved in the tolerance to sugar-mimic alkaloids by comparative transcriptome analysis between mulberry feeders and nonmulberry feeders.

To know how mulberry-feeding worms can digest mulberry leaves and grow healthy, we performed deep sequencing of RNAs (RNA-Seq) isolated from midguts of 6 mulberry feeders (Bombycidae: *Bombyx mori*, *Bombyx mandarina*, *Rondotia menciana*, Arctiidae: *Hyphantria cunea*, Noctuoidea: *Mamestra brassicae*, Crambidae: *Glyphodes pyloalis*) and 4 nonmulberry feeders (Bombycidae: *Trilocha varians*, *Ernolatia moorei*, Saturniidae: *Antheraea pernyi*, *Samia cynthia ricini*) using illumina GAIIx. *De novo* assembly was carried out using Trinity assembler. Short reads were assembled into 34,343-54,717 contigs, with average lengths of

668-1,046 bp in each insect (All contigs will be deposited in SilkBase: http://silkbase.ab.a.u-tokyo.ac.jp). We listed orthologous genes based on 31,758 *B.mori* unigenes by BLAST program. Some differentially expressed genes in mulberry feeders were identified by comparative gene expression analysis of orthologous genes between mulberry feeders and nonmulberry feeders. Two independent methods, i.e. pattern matching method and weighted average difference (WAD) method, showed that a beta-fructofuranosidase gene (*Suc1*) and a maltase gene (*Mal1*) are highly expressed in all mulberry-feeding worms' midgut. It has been reported that the sucrase activity of beta-fructofuranosidase is not inhibited by sugar-mimic alkaloids. Furthermore, enzymatic analysis using recombinant proteins expressed by baculovirus expression system showed that the activity of Mal1 is less sensitive to sugar-mimic alkaloids in *Bombyx mori* than in *Trilocha varians* and *Samia cynthia ricini*. These results suggest that high expression of disaccharidase genes whose activities are insensitive to sugar-mimic alkaloids contribute to mulberry-feeding.

Key words: Midgut, Mulberry-feeding, Sugar-mimic alkaloids, Transcriptome analysis

Anticancer activities of synthetic analogs of coprisin, an antimicrobial peptide from the dung beetle *Copris tripartitus*

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An insect defensin, named coprisin, was identified from the dung beetle, *Copris tripartitus* immunized against *E. coli*. The peptide was composed of forty-three amino acid residues containing conserved six cysteines with three intramolecular disulphide bridges. Then, we generated coprisin analogs based on structural analysis and selected α-helical region of coprisin. Among the several synthetic analogs, dimeric form of CopA3 has been previously confirmed to have antimicrobial activity. In the present study, we have assessed the anticancer activity of CopA3 dimer peptide against human gastric cancer cell lines. As a result, we determined that the cell viability and cytotoxicity of gastric cancer cells. We also conducted an investigation into CopA3's mechanism against SNU668 cancer cell line. CopA3 was shown to induce necrotic and apoptotic cell death of gastric cancer cells by acridine orange/ethidium bromide staining and flow cytometry analysis. In addition, CopA3 bound to the surface of cancer cells via a specific interaction with phosphatidylserine, which is one of cancer cell membrane components. Intratumoral inoculation of D-CopA3 resulted in a significant decrease in the SNU668 gastric cancer tumor volume in a xenograft mice model. Moreover, histologic analysis revealed that D-CopA3 caused tumor suppressive effect in tumor tissues after peptide treatment compared with the untreated tumors. Collectively, the results suggest potential utility of CopA3 as a cancer therapeutic agent.

Key words: Antimicrobial peptide, Anticancer activity, Necrosis, Phosphatidylserine, CopA3

A peroxiredoxin 5 from common cutworm (*Spodoptera litura*) acts as a potent antioxidant enzyme

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Peroxiredoxins (Prxs) represent an expanding superfamily of antioxidative proteins that protect organisms against various oxidative stresses and are involved in a variety of physiological processes. Here, we describe the cloning and characterization of a Prx from the common cutworm *Spodoptera litura* (SlPrx5). The SlPrx5 cDNA contains an open reading frame of 477 bp encoding a predicted protein of 159 amino acid residues, 16.902 kDa, and an isoelectric point of 7.68. Furthermore, the deduced amino acid sequence of the SlPrx5 cDNA showed 86% identity to *Papilio xuthus* Prx5, 72% to *Aedes aegypti* Prx5, and 64-67% to other insect Prxs. A phylogenetic analysis further revealed that the deduced amino acid sequence of SlPrx5 groups within the atypical 2-Cys Prx cluster. Recombinant SlPrx5 (20 kDa) purified from baculovirus-infected insect cells was found to reduce H_2O_2 in the presence of electrons donated by dithiothreitol and protect super-coiled DNA from damage by metal-catalyzed oxidation *in vitro*. During *S. litura* development, *SlPrx5* is constitutively expressed in the epidermis, fat body, and midgut, with the highest expression occurring in the sixth-instar larval stage in the fat body and midgut. Additionally, SlPrx5 mRNA expression was up-regulated after injection with H_2O_2, cumene hydroperoxide, indoxacarb, and metaflumizone. A disc diffusion assay indicated that recombinant SlPrx5 can play a functional role in protecting cells from oxidative stress *in vivo*. These results provide insight into the role of SlPrx5 during development and the oxidative stress response of *S. litura*.

Key words: Antioxidant enzyme, *Spodoptera litura*, Common cutworm, Oxidative stress, Peroxiredoxin, Reactive oxygen species

Chitinase 7 (TcCHT7) is required for chitin deposition and cuticle morphology of *Tribolium castaneum*

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Insect chitinases (CHTs), which belong to family 18 glycosylhydrolases (GH-18), have been detected in molting fluid and gut tissues and are predicted to mediate the digestion of chitin present in the cuticle/exoskeleton and peritrophic matrix (PM) in the gut. Based on amino acid sequence similarity and phylogenetic analysis, insect CHT family proteins have been classified into eight groups (group I to VIII).

CHT7s belong to Group III chitinase contain two catalytic domains and one chitin binding domain (CBD) at the C-terminus. The catalytic domain 1 of this group of chitinases exhibits greater sequence similarity to one another than to the catalytic domain 2 in the same protein(s), suggesting distinct functions and/or evolutionary origins for each of these two catalytic domains. This group of chitinases, unlike most insect CHTs, possesses a predicted transmembrane segment at the N-terminal region. The recombinant *Tribolium castaneum* CHT7 (TcCHT7) protein expressed in Hi-5 insect cells was bound to the cell membrane. Apparently, the catalytic domains of this CHT face the extracellular space as revealed by its ability to hydrolyze an artificial chitin substrate added to the medium.

DsRNA-based functional studies (RNAi) for several *CHT* genes in *T. castaneum* indicated that CHTs belong to groups I (TcCHT5) and II (TcCHT10) are critical for molting and turnover of chitin in the old cuticle during molting and/or metamorphosis. In other hand, RNAi for *TcCHT7* did not affect any types of molting such as larval-larval, larval-pupal and pupal-adult. The resulting pupae or adults, however, failed to wing-expansion and abdominal contraction. Immunohistochemical analysis revealed that TcCHT7 protein is localized in newly synthesized procuticle, suggesting that TcCHT7 could be released from the plasma membrane of epidermal cells by proteolysis. Cuticular chitin appears to accumulate inner region of the procuticle in TcCHT7-deficient animals. Transmission electron microscopy revealed that RNAi for *TcCHT7* resulted in disorganization of horizontal chitin laminar in both rigid cuticle (e.g. elytron) and soft cuticle (e.g. hindwing). In former cuticle, TcCHT7 is

also critical for formation of the vertical oriented pore canals. These results suggest that TcCHT7 have critical roles in the laminar assembly and synthesis and/or deposition of cuticular chitin.

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Key words: *Tribolium castaneum*, Chitinase, Chitin, RNAi, Cuticle/Exoskeleton

Branch Session II

The 4th Asia-Pacific Congress of Sericulture and Insect Biotechnology

Friday, 24 April

Sericulture II (Room 1, 2F)

Activities of the national bioresource project silkworm Japan

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The National BioResource Project (NBRP) started in 2002 with support by the government of Japan. The purpose of NBRP is the collection, preservation and distribution of bioresources that are basic materials for life science researches worldwide. Currently, 29 species, including the silkworm are available for distribution. The following resources are available by contacting the Silkworm NBRP (http://silkworm.nbrp.jp/). (1) Domesticated silkworm (*Bombyx mori*) mutant, consomic, and transgenic strains; (2) eight species of wild silkworm representing the *Bombyx, Antheraea, Samia, Rhodinia, Actias, and Trilocha* genera; (3) cDNA clone of *B. mori* and *S. cynthia ricini*, Fosmid clone of *B. mandarina*; and (4) assembled RNA-seq data of *B. mori*, *S. cynthia ricini,* and *Trilocha varians.* The progress of the Silkworm NRBP project makes it possible to provide *B. mori* strains throughout the seasons. Development of genome editing technology in *B. mori* has resulted in the rapid increase in the number of strains, which has created an urgent need to establish efficacious systems for the preservation of increasing strains. All strains should be reared once a year, because diapause egg of *B. mori* cannot be preserved for more than one year. There are two kinds of long-term preservation methods in *B. mori*. One uses frozen sperm and the other uses frozen ovaries. The former are artificially inseminated into female moths and the latter are implanted into castrated female larvae. We are testing the freeze tolerance of sperm and ovaries in inbred strains. Preliminary results showed that 43% (102/233) and 71% (202/284) of inbred strains have tolerated sperm-freezing methods and ovary-freezing methods, respectively. On the other hand, transgenic strains have same genetic background with high freeze tolerance. The ovary-freezing method has been applied to the cryopreservation of transgenic strains because it is easier than sperm-freezing methods. Seventy percent of transgenic strains ovaries

(100/140) have been cryopreserved, making it unnecessary to rear 40 of these 100 strains annually for the preservation of diapause eggs. In 2013, we began to provide transgenic strains that were restored from frozen ovaries. Practical applications of cryopreservation help us to reduce both the labor involved in rearing silkworms and the risks of losing particular strains through unexpected accidents such as disease or contamination.

Key words: Domesticated silkworm, Transgenic silkworm, Wild silkworm, Cryopreservation

Systematic preservation of silkworm genetic resources

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Genetic resources are current and future ground for evolution of new properties. Fortunately, more than 3000 silkworm (*Bombyx mori*) strains are still maintained in Europe and Asia. These strains are annually reared, and scores from indoor rearing are analyzed for consistent character maintenance. Thus, individuals with unstable heritable characters are discarded for better keep pure lines. Nevertheless, still much confusion on the genetic stock exists. Furthermore, recent advance in molecular techniques again requires reexamination of once well established strains. In this study, we selected eight available microsatellite markers that were previously utilized and genotyped silkworm strains preserved in Korea, in order to determine the utility of the markers in detecting DNA polymorphism and to assess their potential for use in strain discrimination. The eight markers was applied to identify more than 340 silkworm genetic stocks preserved in korea. In conclusion, the strains with same traits have been classifying whether or not duplicate genetic resources by the eight discriminal microsatellite markers.

Key words: Silkworm strain, *Bombyx mori*, Microsatellite markers, Genetic resources

Cloning and characterization genes in hedgehog signaling pathway in silkworm, *Bombyx mori*

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Hedgehog (Hh) signaling pathway is one of the classical signaling pathways that govern the regulated developmental processes in multicellular organisms. However, the role of the pathway in silkworm remains poorly understood. In this report, we found that Hh pathway components are expressed in most tissues of silkworm larvae, Which includes only one ligand named *BmHh*, *Bmptch* receptor, signal transducer *BmSmo*, and transcription factor *BmCi*. In response to *Escherichia coli* induction, Hh signaling pathway is activated, the key moleculars involved in Hh signaling pathway are all upregulated . Conversely, repressing the Hh signaling pathway transduction by Cyclopamin, the expression level of the four key genes involved in Hh signaling pathway are reduced. Overall, these findings provide us a totally new insight into the component and function of Hh signaling pathway in silkworm, *bombyx mori*.

Key words: Hedgehog signaling pathway, Expression patterns, qRT-PCR

Effect of non-susceptibility gene to *Bombyx* densovirus type 1, *Nid-1*, to the virus infection mechanism

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Bombyx mori densovirus type 1 (BmDNV-1) is a pathogen that causes flacherie disease in the silkworm *B. mori*. The absolute nonsusceptibility to BmDNV-1 among certain silkworm strains is determined independently by two genes, *nsd-1* and *Nid-1*. Previously, we investigated the viral transcript by RT-PCR in inoculated silkworms carrying different sets of *nsd-1* and *Nid-1* genotype, suggesting that *nsd-1* and *Nid-1* block the early and late steps of virus infection in the silkworm, respectively. In this study, we will report the function of NID-1 protein to virus infection mechanisms. We investigated the sequential change of transcripts and proteins derived from BmDNV-1 in silkworms carrying *Nid-1* genotype after inoculation of the virus. RT-PCR showed that virus transcript was detected immediately after the virus infection, but clearly decreased as time goes by. On the other hand, western blotting analysis using three antibodies corresponding to the one BmDNV-1 structural protein and two BmDNV-1 non-structural proteins demonstrated that all virus proteins were not detected from the silkworms carrying *Nid-1* genotype. These results suggested that NID-1 protein might block the *translational* step of virus proteins. Now we are attempting to isolate *Nid-1* by positional cloning using the *Bombyx* genome information. We will discuss the relationship between translational inhibitors and *Nid-1* candidate genes.

Key words: *Bombyx mori*, Densovirus, Nonsusceptibility, *Nid-1*

Engineering of silkworm-baculovirus expression system for efficient production of G protein-coupled receptors

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The baculovirus expression system (BES) has been extensively utilized for the high-level expression of sufficient quantities of recombinant proteins in a broad taxonomic range of insect cell lines and indivisuals. The proteins expressed by BES are correctly folded and biologically active in many instances. Several recent studies have greatly contributed to reducing the amount of time and labor required to generate recombinant viruses. Additionally, modifications of virus vectors have made this system a more attractive alternative for protein scientists. Among the many protein species, however, membrane proteins were known to be targets with the great difficulty of high yield production. There are few reports on the screening and improvement of the silkworm, *Bombyx mori* as a host insect for efficient membrane protein production. G protein-coupled receptors are seven transmembrane domain membrane proteins, which are regulating many basic physiological processes, such as neurotransmission, growth, development, cellular differentiation, inflammation and the immune response. Here we chose five silkworm GPCRs, named GPCR1, GPCR2, GPCR3, GPCR4, and GPCR5, as the targets for mass-production using silkworm baculovirus expression system. Combination with other study result, we can know the basically function of these GPCRs in the silkworm.

Key words: Baculovirus expression system, G-protein coupled receptor, *Bombyx mori*

The dual role of the prophenoloxidase-activating system in cuticular melanization and innate immunity in the silkworm, *Bombyx mori*

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Insect cuticular melanization is regulated by the prophenoloxidase (proPO)-activating system, which is also involved in the innate immune reaction. Here, we demonstrate how the differentiation of the proPO-activating system is regulated toward a cuticular melanization or innate immunity function in silkworm (*Bombyx mori*) pupae. Our results indicate that the differential and spatial regulation of key components, such as the proPO-activating factor, tyrosine hydroxylase, and proPOs, primes the proPO-activating system for either cuticular melanization or innate immunity. This dual strategy for cuticular melanization in development and innate immunity upon infection demonstrates a two-pronged defense mechanism that is mediated by the priming of the proPO system.

Key words: Cuticular melanization, Development, Innate immunity, proPO-activating system, Silkworm

SUMOylation signaling in the regulation of Polo-like kinase 1 localization during the cell cycle

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It has been shown that the disruption of the SUMOylation system in silkworm induced the defects of holocentric chromosome congression and segregation during mitosis. How the SUMOylation signaling regulates cell division is largely unknown. Here, we identified one potential target of the SUMOylation, Polo-like kinase 1 (Plk1), which is able to regulate the cell division in silkworm. We showed that the Plk1 could be localized to centrosome, mitotic spindle, midzone, and midbody together with the SUMOylation protein Smt3 following the progress of cell cycle. RNAi-mediated knockdown of the SUMOylation genes including Smt3, Ubc9, and Ulp1 led to the mislocalization of Plk1 on mitotic spindle and midzone during mitosis. Furthermore, we demonstrated that the correct localization of Plk1 on centrosome requires a proper SUMOylation at Lys466 by Smt3 during interphase, and mutation of Plk1 at Lys466 abolished its interaction with Smt3 and Ubc9. The conserved role of silkworm Plk1 SUMOylation at Lys466 was further confirmed by its *Drosophila* and human homologues. Altogether, the present data provides a novel regulatory layer for cell division by Plk1 via the SUMOylation system.

Key words: Silkworm, SUMOylation, Polo-like kinase 1, Cell cycle regulation

BmLanB1-w, a new laminin beta gene evolved for the wing-specific cell adhesion in silkworm, *Bombyx mori*

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Laminins, composed of different α, β and γ chains, are extremely important basement membrane components with crucial roles in development. The number of the laminin isoforms and their coding genes are variable across spieces. To date, two α, one β, and one γ chain have been identified in insects. Here, we isolated a novel laminin beta gene, *BmLanB1-w*, by positional cloning of the recessive mutant (*crayfish*, *cf*) with blistered wings in pupal and adult stages in silkworm. Gene structure analysis showed that a 2-bp deletion *of BmLanB1-w* gene in the *cf* mutant caused a frame-shift in ORF and generated a TGA premature stop codon. Konckdown of *BmLanB1-w* gene induced individuals exhibiting blistered wings, indicating that it was required for cell adhesion during wing development. By identifying laminin homologs in different species, we proved that two laminin β copies, *BmLanB1-w* and *BmLanB1*, were originated in Lepidoptera during evolution. Furthermore, phylogenetic analysis and expression profile analysis of silkworm laminin genes revealed that the *BmLanB1-w* gene was newly evolved, and was required for the wing-specific cell adhesion. To our knowledge, this is the first report showing the tissue specific distribution and functional differentiation of laminin β in insects.

Key words: laminin, blister, wing, *Bombyx mori*

Mass production of bioactive recombinant human acidic fibroblast growth factor (r-haFGF) in transgenic silkworm cocoons and its application in silk fiber genetical modification

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The silk gland (SG) of the silkworm has significant potential for mass production of valuable recombinant proteins due to its convenient synthesis and secretion of silk proteins. In this study, we provided an alternative strategy to modify the original sericin-1 expression system. Transgenic silkworms were generated and the results showed that the DsRed was successfully expressed in MSG and secreted into the sericin layer; the modification of the vector resulted in a 4-fold increase of the recombinant RFP in transgenic silkworm, which leading the contents of recombinant RFP account for approximately 9.5% (w/w) of the cocoon weight. Then, the sequence-optimized Human acidic fibroblast growth factor (haFGF) was inserted into the modified sericin1 expression system to generate the original transgenic silkworm line, which was further crossed with a PIG jumpstarter line to achieve the remobilization of the expression cassette to the “safe harbor” locus of genome for the efficient expression of the r-haFGF. As a result of that, the expression of r-haFGF protein in the remobilized line achieved a further 5.6 folds increase comparing to the original line, which accounted for almost 30% weight of total extracted cocoon proteins. The high content of r-haFGF facilitated its purification that ultimately led 350μg r-haFGF with over 99% purity could be purified from 1g the cocoons. Mitogenic activity and wound healing assays showed that the r-haFGF protein was bioactive to promote the growth, proliferation and migration of NIH/3T3 cells, which was equivalent to the commercial haFGF standard. Remarkably, the transgenic raw silk also significantly stimulated the cell growth and proliferation of mouse embryonic fibroblast cell (NIH/3T3), suggesting the mitogenic activity of recombinant hFGF1 was well maintained and functioned in the sericin layer of transgenic raw silk and the genetically engineered raw silk could be directly as a fine biomedical material for mass application. In conclusion, silk

gland of silkworm combining with the jumpstarter-mediated remobilization could be as an efficient bioreactor strategy for recombinant production of bioactive proteins in the cocoons and the strategy by expression of functional recombinant proteins in the sericin layer of silk might be applicable to create more genetically engineered silks with various bio-functions and applications.

Key words: Silkworm, Ttransgene, Bioreactor, Silk genetical modification

Branch Session II

The 4th Asia-Pacific Congress of Sericulture and Insect Biotechnology

Friday, 24 April

Insect Biotechnology II (Room 2, 2F)

Entomopathogenic fungal resources: Pest management and functional genomics

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Application of pesticides for a plenty period of time resulted in insect resistance and residual adverse effect in environment, thus alternative insecticides with different mode of actions and safety, for example entomopathogenic microorganisms are now seriously considered. In particular, entomopathogenic fungi have relatively broad spectrum including lepidopteran, coleopteran, hemipteran, thysanopteran, dipteran insects and, some mites, compared to entomopathogenic bacteria and viruses. The fungi are facultative microorganisms, dwelling in soil or infecting host insects, and some of the genera have been used as biological control agents worldwide. In this work, we are under construction of entomopathogenic fungal library, which serve resources for the research and development of biopesticides to control some serious pests, such as whiteflies, mites, diamondback moths, aphids, stink bugs, thrips, and mosquitoes. Fungal colonization is an essential requirement for the successful management of insect pests. Molecular biotechnology provides a deep insight on how the fungal entomopathogens infect and kill the target insects and what factors are mainly involved in the pathogenesis. This work reports the importance of entomopathogenic fungal library and its usefulness in pest management with ecological colonization, followed by the characterization of virulence factors.

Key words: Insect resistance, Entomopathogenic fungi, Broad spectrum, Pest management, Entomopathogenic fungal library

Application of insect molecular pathology on beneficial insect: Honeybee (*Apis mellifera*) microsporidium (*Nosema ceranae*) detection

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The microsporidium, *Nosema ceranae*, is considered to be one of most serious pathogens of the honeybee (*Apis mellifera*). However, the pathogenesis on the infected honeybees caused by *N. ceranae* is not well understood. Herein, suppressive subtractive hybridization (SSH) was performed to evaluate the differential expression of midgut genes of the infected honeybees with *Nosema ceranae*. A total of 248 differentially expressed sequence tags (ESTs) was identified from the subtracted libraries. Among them, 56% of these ESTs (140 ESTs) are belonging to insect hosts. 23% (57 ESTs) are microbial related genes, including genes of microsporidia (11%; 27 ESTs) and deform wing virus (1% ESTs). A nosema specific gene, *sr22*, a putative structural gene, was selected and further investigated. Based on the detection of the experimental infections of honeybees with *N. ceranae* by PCR with a designated *sr22* specific primer set, the gene expression of *sr22* was initially detected at 3.5 days post inoculation (dpi) and reached a peak at 14 dpi, then declining slightly until 21 dpi.. Furthermore, *sr22* was found that this gene is a highly conservative gene within the genus *Nosema*. The efficiency of this specific primer set was also evaluated and found that up to the DNA from a purified spore and from the infected midguts at 3 dpi. could be detected. Moreover, by absolute quantitative PCR, the copy number of *sr22* could be represented the number of parasites. We suggest this diagnosis platform, based on *sr22*, can not only be applied to detect *N. ceranae* infection but also the level of infection (copy number determination) in the infected tissue and also be used for other *Nosema* spp.

Key words: Microsporidia, *Nosema ceranae*, Honeybee, cDNA subtraction, sr22, quantitative PCR, Diagnosis platform

Arthropod-pathogenic fungi entomophthorales occurrence in Korea

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Entomopathogenic fungi are categorized into two groups of orders, Hypocreales and Entomophthorales. Consideration has been given to members of the Hypocreales order, such as *Beauveria*, *Metarhizium*, *Lecanicillium*, and *Isaria* because of their high virulence and broad spectrum. In contrast, little interest has been given to the Entomophthorales although this order includes many genera having pathogenicity against agriculturally serious pests. Hosts of Entomophthorales are usually contained mites, aphids, lepidopteran larvae which are economically important pest arthropods. Although many studies focus on the morphological and cultural characterization of the genera of the Hypocreales, few studies examined genera of the Entomophthorales even though these fungi infect some serious agricultural pests in our country. Entomophthorales have been required solutions of some problems including cultivation and production for using IPM factors. Nevertheless, one of particularities of the Entomophthorales, which enable them to spread fast, is an active projecting of conidia from conidiophores, and, they have formed natural epizootics sometimes. As though entomopathogenic fungi are very sensitive to abiotic environmental factors in pathogenesis, Entomophthorales have been influenced by temperature, precipitation and humidity. This work reports characteristics of Entomophthorales, including *Entomophaga aulicae* infecting *Aedia leucomelas* in sweetpotato, in our country.

Key words: Entomopathogenic fungi, Entomophthorales, *Entomophaga aulicae*

Investigation of insect pathogenic fungal bioactive substances

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Insects constitute the largest and most diverse group of animals on Earth. They also serve as the hosts or nutrient sources for an immense assemblage of parasites, pathogens, and predators, ranging from bacteria and fungi to plant and animals. More than 700 known fungal species from 100 genera have adopted an entomopathogenic lifestyle. These fungi are widely distributed, occurring in aquatic, forest, and agricultural habitats, and are often used as active ingredients in microbial insect pest control agents. Their mode of action against insects involves the attachment of conidia to the insect cuticle, followed by germination, cuticle penetration, and internal dissemination throughout the insect. During this process, secreted enzymes, protein toxins, and secondary metabolites can be used by the fungus to overcome the host immune system, modify host behavior, and defend host resources against competing pathogens and saprophytes. In some cases, the host insect relationship has been found to be associated with bioactive fungal metabolites. These metabolites exhibit a wide variety of insecticidal, antibacterial, antifungal, anticancer, antioxidant, and antiviral activities. Using molecular techniques and phylogenetic analyses, both the asexual (anamorphic) stages and sexual (teleomorphic) stages of entomopathogenic fungi have recently been shown as linked together. Therefore, entomopathogenic fungi, especially in complex with the host insect, might be a promising source of bioactive molecules of pharmaceutical and various industrial interests. Here, we evaluated the antimicrobial activity of entomopathogenic fungi metabolites against plant pathogenic bacteria and fungi for the use in agriculture. The anticancer activity was also evaluated by cadaver infected fungi for pharmaceutical interests.

Key words: Entomopathogenic fungi, Secondary metabolites, Insect cadaver infected with fungi-derived substances

Development of mycopesticides using entomopathogenic fungi for control of whiteflies and aphids

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During crop production various insect pests including aphids and whiteflies infest greenhouse vegetables or ornamentals world-wide. Aphids and whiteflies are very difficult to control using chemical pesticides because of the development of insecticide resistance resulting in rapid population increases. Entomopathogens offer promising alternative control methods. Most must be ingested in order to initiate infection and subsequent death of the pest. However, because entomopathogenic fungi can infect without ingestion, they are good candidates for control of plant sucking insects such as aphids, whiteflies and mites. Conidia of entomopathogenic fungi are influenced by environmental conditions such as temperature and relative humidity and cause slow and fluctuation in mortality. These factors are preventing wider application and use of these biocontrol agents. To investigate means to mitigate such problems, we conducted a study with fungal culture filtrates to determine if secondary metabolites produced by entomopathogenic fungi have the potential for use in aphid control. Culture filtrates of an isolate of *Beauveria bassiana* produced high mortality in cotton and green peach aphids. Filtrate of the isolate cultured in Adamek's medium showed highest toxicity at 100% in third instar nymphs of the aphid compared with seven other filtrates cultured in different broths amended with colloidal chitin or oil. The fungal culture fluid or culture filtrate of the *B. bassiana* cultured in Adamek's medium has potential for development as a mycopesticide for aphid control. This isolate has been transferred to companies in Korea to develop commercial microbial pesticide for aphid control. We also conducted research using domestic fungal isolates to develop a mycopesticide for control of the sweet potato whitefly. An isolate of *Isaria javanica* was selected due to its high virulence to the sweet potato whitefly. It infects all developmental stages of *B. tabaci* including the egg stage and is effective under various environmental conditions. It has also been transferred to companies for commercialization in Korea and has been registered as an eco-friendly pesticide.

Key words: Aphid, Entomopathogenic fungi, Microbial pest control, Whitefly

Identification and functional characterization of autophagy-related genes in response to *Listeria monocytogenes* infection in the coleopteran model insect, *Tenebrio molitor*

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Autophagy is a lysosomal self-eating process against unused or damaged macromolecules, cellular components and whole organelles. Currently 36 autophagy-related gene (ATG) homologues have been characterized in yeast and higher eukaryotes including insects. Autophagy signals can be induced by extra- or intracellular stressors and signals such as starvation, growth factor deprivation, ER stress, and pathogen infection. In this study, we have screened and identified a comprehensive set of *Tenebrio molitor* autophagy-related genes using transcriptome sequencing and EST analysis that may operate at different levels in the autophagic process. To study autophagy in response to microbial infection, anti-TmAtg8 polyclonal antibody, the marker for autophagy signaling was generated. We investigated expression patterns of autophagy-related genes in different developmental stages and different tissues, and induction patterns of autophagy-related genes against pathogenic infection. Depletion of autophagy-related genes (*TmAtg3*, *TmAtg5* or *TmAtg8*) led to a significant reduction in survival ability of *T. molitor* larvae against an intracellular pathogen, *Listeria monocytogenes*. These data suggested that *Tenebrio* autophagy-related genes may play putative role in mediating autophagy-based clearance of *L. monocytogenes* in *T. molitor* model. We are currently working on potential function of *microtubule-associated protein 1a/1b light chain 3* (*TmLC3*) which is another member of ATG8 gene family.

Key words: RNA-seq, *Tenebrio molitor*, Autophagy-related genes, RNA interference, *Listeria monocytogenes*

Enhanced production of recombinant proteins in partial-polyhedrin fusion baculovirus expression system

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The baculovirus expression vector system (BEVS) is an effective and widely used method for the production of recombinant proteins in insect cells or larvae. However, the expression efficiency of foreign proteins using the polyhedrin promoter could not obtain the protein yields observed for native polyhedrin. To enhance the expression level of baculovirus vector system, we constructed several fusion vectors using various fragments of the polyhedrin. The polyhedrin fragments were genetically fused to the various recombinant proteins under the control of polyhedrin promoter, and their expressions were analyzed in insect cells. The distribution of these fusion proteins in infected insect cells was determined by using a detergent-based biochemical fractionation procedure and confocal laser scanning microscopy. Expression of the fusion protein was identified by SDS-PAGE and Western blot analysis. As a result, the several proteins expressed by the partial polyhedrin-fused expression system was markedly increased. However, we identified that hyper-expression of target protein varied depending on the partial polyhedrin. This study provides a new option for the higher and stable expression of useful foreign recombinant protein by using the Partial-polyhedrin Fusion Baculovirus Expression System (PFBES).

Key words: Baculovirus, Expression system, Partial polyhedrin, Enhanced production

Autographa californica multiple nucleopolyhedrovirus ORF11 and ORF78 are essential for budded virus production, occlusion-derived virus envelopment, and occlusion body formation

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ORF11 and ORF78 of *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) are highly conserved genes of unknown function. To determine the role of *ac11* and *ac78* genes in baculovirus life cycle, two AcMNPV mutants, Ac11KO and Ac78KO with *ac11* and *ac78* deleted, were constructed, respectively. By Northern blot and qPCR, it was revealed that *ac11* and *ac78* are early and late genes, respectively. Microscopy, titration assays, and Western blot analysis revealed that budded viruses (BVs) were not produced in Ac11KO-transfected Sf9 cells. However, qPCR analysis demonstrated that the deletion of *ac11* did not affect viral DNA replication. Electron microscopy showed that there was no nucleocapsid in the cytoplasm or plasma membrane of Ac11KO-transfected cells, which demonstrates that the defect in BV production in Ac11KO-transfected cells is due to the inefficient egress of nucleocapsids from the nucleus to the cytoplasm. Also it was observe that the nucleocapsids in the nucleus were not enveloped to form occlusion-derived viruses (ODVs) and their subsequent embedding into occlusion bodies (OBs) was also blocked in Ac11KO-transfected cells. These results demonstrate that *ac11* is essential for BV production and ODV envelopment. Ac78KO-transfected cells produced a single-cell infection phenotype, indicating that no infectious BVs were produced. The defect in BV production was also confirmed by both viral titration and Western blotting. However, viral DNA replication was unaffected, and OBs were formed. An analysis of BVs and ODVs revealed that AC78 is associated with both forms of the virions and is an envelope structural protein, and also plays an important role in the embedding of ODV into the OB, which is essential for the viral life cycle.

Key words: AcMNPV, Ac11, Ac78, Virus replication

Interaction and Assembly of Two Novel Proteins in the Spore Wall of the Microsporidian Species *Nosema bombycis*

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Microsporidia are obligate intracellular parasites with rigid spore walls that protect against various environmental pressures. Despite an extensive description of the spore wall, little is known regarding the mechanism by which it is deposited or the role it plays in cell adhesion and infection. In this study, we report the identification and characterization of two novel spore wall proteins, SWP7 and SWP9, in the microsporidian species *Nosema bombycis*. SWP7 and SWP9 are mainly localized to the exospores and endospore of mature spores and the cytoplasm of sporonts, respectively. In addition, a portion of SWP9 is targeted to the spore wall of sporoblasts earlier than SWP7 is. Both SWP7 and SWP9 are specifically colocalized to the spore wall in mature spores. Furthermore, immunoprecipitation, far-Western blotting, unreduced SDS-PAGE, and yeast two-hybrid data demonstrated that SWP7 interacted with SWP9. The chitin binding assay showed that, within the total spore protein, SWP9 and SWP7 can bind to the deproteinated chitin spore coats (DCSCs) of *N. bombycis*. However, binding of the recombinant protein rSWP7-His to the DCSCs is dependent on the combination of rSWP9–glutathione *S*-transferase (GST) with the DCSCs. Finally, rSWP9-GST, anti-SWP9, and anti-SWP7 antibodies decreased spore adhesion and infection of the host cell. In conclusion, SWP7 and SWP9 may have important structural capacities and play significant roles in modulating host cell adherence and infection *in vitro*. A possible major function of SWP9 is as a scaffolding protein that supports other proteins (such as SWP7) that form the integrated spore wall of *N. bombycis*.

Key words: *Nosema bombycis*, *Bombyx mori*, Spore wall protein, Microsporidia, interaction.

Branch Session III-1

The 4th Asia-Pacific Congress of Sericulture and Insect Biotechnology

Friday, 24 April

Sericulture III-1 (Room 1, 2F)

Effect of water deficit stress on anthocyanin accumulation in mulberry fruits

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Mulberry is widely grown in the northeastern part of Thailand for its foliage, which is a primary source of food for silkworms (*Bombyx mori*). Mulberry belongs to the *Morus* genus of *Moraceae* family. Several studies have demonstrated that mulberry fruits are rich in anthocyanins, which can be used in several types of industries, including food, beverages and cosmetics. Anthocyanins are a large class of water-soluble pigments synthesized from the flavonoid biosynthetic pathway via the phenylpropanoid pathway. These compounds are of considerable interest to the researchers due to their strong antioxidant properties. Therefore, the objectives of this study were to determine anthocyanin content in the fruits of different mulberry cultivars and investigate the relationship between water deficit stress and anthocyanin accumulation. The fruits of Kamphaengsaen (KS), Chiangmai (CM) and Buriram (BR) cultivars were harvested from the plants grown under well-watered (WW) and water deficit stress (WS) conditions. Anthocyanins were then extracted from the fruits and analyzed by high performance liquid chromatography (HPLC). Furthermore, the antioxidant activities of mulberry fruit extracts were evaluated through the free radical-scavenging effect on DPPH radical and the ferric reducing ability of plasma (FRAP) assay. The results obtained from this study may provide a basis for selecting mulberry cultivars with high anthocyanin content and antioxidant activity as functional fruits.

Key words: Anthocyanin, Mulberry, Water deficit stress, Antioxidant activity

Bioactive components, functional properties and processing of mulberry fruits as food resources

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Sericulture is an important industry for silk production world-wide and mulberry(*Morus alba* L.) is cultivated for silk worm's dietary food. So mulberry fruit has been regarded as undesirable byproduct. However, sericulture began to decline in the 1990's, and it is maintained with silk worm's powder for diabetic patients and DongChungHaCho in South Korea. On the other hand, the researchers of mulberry were force to change the breeding purpose and field. They were interested in mulberry fruit and its using possibility as resources of functional food. For the first time, we analyzed bioactive components with mulberry fruits. As a result, we confirmed that there were cyanidine-3-glucoside(C3G), free sugars, fatty acids, rutin, amino acis and resveratrol in mulberry fruits. Next we investigated the protective effect against neuronal cell and inhibitory activity against bacteria of mulberry fruit extracts.

Also, we studied about the high value-added processing method using the mulberry fruit. The study on extraction and color characteristics of mulberry fruit pigment(C3G; cyanidin-3-glucoside) were performed to increase utilization as new source of natural food colorant. C3G was extracted with 0.1% citric acid-70% EtOH. Then it was evaporated with large scale evaporation system. After adding dextrin to C3G concentration materials, we made pigment powder with freezing dryer. This method about natural food colorant from mulberry fruit was obtained patent. Finally, we developed the manufacturing method for semi-dried mulberry fruits having improved stability of cyanidin-3-gluoside and maintaining the shape according to the dryer.

Key words: Bioactive components, Functional propertie, Processing, Mulberry fruits, Food

The basic chromosome number of mulberry plants (*Morus* L) is seven

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Mulberry (*Morus* L), of the Moraceae family, is a deciduous tree and an important cultivated plant used for rearing the domesticated silkworm. It has long attracted the attention of biologists interested in sericulture. Determination of the basic chromosome number of *Morus* is especially important to identify the polyploidy of mulberry species and clarify the evolution route of these plants.

Mulberry was believed to have a chromosome base number of 14, which is still widely cited in literatures. *Morus notabilis* C. K. Schneider is a wild mulberry species and was first recorded by a German preacher Schneider in 1916. It has a chromosome number of 14 based on the cytological studies in the 1980s. Since it is a species with a minimal chromosome number, *M. notabilis* was used for the sequencing of genome. Prior to genome sequencing, we confirmed the chromosome number of *M. notabilis* using conventional cytological techniques. Karyotyping distinctly illustrated that the 14 chromosomes of *M. notabilis* forms seven pairs, supporting a base number of 7 rather than 14. Recently, the germination studies of *M. notabilis* seeds were performed. The *M. notabilis* seeds were germinated after manually peeling the seed coats and cultured on the MS media. The germination rate of *M. notabilis* seeds was up to 85.7%. The young leaves and roots from the obtained seedling were used to prepare chromosome samples using wall removal and hypotonic treatment for karyotypic analysis. The result showed that the chromosome number of somatic cells of *M. notabilis* was 14 (2n = 14).

In addition, the results of karyotype analysis of *Morus yunnaneneis* also showed that the somatic chromosome number of *M. yunnanensis* is 14 and its formula is K(2n) = 2x = 2St + 12m. Moreover, the seedlings of two mulberry resources, which were named Yun 6 and Yun 7, were germinated from the seeds of *Morus mongolica Schneid*. No.6 and *Morus wittiorum Handel-Mazzetti* No.7, respectively. The young leaves of two seedlings were used for

chromosome number observation. The chromosome number of Yun 6 and Yun 7 are 35 and 49, respectively, still suggesting the basic chromosome number of mulberry plants is 7.

Taken together, our analysis proved that the basic chromosome number of mulberry plants is 7. It provides important information for the study of system evolution, genetic relationship, the origin of species and genetic characteristics of these plants.

Key words: Mulberry, Karyotypic analysis, Basic chromosome number, Germination

Branch Session III-2

The 4th Asia-Pacific Congress of Sericulture and Insect Biotechnology

Friday, 24 April

Sericulture III-2 (Room 2, 2F)

Alkaline protease from midgut of silkworm, *Bombyx mori*

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Alkaline protease digests protein into a small peptide in an alkaline condition. In silkworm, *Bombyx mori*, alkaline protease has been reported to involve in silk fibroin digestion. Therefore, production of alkaline protease from midgut of silkworm could be useful for silk application such as cosmetic industry, bandage and hydrogel. The deduced protein sequences showed 81-98% identity to other insect cathepsin Fs. The deduced amino acid sequence consists of three conserved domain including cystatin-like domain (CY), cathepsin propeptide inhibitor domain (I29), and cysteine peptidase domain (CPD). In this study, the pET28b-BmCPD was successfully constructed. The recombinant BmCPD protein was expressed by addition IPTG. Unfortunately, the recombinant protein was produced an insoluble form. Moreover, refolding of the BmCPD was unsuccessful, therefore, pPICZαB-BmCPD was constructed for protein expression in *Pichai pastoris*. After induction with an appropriate amount of methanol, the recombinant protein was successfully produced and secreted into a culture medium. Then, an enzyme activity was elucidated by digestion of azocasein. The result showed that the recombinant protein contained protease activity with an expected size of 26 kDa on SDS-PAGE. Optimum activities in temperature and pH were also determined. The results from this study could be a clue for further applications using a recombinant enzyme in a related industry.

Key words: *Bombyx mori*, Alkaline protease, *P. pastoris*

Crystal structure of an aldo-keto reductase with 3-dehydroecdysone reductase activity from the silkworm, *Bombyx mori*

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Aldo-keto reductases (AKRs) constitute a large superfamily and are widely distributed in plants, animals, and prokaryotes. These enzymes catalyze the NAD(P)(H)-dependent reduction of compounds containing ketones and/or aldehydes to the corresponding alcohols. Physiological functions for AKR include hydroxysteroid dehydrogenase, mannose/xylose reductase, prostaglandin synthesis, xenobiotic detoxification. So far, no structural data for insect AKRs have been available, and therefore little information is known regarding enzymatic properties of AKRs.

In this study, an mRNA coding for a previously unknown AKR was found during the course of identifying genes induced by the insecticide diazinon in silkworms. The sequence contains an open reading frame of 927 bp that encodes 308 amino acid residues. Sequence similarities with members of AKR family 2, subfamily E led to the systematic name of AKR2E4. To determine the substrate specificity of AKR2E4, recombinant AKR2E4 was expressed, purified to near homogeneity, and kinetically characterized. AKR2E4 reduces carbonyl-containing compounds such as DL-glyceraldehyde, phenylglyoxal, isatin and 17α-hydroxy progesterone using NADPH as a cosubstrate. However, AKR2E4 was not able to reduce the common AKR substrates p-nitrobenzaldehyde and 3-hydroxybenzaldehyde. Although some members of the AKR superfamily have been shown to utilize NADH as an alternative co-substrate, no NADH-dependent AKR2E4 activity was detected. Notably, activity toward 3-dehydroecdysone was observed, which suggests that this enzyme plays a role in regulation of the important molting hormone ecdysone. The binary structure of the enzyme in complex with $NADP^+$ was refined at 1.3 Å resolution to elucidate substrate binding and catalysis. The enzyme is a 33-kDa monomer and shares a common TIM- or $(\beta/\alpha)_8$-barrel fold. Bound NADPH is located at the center of the barrel, and residues (Thr23, Gly24, Gly26, Lys29,

Asp53, Ser158, Asn159, Gln180, Tyr206, Ser207, Arg215, Phe209, Ile257, Lys259, Ser260, Arg265, and Asn269) involved in catalysis are conserved. This structure constitutes the first insect AKR structure determined. Kinetic and structural results indicate that AKR2E4 is capable of functioning in *B. mori* ecdysone metabolism and in xenobiotic degradation.

Key words: Crystal structure, Enzyme specificity, NADH, NADPH

Land Utilization, Insect Industry and Sericulture

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1. Land Utilization: In historical surveys, Japanese farmers utilize their farmland for wide variety uses to help to increase their income. In addition to land cultivating for rice and vegetables, there are many types of land utilization like mulberry fields, real estate for building houses and solar power generating system recently. Farmer's activity is rational to maximize their income through their farmlands. Because growing industry changes depending on requirements of that times. Historically farmer managed their portfolio through the changing the uses of their own farmland.

2. Insect Industry (Current trend mainly in Japan): Divide Insect Industry into two types by their business, mainly on traditional needs in the society and cutting-edge technology. But for technological innovation going on and natural environment change, business model of Insect Industry likely to transform depending on market needs.

(1) Traditional role in social needs: Sericulture, Apiculture, Insect foods, Pesticide, Pest control, etc. (2) Cutting-edge technology: Medicine and Medical manufacture, Chemistry, Fiber, Biomimicry, etc.

3. Venture enterprise and established company and technological innovation of silkworm in the molecular biology: Various companies starting a business that based on molecular biological technology of silkworm. Their business is capital intensive compared to traditional sericulture industry, and many researcher mainly concern with this fields. Financial markets encourage capital inflows into this business fields as the investment target.

Key words: Land utilization, Portfolio management, Insect industry

Young Scholar Session

The 4th Asia-Pacific Congress of Sericulture and Insect Biotechnology

Friday, 24 April

Young Scholar Session I (Room 1, 2F)

Digital gene expression (DGE) analysis to reveal genes related to the negative temperature coefficient of deltamethrin in the silkworm, *Bombyx mori*

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The silkworm (*Bombyx mori*) is an important insect for various industrial applications and is also a model insect for the order Lepidoptera. It has to be found that the silkworm is more sensitive to deltamethrin at low temperatures than at higher temperatures. To elucidate the role of the negative temperature coefficient of deltamethrin in the silkworm, digital gene expression (DGE) analysis was performed for identification of differentially expressed genes in silkworms challenged with deltamethrin at different temperatures. Compared with deltamethrin exposure at 30℃, silkworms treated at 20℃ exhibited more severe stress responses, and gene sets associated with heat-shock proteins (HSPs) and antimicrobial peptides (AMPs) were down-regulated dramatically. Similarly, a decrease in genes related to reactive oxygen species scavenging was also detected, which may have resulted in an imbalance between radical-generating and radical-scavenging systems. Taken together, these findings suggested that the higher mortality rate after deltamethrin treatment at 20℃ was caused by a series of gene alterations in HSPs, AMPs, CYP450, and glutathione *S*-transferases.

Key words: Silkworm, Deltamethrin, DGE, Negative temperature coefficient

Tanaka's mottled translucent (*otm*) mutant of the silkworm is caused by an insertion of *Organdy* into the gene encoding a BLOC1 subunit

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The silkworm, *Bombyx mori*, accumulates the uric acid, one of the end products of nitrogen metabolism in most insects, as urate granules in the epidermal cells to keep its skin white and opaque during all larval stages. Interestingly, any of the failure of biosynthesis, transportation, or accumulation of uric acid causes a striking abnormal phenotype with oil-paper-like translucent larval skin, so-called the “oily” mutant. In the present study, we performed the positional cloning of the gene responsible for the translucent phenotype of *otm* (Tanaka's mottled translucent), which has been maintained in strains w05 and o26. We finally mapped it onto a 364-kb region of nscaf 2529, chromosome 5. The 364-kb region contained 22 hypothetical protein-coding genes. We performed the RNA-seq analysis of tissues of *otm* mutant larvae, and found that the gene BGIBMGA002619 was abnormally spliced and produced a transcript lacking 90 bp length part in 5’ end. BGIBMGA002619 potentially encodes the biosynthesis of lysosome-related organelles complex 1 (BLOC1) subunit 5, whose ortholog is responsible for the *Muted* mutant in mouse. We discovered that the expression of this gene in epidermis and fat body of two *otm* mutants was dramatically suppressed, compared with that in the wild type. On the other hand, siRNA-mediated knock down of this gene caused the partial translucency of the larval skin. These data indicates that the mutation of this gene, which we named as *Bm-Muted*, caused the *otm* phenotype. Then, we determined the nucleotide sequences of the full-length cDNA and genomic region corresponding to *Bm-Muted*. As the result, we found that a 538 bp length DNA sequence, similar to the *B. mori* transposon *Organdy*, was inserted into the 3' end of the first intron of

Bm-Muted in the two *otm* strains. The cDNA lacked exon 2, and accordingly generated a premature stop codon in exon 3. Collectively, we presume that the insertion of *Organdy* caused the splicing disorder of *Bm-Muted* in the *otm* mutant, and resulted in deficiency of the Bm-Muted protein. Probably, it led insufficiency of uric acid accumulation in epidermis.

The *otm* mutant was first found in the og^t strain (Tanaka and Syo, 1939), where *Organdy* was inserted in *Xdh* responsible for og^t (Komoto *et al.*, 2003). Here we report that *otm* was also caused by an insertion of *Organdy* into another gene, *Bm-Muted*, suggesting that the original og^t strain possessed a special potential for transposition of *Organdy*.

Key words: *Bombyx mori*, *otm* mutant, Uric acid accumulation, *Bm-Muted*, *Organdy* transposon

Silkworm HP1 protein BmHP1a exerts two context-dependent modes of action with respect to transcriptional regulation

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Heterochromatin protein 1 (HP1) is an evolutionarily conserved protein across different eukaryotic species and is known to be crucial for heterochromatin establishment and maintenance. Although previous studies in *Drosophila* imply that HP1 proteins may play various context-dependent roles in the regulation of gene expression, the molecular mechanisms underlying the differential functional properties of the HP1 isoforms are largely unknown.

The silkworm, *Bombyx mori*, possesses two HP1 genes, *BmHP1a* and *BmHP1b*. A previous study showed that both BmHP1a and BmHP1b function as transcriptional repressors and can form heterodimers. However, the role of HP1 proteins in transcriptional regulation was still unclear. To investigate the role of BmHP1a, we performed genome-wide survey of the transcriptome (RNA-seq), transcription start sites (TSS-seq), chromatin modification states and BmHP1a binding sites (ChIP-seq) using the silkworm ovary-derived BmN4 cell line.

First, we examined BmHP1a's association with heterochromatic repeat elements, i.e. transposable elements. We previously showed by ChIP-PCR experiments that BmHP1a is enriched for one of the telomeric transposons, *SART1*. In the current study, ChIP-seq analysis revealed that BmHP1a is extensively enriched for telomere-specific transposons and silkworm telomeric repeat sequence of [TTAGG], strongly suggesting the involvement of BmHP1a in the establishment and/or maintenance of the silkworm telomeric regions.

Second, we attempted to understand the situation of non-repeat genomic regions where BmHP1a associates. Using ChIP-seq data, we identified a number of BmHP1a-binding loci throughout the silkworm genome and found that these loci include TSSs and frequently co-occur with neighboring euchromatic histone modifications but without heterochromatic histone modifications. In addition, we observed that genes with BmHP1a-associated TSSs are relatively highly expressed in BmN4 cells. RNA interference (RNAi)-mediated *BmHP1a*

depletion resulted in the transcriptional repression of highly expressed genes with BmHP1a-associated TSSs, whereas genes that are not coupled with BmHP1a binding regions were less affected by the treatment. Putative BmHP1a target genes contained a number of ribosomal genes and genes expressed ubiquitously in the silkworm. These results demonstrate that BmHP1a binds near TSSs of highly expressed euchromatic genes and positively regulates their expression. Our study revealed a novel mode of transcriptional regulation mediated by HP1 proteins.

Finally, RNAi experiments provided a hint on the relationships between *BmHP1a* and *BmHP1b*. The genes whose expression was enhanced were common when each *HP1* gene was depleted. In contrast, the genes that were repressed were quite different. These results strongly suggest that BmHP1a and BmHP1b proteins presumably cooperate in transcriptional repression through their heterodimerization, whereas BmHP1a can activate the transcription of highly expressed genes irrespective of BmHP1b action.

In conclusion, we showed that BmHP1a exerts two context-dependent modes of action with respect to transcriptional regulation. It will be of great interest to identify the factors that determine whether BmHP1a acts in canonical or non-canonical pathways and to determine how BmHP1a binds to the euchromatic regions of the silkworm genome to selectively activate highly expressed genes.

Key words: *Bombyx mori*, Silkworm, ChIP-seq, RNA-seq, HP1, Epigenetics

Synthesis of complex-type N-glycans on recombinant proteins by modifying N-glycosylation pathway in cultured silkworm cells

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N-glycosylation is an important post-translational modification that confers various biological activities, structural stability, and inter-molecular interactions to proteins. Baculovirus expression systems (BES) using lepidopteran insects and cells are widely used to produce recombinant glycoproteins. However, while mammalian cells produce complex-type N-glycans containing galactose and sialic acid, insect cells mostly produce paucimannosidic-type N-glycans. Because of this difference, recombinant proteins produced by BES might be destabilized in human body, and unsuitable for medical use. Therefore, much effort has been expended to synthesize mammalian-like N-glycans in insect cells. A key reason for the difference is the presence of a highly specific N-glycan processing beta-N-acetylglucosaminidase (FDL) in insect cells. Additionally, insect cells lack enzymes for mammalian-like N-glycosylation. In this study, we suppressed *Bombyx mori* FDL (BmFDL) in silkworm cultured cells by RNAi, and introduced some human glycosyltransferases to provide the machinery for the mammalian-like N-glycosylation pathway. Western blotting and MALDI-TOF mass spectrometry demonstrated that synergistic effects of BmFDL suppression and human glycogenes expression converted the paucimannosidic-type structures of the recombinant proteins produced by BES into a complex-type structure.

Key words: Baculovirus expression system, Silkworm, N-glycosylation

Discovery of novel factors involved in biogenesis of urate granules in the silkworm by siRNA-based knockdown

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The silkworm synthesizes uric acid as the end product of metabolism of nitrogen, and transports a part of it to the epidermal cell. The granular organelle called the urate granule in the epidermal cells incorporates uric acid, and consequently the crystals of uric acid reflects the light diffusely, which makes the silkworm larvae look opaque white. The mutant silkworms that lack the urate granules have translucent larval skin because the light gets through their epidermis. Conventionally, these silkworms are called “oily mutants”. It has been shown that responsible genes of some oily mutants such as *od*, *ov* and *oa* encode subunits of “biogenesis of lysosome-related organelles complex” (BLOC)-1 or -2. In mammals and *Drosophila*, BLOC-1 and -2 are known to participate in the formation of lysosome-related organelles (LROs) which share some features with lysosome but have distinctive functions depending on the cell types. Since the defects of subunits of BLOC-1 or -2 result in translucent skin in the silkworm, it is suggested that the urate granule is also an LRO and made through a similar process by which other LROs are made. To prove this hypothesis, we knocked down the genes encoding subunits of BLOC-3 and adaptor protein (AP)-3, both of which contribute biogenesis of LROs together with BLOC-1 and -2. We targeted the genes encording HPS-1 and μ3A, subunit of BLOC-3 and AP-3 respectively. When we performed siRNA-mediated knockdown by electroporation in the second instar larval epidermis, the larval skin was chaged from opaque white to translucent after the third instar. We confirmed that the mRNA expression of targeted genes was reduced in translucent skin by quantitative RT-PCR. This result indicates that the formation of urate granules requires BLOC-3 and AP-3, and strongly supports the hypothesis that the urate granule is one of the LROs.

Key words: Urate granule, Oily mutant, Lysosome-related organelle

Genetic linkage analysis of female sexual behavior mutant in the silkmoth, *Bombyx mori*

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Mating in many animals including mammals and flies induces the drastic change in female reproductive behavior. In the silkmoth, *Bombyx mori*, virgin females are receptive to mating and produce sex pheromone, bombykol, to attract males, whereas mated females are unreceptive, decrease sex pheromone release and lay eggs. Mating gives the different stimulations such as physical and chemical stimuli, and transfer apyrene and eupyrene spermatozoa as well as male-derived seminal fluid proteins, to females. The previous works have been shown that the progression of eupyrene spermatozoa to vestibulum in the female reproductive tract triggers sexual behavioral changes in the silkmoth. However, the molecular mechanism that mediates the behavioral switch in females remains unexplored.

To understand the mechanism underlying the regulation of female sexual behavior, we focused on silkmoth sexual behavior mutant, named constitutive egg-laying (*egl*). In *egl* mutants, virgin females show abnormal behavior alike mated females, which actively lay eggs and decrease the release of sex pheromone. In virgin females, wild type (WT) silkmoths lay a small number of eggs (15.2 ± 2.6 eggs per female), whereas *egl* mutants lay a large number of eggs (443.7 ± 18.4 eggs per female) within 48 hrs after adult eclosion. To identify and characterize the gene responsible for the *egl* phenotype, we performed genetic linkage mapping. The analysis of BC1F females derived from crosses between F1 hybrid females (WT/*egl*) and *egl* males indicated that *egl* locus is significantly linked to chromosome 10. Next, our analysis of 214 BC1M females derived from cross between F1 hybrid males and *egl* females, and 576 F2 femaless narrowed the *egl* locus to a 854 kb-region on chromosome 10, where there were 18 putative protein coding-gene. To identify the tissue responsible for the *egl* phenotype, clonal analysis using the genetic mosaic (*mo*) strain was performed by the marker on chromosome 10. The result indicated the defect of either the female reproductive tract or the ventral nerve cord but not brain caused the *egl* phenotype. The quantitative RT-

PCR experiment in each tissue in 2 hrs after adult eclosion showed that one candidate gene was only significantly decreased its expression at the transcript level in adult reproductive tract in *egl* females. In sum, the functional defect of the female reproductive tract in the *egl* mutant may cause the abnormality in sexual behavior. To further investigate this gene, we are generating a loss-of-function allele by CRISPR-Cas9-mediated knockout.

Key words: Sexual behavior, Egg-laying, Genetic linkage analysis

Identification of the region of *Autographa californica* MNPV P143 responsible for ribosomal RNA degradation in *Bombyx mori* cells

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BM-N cells derived from the silkworm, *Bombyx mori*, are permissive for homologous *B. mori* NPV (BmNPV), while non-permissive for heterologous NPVs, including *Autographa californica* multiple NPV (AcMNPV), *Hyphantria cunea* MNPV, *Spodoptera exigua* MNPV and *Spodoptera litura* MNPV. We previously showed that the ribosomal RNA (rRNA) degradation is induced in BM-N cells infected with these heterologous NPVs, and triggered by P143s of the heterologous NPV.

In this study, we analyzed AcMNPV P143 (Ac-P143) to identify the region responsible for rRNA degradation. P143 is a DNA helicase essential for viral DNA replication. To determine whether DNA helicase activity of P143 is involved in the rRNA degradation, we examined rRNA of BM-N cells infected with AcMNPV temperature sensitive mutant 8 (ts8) defective in DNA helicase activity of P143 at non-permissive temperature (33℃). The result showed that rRNA degradation was induced at both permissive and non-permissive temperature, indicating that DNA helicase activity of P143 is not associated with rRNA degradation in AcMNPV-infected BM-N cells. Next, we constructed mutated Ac-P143 containing amino acid residue substitutions with corresponding those of BmNPV P143 that does not trigger the rRNA degradation, and analyzed by transient expression assay. The results demonstrated that six amino acid residues of Ac-P143 are responsible for the rRNA degradation of BM-N cells. These amino acid residues are contained within the region related to restriction of AcMNPV replication in *B. mori* cells. Further experiments are on the way to explore the relation between rRNA degradation and NPV multiplication in BM-N cells.

Key words: *Bombyx mori*, Nucleopolyhedrovirus, RNA degradation, P143

Characterization of BmNPV promoters for improving exogenous genes expression in *Bombyx* cells

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Bombyx mori nucleopolyhedrovirus (BmNPV) promoters are important tools to express exogenous genes in cultured silkworm cells. Immediate early promoters, such as ie1 and ie2, possess extremely high transcriptional activities, mediating the expression of early genes of BmNPV by using host RNA polymerases. However, if the same promoters are repeatedly used to initiate multiple expressions of several genes from a single plasmid or construct inserted into single locus, their promoter activities will significantly be decreased and the repeated sequences may become instable. To overcome these problems, it is necessary to obtain short promoters of BmNPV with various transcriptional activities. Here, we have cloned 142 promoters of BmNPV with a length of 200 bp from transcription initiation site, and inserted into the plasmids with luciferase gene as a reporter. Subsequently, luciferase activities were measured at 3 days after transfection. In these promoters tested, 10 promoters had transcription initiation activities but not higher than that of ie1 promoter. It was found that virus infection or ie1 co-transfection significantly increased the activities of many promoters. Their transcription initiation activities will be further confirmed in the future studies.

Key words: BmNPV, Promoter, Transcriptional activity

Distribution of a gustatory receptor expressing cells in the midgut of the silkworm, *Bombyx mori* larvae

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Insects taste non-volatile chemicals and make choices about foods, mates, and egg deposition sites by gustatory receptors which are a member of large family of seven transmembrane proteins, with 69 constituents in the silkworm, *Bombyx mori*. Despite a massive research efforts in many species of insects, little is known about the distribution of gustatory receptors expressing cells in this insect. Here, we report a gustatory receptor (BmGr6, which belongs to the sugar receptor subfamily) expressing cells from *B. mori* larvae. Reverse transcriptase PCR (RT-PCR) revealed that BmGr6 is expressed in the foregut, (anterior, middle, posterior) midgut and hindgut of various instar larvae. Immunofluorescence was used to map the distribution of BmGr6 expressing cells in different regions of the larvae midgut, and we found these immunoreactive cells were mainly observed in the middle and posterior regions, only a small number of positive cells in the anterior region. According to the morphological character of these immunoreactive cells, we hypothesize that these cells are midgut enteroendocrine cells. Since previous studies have shown that the passage of food through the midgut of insect is controlled by enteroendocrine cells, it is possible that BmGr6 plays an important role in the sensing of sugar in the lumen to control midgut motility and feeding behavior through hormonal secretion. Furthermore, we found that BmGr6 also expressed in several neurons in the brain and ventral nerve cord of larvae. Collectively, our findings could provide important information in understanding the functional roles of this receptor in *B. mori*.

Key words: Gustatory receptor, *Bombyx mori*, Midgut, Distribution

GFP-p62 degradation is an excellent marker of autophagic flux in *Bombyx*

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Lipidation of LC3/Atg8, a ubiquitin-like protein, is used to detect the initiation of autophagy in many species, but molecular mechanism of the Atg8 modification is still not fully understood in the silkworm, *Bombyx mori*. To understand autophagy, we have cloned several genes related to autophagic induction in silkworm, and evaluated their availability as a new marker to monitor autophagy. Firstly, cDNA encoding p62, a substrate of autolysosome, was cloned from silkworm BmN4 cells. The ORF of silkworm p62 is 1839 base pairs long with a molecular weight of ~ 67.9 kDa. To visualize the subcellular location of p62, GFP was fused to its N-terminus and introduced into BmN4 cells. GFP-p62 was localized to the cytoplasm by forming small or large puncta, indicating that it intensively combined with aggregates mediated by ubiquitin in the cells. GFP-Atg8 was co-localized with DsRed-p62. Interestingly, the DsRed-p62 aggregates were surrounded by GFP-Atg9, the only transmembrane protein of autophagosome. It is reported that p62 has many functional domains, such as PB1, ZZ, AIM and UBA in other species. Mutation of these domains revealed that PB1 and UBA were important for p62 to form the intensive puncta, and the amino acid residues critical for the puncta formation were conserved among human, fruit fly and silkworm. In addition, the mutations in AIM and UBA domains negatively affected GFP-p62 degradation induced by Rapamycin. Treatment by Rapamycin at different concentrations resulted in the degradation of GFP-Bmp62 and increase of free GFP, but RNAi of Atg1 and Atg8 significantly attenuated free GFP generation, suggesting that free GFP was a dynamic reflection of autophagic flux in *Bombyx*. Using this monitor system, it was clearly demonstrated that RNAi of Tor2 or both Tor1 and Tor2 caused higher autophagic flux compared with the RNAi of Tor1, implying that Tor2 involved in the formation of repressive complex of autophagy in silkworm.

Key words: Insect, Silkworm, *Bombyx mori*, Autophagy, p62

Young Scholar Session

The 4th Asia-Pacific Congress of Sericulture and Insect Biotechnology

Friday, 24 April

Young Scholar Session II (Room 2, 2F)

A novel negevirus isolated from *Aedes* lavae collected in Japan

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Arthropod-borne diseases transmitted by mosquito are serious problems on the public health that needs to be controlled effectively. Mosquito is a principal vector that transmits a lot of diseases such as malaria and dengue fever. Vector control, especially at the larval stage, is an essential strategy for reducing mosquito-borne diseases transmission since no effective vaccine for malaria and dengue fever has been developed. Some mosquito-specific viruses have been isolated from mosquito larvae, but unavailability of cell line suitable for detailed studies of most of them impedes the progress in the study for developing viral mosquitocides. We isolated a novel positive single-stranded RNA virus from *Aedes* larvae collected in Okushiri Island, Hokkaido, Japan. This virus designated Okushiri virus (OKV) replicated in *Aedes albopictus* cell line C6/36 cells, caused severe cytopathic effects on the mosquito cultured cells and produced a large amount of spherical viral particles with a size of 50 to 70 nm in diameter. The OKV genome consisted of 9,706 nts containing three open reading frames (ORF1, ORF2 and ORF3). ORF1 encoded a large protein with MW of about 268 kDa including methyltransferase, FtsJ-like methyltransferase, helicase and RNA-dependent RNA polymerase domains. The proteins encoded in ORF2 and ORF3 were function unknown. Phylogenetic analysis of the translated amino acid sequences suggested that OKV was a member of Negevirus, a proposed new group of insect-specific viruses isolated from adult mosquitoes and predicted an evolutionary relationship to some plant viruses.

Key words: Mosquito-borne diseases, Okushiri virus, Negevirus, Mosquito-spesific virus

Study on in vitro excystation of *Farinocystis* sp. isolated from the West Indian sweet potato weevil, *Euscepes postfasciatus*

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Farinocystis sp. is an entomopathogenic apicomplexan protozoan parasite, which was isolated from the West Indian sweet potato weevil, *Euscepes postfasciatus* in Okinawa Japan in 2006. Apicomplexan protozoans characteristically form resistant spores called oocysts. After being ingested by the susceptible hosts, oocysts release the sporozoites, the first infective stages, a process of which is known as excystation. In other human pathogenic apicomplexans such as *Cryptosporidium* and *Eimeria*, there are some reports that in vitro excystation was induced by trypsin and a bile salt (sodium taurocholate). On the other hand, the excystation of entomopathogenic apicomplexans remains poorly understood, and *Farinocystis* sp. did not excyst by the same treatment in our previous study. Furthermore, it is known that apicomplexan oocysts are highly resistant to environmental stress and a part of it comes from the impermeability of oocyst wall. A recent study suggests that the lipid-rich layer present in the oocyst wall of *Cryptosporidium* and *Eimeria* may be responsible for oocyst impermeability. In the present study, we investigated an association between components of the oocyst wall and in vitro excystation of *Farinocystis* sp.

First, to ascertain whether oocyst walls of *Farinocystis* sp. have a lipid layer, the oocysts were treated with some organic solvents: ethanol, acetone, and hexane. The treated oocysts were stained with fluorescein isothiocyanate (FITC) and measured their fluorescent intensity to investigate changes in the impermeability of oocyst wall. In the case that oocysts were treated with a low-polarity solvent, they showed strong FITC fluorescence. It suggests that oocyst wall of *Farinocystis* sp. has lipid layer and the organic solvent treatments altered the permeability of the oocyst wall as a consequence of its disruption. Next, oocysts treated with organic solvents were subjected to trypsin hydrolysis to induce in vitro excystation, and the excystation rate was measured using phase contrast microscopy. Trypsin treatment of the

oocysts which had been exposed to the organic solvent induced efficient excystation of *Farinocystis* sp. Other proteases including papain and chymotrypsin also had an effect on the ability to induce excystation. Furthermore, sporozoites released from the excysted oocysts were collected by Percoll™ density gradient centrifugation, and cell viability of these sporozoites was tested with double staining using fluorescein diacetate (FDA) and propidium iodide (PI). As a result, these sporozoites were FDA-positive and PI-negative, indicating that they remained viable.

Further studies are required to characterize lipids and proteins present in oocyst walls and elucidate their association with in vitro excystation of *Farinocystis* sp..

Key words: *Farinocystis* sp., *Euscepes postfasciatus*, Oocyst wall, in vitro excystation

Antifungal and insecticidal activities of a bee (*Apis cerana*) inhibitor cysteine knot peptide

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Inhibitor cysteine knot (ICK) peptides exhibit ion channel blocking, insecticidal, and antimicrobial activities, but currently, no functional roles for bee-derived ICK peptides have been identified. In this study, a bee (*Apis cerana*) ICK peptide (AcICK) that acts as an antifungal peptide and as an insecticidal venom toxin was identified. AcICK contains an ICK fold that is expressed in the epidermis, fat body, or venom gland and is present as a 6.6-kDa peptide in bee venom. Recombinant AcICK peptide (expressed in baculovirus-infected insect cells) bound directly to *Beauveria bassiana* and *Fusarium graminearum,* but not to *Escherichia coli* or *Bacillus thuringiensis*. Consistent with these findings, AcICK showed antifungal activity, indicating that AcICK acts as an antifungal peptide. Furthermore, *AcICK* expression is induced in the fat body and epidermis after injection with *B. bassiana*. These results provide insight into the role of AcICK during the innate immune response following fungal infection. Additionally, we show that AcICK has insecticidal activity. Our results demonstrate a functional role for AcICK in bees: AcICK acts as an antifungal peptide in innate immune reactions in the body and as an insecticidal toxin in venom. The finding that the AcICK peptide functions with different mechanisms of action in the body and in venom highlights the two-pronged strategy that is possible with the bee ICK peptide.

Key words: *Apis cerana*, Honeybee, Inhibitor cysteine knot fold, Innate immunity, Venom, Antifungal peptide, Insecticidal toxin

Identification and functional analysis of *Masculinizer* in *Trilocha varians* (Lepidoptera: Bombycidae)

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Bombyx mori Masculinizer (*BmMasc*) is the first identified gene that plays the primary role in sex determination of lepidopteran insects. However, whether Masc-dependent sex determination is a shared system in family Bombycidae remains unclear. To address this issue, we configured *Trilocha varians* (Lepidoptera: Bombycidae) as the best organism for comparative study on the sex determination because *T. varians* utilizes the WZ/ZZ (female/male) sex chromosomes the same as *B. mori*. In this study, we identified the *T. varians Masc* homologue (*TvMasc*) from *T. varians* transcriptome data and performed functional analysis of *TvMasc* using embryonic RNA interference (RNAi) and transient expression assays in *B. mori* ovary derived BmN4 cells. All *T. varians* embryos injected with small interfering RNAs for *TvMasc*, expressed the female-specific splicing of *T. varians doublesex*, which is a highly conserved gene whose homologues play a key role in sex differentiation. On the other hand, the transient transfection of *TvMasc* cDNA into BmN4 cells induced male-specific splicing of *B. mori doublesex*. Taken together with these results, we concluded that *TvMasc* is a functional homologue of *BmMasc* and plays a crucial role in the sex determination cascade of *T. varians* as the masculinizing factor.

Key words: Sex determination, *Masculinizer*, *doublesex*, *Bombyx mori*, *Trilocha varians*

Screening and Identification of Spoilage Fungus and Antagonistic Yeast from Mulberry Fruit (*Morus alba* L.)

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Mulberry fruit is a traditional Chinese edible fruit that is used effectively in folk medicines. However, the commercialization of fresh mulberry fruit is limited due to the short harvesting season, the high water content (more than 70%), fragile structure and sensitivity to storage. In an attempt to prolongation the shelf-life of mulberry fruit, the micororganisms in the fruit were isolated and characterized. Two fungi *Sclerotinia sclerotiorum* and *Alternaria sp*. and one antagonistic yeast *Sporidiobolus pararoseus* were consistently detected from mulberry fruit confirmed by 18S rRNA sequence for fungi and 5.8S rDNA for yeast. The yeast *Sporidiobolus pararoseus* strongly inhibits the growth of *Sclerotinia sclerotiorum* and *Alternaria sp*. *Sporidiobolus pararoseus* could ferment glucose, maltose, sucrose and mannose, but not galactose, xylose, lactose, raffinose and starch, and assimilate glucose, maltose, sucrose, mannose, xylose, lactose, raffinose and starch, but not galactose. Our results suggest that the yeast *Sporidiobolus pararoseus* might be a potential microorganism to develop a biocontrol agent for mulberry fruit or other fruits.

Key words: mulberry fruit; fungi; yeast; isolation; identification

Lipopolysaccharide contributes to the virulence of *Serratia liquefaciens* against *Bombyx mori*

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Serratia spp. are Gram-negative bacteria of the family Enterobacteriaceae. *Serratia* spp. are ubiquitous environmental bacteria, and they infect a wide range of species from animals to plants. Unfortunately, *Serratia* spp. have been reported to kill useful insects, such as the silkworm, *Bombyx mori*, and the honeybee, *Apis mellifera*. Therefore, in order to protect these useful insects from diseases caused by *Serratia* spp., it is important to elucidate their pathogeneses. In our previous work, *S. liquefaciens* isolated from an antlion larva was highly virulent against the silkworm. Since the virulence of *S. liquefaciens* remains poorly understood, a comprehensive analysis using transposon-induced mutants was carried out to investigate its virulence against the silkworm in the present study. Firstly, a transposon insertion library of *S. liquefaciens* FK01 was constructed. Subsequently, 1,200 transconjugants were injected into the hemocoel of silkworm larvae. Then, four transposon mutants showing a reduced virulence were screened. A complementation test indicated that the virulence of the four transposon mutants were recovered. Sequences flanking both sides of the transposons indicated that they were inserted into the lipopolysaccharide (LPS) biosynthesis genes in these transconjugants. These results suggest that LPS markedly contributes to the virulence of *S. liquefaciens* against the silkworm. Gram-negative bacteria including *S. liquefaciens* have an outer membrane covered in LPS. LPS is known to contribute to protection of bacterial outer membrane from degradation. In *Escherichia coli*, deficiency in LPS biosynthesis pathway enhanced sensitivity to sodium dodecyl sulfate, novobiocin and polymyxin B. In this work, LPS deficient mutants of *S. liquefaciens* were subjected to antimicrobial sensitivity testing with these reagents as a measure of outer

membrane stability. As a result, the sensitivity of the mutants did not greatly increase in comparison with a parent strain. It suggested that decreased virulence observed in LPS mutants was not caused by increased outer membrane permeability. The role of LPS in the virulence of *S. liquefaciens* against the silkworm remains to be determined in future.

Key words: *Serratia liquefaciens*, Transposon, Virulence, Lipopolysaccharide

Polycaprolactone (ε-caprolactone) and silk fibroin composite nanomatirx for artificial dermis using cold-plate electrospinning technique

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Synthetic polymers like poly (ε-caprolactone) (PCL) had been considered as most favorable polymer used to create scaffolds for tissue engineering. On the other hand, intrinsic hydrophobicity and comparatively toxic behavior than natural polymers limits its applicability. In this work, silk fibroin particles (SP) had been prepared by simple ball-milling technique, and further blended with PLC to form a colloidal solution containing silk fibroin SP (PCL/SP10% and PCL/SP30%) capable of forming nanofibers by cold-plate electrospinning. A comparative study consisting of pristine and silk modified PCL nanofibers had been assayed in the present communication. The pristine nanofibers prepared by cold plate electrospinning experienced collapse in porosity and therefore, resulted in thin membrane-like films. However, nanofibers modified with silk fibroin particles possessed intact pore architecture and therefore retained full-thickness. These nanofibers fabricated had been extensively characterized by various states of art techniques; like TEM, VP-FE-SEM, TEM, Contact angle, FT-IR and TGA. The results from these experiments revealed that silk particles can be successfully introduced in/on nanofibers. The cytotoxicity and cell infiltration studies were carried after culturing NIH3T3 fibroblasts in presence of nanofibers. These results confirmed hybrid nanofibers exhibits better cell viability and good cell infiltration than those of pristine PCL nanofibers. Moreover, *in vivo* studies were conducted on back of experimental rat models to determine the usability of these nanofibers as dermal analogue than commercially available Matriderm®. The histological examination using H&E and MT staining after (5, 10, 15 and 20 days), revealed that these nanofibers can moderately be converted to artificial dermis compared than Matriderm®, However, the results from gross findings indicated that nanofibers results in low contraction and less scar formation than the commercially available Matriderm®.

Key words: Biocompatible polymers, Cell viability, Cell attachment, Cell infiltration, Dermis

Osteoinductive silk fibroin/titanium dioxide nanoparticle/hydroxyapatite hybrid scaffold for bone tissue engineering application

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The present study demonstrated the fabrication that incorporation of titanium isopropoxide (TiO_2) and hydroxyapatite (HA) nanoparticles into the silk fibroin (SF) scaffolds. In this process, we prepared TiO_2 nanoparticles using sol-gel synthesis and the porous structure was developed by salt-leaching process. Homogeneous distribution of TiO_2 and HA nanoparticles were confirmed by images of VP-FE-SEM and those equipped with energy dispersive X-ray spectrometer. Structural characteristics of the porous SF/TiO_2/HA hybrid scaffold were also determined using FTIR analysis and X-ray diffractometer. In this study, the porous SF/TiO_2/HA hybrid scaffold showed similar porosity, enhanced mechanical property, but decreased water binding abilities, compared with the porous SF scaffold. For evaluation of the osteogenic differentiation of rat bone marrow mesenchymal stem cells, alkaline phosphatase activity and osteogenic gene expression were employed. Our results revealed that the porous SF/TiO_2/HA hybrid scaffold had improved osteoinductivity compared with the porous SF scaffold. These results suggest that the osteogenic property as well as mechanical property of the porous SF/TiO_2/HA hybrid scaffold could be better than the porous SF scaffold. Therefore, the porous SF/TiO_2/HA hybrid scaffold may be a good promising biomaterial for bone tissue engineering application.

Key word: Silk fibroin, Titanium isopropoxide, Hydroxyapatite, Scaffold, Tissue engineering

Construction the system of establishing new silkworm cell lines by using cell fusion

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Cultured cell lines are very important bioresources for basic and applied researches since they could be used as substitute of individual animals. Generally, unlike tumor-derived cell lines, e.g. Hela, tissue-derived cells have limited cell division number. Therefore, immortalization is essential to establish cell lines. It is known that mammalian cells can be immortalized by the exogenous expression of active telomerase. In case of insect cells, however, the similar method could not be applied. Until now, most of insect cultured cell lines established are derived from embryo or ovary. This lead us to difficult circumstance to investigate tissue- or organ-specific gene or protein expression using cultured cell lines. It is of great interest to obtain various cell lines maintaining tissue-specific characters. In order to obtain these insect cell lines, we employed cell fusion technology in the current study. As the first step, we fused two silkworm cultured cell lines, BmN4 and Bme21, by using PEG induced cell fusion, and new cell lines were established by drug selection. Genomic PCR analyses using parental cell line-specific primers confirmed the cell fusion event between two cell lines. Interestingly the fused cell line exhibited an intermediate sensitivity between their parental cell lines against BmNPV infection. Taken together, these results provide fundamental knowledge for further experiments, such as direct cell fusion between silkworm tissue derived cells and cultured cell lines.

Key words: Cultured cell line, Cell fusion, Silkworm

Characterization of silkworm replication related proteins for making an artificial chromosome

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The artificial chromosomes have been made and applied in bacteria, yeast and human because they are capable to transfer large DNA sequences into cells. In addition to this, artificial chromosomes do not cause unanticipated mutations by insertions and their copy number is controllable. At the same time, the expression of genes locating on artificial chromosomes are under the control of host cell system, if the specific chromatin regulators are not incorporated, it is very useful to investigate the molecular mechanisms of gene transcription. Due to these reasons, we try to develop an artificial chromosome system working in silkworm cells. As the first step, the factors crucial for replication in the silkworm cells were screened comprehensively from 66 candidate genes using soaking RNAi sensitive BmN4-SID1 cells. The effects of depletion of these genes on cell cycle progression were examined by flow cytometory. At a result, the knockdown of 17 genes, c-Myc, Cdc6, Cdc45, Cdk1, Cdk2, CyclinE, DNA polymerase alpha, DNA polymerase delta1, DNA polymerase epsilon, Dp1, E2F1, Fen1, Geminin, Mcm7, RFC4, Rpa1 and Spt16 exhibited significant impairment on cell cycle progression. Tethering of these factors to DNA fragment is possible to induce an initiation of DNA synthesis in the S-phase and some of which can be used as tools for constructing silkworm artificial chromosomes.

Key words: An artificial chromosome, Replication, Silkworm

Poster Session

The 4th Asia-Pacific Congress of Sericulture and Insect Biotechnology

Friday, 24 April

Sericulture (Room 3)

PS01

Functional analysis on RGD motif of *Bm126* from BmNPV

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Baculoviruses, the pathogens that infect Lepidoptera insect, was applied widely as biological pesticide and expression tool in biological medicine. Previous study revealed that BmNPV *orf126* (*Bm126*) contained a RGD motif. To investigate the role of RGD of *Bm126-GD* played in the infection, RGD mutant transfer vectors were constructed by point mutant and were transposed into BmNPV Bac-to-Bac system to construct recombinant bacmids, the recombinant viruses were achieved by transfection and infection. The occlusion bodies (OBs) productivity of RGD mutant viruses were investigated in infection to confirm the relationship between RGD and OBs productivity, *t* test statistics indicated that OBs of mutant viruses showed no significant difference with that of control virus. Competitive peptide Cyclo(RGDf-N(Me)V-) was also applied to investigate OBs productivity, and it showed no effect on OBs productivity with control virus. These results suggest that RGD of *Bm126-GD* was not involved in virus replication process directly, which might facilitate virus-host interaction of other factors.

Key words: *Bombyx mori* nucleopolyhedrovirus, Mutation, *orf*126, RGD motif, Occlusion body

PS02

Structural insights into the unique inhibitory mechanism of the silkworm proteinase inhibitor serpin18

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Serpins (serine protease inhibitors) are inhibitors of serine proteinases and/or cysteine proteinases. These inhibitors are expressed in the different segment and involved in regulating proteolytic activity. Here, we present the crystal structures of the serpin18 from *Bombyx mori* in active-form at 1.60 Å resolution. Serpin18 forms a monomer, which contains one helical subdomain, one β-sheet subdomain and one reactive center loop. Structural comparison demonstrated that the RCL of serpin18 adopt one unique conformation which determines a specific inhibitory. Activity analysis showed that the inhibitory target of serpin18 is a cysteine proteinase rather than a serine proteinase. Notably, this inhibitiory reaction results from the formation of an intermediate complex, which then follows for the digestion of proteinase and inhibitor into small fragments. This activity differs from previously reported modes of inhibition for serpins. Our findings have thus provided novel structural insights into the unique inhibitory mechanism of serpin18. Furthermore, proteomic analysis showed that there is a kind of cysteine proteinase called fibroinase in the lumen of silk gland. The purified fibroinase from silk gland can catalyze the digestion of fibroin, whereas the hydrolytic activity of fibroinase can be decreased by serpin18. Thus, these results combined with the expression profiles of serpin18 and fibroinase indicated that serpin18 is involved in regulating fibroinase activity and protecting fibroin from degradation.during *B. mori* development.

Key words: *Bombyx mori*, Silk gland, Serpins (serine protease inhibitors), Crystal structure, Enzymatic analysis

PS03

Homeodomain POU regulates vitellogenin transcription by binding with Broad complex in the silkworm *Bombyx mori*

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Vitellogenin (Vg) is the precursor protein of vitellin (Vn), which is one of the important nutrition for the formation of egg and the development of embryo in the silkworm. In this study, cell transfection assay and EMSA showed BmPOUM2 transcriptional factor could bind with POU CRE (near the -186~-211 BrC-Z2 CRE) of *BmVg* gene promoter. qPCR and immunohistochemistry showed the transcriptional trend of *BmPOUM2* is consistent with that of *BmVg* during the metamorphosis of female silkworm. When BmPOUM2 and BmBrC-Z2 were overexpressed in the BmE-SWU1 cells, the *BmVg* promoter activity was higher than the control under the treatment of ecdysone. GST-pull down and co-immunoprecipitation showed BmPOUM2 and BmBrC-Z2 could bind each other as a functional complex. These data revealed that edysone signal would be firstly transmitted to BmBrC-Z2 transcriptional factor by ecdysone receptor complex (EcR/USP), and then BmBrC-Z2 binds to the BrC-Z2 CREs on the *BmVg* gene promoter together with BmPOUM2, which participates in the transcriptional regulation of *BmVg* gene. These results thus indicated that ecdysone signaling plays an important role in the stage of vitellogenesis.

Key words: Silkworm, Vitellogenin, BmBrC-Z2, POUM2

PS04

1-methylcyclopropene combined with chlorine dioxide for the postharvest shelf-life of mulberry fruit (*Morus alba* L.)

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Mulberry fruits are delicious, fleshy, succulent berries, which are low in calories and contain health promoting phyto-nutrient compounds. But it shorts in market due to its perishability. To postpone the postharvest shelf-life of this berry, combination of 1-methylcyclopropene (1-MCP) and chlorine dioxide (ClO_2) are used for this study. Changes of the quality and physiological properties in berry were investigated. Dose test of 1-MCP and ClO_2 indicated that the ideal concentrations were 1μl/L for 1-MCP and less than 50 mg/L for ClO_2. Further composite treatment shown that combination of 1μl/L 1-MCP and 30 mg/L ClO_2 was the optimal doses condition. Under this condition, the postharvest shelf-life could postpone to 5th day, while control group lost the edible value at the third day. The main quality properties, amount of vitamin C (Vc), total sugar (TS) and titratable acid (TA) were not changed significantly, and malonaldehyde (MDA) is increased.

Key words: Mulberry fruit, 1-MCP, ClO_2, Postharvest shelf-life

PS05

Comparison of 1-deoxynojirimycin and flavonoids among 86 *Bombyx mori* varieties

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Mulberry left is the sole food of *Bombyx mori*. 1-Deoxynojirimycin (DNJ) and total flavonoids are main secondary metabolites in the left. In this work, 86 different silkworm varieties were selected from the National Center for Silkworm Genetic Resource Preservation, China. DNJ and total flavonoids are determined at the day 3 of 5th instar larva. The results showed that DNJ in the larvae is significantly different from 0.3947% to 0.034% among those varieties. DNJ content in bivoltine race is higher than univoltine race. Among Chinese race, Japanese race and European race, Japanese race contents the highest DNJ than others. Meanwhile, total flavonoid in the larvae is also significantly different from 1.397% to 0.376% among those varieties. Total flavonoid content in bivoltine race is higher than univoltine race. Chinese race and Japanese race content total flavonoid are almost similar, but higher than European race.

Key words: *Bombyx mori*, Variety, 1-Deoxynojirimycin, Flavonoid

PS06

Cordyceps militaris polysaccharide triggers apoptosis and G_0/G_1 cells arrest in tumor cells

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Although many studies have shown the antitumor properties of *Cordyceps militaris* (artificial cultivated from *Bombyx mori* pupa) polysaccharide, little is known regarding the mechanism of its effects. This study was conducted to determine the mechanism of antitumor effects of *C. militaris* polysaccharide extract by evaluating apoptosis rate and cell cycle progression status in human liver cancer cell SMMC-7721, stomach cancer cell BGC-823 and breast cancer cell MCF-7. SMMC-7721, BGC-823 and MCF-7 cells were cultured in the presence of *C. militaris* polysaccharide at various concentrations for 24 hours. The percentage of cell viability was determined by 3- (4, 5-dimethylthiazol-2-yl)-2, 5-di phenyl tetrazolium bromide (MTT) assay. Our results showed that *C. militaris* polysaccharide inhibited proliferation of SMMC-7721, BGC-823 and MCF-7 cells with an IC_{50} of 192 ± 23.2 μg/ml, 237 ± 12.7 μg/ml and 165 ± 16.3 μg/ml respectively. We also found that *C. militaris* polysaccharide at increasing concentrations induced apoptosis dose dependently in those cancer cells: apoptosis rates were 48.3 %, 59.4 % and 70.9 % for SMMC-7721, 41.3 % and 57.0 %, 72.2 % for BGC-823 and 61.3 %, 66.9 % and 80.6 % for MCF-7 at 200, 250 and 375 μg/ml of *C. militaris* polysaccharide respectively. *C. militaris* polysaccharide arrested SMMC-7721, BGC-823 and MCF-7 cells at G_0/G_1 and G_2/M phases with corresponding decreased in S-phase. This study suggests that *C. militaris* polysaccharide may exert its antitumor effects in those cancer cells by suppressing its growth, arresting the G_0/G_1-phase, reduced DNA synthesis and induced apoptosis.

Key words: *Cordyceps militaris*, Polysaccharide, Tumor cells, G_0/G_1 phase, Apoptosis

PS07

Inhibitory effect of hydrolysates on the silkworm pupa (*B. mori*) protein hydrolysis with alcalase

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In order to investigate the inhibitory effect of hydrolysates on process of alcalase hydrolysis for silkworm pupae (*B. mori*) protein, the inhibitory rates of hydrolysates obtained at different degree of hydrolysis (DH) against alcalase reaction were analyzed and compared. In addition, the inhibitory mechanism of hydrolysates against alcalase reaction was preliminarily studied based on the analyses of Lineweaver–Burk plots, ultraviolet spectrum, fluorescence spectrum and circular dichroism. The results showed that the hydrolysates could inhibit alcalase hydrolysis of silkworm pupae (*B. mori*) protein. The inhibitory rate increased with increasing DH when the DH is less than 9.2%, and keeped invariable when the DH exceed 9.2%. Fraction 2, an inhibitor with strong inhibition alcalase reaction, was isolated from hydrolysis of silkworm pupae (*B. mori*) protein by ultrafiltration and sephadex, its inhibitory rate was 18.6% (2.0mg/mL) and it present a mixed inhibition pattern. After fraction 2 inhibition, alcalase molecule was folded. In the secondary structure of inhibited alcalase, the relative content of α-helix was increased, while the relative contents of β-structure and random coil were decreased. In conclusion, the inhibition of hydrolysates causes some changes of alcalase molecular structure.

Key words: Silkworm pupae (*B. mori*), Enzymolysis, Inhibitory effect, Molecular structure

PS08

Isolation and characterisation of a novel angiotensin-I converting enzyme inhibitory peptide from silkworm pupa (*B. mori*) protein hydrolysate

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In this study, the ultrasonic-pretreated technology was applied to pretreat silkworm pupa (*B. mori*) protein (SPP) in order to improve the angiotensin-I converting enzyme (ACE) inhibitory activity of hydrolysate. The present results confirm that ultrasonic pretreatment was a good means for improving the ACE inhibitory activity of alcalase hydrolysate of SPP. The ultrasonic-pretreated silkworm pupa protein hydrolysate (USPPH) with the highest ACE inhibitory activity was prepared using alcalase at ultrasonic power of 410 W, ultrasonic time of 32 min and hydrolysis time of 50 min. The prepared USPPH had an increase of about 57.8% in ACE inhibitory activity compared to that of unpretreated SPP. One peptide, Lys-His-Val, was successfully purified from USPPH using ultrafiltration, gel filtration chromatography and RP-HPLC. The purified tripeptide exhibited a potent ACE inhibitory activity with an IC_{50} value of 12.82 μM, and was stable against gastrointestinal proteases of pepsin, trypsin and α-chymotrypsin. Molecular docking simulation of the purified tripeptide at the ACE active site indicated that nine amino acids from ACE active site (Asn277, Gln281, Thr282, His383, Asp415, Lys454, Ser526, Phe527 and Gln530) greatly contributed to the docking complex stabilization. In addition, our results showed that the ACE inhibition of the tripeptide could attribute to forming a distorted tetrahedral geometry of Zn(II) in the ACE active site after docking. The results of this study suggested that this Lys-His-Val from USPPH could be used as a functional food ingredient to prevent and/or treat hypertension. However, further study should be done to evaluate in vivo the antihypertensive activity of the tripeptide.

Key words: Silkworm pupa (*B. mori*), ACE inhibitory peptide, Purification, Molecular docking

PS09

Response surface optimization of ACE inhibitory activities of silkworm pupa (*B. mori*) protein hydrolysates after coupled ultrasound-ionic liquids pretreatment

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In the present research, studies were conducted on coupled ultrasound-ionic liquids pretreatment of silkworm pupa (*B. mori*) protein before enzyme hydrolysis in order to produce hydrolysates with potent ACE inhibitory activity. The effect of pretreatment conditions on the production of ACE inhibitory peptide from silkworm pupa (*B. mori*) protein with alcalase was investigated using response surface methodology. In addition, the molecular weight changes of silkworm pupa (*B. mori*) protein and its hydrolysate after pretreatment were studied by Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The results indicated that the effect sequence of three main factors for ACE inhibitory activity from big to small was as follows: liquid-solid ratio, ultrasonic power, pretreatment time. The optimal pretreatment conditions were as follows: liquid-solid ratio 27.2 mL/g, pretreatment time 31.9 min, ultrasonic power 407 W. Under the optimal pretreatment conditions, the average ACE inhibitory rate of silkworm pupae protein hydrolysate was 75.7% (IC_{50}, 0.071 mg/mL). Compared with the un-pretreatment and ultrasound pretreatment, the pretreatment of the coupled ultrasound-ionic liquids has obvious advantages in increasing ACE-inhibitory activity of silkworm pupae protein hydrolysate. After the coupled ultrasound-ionic liquids pretreatment, the molecular weight of silkworm pupae protein has no significant change, while the molecular weight of its hydrolysate (<1.43kDa) becomes smaller.

Key words: Silkworm pupa (*B. mori*), ACE inhibitory peptide, Response surface methodology, Coupled ultrasound-ionic liquids

PS10

Screening and identification of spoilage fungus and antagonistic yeast from mulberry fruit (*Morus alba* L.)

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Mulberry fruit is a traditional Chinese edible fruit that is used effectively in folk medicines. However, mulberry fresh fruit is hardly commercialized because of the short harvesting season, the high water content, fragile structure and sensitivity to storage. In an attempt to prolongation the shelf-life of mulberry fruit, two fungi *Sclerotinia sclerotiorum* and *Alternaria sp*. and one antagonistic yeast *Sporidiobolus pararoseus* were consistently isolated from mulberry fruit and identified by 18S rRNA sequence for fungi and 5.8S rDNA for yeast. The yeast *Sporidiobolus pararoseus* inhibits strongly the growth of *Sclerotinia sclerotiorum* and *Alternaria sp*. *Sporidiobolus pararoseus* could ferments glucose, maltose, sucrose and mannose, but not galactose, xylose, lactose, raffinose and starch, and assimilates glucose, maltose, sucrose, mannose, xylose, lactose, raffinose and starch, but not galactose. Results show that the yeast *Sporidiobolus pararoseus* is a potential microorganism to develop a biocontrol agent for mulberry fruit or other fruits.

Key words: Mulberry Fruit, Fungi, Yeast, Isolation, Identification

PS11

Stabilities and inhibition kinetics of ACE inhibitory peptides from silkworm pupa (*B. mori*) protein hydrolysate

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The effects of temperature, pH, drying methods and *in vitro* digestion of intestinal enzyme on the stabilities of angiotensin-I converting enzyme (ACE) inhibitory peptides from silkworm pupa (*B. mori*) protein hydrolysate were studied. Furthermore, the inhibitory mechanism of ACE-inhibitory peptides from silkworm pupae against ACE was preliminarily studied based on the analyses of Lineweaver–Burk plots and ultraviolet spectrum. The results showed that, ACE inhibitory peptides from silkworm pupae (*B. mori*) protein hydrolysate were instability and easy inactivation under the conditions of high temperature, acidic or alkaline. The effects of freeze-drying and spray-drying on the peptides activity were smaller. The peptides were resistant to digestion by pepsin, trypsin and α-chymotrypsin. After co-digestion by pepsin, trypsin and α-chymotrypsin, the peptides activity could maintain 94.0% of their initial activity. Moreover, the inhibition pattern against ACE revealed that the peptides were competitive inhibitors with inhibition constants (K_i) 0.06 mg/mL. After the inhibition by the peptides, the ultraviolet absorbance values of ACE at 240-280 nm were significant increase, and these changes of ultraviolet absorbance values initially revealed that ACE molecular structure has been changed.

Key words: Silkworm pupae (*B. mori*), ACE inhibition, Stabilities, Kinetics

PS12

Studies on kinetics model of controllable enzymatic hydrolysis for *Bombyx mori* pupal protein

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Bombyx mori pupal protein has a high nutritional value. Due to the poor processing properties, it could not be widely applied to production. In this paper, the alcalase alkali protease enzymatic kinetics model with the substrate of *Bombyx mori* pupal protein has been studied. The model's parameters and formulas were obtained by the nonlinear regression fitting through origin 8.0. The results shown that, R (the hydrolysis rate) = $(27.582E_0\text{-}0.0695S_0)$ exp [-0.274(DH)], DH (the degree of hydrolysis) = 3.649 ln[1+$(7.557E_0/S_0\text{-}0.019)$t], k_2 (the kinetic constants) = 50.103 min^{-1}. The hydrolysis rate (R) of alcalase alkali protease on *Bombyx mori* pupal protein is increased with the initial enzyme concentration, and decreased with initial concentration of substrate and DH. Further experiments verified that this model is good coincided with study result. This kinetic model can better explaining the hydrolysis process of *Bombyx mori* pupal protein.

Key words: *Bombyx mori* pupal protein, Enzymatic hydrolysis, Kinetics model

PS13

Studies on process optimization and functional properties of modified protein in silkworm pupa by ultrasonic

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In this paper, effects of the protein concentration, ultrasonic power, temperature and time on the solubility of silkworm (*Bombyx mori*) pupal protein were studied. Under this experiment, response surface analysis is used to optimize the conditions of ultrasonic modification for silkworm pupal protein. The lack of fit test of the model is P = 0.0663 (>0.05), and the difference was not significant. The model value is P < 0.0001 extremely significant. The coefficient of the equation (R^2) is 0.9206, and model adjustment coefficient of determination ($AdjR^2$) is 0.8279. The accuracy, reliability and regression are quite good. The optimize conditions are ultrasonic power 81.6%, protein concentration10.84%, temperature 37.3℃ and time 96.4 min. Those factors ordered as protein concentration, ultrasonic power, temperature and time. Under the optimize condition, the solubility, emulsibility and foaming properties of modified silkworm pupal protein increased 4.02, 1.87 and 1.41 times respectively than before. The amount of essential amino acids increased 23%. It may suggest that the functional properties of silkworm pupal protein are related to the amount of essential amino acids.

Key words: Silkworm pupal protein, Ultrasonic, Functional properties

PS14

A hemocyte-specific cathepsin O is related to bacterial response in the silkworm *Bombyx mori*

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Cathepsins are a group of protease predominantly located in lysosomes and play important roles in many physiological and pathological processes. In the present study, a full-length DNA of Cathepsin O from *Bombyx mori* was first cloned by the rapid amplification of cDNA ends (RACE). The genomic DNA was 6131bp long including a total of six exons and five introns, and its pre-mRNA was spliced to produce two spliceosome. By comparisons with cathepsin O from other species, we found that the identity among them ranges from 29 to 39%. qRT-PCR results showed that *BmCathepsin* O was highly expressed in hemocytes, and reached a peak at the 4^{th} molting and metamorphosis stages. Immunofluorescence assay demonstrated that BmCathepsin O was specifally expressed in granuocytes and plasmatocytes. Further analyses indicated that *BmCathepsin* O was significantly up-regulated after challenged with *E.Coli*, indicating its potential role in the innate immune system. In summary, our studies provide a new insight into the functional features of Cathepsin O.

Key words: *Bombyx mori*, Cathepsin O, Infection

PS15

Cloning and identification of a novel hemocyte-specific gene *BM04862* in silkworm *Bombyx mori*

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A novel hemocyte-specific gene *BM04862* was first cloned and identified in silkworm. The full-length cDNA sequence of *BM04862* was obtained by the rapid amplification of cDNA ends (RACE), which was predicted as a transmembrane protein, including 819bp, encoding 273 amino acid residues. Semi-quantitative RT-PCR was applied for its' temporal and spatial expression profiles. The result indicated that it was specifically expressed in hemocyte, and reached a peak at L4M and L5M stage. Further, we established a overexpressed vector to transform SF9 cell line, and found that BM04862 was localizated in cytoplasm and nuclei membrane. In addition, after challenged with E.*coli* for 24h, The qRT-PCR expriment showed that BM04862 expression was doubled, which suggested *BM04862* might play a certain role in defense response to bacteria invasion.

Key words: *Bombyx mori*, Hemocyte, Expression profile, Subcellular localization

PS16

Friend of GATA factor U-shaped regulates the proportion of plasmatocyte in silkworm *Bombyx mori*

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Friend of GATA (FOG) protein, a partner of GATA protein family, regulates the development and differentiation of hemocytes in vertebrate and invertebrate. To identify the function of *U-shaped* (*Ush*) in silkworm which usaually used as an invertebrate model organism, we gained the full-length cDNA of *BmUsh* by RACE method. The gene expression profile on day 3 of 5th (L5D3) instar larvaes illustrated that *BmUsh* was highly expressed in hemocytes. Immunoflurecence staining showed that BmUsh nuclear localization signal (NLS) was between 450th to 459th amino acid, and the key amino acids were the two arginines on 456th and 458th site respectively. In addition, we found that over-expressed *BmUsh* in vivo reduced proportion of plasmatocyte significantly. However, down-regulation of *BmUsh* by RNA interference increased the proportion of plasmatocyte dramatically. These findings suggested that *BmUsh* might play an important role during plasmatocyte development.

Key words: Friend of GATA (FOG), *Bombyx mori*, *BmU-shaped*, Plasmatocyte

PS17

Identification and characterization of the RUNX family in silkworm *Bombyx mori*

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Runt-related transcription factors are evolutionarily conserved either in vertebrate or invertebrate. RUNX family function as important transcription factors regulating the cells or tissues development and differentiation. We identified and characterized RUNX family in silkworm, which is an invertebrate animal model. Through bio-information analysis, we found there are three RUNX genes (*BmRunt*, *BmLozenge*, *BmRunt3*) in silkworm. The full-length cDNA of them was obtained by rapid amplification of cDNA ends (RACE) technique. The gene expression analysis illustrated that *BmRunt* and *BmRunt3* were almost expressed in every tissue, but *BmLozenge* was only highly expressed in hemocytes and wing imaginal disc. Further, up-regulation and down-regulation of *BmLozenge* in vivo showed that *BmLozenge* would regulate the melanization process of hemolymph by inducing polyphenol oxidases (PPOs) expression, which were key enzymes in the melanization process. These findings suggested that *BmLozenge* played a critical role in innate immunity defense in silkworm.

Key words: Runt-related transcription factor (RUNX), *BmLozenge*, Melanization, Polyphenoloxidases, *Bombyx mori*

PS18

Differential genes expression analysis of BmN cells infected variant *Bm126*-contained BmNPV

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Bombyx mori nucleopolyhedrovirus (BmNPV) is a serious viral pathogen of silkworm. Although functions of most BmNPV genes were depicted in recent years by gene knocked-out technology, deep-researches were needed to further understand the mechanism of virus infection. Previous study showed that *Bm126* was not an essential gene for viral replication, however, the mean survival time of the larvae infected by virus contained *Bm126-GD* was significantly delayed compared with that of the virus *Bm126-SX*, and showed significant increases in OB yield. In this report, we further explored the infection difference between the cells infected by variant *Bm126* through digital genes expression profiling. Totally112, 253, and 380 genes were identified in v*Bm126-GD* infected samples at 6, 12, and 24 h p.i. compared with that of v*Bm126-SX*, which including 40, 97, and 114 up-regulated genes, and 72, 156, and 166 genes were down-regulated respectively. Among these different genes, 9, 44, and 66 were identified as viral genes, respectively. The most significant gene at 6 h p.i. was *DNA pol*, a necessary gene for virus replication, and other virus early genes involved in also were identified, such as *DNA helicase*, *DBP*, *etc.*, and more viral genes were identified on other two time point, theses result was further verified by Q-PCR. GO analysis revealed most identified host genes were the components of ribosome that involved in the gene expression and some other gene were associated with nuclear acid binding. Taken together, these studies revealed that the infection procedure of virus contained *Bm126-GD* was postponed by inhibition form host cell and this inhibitory mechanism was still exploring.

Key words: *Bombyx mori* nucleopolyhedrovirus, *orf126* (*Bm126*), Digital genes expression, Replication

PS19

Identification of the silkworm (*Bombyx mori L.*) strain by RAPD method

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To investigate in specific biotype or breed, usually were performed SNP, Microsatellite (SSR, STR) and RFLP Analysis. However, It takes long time to perform these methods because it has to require searching for specific nucleotide sequence.

We performed RAPD analysis using DNA fragment marker to identify the specific strain of silkworm (*Bombyx mori. L.*) usually, RAPD is used to not only analyze the genetic relationships of the species, but also identify specific breed or strain due to the simplicity of the analysis.

We divide breeding strains into 4 groups, (Group A (301, 302, 303, 313), B (304, 305, 306), C (308, 309), D (310, 311, 314)), and use (operon kit, OPA 1 ~ 20, OPB 1 ~ 20, OPC 1 ~ 20, OPD 1 ~ 20, OPE 1 ~ 16) as RAPD primers. As a result, we found the 35 molecular indicators (OPA 7 pieces, OPB 6 pieces, OPC 7 pieces, OPD 8 pieces, OPE 2 pieces) that are selected from each group of 30 primers.

Key words: *Bombyx mori*, RAPD, Strain identification

PS20

Analysis of core fucosyltransferase gene of the silkworm cell line for metabolic engineering of *N*-glycosylation pathway by genome editing

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The baculovirus expression vector system (BEVS) has been frequently used for high-level production of biologically active proteins with eukaryotic posttranslational modifications by insect cells. Several differences in *N*-glycan structure between insect and mammalian cells, however, make it difficult to produce safe and effective glycoproteins for medical and veterinary uses by BEVS. One of the most problematic differences is the addition of fucose to the inner most core GlcNAc by insect fucosyltransferase (FucT3). Therefore, complete suppression of FucT3 activity in insect cells is required for therapeutic glycoprotein production.

To generate insect cells without FucT3 activity by permanently knocking out of FucT3 gene with the CRISPR/Cas9 system, we have analyzed genome DNA of the silkworm cell line BmN4. As predicted by the genome sequence of *Bombyx mori* in the database, three cleavage target sequences for CRISPR/Cas9 were identified in exons 3, 4 and 8 of the BmN4 FucT3 gene. In addition, another FucT3 sequence with a longer intron between exons 7 and 8 was identified, and its transcripts with unspliced introns were predominantly amplified by RT-PCR. Strand-specific RT-PCR analysis has indicated that they are antisense transcripts, although their functions in the *N*-glycosylation pathway of BmN4 cells are unclear.

Based on the results, we have constructed plasmids to synthesize guide RNA for CRISPR/Cas9 to cleave target sites in exons 3 and 4, and knocking out of BmN4 FucT3 gene as well as further analysis of the antisense RNA are underway.

Key words: *N*-glycosylation, Fucosyltransferase, Silkworm, Insect cell line, CRISPR/Cas9

PS21

Comparative whole genome analysis of nucleopolyhedroviruses infecting saturniid silkworms by next-generation sequencing

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Nucleopolyhedroviruses (NPVs) of saturniid silkworms are important in both insect pathology and insect-based technology, because they cause severe damage to wild silk production in Asian countries and are also utilized as expression vectors for production of valuable proteins in wild silkworms larger than the domesticated silkworm, *Bombyx mori*. Recently, whole genome sequences of NPVs isolated from *Antheraea pernyi* (AnpeNPV) and *Philosamia cynthia* (Phcy (=Sacy) NPV) in China indicated that they are variants each other and some mutations may be responsible for their host specificities. For extending our knowledge on virus-host interactions and utilizing it to virus disease prevention and protein production, we have conducted comparative whole genome sequence analysis of NPV isolated from *A. yamamai* (AnyaNPV) in Japan (Nagano).

Whole genome sequence of AnyaNPV derived from infected five fifth-instar larvae of *A.yamamai* was determined with the next-generation sequencer (Ion Torrent PGM). The output reads were mapped against AnpeNPV genome (GenBank: NC_008035) used as a reference and a consensus sequence was constructed.

The resulted AnyaNPV consensus sequence showed very high homology (99.74%) with the AnpeNPV reference sequence, indicated AnyaNPV is also a variant of the saturniid NPV group. Precise sequence comparison revealed 214 SNPs and 58 INDELs between AnyaNPV and AnpeNPV. Among 10 INDELs causing frame-shift mutation in AnyaNPV ORFs, those in homologs of AnpeNPV ORF6 and ORF48 (*pcna*) altered the C-terminal half amino acid sequences, suggesting loss of function in their products. In addition, 35 SNPs and 16 INDELs were detected within AnyaNPV.

We are further analyzing the genomes of other NPV isolates from *A. pernyi* (China) and *S.*

cynthia (China, Vietnam and Cambodia) to obtain comprehensive information on inter- and intra-virus mutations by deep sequencing and understand their importance in virus divergence and virus-host interaction.

Key words: Saturniid silkworm, Nucleopolyhedrovirus, Next-generation sequencing

PS22

Genetic analysis of variable pupal period (bet-hedging strategy) in the wild mulberry silkworm *Bombyx mandarina*

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The wild mulberry silkworm *Bombyx mandarina* possesses several genetic traits considered to be lost in *B. mori* during domestication. In particular, variable pupal period have adaptive significance as a bet-hedging strategy to survive unpredictable environmental changes in the field. Since *B. mori* and *B.mandarina* can copulate and produce fertile hybrid progenies, it is expected that analysis of the hybrids will enable to identify genes responsible for the variable pupal period and reveal molecular mechanisms of the bet-hedging strategy.

We first selected hybrid lines whose pupal periods are highly variable from hybrid progenies between *B.mori* and *B.mandarina*, and analyzed pupal periods of back crosses (BC_1) obtained by crossing of a female of each reciprocal hybrid (F_1) between a hybrid line and *B.mori* (Daizo) with a male of the hybrid line. The result indicated that pupal period become variable when putative recessive genes of the hybrid line are homozygous. PCR analysis of BC_1 genome DNA using a set of chromosome-specific primer pairs, however, fail to specify any hybrid line-derived chromosomes completely related to the length of pupal period, suggesting that multiple genes with additive effects on the pupal period may disperse among chromosomes. Furthermore, analysis of correlation between pupal period and homozygous rate of hybrid line-derived chromosomes identified more than 10 positively correlated chromosomes including those likely to have hybrid line-specific and sex-specific effects. All of the results indicate that the variable pupal period seems to be a polygenetic trait controlled by many minor genes.

We are now making a set of chromosome-substitution *B. mori* lines, one pair of whose homologous chromosomes is displaced with those of *B. mandarina*, to specify the

combination of chromosomes required for the variable pupal period and identify the responsible genes.

Key words: *Bombyx mandarina*, Pupal period, Bet-hedging, Domestication

PS23

An electrical source and acceptor between the silkworm moth and the food leaf

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Some moths and worms are flying around the many places. The bonfire, fluorescent tube and heated metal pan let them jump in, turn round and escape by the intonation of the sources. The heated metal pan used in common about 200℃ by the gas cooking stove. They were tricked into the way by the artificial intonation. The electromagnetic induction and inhibition by the optical stimulation to the insect may have been studied. There is a moth it has the head looks like two coils, too.

My own making condenser could make like a sandwich structures. (chewing gum papers effective) One small Mulberry leaf measured from 39.0 to 109.0 nF. One small Japanese ork leaf measured from 7.0 to 19 nF for 200h, in the same summer. But this case, I ignored the thickness and the square to measure the capacitance (nF). It would work to function as the antimagnetic and the insulator in the thin film and small tube protoplasm. It has been rolling a source and acceptor electrically. How interest an experiment to use heated metal pan and a little water. (about 0.2ml)

Hot water antimagnetic phenomenon might maintain to the low vapor and low movement speed. It must be dedicated to up and down leaf capacitance. By the way, the unsatisfied fatty acids and satisfied fatty acids in the leaf would make of the molecular single layer structures (like Langmuir Bourget film). It seems like an electric condenser. Their double bonds of the unsatisfied fatty acid layers must work like graphite. It is easy to measure their oscillation of capacitance and smell, too. Several charge structures would remember to Self Complementary Antennas (SCA) like a moth on the move. It was suggested to the communication between the silkworm and the food leaf. A SCA shaped head flying moth would take a certain partner and same food, too. The crystal radio is one acceptor , for example.

I dedicated the source and acceptor terminals between the food leaf and insect head. The fetal food of the essential materials makes up the insect body and antenna. These distance and

distribution terminals between insulated each source and acceptor layers were stimulated in its by the restricted circumstances. An oscillated leaf capacitance would generate to the frequency response. A physical nutrition of the leaf and silkworm would repeat the alternation of generation and survive. Okinawa-Is and Shodoshima-Is some arias in Japan didn’t use any agricultural chemicals in their circumstances. There is no harmful insect.

I would like to consider for the physical idea and original self supporting food technique and study how to drive a silence minority independently. It is important to maintain the radio low role and their interference in their electromagnetic field. We have to control the frequency response for our public wealth.

Key word: Source Acceptor Condenser Antimagnetic Inter

PS24

Insect water-specific aquaporins in developing ovarian follicles of the silk moth *Bombyx mori*: role in hydration during egg maturation

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Egg formation in terrestrial insects is an absorptive process, accommodated not only by packing proteins and lipids into yolk but also by filling chorions with water. An osmotic swelling of ovarian follicles takes place during oocyte maturation. This study investigated the role of the aquaporin (AQP) water channel in the osmotic uptake of water during oogenesis in the silk moth *Bombyx mori*. Using the antibodies specifically recognizing previously characterized AQPs, two water-specific subtypes—AQP-Bom1 and AQP-Bom3— belonging to the *Drosophila* integral protein (DRIP) and *Pyrocoelia rufa* integral protein (PRIP) subfamilies of the insect AQP clade, respectively, were identified in the developing ovaries of *B. mori*. During oocyte growth, *Bombyx* PRIP was distributed at the oocyte plasma membrane where it likely plays a role in water uptake and oocyte swelling and may be responsible for oocyte hydration during fluid absorption by ovarian follicles. During the transition from vitellogenesis to choriogenesis during oocyte maturation, *Bombyx* DRIP expression became abundant in peripheral yolk granules underlying the oocyte plasma membrane. The restricted DRIP localization was not observed in non-diapause-destined follicles, in which DRIP was evenly distributed in medullary yolk granules. There was no difference in PRIP distribution between diapause- and non-diapause-destined follicles. The diapause-destined oocytes encase DRIP protein into the peripheral yolk granules, where DRIP might be inert and this would be reflected in the metabolic arrest associated with diapause after fertilization and egg oviposition.

Key words: Aquaporin, Water channel, Oogenesis, *Bombyx mori*, Egg diapause

PS25

Actualizing mechanisms for nodule specific melanization in the hemolymph of the silkworm, *Bombyx mori*

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Nodule which is well known as a product of the cellular response against microorganisms in insect immune system is considered to be an preferential target for melanization. However, the mechanism of nodule-preferential melanization remains to be explored. Here, we show that the mechanism of nodule-preferential melanization by analyzing several factors which are members of prophenoloxidase (proPO) activating system in the silkworm, *Bombyx mori*. By binding assay, the pathogen-associated molecular patterns (PAMPs) recognition proteins, BmβGRP1, BmβGRP2 and BmβGRP3 of βGRP group, BmMBP of C-type lectin, and BmPGRPS1 of PGRP group were analyzed regarding the binding ability to microorganisms. PAMPs recognition proteins originated from two different groups were observed to bind to the same microorganism. In addition, BmβGRP2, BmβGRP3 and BmMBP can recognize two different types of microorganisms. From these, outline of intricately overlapped surveillance networks for immunity against three typical microorganism groups in the *B. mori* hemolymph became clear. However, BmMBP seemed to have a different roll, since they were found not only bind to *Saccharomyces cerevisiae* and *Micrococcus luteus* cells, but by pull-down assay to form complex directly with the serine protease homologs (BmSPH1 and BmSPH2) in the hemolymph. BmSPH1 and BmSPH2 in hemolymph were thought to be brought into primary nodule formed against *S. cerevisiae* and *M.luteus* cells by BmMBP for prophenoloxidase (proPO) activation.

A hemolymph serine protease BmHP14 which is a homologue of *Manduca sexta* HP14 (MsHP14), was found to bind directly to *S. cerevisiae* cells as 70 kDa proenzyme and 30 kDa active form in the hemolymph. According to previous studies, MsHP14 was reported to be activated by MsβGRP1 and activate next serine protease of the proPO activating cascade. All

these things indicated that activated serine protease homologs and serine proteases were collected on or around *S. cerevisiae* cells to meet prophenoloxidase which is thought to be brought by hemocytes. But, the serine protease which act as BmHP14 in *M.luteus* and *E.col* cascades still unknown. By the way, all the factors that we surveyed (BmβGRP1, BmβGRP2, BmβGRP3, BmLBP, BmMBP, BmPGRPS1, BmSPH1, BmSPH2, BmHP14, BmHP21 and BmHP8) in this research existed not only in the hemolymph but in hemocytes, indicating that nodule should include two routes to congregate factors for proPO activation, the hemolymph route and the hemocytes route. This system make the melanization reaction in nodule more quick and specific than in hemolymph.

Key words: Nodule, Melanization reaction, PAMPs recognition proteins, proPO activation, *Bomyx mori*

PS26

Mechanisms of apoptosis regulation by apsup during *Lymantria dispar* MNPV infection

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Lymantria dispar M nucleopolyhedrovirus (LdMNPV) encodes *apsup* (*apoptosis suppressor*, *ld109*) to suppress host apoptosis during infection. *apsup* encodes a polypeptide of 336 amino acid residues with a predicted molecular mass of 39 kDa. Apsup does not have any homology with IAPs and P35 baculovirus anti-apoptotic proteins, and any characteristic domain that suggests its function. Previously we showed that Apsup prevented apoptosis through inhibition of *L. dispar* initiator caspase Ld-Dronc activation, based on the result that Apsup suppressed Ld-Dronc proteolytic processing in Ld652Y cells induced by overexpression of Ld-Dronc. In this study, the mechanisms of suppression of the proteolytic processing of Ld-Dronc by Apsup were further studied. *Autographa californica* MNPV (AcMNPV) 112/113 (Ac-Apsup) is a homologue of Ld-Apsup and deficient in 79 amino acid residues at the C-terminal region compared to Ld-Apsup. Expression of Ac-Apsup in Ld652Y cells did not suppress apoptosis and proteolytic processing of Ld-Dronc in Ld652Y cells induced by overexpression of Ld-Dronc. Ac-Apsup fused with C-terminal 79 amino acid sequence of Ld-Apsup suppressed apoptosis and proteolytic processing of Ld-Dronc in Ld652Y cells, while Ld-Apsup with C-terminal 79 amino acid sequence deletion did not suppress, indicating that C-terminal sequence of Ld-Apsup has indispensable role for apoptosis suppression. Further analyses concerning the functional characterizations of Ld-Apsup domains are underway.

Key words: LdMNPV, Apoptosis suppressor, Apsup, Ld-Dronc, Ld652Y

PS27

Relationship between cocooning abnormality and matured stages in the silkworm *Bombyx mori* at the high humidity condition

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The humidity during at mounting is the one of the important factor to obtain high-quality cocoon in the sericulture. Many studies have been investigated the relationship between cocooning and humidity so far, however, there is no research for cocooning at nearly 100% high humidity condition. In this study, we investigated the effect of high humidity on cocooning in the silkworm. At nearly 100% high humidity condition, N17 (Chinese race) and N43 (Japanese race) larvae could not make cocoons and then died. This result indicated that high humidity condition cause the cocooning abnormality. Additionally, bioassay using larvae at four matured stages (premature, mature, overmature, and undergrown) at high humidity showed that N17 and J124 (Japanese race) larvae could hardly make cocoons and then died in all examined stages. However, the percentage of cocooning abnormality was high in undergrown larvae and low in overmuture larvae, it was suggested that matured stages related abnormality at the high humidity condition. Now we are investigating to whether these characters are suitable for other silkworm strains or not.

Key words: Silkworm, Humidity, Cocooning abnormality

PS28

Mulberry (*Morus* L.) ribosomal protein S3a（RPS3a）gene cloning and expression analysis under abiotic stresses and different resistant mulberry varieties

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A full-length cDNA sequence coding ribosomal protein S3a of mulberry tree, which we designated *MmRPS3a,* was cloned based on mulberry expressed sequence tags (ESTs). Sequence analysis showed that the *MmRPS3a* is 1089bp long and contains a 80 bp 5'-UTR (untranslated region) and a 220 bp 3'-UTR. It's open reading frame (ORF) is of 789 bp, encoding 262 amino acids with a predicted molecular weight of 30.053 kD and an isoelectric point of 9.84. Homology analysis revealed that *MmRPS3a* gene is highly conservative in mulberry and other species including *Morus notabilis*, *Theobroma cacao* and *Ricinus communis*. Phylogenetic analysis based on MmRPS3a with other species showed that mulberry had a closer relationship with *Prunus persica, Arabidopsis thaliana, Solanum tuberosum, Solanum lycopersicum* and *Vitis vinifera*. The results of quantitative PCR analysis showed that the transcriptional level of *MmRPS3a* mRNA changed significantly under the conditions of hypothermia, arid, salt stress, and different resistant and susceptible varieties. However, the gene has the characteristics to be further studied, because studies of plants to abiotic stress adaptation should adopt a multi-factor test analysis.

Key Words: *Morus multicaulis*, *MmRPS3a,* Gene clone, Stress-induced expression

The first two authors contributed equally.

PS29

Mulberry Δ1-pyrroline-5-carboxylate synthase (M*P5CS*) gene cloning, sequence analysis, and determination of abiotic stress patterns of M*P5CS* gene expression

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Drought is one of the important factor limiting plant growth. Δ1-pyrroline-5-carboxylate synthase is the key enzyme closely related in the synthesis of proline under drought stress. Cloning and function analysis of mulberry gene *P5CS* has a very important significance. In this report, we cloned the *P5CS* gene of mulberry by means of homology cloning, RT-PCR and RACE (rapid amplification of cDNA ends), and we got the full sequence of cDNA named M*P5CS* (GenBank accession number：KC202259). Homology and phylogenetic analysis of *P5CS* were carried out using Clustal X and MEGA4.1 software. In addition, the expression level of *P5CS* in drought and salt stresses were performed by qRT-PCR. Result shows that the expression level of *P5CS* is up-regulated.

Key Words: Mulberry, *P5CS*, Gene cloning, Expression analysis, Drought stress

The first two authors contributed equally

PS30

Optimization of ultrasonic-assisted extraction of total polysaccharide from mulberry leaves by response surface methodology

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Based on single factor experiments, a three level, three variable central composite designs were carried out to establish a quadratic regression model for the extraction efficiency of total polysaccharides as a function of extraction time, extraction temperature and water-material ratio. The optimum extraction conditions were obtained as follows: extraction temperature 70℃, water-material ratio 1:30 (g/ml), and extraction time 40 min. Under these conditions, the predicted total polysaccharides extraction efficiency was 3.6%, while the experimental value was 3.56%. The result indicated that the established model well predicted the extraction efficiency of total polysaccharides from mulberry leaves.

Key words: Mulberry leaves, Total polysaccharides, Extraction conditions, Response surface methodology, Optimization

# The first two authors contributed equally

PS31

A hypothetical model of crossing *Bombyx mori* nucleopolyhedrovirus through its host midgut physical barrier

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Bombyx mori nucleopolyhedrovirus (BmNPV) is a primary pathogen of silkworm (B. mori) that causes severe economic losses each year. However, the molecular mechanisms of silkworm-BmNPV interactions, especially the silkworm proteins that can interact with the virus, are still largely unknown. In this study, the total and membrane proteins of silkworm midguts were displayed using one- and two dimensional electrophoresis. A virus overlay assay was used to detect *B. mori* proteins that specifically bind to BmNPV particles. Twelve proteins were located and identified using mass spectrometry, and the different expression of the corresponding genes in BmNPV susceptible and resistant silkworm strains also indicated their involvement in BmNPV infection. The 12 proteins are grouped based on their potential roles in viral infection, for example, endocytosis, intracellular transportation, and host responses. Based on these results, we hypothesize the following: I) vacuolar ATP synthase catalytic subunit A and subunit B may be implicated in the process of the membrane fusion of virus and the release of the nucleocapsid into cytoplasm; II) actin, enolase and phosphoglycerate kinase are cytoskeleton associated proteins and may play an important role in BmNPV intracellular transportation; III) mitochondrial prohibitin complex protein 2, ganglioside-induced differentiation-associated protein, calreticulin, regucalcin-like isoform X1 and 60 kDa heat shock protein are involved in cell apoptosis regulation during BmNPV infection in larvae midguts; IV) ribosomal P0 may be associated with BmNPV infection by regulating gene expression of BmNPV; V) arginine kinase has a role in the antiviral activities against BmNPV. Our work should prove informative by providing multiple protein targets and a novel direction to investigate the molecular mechanisms of the interactions between silkworms and BmNPV.

Key words: *Bombyx mori*, *Bombyx mori* nucleopolyhedrovirus (BmNPV), Silkworm-BmNPV interactions, BmNPV Binding Proteins

PS32

Superoxide dismutase, SOD1 and SOD2, response to a phototoxic damage in the fat body of silkworm *Bombyx mori*

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Superoxide dismutase (SOD) is a group of metalloenzymes that play an essential role in the cellular anti-oxidant defense system. SOD is widely distributed in eukaryotic cells, categorized into three classes. We characterized SOD1 and SOD2 of *Bombyx mori* isolated from the fat body of larvae. Immunological analysis demonstrated the presence of BmSOD1 and BmSOD2 in the silk gland, midgut, fat body, Malpighian tubules, testis and ovary from larvae. The anti-oxidative functions of BmSOD1 and BmSOD2 were assessed by exposing larvae to insecticide rotenone or vasodilator isosorbide dinitrate, which is an ROS generator in BmN4 cells; however, exposure to these compounds had no effect on the expression levels of either BmSOD protein. Next, we investigated the physiological role of BmSOD1 and BmSOD2 under environmental oxidative stress, applied through whole-body UV irradiation and assayed using quantitative RT-PCR, immunoblotting and microarray analysis. The mRNA expression level of both BmSOD1 and BmSOD2 was markedly increased but protein expression level was increased only slightly. To examine the differences in mRNA and protein level due to UV irradiation intensity, we performed microarray analysis. Gene set enrichment analysis revealed that genes in the insulin signaling pathway and PPAR signaling pathway were significantly up- regulated after 6 and 12 hours of UV irradiation. Taken together, the activities of BmSOD1 and BmSOD2 may be related to the response to UV irradiation stress in *B. mori*. These results suggest that BmSOD1 and BmSOD2 modulate environmental oxidative stress in the cell.

Key words: Superoxide dismutase, Oxidative stress, UV

PS33

Transgenic expression of the innate immune response transcription factor BmRelish induces high level antimicrobial peptides in transgenic silkworms

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To artificially enhance expression of antimicrobial peptides in *Bombyx mori*, we genetically engineered silkworms that overexpressed Rel family transcription factor, truncated BmRelish1 (BmRelish1t) lacking the ankyrin repeats domain (ANK) and BmRelish2 gene under the control of *B. mori* cytoplasmic actin 3 promoter (BmA3) using piggyBac transpon vector, respectively. A transgenic silkworm with EGFP expression was obtained and chromosome analysis of the G1 generations confirmed the stable insertion of BmRelish1t and BmRelish2 in the genome. Overexpression of the BmRelish1t in transgenic silkworm showed higher mRNA expression levels of the *B .mori* antimicrobial peptides such as lebocin(~19.6-fold), moricin (~9.8-fold) and nuecin (~16.4-fold) than normal silkworms. But, transgenic silkworms overexpressing BmRelish2 showed similar mRNA expression patterns of antimicrobial peptides compare to normal silkworms, excluding lebocin expression levels (~3.9-fold). Moreover, transgenic silkworms expressing BmRelish1t showed antibacterial activity against E.coli. But, BmRelish2 gene-introduced transgenic silkworms have no antibacterial activity. Therefore, we suggest that transgenic expression of BmRelish1t can be very useful for the production of various antimicrobial peptides at the same time in transgenic silkworms.

Key Words: *Bombyx mori*, Relish, Antimicrobial peptides, Transgenic silkworm, Immune

PS34

Quantitative analysis of functional ingredients of sex-limited silkworm strains

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Silkworm including functional ingredient such as DNJ, Rutin, GABA has avariety of effects. Commercialization of sericultural products based on the research results are being promoted recently. This study analyzed the functional ingredient contents of sex-limited silkworm strains in order to improve the added value. The antioxidant activity of sex-limited silkworm strains was measured by DPPH. Yangweonjam was 32.1~32.4% in DW, 83.6~84.2 in 70% ethanol and 84.6~85.3% in 100% ethanol. Hansaengjam was 32.0~32.4% in DW, 81.7~82.6 in 70% ethanol and 82.5~83.1% in 100% ethanol. Both varieties were higher in 100% ethanol extract. Estrogens were analyzed by HPLC. Estrogens were contained 10.65 mg% in Hansaengjam female, 19.95 mg% in Hansaengjam male, 21.95 mg% in Yangweonjam female, 14.70 mg% in Yangweonjam male, and 17.50 mg% in Baekokjam. Estrogen contents of Yangweonjam female were higher than others.

Key words: Silkworm, Functional ingredient, Antioxidant activity, Estrogen

PS35

Microsatellite markers development from NGS (Next-Generation Sequencing) in *Antheraea yamamai*

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An Illumina paired-end library with a mean insert size of 556bp was made using an isolate of *Antheraea yamamai* collected from jeju island in korea. The *Antheraea yamamai* library shared a single lane on a flow-cell with another library and was sequenced on the Illumina Mi-SEQ platform to produced 250bp x2 paired-end reads. A total of 40,182,580 reads, including 20,091,290 x2 in pairs were generated for the *Antheraea yamamai* library. Also the total number of contigs was 149,317 and the length of average contigs was 2,848bp. Tri-nucleotide repeats were the most abundant class of microsatellites (57,864 regions) detected in the partially assembled *Antheraea yamamai* genome, followed by di-nucleotide (14,639 regions), tri-nucleotide (10,983 regions) and tetra-nucleotide (21,501 regions). The most frequent tetra-nucleotide type in the *Antheraea yamamai* genome was CTGT repeats (21.1%), followed by ACAG (20.9%), AAAC (1.8%) and GTTT (1.7%).

Polymorphic microsatellite markers from *Antheraea yamamai* were successfully identified using NGS without any prior sequence information and deposited into the public database. Thus, the methods described herein represent a rapid and low-cost way to investigate the population genetics of endangered/non-model species.

Key words: Next-Generation Sequencing, *Antheraea yamamai*, Microsatellite

PS36

Character of silkworm strains registered as genetic stocks in Korea

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In order for further systematic maintenance of silkworm stocks kept in Korea we analyzed character quality of a diverse array of silkworm strains originated from several sericulture-practicing countries. The analysis of about ten qualitative characters from 67 strains (13 of Japanese strains, 15 of Chinese strains, 14 of European strains, 6 of Korean and Tropical strains, and 19 of unknown origin) revealed a significant difference in the ten different qualitative characters among silkworm strains. In the analysis of quantitative characters, Japanese and European strains were highest in hatchability, the Korean and Tropical strains were highest in pupation rate, and unknown origin and Chinese strains were highest in cocoon yield and number of egg laid. With the connection of molecular genetic analysis the current data may provide the advanced ground for further systematic maintenance of valuable genetic resources of silkworms, although more breeds should be investigated for further complete pictures.

Key words: Silkworm strain, *Bombyx mori*, Qualitative characters, Quantitative character

PS37

Development and characterization of twelve novel microsatellite markers in the medicinal mushroom, *Cordyceps militaris* (Ascomycota: Clavicipitaceae) for its strain identification

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Cordyceps militaris (a caterpillar fungus), which belongs to the class *Ascomycetes*, has extensively been used for medicinal purposes in East Asia. Here, we isolated and characterized 12 microsatellite loci from the medicinal mushroom, *C. militaris*. Forty-two individuals were sampled from Korea and used to characterize the developed markers. The number of alleles of these polymorphic loci ranged from 3 to 13. Observed heterozygosity and expected heterozygosity in the population ranged from 0.034 to 0.880 and from 0.033 to 0.870, respectively. Tests of genotypic linkage disequilibrium between the 12 loci showed no significant association of alleles except for five pairs of loci. These microsatellite markers will provide valuable tools for genetic analyses for the strain identification of *C. militaris* as well as for the resources conservation of this species.

Key words: *Cordyceps militaris*, Microsatellite, Population

PS38

The enhance blue fluorescent protein (EBFP) in the cocoon by the transgenic silkworms

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We produced the transgenic silkworm that expressed the enhanced blue fluorescent protein (EBFP) in the cocoon of silkworms. The EBFP fusion protein formed each with N- and C-terminal sequences of the fibroin H-chain was designed to be secreted into the lumen of the posterior silk glands. The expression of the EBFP/H-chain fusion gene was regulated by the fibroin H-chain promoter. The use of the 3xP3-driven DsRed2 cDNA as a marker allowed us to rapidly distinguish transgenic silkworm. A mixture of the donor and helper vector was micro-injected into 300 eggs of silkworms, Baegokjam. As a result, we obtained 5 broods. The cocoon showed blue fluorescence, proving that the fusion protein existed in the cocoon. Also, the existence of fusion proteins in cocoons was demonstrated by SDS-PAGE and western blot analysis. Accordingly, we suggest that the EBFP fluorescence silk will enable the production of the silk-based biomaterials.

Key words: Transgenic silkworm, Fluorescent silk, Blue fluorescent protein, EBFP

PS39

Effect of cocoon extracts on cytotoxicity in silkworm varieties

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Silkworm cocoon has been used as textile fiber, surgical sutures, and so on. Now a day, novel applications using silkworm cocoon has been studied as functional food, biomedical materials, and so on. The most important thing for developing medical material is cell toxicity of the materials. Therefore, we investigated cytotoxicity of cocoon extract in silkworm varieties. To investigate the toxic effects, we obtained extract from silkworm cocoons in different Korean silkworm varieties. Silkworm cocoon was cut into 0.5 mm of squares, divided into 2 groups and then immersed in prepared solution. After absolutely dried, cocoon was immersed in 1X phosphate buffered saline (PBS) on 37^{o}C for 72 h or 72^{o}C for 24 h, respectively. Cocoon extracts were filtered by 0.2 um syringe filter and the quantity of extracted silk protein was determined by BCA protein assay. Also, we checked the electrophoresis patterns with protein extracts by SDS-PAGE. Four ug of protein extracts was tested to elucidate the toxicity on L929 mouse fibroblast cells. 3-species silkworm extracts showed no toxicity on L929 cells, and some groups of the extracts proliferated fibroblast cells. Our results suggested that silkworm cocoon could be used as biomaterials.

Key words: Silkworm, Cocoon, Cell toxicity, L929, Fibroblast

PS40

Effects of immunized silkworm larvae as a feed additive on growth performance of broiler chickens

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We were recently mass-produced of immunized silkworm (*Bombyx mori* L.) powder with antibacterial activity by injected immune stimulant containing the lactobacillus peptidoglycan into the silkworm larvae. This study was conducted to investigate the effects of immunized silkworm powder as replacement for antibiotic in broiler feed on growth performances, intestinal microbiota, and blood characteristics. Two hundred forty male broiler chicks (Ross) were fed diets for five weeks containing 0.01, 0.05, 0.1, and 0.5% of immunized silkworm powder. Feed and Body weight (BW) were measured at 0, 21, and 35 d. The results showed that BW gain and feed conversion ratio (FCR) were improved when compared with negative control (NC) group ($p<0.05$). Diet with 0.05% of silkworm powder increased BW gain (5.2%) and reduced FCR (6.2%) in compared with NC group. In cecal microbiota, tested groups showed decreased total microbe population, coliform bacteria and salmonella sp. compared to NC group. However, lactic acid bacteria counts showed no significant difference ($p<0.05$) in all groups. Antioxidant activity of tested group were higher than NC group, though there was no significant difference ($p<0.05$). The level of cortisol in the blood decreased significantly by feeding diets containing 0.01% to 0.5% of immunized silkworm powder (37~45%) compared to NC group. In conclusion, this silkworm powder additive improved growth performance of broiler chickens. It also demonstrated inhibitory effects on intestinal pathogenic bacteria as a dietary antibiotic alternative.

Key words: Silkworm, Broiler chicken, Feed additive, Antioxidant activity, Cortisol

PS41

Hac1/UPRE expression system for production of useful material using silkworm

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To develop an efficient system for producing recombinant protein in the silkworm, we investigated the feasibility of using the HAC1/UPRE system of *Saccharomyces cerevisiae*. HAC1 is transcriptional activator, involved in the unfolded protein response (UPR) pathway. Recognizes and binds to the UPR element (UPRE) in the promoter of UPR-regulated genes such as KAR2, PDI1, EUG1 and FKB2. Increases the synthesis of endoplasmic reticulum-resident proteins required for protein folding as well as components of the secretory pathway. In initial tests of the protein expression of the HAC1/UPRE system, we used the the *B. mori* actin A3 promoter to drive the HAC1 gene and luciferase gene as the reporter. In the dual luciferase analysis, we could show that the HAC1/UPRE system was more effective gene expression than the Gal4/UAS system.

Key words: Transgenic silkworm, HAC1, UPRE, Protein expression, Binary system

PS42

The antimicrobial activity of recombinant papiliocin/jelleine hybrid peptide

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In order to develop new antibiotic peptide, the hybrid peptide PAJE (RWKIFKKPFKISIHL-NH2) was rationally designed incorporating the N-terminal α-helical segments of papiliocin (amino acid 1-7) and jelleine (amino acid 1-8) according to the structure activity relationship of the amphipathic and cationic peptides. The 15-residue PAJE was chemically synthesized on-solid phase, purified and evaluated for direct antimicrobial activity. The synthetic PAJE showed good antimicrobial activity in the concentration range 1-4 μM against a wide range of microbes from Gram-negative bacteria, Gram-positive bacteria and yeast. This hybrid peptide was a 4 to 8-fold more active than jelleine and melittin against Gram-negative bacterial species. It was also a 4-fold more active than jelleine against yeast and Gram-positive bacterial species. The time-kill assay showed that PAJE displayed a time-dependent bactericidal activity, as was also seen after treatment with melittin. These results indicate that PAJE has a fast microbicidal effect on the E. coli and S. aureus. Therefore, this peptide will be a significant potential for future development as antimicrobial agent.

Key words: Antimicrobial peptide, Hybrid peptide, PAJE, Gram-negative bacteria, Gram-positive bacteria

PS43

The yellow fluorescent cocoon production by transgenic silkworm

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To express yellow fluorescent protein (YFP) in the cocoon of silkworm, we were fused YFP cDNA to the heavy chain gene and injected the gene into a silkworm eggs. The YFP fusion protein, each with N- and C-terminal sequences of the fibroin H-chain, were designed to be secreted into the lumen of the posterior silk glands. The expression of the YFP/H-chain fusion gene was regulated by the fibroin H-chain promoter. The 3xp3-driven DsRed2 cDNA was used as a marker and it allowed us to rapidly distinguish transgenic silkworms. The recombinant transformation vectors were injected with helper plasmid into 3060 Kumokjam, bivoltin silkworm eggs. We obtained 120 broods and selected 8 broods with red fluorescence positive. The cocoon was displayed yellow fluorescence, proving that the fusion protein was present in the cocoon. Therefore, we suggest that the yellow fluorescence silk can provide material for many industries such as textile, apparel and fashion industries.

Key words: Transgenic silkworm, Fluorescent silk, YFP fluorescent protein

PS44

Production of 1-deoxynojirimycin using sericultural sources from a various of microorganisms

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1-deoxynojirimycin (1-DNJ), a potent a-glycosidase inhibitor, has therapeutic applications in treatments of HIV, Gaucher's disease, and diabetes. 1-DNJ has been extracted from natural sources (mulberry leaves) for therapeutic purposes; however, 1-DNJ ingredients are in limited supply and are costly to obtain on a large scale. Since certain strains of *Bacillus* and *Streptomyces* species reportedly produce 1-DNJ, they may serve as potential sources for high-yield 1-DNJ production. In this study, we obtained evidence for four bacteria that produce 1-DNJ in large quantities by high performance liquid chromatography and thin layer chromatography. One of them, *B. subtilis* JS had a high yield of 1-DNJ. Investigation of the effect of mulberry leaves powder concentration(1~5%), using the 1-DNJ high-production *B. subtilis* JS, provided evidence for microbial mass production of 1-DNJ.

Key words: 1-deoxynojirimycin (1-DNJ), Mulberry leaves, Silkworm, *Bacillus subtilis*

PS45

Survey of damage by mulberry popcorn disease and development of integrative control method

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Though mulberry fruit is known to a by-product that was produced from mulberry tree after harvesting leaves for silkworm rearing, as a yield and consumption of mulberry fruit was increased, it has been fixing to a new income crop. Mulberry fruit has been effectively in natural medicine for the treatment of sore throat, fever, hypertension and anemia. Mulberry fruit also contains not only high amounts of anthocyanins, but non-anthocyanin phenolics including rutin and quercetin known to have multi-bioactive functions including neroprotective effects. Nevertheless, through global warming, the popcorn disease caused by sclerotia forming fungi reduces the productivity of mulberry fruits in worldwide.

So, in this study, we investigated damage ratio of mulberry popcorn disease in mulberry fruit production farm (Buan, Jeongueb, Sangju, Gochang in Korea). In Jeonbuk Buan, popcorn disease rate was the highest on 30%, on the other hand, in case of Gyungbuk Sangju and Jeonbuk Gochang, not damage. Also, we investigated about popcorn disease prevention by a various of chemical treatment methods.

Key words: Mulberry, Mulberry fruits, Popcorn disease

PS46

Quantitative analysis of rutin with mulberry leaves

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We analyzed rutin content using mulberry genetic resources. They were grown under the same environment and conditions. Mulberry leaves were collected and then freeze-dried and powdered for rutin test. As a result, mean content of mulberry strains was 0.47±0.23 %, and the coefficient of variation (CV) was 49.7 %. The variation between the strains was greatly severe. Among the tested 338 strains(varieties)(322 strains and 16 varieties), 'Buyoungsang' was showed the highest content of 1.37 %, whereas 'Sungsu 3' was showed the lowest content of 0.01 % respectively. The content of rutin of 16 mulberry varieties for silkworm rearing were compared. 'Cheongolppong' was showed the highest content of 0.69%, whereas 'Cheongilppong' was showed the lowest content of 0.14 %. Finally we selected rutin high-containing 11 strains. They are as follows. 'Buyoungsang', 'Yeulbon', 'Dangsang 5', 'A8', 'Aja', 'Seongsu 5', 'Sawonppong 12', 'Hwanyoupjosaengnosang', 'Hwansipjosaeng', 'Jangloe', and 'Pumbo 6' which are more than twice of the overall average content.

Key words: Rutin, Mulberry leaves

PS47

Manufacturing and characterization evaluation of mulberry concentrate for food additive

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Study on extraction and concentration of mulberry leaf were performed to increase utilization as new source of food additives. We analyzed extraction method in EtOH, sugar and hot water solution. The desirable method was 70% alcoholic extraction. Color of concentrate was comparatively stable in 70% alcoholic extraction solution and sugar solution. But hot water extraction was showed color change with brown. By filtering of concentrate in 70% alcoholic extraction and sugar extraction, we removed a deposits. Also, we investigated characteristics of the concentrate from mulberry leaf.

Key words: Mulberry, Food, Additive

PS48

Silk fibroin hydrogel fabricated by molecular weight manipulation

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Silk fibroin (SF) is the fibrous protein that it plays the critical role in the structural features of cocoons. This SF can be isolated by removing SS at the boiled weak alkaline solution conditions, so we called degumming. Among many natural polymers, SF has the excellent biocompatibility and biodegradability in addition to superior mechanical strength. In this reasons, many researches were progressed to use SF for applying biomaterial fields.

Hydrogel has a network structure made by physical or chemical crosslinking of hydrophilic polymer chain and shows high water contents. Hydrogel has been studied in tissue engineering filed due to its pore structure which promotes exchange of small molecules, such as water, nutrients, wastes and ions, and its structural similarity to extracellular matrix. SF can form hydrogel by physical crosslinking induced by structural transition from random coil to β-sheet. There have been many researches on the effects of gelling conditions (temperature, pH and additives) and chemical or mechanical stimuli (alcohol treatment or sheer stress like ultrasonication) on the physical properties and gelling behavior of SF hydrogel. However, until now, there is no study about the effect of molecular weight on physical properties of SF hydrogel. In this study, we fabricated the various molecular weight ranges of SF hydrogel. Also, we investigated the effect of molecular weight on the physical properties of SF hydrogel.

Key words: Silk, Hydrogel, Molecular weight, Physical properties

PS49

Construction of the *Bombyx mori* fibroin H-chain gene core promoter in the fibroin H-chain 5'-UTR region

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BmA3 is a main substance that combines filament protein of cell and muscles, can be found in most eukaryotic cells. So far, no strong promoter is available for ubiquitous expression in *B. mori*, excluding BmA3 promoter. In this study, we isolated 8 clones which showed strong signal compared to *B. mori* cytoplasmic actin gene (BmA3) from *B. mori* fibroin H-chain 5'-UTR region (-8201~-1). Among the 8 clones, F5R1(-561~-1) was selected the maximum activity region of fibroin H-chain 5'-UTR region. To determine core promoter region, F5R1(-561~-1) was segmented 4 deletion mutants. As result of promoter assay using dual luciferase assay system within 4h after oviposition eggs, we found the highest transcription activity core promoter region (-468/-1) in the *B. mori* fibroin H-chain 5'-UTR region of F5R1-5'-1 gene, which has 6.6 times higher activity than BmA3 promoter. Therefore, we suggest that F5R1-5'-1 core promoter may be used more useful and effectively for making recombinant proteins in transgenic silkworms.

Key words: Fibroin H-chain, Promoter, Luciferase assay, 5'-UTR

PS50

Silk fibroin nanofiber wound dressing immobilized with antimicrobial peptide KR12

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Silk fibroin (SF) is one of the most attractive materials, which has good mechanical property, biocompatibility, and biodegradability. Therefore, the SF has been studied for biomedical applications such as tissue engineering scaffold, wound dressing and surgical suture. Electrospinning is a fascinating fabrication method of scaffold because it enables to mimic the extracellular matrix (ECM). However, the biomaterial-related bacterial infection is one of the most major clinical complication. There were several attempts to reduce bacterial infections, such as antibiotic treatment, loading silver nanoparticle or silver ions, and chitosan nanoparticle. However, in many cases, there were some side effects, such as the emergence of resistant bacterial strain and cytotoxicity to human body, and limited efficacy. Antimicrobial peptides are innate host defense peptides and exist in many mammals, amphibians, and insects. KR12 (residues 18-29 of LL37) was known to be the shortest peptide of human cathelicidin LL37, which is cationic, amphiphilic, and α-helical peptide. LL37 has an antimicrobial activity against gram positive and gram negative bacteria and wound healing ability and reduce inflammatory reaction. Therefore, in this study, we developed the immobilization method of antimicrobial peptide KR12 onto the electrospun SF nanofiber wound dressing using EDC/NHS and thiol-ene click chemistry to obtain antimicrobial activity of SF nanofiber wound dressing. The immobilization of KR12 and the amount of immobilized KR12 onto the SF nanofiber was confirmed by Ellman's assay. Antimicrobial activities of KR12 in solution and KR12-immobilized SF nanofiber wound dressing were tested against both gram positive and gram negative bacteria, using JIS Z-2801 method. Cell proliferation and cytotoxicity of KR12 in solution and KR12-immobilized SF nanofiber wound dressing were tested against fibroblast and keratinocyte cell using MTT assay.

Key words: Silk, Nanofiber, Antimicrobial, Wound dressing, Tissue regeneration

PS51

Effect of heat treatment on the mechanical properties and elasticity of silk textile woven with highly twisted yarns

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Silk has been known as the best textile material for a long time because of its excellent touch feeling and luster. However, silk textile has limited mechanical properties with less elasticity resulting in restricted application in textile fields. In this study, to introduce new function (elasticity) of silk textile, the silk textile was woven with highly twisted yarns, first. After that, heat was applied to the silk textile during water treatment and drying process and the effect of heat treatment on the mechanical properties and elasticity of silk textile was examined. As the water treatment temperature and drying temperature were increased, the shrinkage ratio, the elongation and the elastic recovery ratio of silk textile were increased, while the breaking strength of textile was decreased. By controlling heat treatment condition during water treatment and drying process, a highly elastic silk textile with more than 100% elongation and 100% elastic recovery ratio could be fabricated successfully.

Key words: Silk textile, Elongation, Elasticity

PS52

Preparation, structural characteristics and properties of cellulose nano fibril/silk sericin composite film

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Silk is a natural fiber composed of fibroin and sericin. Silk sericin protein contains 18 amino acid and most of them have strong polar side groups. Owing to its good biocompatibility, non-toxic and antibiotic properties, silk sericin has attracted the researcher's attention, recently. However, when silk sericin was fabricated into film, it has poor mechanical properties leading to the difficulty of its practical application. In this study, cellulose nano fibril (CNF) was used as a reinforcing material to enhance mechanical properties of silk sericin film. Structural characteristics and properties of CNF/silk sericin composite film were examined. By increasing ultrasonication time, the turbidity of CNF/silk sericin formic acid solution was decreased implying more homogeneous dispersion of CNF in solution. However, the tensile strength of resultant CNF/silk sericin composite film could not be improved by addition of CNF. FTIR measurement results showed that Beta sheet crystallization of sericin could not be intervened by addition CNF.

Key words: Silk sericin, Cellulose nano fibril, Structural characteristics

PS53

Structural characteristics and properties of natural silk fibroin nanofiber/regenerated silk fibroin composites film

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Silk has been studied to apply it to various biotechnological fields owing to its good bio-compatibility and excellent cyto-compatibility. Many researchers have tried to improve the mechanical properties of regenerated silk film because the regenerated silk film is too brittle, thereby, it is hardly use it in biomedical and cosmetic applications. In this study, the new silk fibroin nano fiber (SFNF) with high crystallinity was used a reinforcing material to improve the mechanical properties of regenerated silk fibroin (RSF) films. The effect of SFNF contents on structural characteristics and mechanical properties of SFNF/RSF composite film were examined. FTIR results showed that the crystallinity index was increased by increasing SFNF contents. Also, the tensile strength, tensile elongation and Young's modulus of SFNF/RSF composite film could be increased by increasing the additional amount of SFNF indicating SFNF can be utilized as a natural reinforcing material to enhance other biomaterials.

Keywords: Regenerated silk fibroin, Silk fibroin nano fiber, Composite film, Mechanical properties

PS54

Characteristics of Korean *Antheraea yamamai* silkworm cocoon

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Wild silkworm makes cocoon with different size and color. Among them, *Antheraea yamamai* Guerin-Meneville has been cultured in Korea for several thousand years. However, the general characteristics of Korean *Antheraea yamamai* Guerin-Meneville had not been reported. The authors cultured *Antheraea yamamai* silkworm in Suwon and harvested to study. The yellow-green silkworm makes yellowish green cocoon. It can be peeled out several layer with different color. The outmost layer showed yellowish green, but the innermost layer showed white color. The average weight and thickness of *Antheraea yamamai* cocoon shell is 0.528 g and 0.424 mm. Amino acid composition of *Antheraea yamamai* cocoon was analyzed. The main amino acid composition of *A. yamamai* is alanine, glycine, serine, aspartic acid, tyrosine and arginine. It showed strong scattering peaks around 2 theta = 17 and 20° and specific sharp peaks. Electron microscopic analysis showed that the surface of *Antheraea yamamai* cocoon has some crystals through the cocoon regardless of inner or outer shell. Thermogravimetric analysis showed that *Antheraea yamamai* cocoon showed thermal stable until 200^{o}C.

Key words: Silkworm, *Antheraea yamamai*, Cocoon, Structure, Degumming

PS55

Chromium (VI) removal by methylated sericin beads

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The recovery and utilization of silk sericin (SS) have attracted much interest among scientists in the field of sericulture, since they can provide an additional benefit to the sericulture industry. SS can be recovered from degumming waste using membrane techniques. However, without an appropriate application of the recovered SS, this technique will not be applied in the industry. Therefore, there has been much research on new applications of SS besides in cosmetics, where SS has been used widely for a long time. We have previously reported that SS can be fabricated into beads and microspheres using a 1 M LiCl/DMSO solvent.

Among the various heavy metal ions, chromium is a common pollutant resulting from processes in a variety of industrial fields, such as textile dyeing, mining, leather tanning and metal finishing industries. In aqueous solutions, depending on the pH and redox conditions, two main species of chromium ion exist: trivalent chromium (Cr(III)) and hexavalent chromium (Cr(VI)). While the tiny amount of Cr(III) ions are necessary for the metabolism of microorganism, Cr(VI) is toxic, and currently, it is the cause of serious environmental problems. Many of studies about Cr(VI) removal focused on the adsorption of Cr(VI) under strongly acidic conditions (pH 1–3) because the adsorption amount of Cr(VI) onto these biomaterials steeply decreased above pH 3.

In this study, we prepared methylated sericin beads for Cr (VI) removal at near-neutral pH. Methylated sericin was prepared by hydrochloric acid (HCl) catalyzes the reaction, which forms an ester bond between the hydroxyl group of methanol, and the carboxyl group of sericin, under mild reaction conditions. Confirmation of methylation was achieved with Fourier transform infrared spectroscopy (FTIR) and elemental analysis. Using 1 M lithium chloride (LiCl) and a dimethyl sulfoxide (DMSO) solvent system, methylated sericin beads were prepared. Finally, adsorption experiments were carried out in the pH range 7–8, in order to evaluate the effect of methylation on the Cr (VI) adsorption capacity in the absence of any hydrolysis reaction.

Key words: Sericin, Methylation, Chromium (VI), Biosorption

PS56

Preparation of a bead-type methylated sericin drug delivery carrier for the treatment of helicobacter pylori infection

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Helicobacter pylori is a gram-negative, spiral microaerophilic bacterium that is commonly found in the stomach. Infection with *H. pylori* causes more than 90% of duodenal ulcers and up to 80% of gastric ulcers.

The incomplete efficacy of antimicrobial agents against *H. pylori* may be attributed to the short drug residence times and degradation by gastric acid; this prevents the maintenance of sufficient drug concentration in the gastric mucosa layer during constant time. In order to release the drug at the desired concentration for a long time in a specific region, a targeted local drug delivery carrier is needed.

In the present study, methylated silk sericin (MeSS) was prepared and a bead-type drug delivery carrier was formulated for the potent treatment of *H. pylori* infection. Sericin was reacted with methanol in the presence of hydrochloric acid as a catalyst under mild conditions to form an ester bond between the carboxyl group of sericin and the hydroxyl group of methanol. The methylation of silk sericin was confirmed by Fourier transform infrared spectroscopy (FTIR) and point of zero charge (pHpzc) studies. The formulated MeSS beads swelled more under acidic conditions, which is below the pHpzc of the MeSS. The release behavior of the model drug from the MeSS beads in the medium was extremely pH-sensitive. These results suggest that the formulated MeSS beads have the potential to be an effective antimicrobial agent carrier for the treatment of H. pylori infection.

Key words: Silk sericin, Esterification, Methylation, *Helicobacter pylori*, Drug delivery

PS57

Characteristics of dissolution about *Antheraea yamamai* degummed silk with molten calcium nitrate tetrahydrate

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Silk is a popular but expensive fiber that is used in formal Korean garments and high-end fashion. Recently, several researchers have investigated silk fibroin as one of promising resources of biotechnology and biomedical materials due to its unique properties including non-toxic, little immunogenic properties, and so on. *Antheraea yamamai* silkworm, one of typical wild silkworms, spins a greeny color cocoon. In this study, dissolution condition using molten calcium nitrate for *Antheraea yamamai* silkworm cocoon has been examined. Regenerated *Antheraea yamamai* silk were measured by FTIR, SDS-PAGE. From the results of our experiment, *Antheraea yamamai* silk might be used as novel materials for non-textile applications.

Key words: Wild silkworm, Calcium nitrate, Solubility, Structure, Molecular weight

PS58

Expression of *Bombyx mori* Dpp protein using baculovirus expression vector system (BEVS) in Sf9 insect cell

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Decapentaplegic (Dpp), a member of the transforming growth factor-β (TGF-β) superfamily similar to vertebrate bone morphogenetic protein-2 (BMP-2) and BMP-4, has been implicated in developmental processed in drosophila. The *Bombyx mori* (*B. mori*) *Dpp* gene shares genetic homology with human *BMPs* and drosophila *Dpp*. However, few studies have been executed the functions of *B. mori* Dpp and its function is not well understood. For the practical use of *B. mori* Dpp protein, therefore, we have tried to overexpress *B. mori* Dpp in Sf9 insect cells, which are widely used as host cell for recombinant protein using baculovirus expression vector system (BEVS). Above all, the *B. mori* Dpp cDNA was inserted into pBacgus4x-1-EGFP vector derived from autographa californica nuclear polyhedrosis virus (AcNPV). The pBacgus4x-1-EGFP vector was designed to be expressed through the combination of the C-terminal 6x His of recombinant protein to an effective and convenient protein purification and confirmation. The *B. mori* Dpp cDNA-inserted vector was expressed in Sf9 cell thereafter. Expression of *B. mori* Dpp cDNA and the Dpp protein were validated by RT-PCR and Western blot analysis in BEVS. It would be able to take advantage of the *B.mori* Dpp protein isolated from BEVS as medical part by overexpression from using this system.

Key words: Decapentaplegic (Dpp), *Bombyx mori*, Baculovirus expression vector system (BEVS), Bone morphogenetic protein (BMP), Sf9 insect cell

PS59

Expression of a biologically active recombinant luteinizing hormone of Japanese eel *Anguilla japonica* using silkworm, *Bombyx mori*

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In the Japanese eel *Anguilla japonica*, the administration of exogenous gonadotropin (GTH) is necessary for the artificial induction and completion of gonadal maturation due to its GTH deficiency under captive conditions. Here, we report on the production of biologically active recombinant luteinizing hormone (rLH) in Japanese eel using silkworm expression system. The two composing LH, i.e. common glycoprotein, alpha polypeptide (CGa) and hormone-specific beta polypeptides (LHb), were produced with (JeLH·eCG) and without (JeLH) caorboxyl-terminal peptide (CTP) of equine chorionic GTHb, and were proven to be glycosylated and secreted as the mature peptides. The rLH of the silkworm hemolymph and BmN cell culture supernatant was separated and a band showed positive reaction with anti-His. In addition, the activity of rLH was confirmed via cAMP level in CHO cells with mouse LH receptor gene. The availability of two type rLHs has allowed us to study the biological function of this interesting factor in detail.

Key words: Silkworm, Japanese eel, Rrecombinant protein, Glycosylation

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PS60

Cultural characteristics of *Paecilomyces tenuipes* according to various silkworm breeds

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There is no specific data on Entomogenous fungus (DongChungHaCho) in accordance with the silkworm breeds, despite of very high value industrial use as functional materials at domestic and abroad. In this study, we investigated cultural condition and characteristics of *Paecilomyces tenuipes* in 3 silkworm cultivars and 2 specific silkworm breeds. Infection rate of *P. tenuipes* was the highest Kumokjam, followed by Baegokjam, Daeseongjam, Golden silk, YeonNokjam in that order. The Optimum culture conditions were as follows: 3~5 cm2 in Planting density per pupa, dark condition, 22℃±1. Also, we have developed annual production technologies of *P. tenuipes*. First, in order that the moisture content of the infection pupa became 3% or less, that was dried in the natural condition. Second, the dried pupa had the vacuum packing and was kept under 4℃. Finally, by restoring the moisture content of the dried pupa, annual production of the *P. tenuipes* was achieved. Therefore, as a breed selection and annual production is expected to contribute significantly to the income of farmers.

Key words: *Paecilomyces tenuipes*, Silkworm, Cultivars, Cultural characteristics

PS61

The effect on cell proliferation according to various silkworm breeds

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Silk protein, a natural protein produced by silkworm, is a good biomaterial which has biocompatibility. To investigate effect on cell growth of this silk protein, we isolated silk fibroin and sericin, from 3 silkworm cultivars cocoons (A, B, and C). Silk sericin was obtained by high- temperature and high-pressure method. Silk fibroin was dissolved in the ternary solvent system of $CaCl_2$/EtOH/H_2O (1:2:8 in molar ratio). The molecular weight of obtained proteins was identified by SDS-PAGE. In the sericin proteins, there were not observed the differences of molecular weight between the varieties. But fibroin proteins showed a difference between the varieties, depending on the dissolving time. There was not difference of molecular weight, depending of dissolving time in A fibroin. B was still protein having a molecular weight greater than 100 KDa, despite of 5 hours dissolution. C was composed of proteins having a molecular weight of not more than 50 KDa by dissolution for 5 hours. The tested all fibroin proteins were no apparent effect on cell proliferation, depending on the concentration. Cell proliferation in the B sericin has increased significantly with the concentration. There was no significant effect in the others of sericin. Also, the expression levels of cell growth-related genes such as EFG, FGF, and PDGF were increased in B sericin. Therefore, there is a need to study the silkworm varieties for the development of materials for biotechnology.

Key words: Cell proliferation, Fibroin, Sericin, Silkworm variety

PS62

Novel splicing form of *decapentaplegic* (*Dpp*) gene in wild silkworm, *Bombyx mandarina*

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Decapentaplegic (Dpp) is a member of the transforming growth factor-β (TGF-β) superfamily, the homolog of vertebrate bone morphogenetic proteins (BMPs), and functionally interchangeable BMPs. The *Bombyx mori* (*B. mori*) and *Bombyx mandarina* (*B. mandarina*) *Dpp* genes share genetic homology with human *BMPs* and drosophila *Dpp*, but few studies have been executed to examine the functions of *B. mori* and *B. mandarina* Dpp and its function is not well understood. To date, there was also no reported splicing form of Dpp in silkworm. In this study, we investigated *Dpp* expression using synthesized cDNA from midgut tissue of *B. mandarina* by RT-PCR. Interestingly, lower band was discovered with band of full-length Dpp cDNA and it was identified as novel splicing form that a part (333 bp) of *B. mandarina* Dpp was deleted through DNA sequencing analysis. In addition, we found that the delated part in the variant was a portion of proprotein region compared to human BMP-2 and 7 candidate single nucleotide variants (SNVs) were able to affect formation of novel splicing form using variant calling analysis. To the best of our knowledge, this is the first approach to address the novel splicing form of *Dpp* in *B. mandarina*, which have been not found in *B. mori*. These results suggest that discovered splicing form of *B. mandarina* was degenerated in evolution process toward more advanced and domesticated *B. mori*.

Key words: Decapentaplegic (Dpp), *Bombyx mandarina*, Splicing variant, Bone morphogenetic proteins (BMPs), Single nucleotide variants

PS63

Toxicology and safety assessment of tussah larvae in mice

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Tussah larvae have many kinds of nutrition, for example γ-linolenic acid, α-linolenic acid and proteins. The γ-linolenic acid plays an important role in cerebral and retina development in infants. The α-linolenic acid in tussah larvae accounting for 45.33% of total fatty acids, which 3.3 times higher than that in grass carp, and over 50 times than that in other ordinarily animal foods (Lanying Tian et al., 2011). As green and natural food with rich nutrition, Tussah was used to make delicious dishes (Xiurong Su et al., 1996), especially in Liaoning Province in China.

We study the toxicology and safety assessment of tussah larvae. This paper would provide a scientific theoretical basis for the comprehensive utilization of tussah larvae. We use acute toxicity test, genetic tests(Bone marrow polychromatic erythrocyte micronucleus test, Sperm shape abnormalitytest, Ames test) and thirty days feeding in the present study.

It was shown that the oral maximum tolerated dose (MTD) was higher than 20.0g/kg BW. According to the acute toxicity grading standards, tussah larvae should thus be attributed to the actual non-toxic material. In this paper, bone marrow polychromatic erythrocyte micronucleus test, sperm shape abnormality test and Ames test were negative. The results show that tussah larvae have no genetic toxicity in mice. The results of the thirty day feeding test indicated that no obvious difference in each index was observed compared with the normal group. The study revealed thattussah larvae could be applied as food.

Key words: Tussah larvae, Acute toxicity test through mouth, Genetic toxicity test, Thirty days feeding test

Poster Session

The 4th Asia-Pacific Congress of Sericulture and Insect Biotechnology

Friday, 24 April

Insect Biotechnology (Room 3)

PI01

A combination method of cold treatment and CO2-narcosis for breaking diapause of *Bombus ignitus* and *Bombus terrestris* bumblebee queens

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Bumblebees are important pollinators of crops and wildflowers. Bumblebees generally produce one generation per year. One of the key stages for year-round rearing of bumblebees is breaking diapause. To evaluate the effects of a combination method of CO_2-narcosis and cold treatment to break the diapause of *B. ignitus* and *B. terrestris* queens, we determined whether this method affected their ability to establish a colony after the diapause break. The diapause treatment regimes that were utilized were CO_2 (CO_2–narcosis), CT-1M (cold treatment at 5℃ for 1 month), CT-1M-CO_2 (CO_2–narcosis after cold treatment for 1 month), CT-2M-CO_2 (CO_2–narcosis after cold treatment for 2 months), CT-2M (cold treatment for 2 months), CT-2.5M-CO_2 (CO_2–narcosis after cold treatment for 2.5 months) and CT-2.5M (cold treatment at 5℃ for 2.5 months). In view of the effects on the colony developmental characteristics of *B. ignitus* queens, the most favorable diapause treatment was CT-1M-CO_2. A combination method of CO_2–narcosis and cold temperature treatment yielded better results than that of single CO_2–narcosis or cold temperature treatment on the colony development of diapause-broken *B. ignitus* queens. In the case of *B. terrestris* queens, we concluded that a combination method of CO_2 and cold temperature treatment yielded better results than that of a single cold-temperature (up to 2 months) treatment. In conclusion, the findings of the present study indicated that the combined application of CO_2 and cold temperature was a favorable method for the colony development of diapause-broken *B. ignitus* and *B. terrestris* queens compared with only CO_2–narcosis or cold temperature treatments. A combination method of CO_2 and cold treatment reduced the side effect of CO_2–narcosis and shortened the duration of cold treatment by at least 1 month.

Key words: Bumblebee, *Bombus ignitus*, *B. terrestris*, Diapause break, CO_2–narcosis, Cold temperature, Oviposition, Colony development

PI02

Collecting and rearing of the Korean native bumblebee qeens, *Bombus ignites*

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Many bumblebee species have declined in number in recent decades, particularly in developing regions. Widespread declines of bumblebee species threaten the pollination levels of both wildflowers and crops. Here, we investigated the body weight and colony-developmental characteristics of Korean native bumblebee (*B. ignitus*) queens collected from 2000 to 2010 for conservation of native bumblebees for breeding. The average weight of 6,852 queens was 0.77 ± 0.44 g. The weight of *B. ignitus* queens collected in 2005 was the greatest, 0.87 ± 0.12 g, which was 1.0–1.3-fold heavier than any other year. The average oviposition rate was 81.6 ± 10.7%, and 2004 showed the highest rate, 95.0%. This value corresponded to 1.1-1.6-fold increases over the queens collected in the other years. The average rate of colony foundation was 60.9 ± 11.0%. Queens in 2008 exhibited the best performance, 75.4%, which was 1.0-1.9-fold higher than the other years. The rate of progeny-queen production averaged 27.0 ± 9.4% (Fig. 5) and peaked in 2001 at 43.2%; this value was 1.1–4.7-fold higher than other years. The average number of queens produced and number of generations begotten by queens was 27.6 ± 10.1% and 4.8 ± 2.0, respectively. Queens in 2000 averaged 9 generations of offspring, which was 1.1-3.1-fold greater than other years. These results indicate that the colony-developmental characteristics of the collected queens changed significantly between 2000 and 2010. In addition, there was no correlation between body weight and number of queens collected, although body weight was affected by collection year. Since 2008, the colony-developmental characteristics of queens have worsened.

Key words: Bumblebee, *Bombus ignitus*, Breeding, Colony development, Generation

PI03

Current status of insect pollinators use for horticultural crops in Korea

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It was surveyed the current status of commercial insect pollinators use for horticultural crops in 2011. Bumblebees, honeybees and mason bees were used in 19 horticultural crops. The use rate and number of use farmer of insect pollinators in 19 horticultural crops were 25.7% and 58,256, respectively. The use number of insect pollinators was 348,110 colonies, which included 268,546 colonies for honeybees, 60,251 colonies for bumblebees, 9,761 colonies for mason bees, and 9,551 colonies for mixed bumblebees, honeybees and mason bees. The use rate of pollinators for 10 vegetable crops averaged 48.4%, which was calculated strawberry (99.9%), oriental melon (81.2%), water melon (40.4%), tomato (40.4%), melon (38.4%), pepper (35.4%), paprika (15.2%), egg plants (10.8%), zucchini (5.3%) and cucumber (0.02%). The use number of insect pollinators in 10 vegetable crops was 306,856 colonies, which included 246,398 colonies for honeybees, 52,684 colonies for bumblebees, and 7,774 colonies for mixed bumblebees and honeybees. The use rate of pollinators for 9 fruit tree crops averaged 14.1%, which was calculated blueberry (23.2%), pear (23.0%), apple (40.4%), egg plants (17.8%), persimmon (14.9%), Rubus corearus (9.1), Mulberry fruit (2.8%), plum (2.8%), peach (1.9%) and pomegranate (07%). The use number of insect pollinators in 9 fruit tree crops was 41,011 colonies, which included 21,905 colonies for honeybees, 7,567 colonies for bumblebees, and 9,761 colonies for mason bees, and 1,778 colonies for mixed bumblebees, honeybees and mason bees. Most farmers expressed a positive intention as 80.6% in use of insect pollinators. And also, 93.3% of farmers intend to use continuously insect pollinators.

Key words: Current status, Insect pollinator, Bumblebee, Honeybee, Mason bee, Horticultural crops, Vegetable crops, fruit tree crops

PI04

The optimal method and solution for collecting sperm of bumblebee male

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Artificial insemination is a technique to transfer instrumentally sperm from the male into the female's reproductive system. A key factor among artificial insemination techniques is gathering sperms. Here, a method for collecting sperm of bumblebee male by pressing was firstly developed. This method has stage of separating reprodutive tract of male, separating accessory testis, separating vas deferens, pressing vas deferens with cover-glass and collecting sperm with syringe. The developed method was 2.8 fold higher in rate of collecting sperm than that of existing method. Among 1M NaCl-, Insect ringer solution-, Phosphate buffered saline(PBS)-collecting sperm solutions, PBS was exhibited the best result as 17.2 sperms/cell, which corresponded to 1.6-1.8 fold increased over the result of other collecting sperm solution. Consequently, we think that this method is very important technique to save time for collecting sperm, to keep activity of sperm and to reduce contamination of sperm.

Key words: Bumblebee, *Bombus ignitus*, Artificial insemination, Male, Collecting sperm

PI05

Insecticide resistance monitoring in diamondback moth *Plutella xylostella* in central China

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The diamondback moth(DBM), *Plutella xylostella* L., is a major cosmopolitan pest of cruciferous vegetables all over the world. For controlling DBM, insecticide application has been the most important method. Resistance to major classes of insecticides in field populations has evolved worldwide, especially in tropical areas. The field populations of *P. xylostella* used for resistance monitoring in this study were collected annually from cabbage fields in five different geographical regions of central China from 2013 to 2014. The sites included Luoyang in He'nan Province, Yueyang in Hu'nan Province, Yunmeng and Yichang and Wuxue in Hubei Province. For monitoring their resistance to chlorantraniliprole, spinosad, diafenthiuron, abamectin, chlorfluazuron, chlorfenapyr, indoxacarb, BT (WG-001), and beta-cypermethrin by using leaf-dipping bioassay method. The results indicated that chlorantraniliprole, spinosad, chlorfenapyr, and diafenthiuron, showed low toxicity to *P. xylostella* in central China, with no obvious resistance variation during the two years. The resistance ratios of *P. xylostella* to chlorfluazuron and BT (WG-001) showed a trend of decline from high level to moderate level. Resistance of *P. xylostella* to indoxacarb, abamectin and beta-cypermethrin varied greatly among the five regions. *P. xylostella* exhibited moderate and high level resistance to these three insecticides. The resistance of this pest to beta-cypermethrin was severe in the five regions. Our study provides a foundation for developing more effective resistance management program for *P. xylostella* and farmers should be trained in the rational use of insecticides for pest management.

Key words: *Plutella xylostella*, Insecticides, Resistance monitoring, Central China

PI06

Insecticide resistance in the brown planthopper (*Nilaparvata lugens* Stål) in China

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The brown planthopper (*Nilaparvata lugens* Stål) is one of the most destructive pests of rice crops in Asian countries including China, Vietnam, Thailand, etc. Evolution of resistance in this pest insect to isoprocarb, buprofezin, pymetrozine, imidacloprid and other neonicotinoid insecticides has been reported. In order to investigate the current status of resistance to commonly used insecticides, 18 field populations of *N. lugens* were collected from Central China, East China and South China in 2014. All the 18 field populations collected in 2014 had developed extremely high resistance to imidacloprid, with resistance ratios (RR) ranging from 495.12 to 1455.88. Resistance to imidacloprid was much higher in 2014 than in 2009. The medium resistance to thiamethoxam in *N. lugens* were detected in fourteen of the eighteen populations collected from different sites, in addition four populations of high resistance was found in Hubei province. The RR of nitenpyram varied from 1.17 to 19.53 in 2014, of the 18 field populations, one population in Tianmen showed medium resistance to nitenpyram in 2014. RR for buprofezin varied from 326.50 to 1529.13 in 2014 whereas resistance was at medium level (RR 20.4-39.4-fold) in 2009. RR for pymetrozine ranged from 169.47 to 584.75 in 2014, the pymetrozine resistance in brown planthopper increased obviously compared with previously reported data. As for isoprocarb, RR ranged from 17.11 to 62.91 in 2014. The obvious increase in resistance to widely applied insecticides indicates that insecticide resistance management strategies are urgently needed to prevent or delay further increase of insecticide resistance in *N. lugens*.

Key words: *Nilaparvata lugens* (Stål), Insecticide resistance, Neonicotinoids, Buprofezin, Pymetrozine

PI07

Sublethal effects of diafenthiuron on the diamondback moth, *Plutella xylostella* (L.)

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Diafenthiuron is a kind of thiourea insecticide and acaricide, which is active against a wide range of sucking insects and some lepidopteran insects including the diamondback moth, *Plutella xylostella*(L.). This insecticide provides a new option for controlling the resistant populations of *P. xylostella* to other chemicals. The effects of sublethal concentrations of diafenthiuron, topically applied for 48 h at LC_{10} (3.53 mgL^{-1}) and LC_{30} (9.71 mgL^{-1}), respectively, on the biological characteristics of the diamondback moth, *P. xylostella*, were investigated in parental and F_1 generations. The results showed that the pupal duration in both generations were significantly extended and emergence rate were significantly declined when third instar larvae were exposed to LC_{10} and LC_{30} of diafenthiuron, respectively. The pupal period in the LC_{10} and LC_{30} groups were extended by 10.34 h and 16.43 h respectively in parental generation. Comparing the control group to LC_{10} and LC_{30} treatment groups, there were significant differences in the development periods of oviposition quantity and hatching rate in the parental generation. In addition, in comparison with the control, the pupation rate in the LC_{10} and LC_{30} groups were reduced by 10.00% and 33.34% respectively in F_1 generation. The biological characteristics of both parental and F_1 generations were adversely affected when parental larvae were exposed to LC_{10} and LC_{30} doses of diafenthiuron. Generally, the sublethal effect of LC_{30} dose was more significant than that of LC_{10} treatment.

Key words: *Plutella xylostella*, Diafenthiuron, Sublethal effects, Biological characteristics

PI08

The inhibitory effects of apamin in PDGF-BB-induced vascular smooth muscle cells proliferation and migration

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The increased proliferation and migration of vascular smooth muscle cells (VSMC) are key process in the development of atherosclerosis lesions. Platelet-derived growth factor (PDGF) initiates a multitude of biological effects that contribute to VSMC proliferation and migration. Apamin, a component of bee venom, has been known to block the Ca2+-activated K+ channels. However, the potential role of apamin in regulation of VSMC proliferation and migration has not been identified. In this study, we investigate the inhibitory effect of apamin on PDGF-BB-induced VSMC proliferation and migration. Apamin suppressed the PDGF-BB-induced VSMC proliferation and migration with no apparent cytotoxic effect. In accordance with these findings, apamin induced the arrest of cell cycle progression at G0/G1 phase. Apamin also decreased the expressions of G0/G1 specific regulatory proteins including proliferating cell nuclear antigen (PCNA), cyclin D1, cyclin-dependent kinases (CDK) 4, cyclin E and CDK2, as well as increased the expression of $p21^{Cip1}$ in PDGF-BB-induced VSMC. Moreover, apamin inhibited PDGF-BB-induced phosphorylation of Akt and Erk1/2. These results suggest that apamin plays an important role in prevention of vascular proliferation and migration through the G0/G1 cell cycle arrest by Akt and Erk1/2 signaling pathway. Thus, apamin may be a promising candidate for the therapy of atherosclerosis.

Key words: Apamin, Atherosclerosis, VSMC, Proliferation, Migration

PI09

Enhanced production of foreign protein by the partial polyhedrin-fused expression in *Bombyx mori* cells and larvae

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The baculovirus expression vector system (BEVS) is an widely used method for the production of recombinant proteins in insect cells or larvae. To enhance the expression efficiency of foreign protein using BEVS, the many of attempts have been studied. Among them, the partial polyhedrin-fused expression system using the polyhedrin of *Autographa californica* nucleopolyhedrovirus (AcNPV) is developed recently. This system could enhance significantly the production of foreign protein by the fusion of partial polyhedrin gene to the foreign gene in Sf21 cells. To evaluate and verify the utility of this system in *Bombyx mori* cells (Bm5) and larvae, a number of recombinant BmNPVs to express the enhanced green fluorescence protein (EGFP) with 7 types of partial polyhedrin fragments. Recombinant BmNPVs were also generated as occlusion negative (Occ-) and positive (Occ+) forms for the inoculation to *B. mori* larvae. Bm5 cells and *B. mori* larvae were infected with each recombinant virus, and the expression of recombinant protein was comparative analyzed with that in Sf21 cells. As the result, the enhanced production of EGFP was also shown in Bm5 cells like in Sf21 cells. Especially, the production of EGFP was markedly increased in *B. mori* larvae than Bm5 cells.

Key words: Partial polyhedrin, *Bombyx mori*, Bm5 cells, BmNPV, EGFP

PI10

Developing long-term preservation technique for *Tenebrio molitor* larvae

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The mealworm beetle, *Tenebrio molitor* (Coleoptera: Tenebrionidae), is one of the strong candidates for the high protein resource. The fecundity of this beetle is quite high, and the larval period is short (ca. 15~20 weeks). Moreover, this beetle is rapidly gaining weight during the larval period. Based on these advantages, the mealworm larvae have been highly focused as a protein supplement for animal feed and human diet. However, there is no criterion for preserving this beetle. In addition, the beetle colony is frequently collapsed during winter. Therefore, this beetle needs to be preserved in good condition for continuous production. In this study, we tested different conditions for preserving larvae. To test different preserving conditions, 12^{th} and 15^{th} larval instars were preserved at 5 and 10℃. Every 30 days after low temperature preservation, 300 larvae were placed at 25℃. After recovery from low temperature preservation, we checked the each larval period, pupation rate, pupa weight, adult emerging rate, and adult weight. The larvae were no significant different for developing after preserved 15^{th} larvae at 10℃. However, the larval period was reduced as the low temperature preservation period was extended with 15^{th} larvae at 10℃. The larvae died because of developmental arrest or damage from mite when the low temperature preservation period was more than 250 days. Therefore, we concluded that the maximum low temperature preservation period is 250 days for 15^{th} larvae. In addition, the 12^{th} larvae also can be preserved for 250 days at 10℃. At 5℃, 15^{th} larvae could be preserved only 30 days for normal development. However, 12^{th} larvae could not be preserved at 5℃. Based on these result, we suggest that 12^{th} and 15^{th} mealworm larvae can be preserved for 250 days at 10 ℃.

Key words: *Tenebrio molitor*, Larva, Low temperature preservation

PI11

Developmental characteristics of *Zophobas atratus* larvae (Coleoptera: Tenebrionidae) in different instars

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The giant mealworm beetle, *Zophobas atratus*, is a promising food resource for animals including humans. It was officially introduced to Korea in 2011, and has been commercially maintained. This beetle contains balanced nutriment with high protein, so it is a good food resource as animal feed. However, the characteristics of each life stage are not clearly known especially for the larval stage that can be used as commercial products. Therefore, we counted the number of *Z. atratus* instars, and described its characteristics at each larval stage. *Z. atratus* larvae required eight to nine days of incubation periods before hatching. The 1st instar period took three to four days. Except the 1st instar, there were relatively large variations for the each instar period. Before emerging adults, most of the individuals had 15 to 18 instars. The highest pupation rate, 25.71%, was observed in both 16th instars and the 17th instars. The body length was gradually increased with each successive instar and it was reached its maximum at the 18th instar. The color of larvae was white in the first instar, and gradually turned brown after the 2nd instar.

Key words: *Zophobas atratus*, Larva, Body length, Instar

PI12

Effect by 3 species of pollinators for blueberry field in the green house

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This study was conducted about the 3 species of1 bee, *Bombus terrestris, Apis mellifera* and *Osmia cornifrons* for pollination effect compared to natural condition in net-house of blueberry field. To improvement quality of the blueberry fruit, 3 species of bee, *Bombus terrestris, Apis mellifera* and *Osmia cornifrons* were used for pollination in blueberry field in Pyeongtaek province. This study was also conducted using 3 kinds of methods for pollination effect, natural condition, net-house and control method. Blooming time of blueberry shows always during the May, foraging behavior of bees used in this examination showed different tendency each other. Especially pollinating time of *B. terrestris* was 2.77sec and transfer time between flower was 2.29sec. Rate of blueberry fruit setting pollinated with *B. terrestris*, *A. mellifera*, *O. cornifrons* were showed 87.7%, 89.6%, 66.5% respectively. And, rate of fruit setting of the untreated control and the control (natural condition pollination) were 15.4% and 80.3%. Quality of fruit pollinated with *B. terrestris* and *A. mellifera* showed higher than *O. cornifrons* in natural field condition in fruit weight. The rate of productivity increase obtained using the *B. terrestris* was 9%～17% greater than that obtained using natural pollination.

Key words: *Bombus terrestris*, *Apis mellifera*, *Osmia cornifrons*, Pollination, Pollinator, Blueberry

PI13

Characterization of transformed entomopathogenic *Beauveria bassiana* JEF-007 using *Agrobacterium tumefaciens*-mediated transformation and localization of integrated gene

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The entomopathogenic fungus, *Beauveria bassiana* is widely used in integrated pest management (IPM), however its successful application is often limited by the little effort to explore its functions of unknown genes. In this work, egfp expression cassette was randomly integrated into *B. bassiana* JEF007 using *Agrobacterium tumefaciens*-mediated transformation, and the general features of the mutants and the localization of the integrated genes were explored. To construct a transformation vector, *egfp* expression cassette including *gpdA* promoter and *trpC* terminator was cut from pBARKS1-egfp using *Sac*I and *Hin*dIII and integrated into pCAMBIA containing hygromycin B resistant *hygR* gene, designated as pCAMBIA-egfp. After the transformation, transformants were grown on 1/4 Sabouraud dextrose agar containing 150 μg ml^{-1} hygromycin B. Expression of egfp was investigated by RT-PCR and a fluorescent microscope (400×). Through the genome walking of the transformants using adaptor primers and gene specific primers, unique bands were detected on the egfp-expressing transformants, which were sequenced to figure out the flanking regions. This work provides a platform of methodology to figure out unknown functional genes of *B .bassiana* and possibly suggest an improved strategy to use the entomopathogen in IPM.

Key words: Entomopathogenic fungi, *Beauveria bassiana*, *Agrobacterium tumefaciens*-mediated transformation, Genome walking

PI14

Quality characteristic of muffin powder was added to a rice grasshopper, *Oxya chinensis* Sinuosa

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Rice grasshopper(*Oxya chinensis* sinuosa) lives only in clean areas was used as an emergency food from the past and this is listed as a food in Korean Food Standards Codex. Rice grasshopper contains large amounts of nutrients and is recognized for its value as a high-protein food, similar to the amino acid composition of animal food. However, because the food with Rice grasshopper does not vary until now, food processing development with Rice grasshopper is necessary. This study made the muffin added to a rice grasshopper powder as part of the development of processed foods and analyzed for its quality characteristics. Grasshopper was used in the muffin prepared by drying the adult 40℃. Rice grasshopper powder was used and added to muffin to 1-5% by homogenization in 60mesh. As a result, the muffin made with rice grasshopper powder 2% was excellent in taste, flavor, color and texture preference rating and the greater the amount of this powder showed a dark color.

Key words: Rice grasshopper, Muffin, Quality characteristic

PI15

Development of microsatellite markers and genotyping using next-generation sequencing in *Apis mellifera*

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Microsatellites, a special class of repetitive DNA sequence, have become one of the most popular genetic markers for population and conservation genetic studies. However, it has been impeded by high development costs, a lack of available sequences, and technical difficulties. We adopted next-generation sequencing (NGS) to elucidate the microsatellites markers of *Apis mellifera*. In this study total of 34 individual 6 *Apis mellifera* strains preserved in our research center were typed for 11 polymorphic microsatellite loci. We performed genotyping to determine the efficiency of this method as applied to population genetics. We obtained 205 Mbp of nucleotide information from 45,476 contigs reads. A total of 20,580 repeat motifs were identified; di-repeats were 2-fold more common than tri-repeats. [GT]n repeats were the most frequent di-repeats, respectively. Of the 20,580 repeat motifs, 42,469 microsatellite primer pairs were derived and we selected 50 microsatellite primer pairs. PCR amplification of 50 primer pairs yielded 26 amplicons and 11 polymorphic markers from 6 hive, 63 individual Korean *Apis mellifera*. Polymorphic rates of the 11 new microsatellites varied from 3 to 7 alleles per locus. Polymorphic microsatellite markers from *Apis mellifera* were successfully identified using NGS without any prior sequence information and deposited into the public database.

Key words: Microsatellite, *Apis mellifera*, Genotyping analysis, Next generation sequencing

PI16

Identification and characterization of *Pseudomonas poae* in *Aromia bungii*

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The presence of *Aromia bungii* (Coleoptera: Cerambycidae) was recorded for the first time in one location in Germany, and *A. bungii* is a fruit tree pest originating from Asia recently. On the other hand, this insect was investigated as a hobby and therapy animal because of the musk aroma from the *A. bungii*, therefore insect rearing farms of *A. bungii* have been increasing. We detected *Pseudomonas poae* from the diseased *A. bungii* using PCR and sequencing 16SrRNA of *P. poae* and identified 99% similarity from BLAST alignment. Pseudomonadaceae insect infections are generally known that invasive power if this bacteria pathogen is infected within hemocoel. Insect pathogens of members of the family Pseudomonadaceae are investigated *P. aeruginosa, P. aureofaciens, P. chlororaphis, P. fliorescens, P. noctuarum, P. putida, P. savastanoi, and P. septica.*

Key words: *Aromia bungii* (Coleoptera: Cerambycidae), *Pseudomonas poae*, 16SrRNA, PCR

PI17

Characterization and differential expreession of vitellogenin and glutathione S-transferase (GST) in cockroaches, *Periplaneta americana* and *Blattella germanica*

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Vitellogenins (Vgs) are precursors of the major egg storage protein, vitellin (Vn), in many oviparous animals. Insects Vgs are large molecules (200-kD) synthesized in the fat body in a process that involves substantial structural modifications (e.g., glycosylation, lipidation, phosphorylation, and proteolytic cleavage, etc.) of the nascent protein prior to its secretion and transport to the ovaries. However, the extent to which Vgs are processed in the fat body varies greatly among different insect groups. We were cloned Vgs partial genes PaVgs and BgVgs from *Periplaneta americana* and *Blattella germanic*a. Real-time quantitative PCR shows that PaVgs and BgVgs were differential-regulated with aging. In insects, glutathione S-transferases (GSTs) are enzymes involved in detoxification of insecticides. We were cloned GST partial genes PaGST and BgGST from *Periplaneta americana* and *Blattella germanica*. Real-time quantitative PCR shows that PaGST and BgGST were up-regulated with aging, and the mRNA level of PaGST and BgGST was higher in 4℃nd 37℃han room temperature. The expression level of PaGST and BgGST exposure to temperature stress suggests that PaGST and BgGST are up-regulated after exposure low and hige temperature treatments.

Key words: *Periplaneta americana* and *Blattella germanica*, Vitellogenin and Glutathione S-transferase (GST)

PI18

Mortality rate comparison of three bacterial insect pathogens; *Bacillus thuringiensis*, *Paenibacillus popilliae*, and *Serratia marcescens* in *Protaetia brevitarsis seulensis* (Kolbe)

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Protaetia brevitarsis seulensis (Kolbe) had been focused on a protein alternate food and oriental and functional medicine with pharmacological effect historically, in Korea. According to efficiency of the larvae of *P. b. seulensis* about anti-oxidant, anti-hepatic disorder, and anti-diabetic effect, *P. b. seulensis* were reared increasingly in Korea. We confirmed that concentration of *S. marcescens,* $1x10^4$/50μl, represented significant effect of 99.9% mortality on the third stage of the *P. b. seuluensis* after bacteria intracoleomic injection, within 72hours. However, oral infection of *S. marcescens* to *P. b. seulensis* had not shown significant mortality. Therefore, we are verifying the mortality comparison rate of three kinds of bacteria frequently infected to *P. b. seulensis* ; *S. marcescens, B. thuringiensis, and P. popilliae* by Bioassay. From this study, it is very important and necessary for the sanitary rearing condition and effective methods of prevention against insect disease and optimal environment for healthy insect.

Key words: *Protaetia brevitarsis seulensis* (Kolbe), *Bacillus thuringiensis*, *Paenibacillus popilliae*, *Serratia marcescens*, Bioassay

PI19

Effect on pollinating activities on mango flower by bumblebee (*Bombus terrestris*), honeybee (*Apis mellifera*) and oriental latrine fly (*Chrysomyia megacephala*) in green house

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To substitute for bluebottle fly, *Chrysomyia megacephala* which is being used for pollinator in mango fruit, and improve the pollinating effect of mango fruit which is also being increased as high value added crop recently in Jeju island of korea, 2 kinds of pollinator were used in analyzing and surveying of foraging activities on mango fruit in Seogwipo province. This study was conducted using 3 species of pollinator, *Apis mellifera, Bombus terrestris* and *Chrysomyia megacephala* with 3 treatment in vinyl-house condition respectively. Species of mango fruit, Irwin, was used in this experiment. A number of foraging activity of *Apis mellifera and Bombus terrestris* in hive showed highest 11 AM, and showed normal foraging activity in high temperature condition(28℃). Pollinating ratio of *Bombus terrestris* was shown 100% and over 95% in case of *Apis mellifera.* This ratio suggest that the 2 species of insects is effective as pollinator on mango fruit compared with bluebottle fly. Daily pollinating activity of *Apis mellifera* and *Bombus terrestris* was shown peak in 11 AM, but showed even activity from 9 AM to 3 PM in case of *Chrysomyia megacephala.* Fruit set ratio of mango pollinated by *Apis mellifera* showed higher with 2.1% than by *Bombus terrestris and Chrysomyia megacephala with 1.5%* respectively in 1st time(1 month after blooming), but 3 species of pollinator showed same results with 0.3% in 2nd time. This results suppose that because only 10% of normal pistil and stamen exist on same flower in case of mango flower, fruit set ratio of mango flower shows lower tendency originally than other fruit crops. Abnormal fruit set ratio pollinated by *Bombus terrestris* showed lowest with 12.1% compared with another 2 species of pollinator. In conclusion, method of using *Bombus terrestris* seems to be effective to increase productivity in pollinating mango flowers.

Key words: Bumblebee, *B. terrestris*, *A. mellifera*, *C. megacephala*, Mango, Foraging Activity, Pollinating Ratio, Fruit Set ratio

PI20

Characterization of a β-1,4-Glucanase from *Cellulosimicrobium* sp. DY-8, a plant biomass-degrading bacterium in the digestive track of the earthworm *Eisenia fetida*

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Plant biomass-degrading microorganisms are widely distributed in various environments such as soil, compost, and the guts of herbivorous animals and invertebrates. These microbes play an crucial role in biological recycling of lignocellulose. Of the structural polysaccharides present in lignocellulosic biomass, β-1,4-glucans are the principle components of soft- and hardwoods. In this study, the gene (1,608-bp) encoding a GH family 6 β-1,4-glucanase (GluC) from an *Eisenia fetida*-symbiotic fibrolytic bacterium, *Cellulosimicrobium* sp. DY-8 was cloned, recombinantly expressed, and biocatalytically characterized. GluC (56.0 kDa) was a novel multi-domain enzyme composed of a catalytic GH6 domain (Val57-Pro396) in the N-terminus region, which was 71% homologous to a GH6 enzyme from *Cellulomonas* sp. KRMCY2 and a CBM 2 domain (Cys429-Cys532) in the C-terminus region. The maximum biocatalytic activity of GluC against carboxymethylcellulose (CMC) was measured at 50^{o}C and pH 5.0, and was stable at a pH range of 4.0-10.0. GluC was able to efficiently hydrolyze the cellulosic polysaccharides in the order of β-1,4-D-glucan from barley > CMC > lichenan > Avicel > glucomannan. However, *p*-nitrophenyl sugar derivatives or structurally unrelated polymers including β-1,3-glucan, β-1,4-mannan, and β-1,4-xylan were not degraded by GluC. The enzyme strongly attached to ivory nut mannan, Avicel, and chitin but exhibited weak binding affinity to lignin, poly(3-hydroxybutyrate) granules, or lichenan.

Key words: Endo-β-1,4-glucanase, Gut bacterium, Earthworm, *Cellulosimicrobium* sp. DY-8

PI21

Cloning and sequence analysis of the two endo-β-1,3-glucanase genes of an earthworm-symbiotic bacterium, *Cellulosimicrobium* sp. DY-8

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β-1,3-Glucan is a polysaccharide composed of a backbone of β-1,3-linked glucopyranoside residues, and is present in plants, fungal cell walls, and marine macroalgae. In nature, microbial degradation of the structural polysaccharide in the organisms is generally initiated by cooperative action of various types of extracellular endo-β-1,3-glucanases. Thus, in this study, we cloned the two genes [*glu*A (1335-bp) and *glu*B (1650-bp)] encoding different endo-β-1,3-glucanases from *Cellulosimicrobium* sp. DY-8, which is a lignocellulose-decomposing Gram-positive bacterium isolated from the digestive track of an earthworm, *Eisenia fetida*. GluA (M.W: 47.5 kDa; pI: 8.8) was predicted to be a multi-domain protein composed of a catalytic GH16 domain (Trp69-Tyr300) in the N-terminus region, which was 94% homologous to a GH16 protein (NCBI Ref. Seq.: WP_034604571) from *Cellulosimicrobium cellulans* and a RICIN domain (Gly320-Phe444) in the C-terminus region. However, GluB was predicted to encode a polypeptide consisting of 549 amino acids with a molecular mass of 58.2 kDa and a calculated pI of 6.0. The results of a protein blast survey revealed that GluB was a modular enzyme comprised of an N-terminal catalytic GH64 domain (Ile41-Asp395), which was 97% identical to a GH64 protein (NCBI Ref. Seq.: WP_043655051) from *C. cellulans*, together with a C-terminal RICIN domain (Ile427-Phe549). Studies on the biochemical properties of these new endo-β-1,3-glucanases produced from *Escherichia coli* BL21 are underway.

Key words: Endo-β-1,3-glucanase, Gut bacterium, Earthworm, *Cellulosimicrobium* sp. DY-8

PI22

The influences of insect mental healthcare program on the emotion and social interaction of elementary school students with withdrawal

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Insects are among the most diverse groups of animals on the planet, representing more than half of all known living organisms. These insects are found in nearly every environment. Although humans regard certain insects as pests and attempt to control them using insecticides, most insects perform complex ecological functions, and provide either direct or indirect economic benefits to humans. Recently, the importance of insects used as food sources or as pets has increased in many countries, including Korea. In addition, several insects have a strong influence on people's emotion. Insect mental healthcare program is designed to help people who have disorders with physical, behavior and development. Elementary school students who have mental disorder, the experimental group that was provided with an insect mental healthcare program over a total of 8 sections, one section per week, 60 minutes per section, followed by pre-test and post-test. They responded to therapeutic effect after the completion of the program. Further research on the basis of this study is expected to help children with emotional therapy in other areas.

Key words: Mental healthcare, Emotion, Social interaction, Pet insects

PI23

Pre-treatment conditions on the powder of *Tenebrio molitor* for using as a novel food ingredient

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Although the mealworm larva (*Tenebrio molitor*) is high protein source, aversion feature of the larva made it difficult for consuming as a food. In this study, we established optimal powder manufacturing process for *T. molitor* larva for using as a novel food. For this purpose, it should be feed with the bran sterilized before freeze-drying. The sterilized *T. molitor* was lyophilized and grinded by a blender. A safety of the powder as a food was validated by evaluation of Raw 264.7 macrophage cytotoxicity using MTS assay. As above results, we propose that optimal powder manufacturing process established in this study can be used in industrial production of *T. molitor* as a novel food.

Key Words: *Tenebrio molitor*, Food, Sterilization, Cytotoxicity

PI24

Molecular phylogenetic diversity of *Monochamus saltuarius* (Gebler) based on mitochondrial COI sequence analysis

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Monochamus saltuarius (Gebler), like *Monochamus alternatus*, is a vectors of *Bursaphelenchus xylophilus* Niclke which causes pine wilt disease and lead to huge damage to pine needle trees. *M. saltuarius* is reported that it has a morphological polymorphism, but there is no standard phenotype to distinguish the differences within species. To investigate a molecular phylogenetic analysis, we collect samples of *M. saltuarius* in Chungbuk, Gyeonggi and Gangwon region of Korean Peninsula. Analysis of mitochondrial COI 5' region showed that two distinctive phylogenetic groups with a geographical separation. The first group includes specimens collected in Chungbuk, Kyungwon and Kangwon region while the specimens collected only in Gangwon region makes another group. Two group shows 2.4~2,6 K2P distances but there is no significant difference in morphological character. But a distinctive difference was found in genital organ that the specimens collected only in Ganwon region (the second group) showed a little shorter and wider paramere. More in depth studies will be necessary to clearly distinguish these two groups with more intensive collection of specimens in wider region and try to find out a phenotypic variations.

Key words: *Monochamus saltuarius* (Gebler), Pine wilt disease, Mitochondria, COI, DNA barcode

PI25

Improvement storability of the fresh-cut vegetables with propolis

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Propolis is a health food, known that high antioxidant and antimicrobial effects, fresh cut vegetables that rapidly increasing consumption has recently faced the problem storability fall down after washing. To improve storability of fresh cut vegetables are being carried out various studies. In this study, using the characteristics of propolis we were performed to improve the storability of fresh cut vegetables. There was prepared in 18% solution of propolis extracts, by using this solution, propolis solution prepared diluting 0.001 to 1%, were dipped in fresh vegetables(cabbage lettuce, perilla leaf, and lettuce). Vegetables were measured the sensory evaluation and hardness after each treatment by placing in a certain period of time at room temperature and refrigerator. The results showed that the storage stability is excellent compared to non-treated as if diluted to 0.1-0.01% propolis solution is to improve the shelf life of fresh cut vegetables.

Key words: Propolis, Storability, Fresh-cut vegetables

PI26

Antimicrobial effects on *Propionibacterium acne* of extracts from Korean propolis

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Propolis, or 'bee glue', is a complex resinous mixture of plant-derived products gathered, modified and used by bees as a general purpose sealer, draught excluder and antibiotic in their hives. The presence of propolis within the hive may also provide an environment not suitable for the growth of bacteria and other microorganisms. Propolis composition is directly related to that of bud exudates collected by bees from various trees. The composition of the propolis depends upon the vegetation of the area from where it was collected on the bee species.

Propolis is an excellent material, as known as antimicrobial activity, propolis samples were collected in south Korea, propolis extracted with ethanol(EEP), EEP was fractionated by prepHPLC, and antimicrobial activity against acne bacteria (*Propionibacterium acne*) investigated.

Key words: Propolis, Antimicrobial effect, *P. acne*

PI27

Lipopolysaccharide-induced macrophage inflammatory response is inhibited by *Oxya chinensis sinuosa* ethanol extract

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Although a grasshopper, *Oxya chinensis sinuosa* has been long used as food in Korea, there is little reported its functional effects. In this study, we investigated the anti-inflammatory effect of *O. c. sinuosa* ethanol extract (OCE) in RAW 264.7 mouse macrophage cells treated with lipopolysaccharide (LPS) for induction of inflammation. First, we examined that there is no cytotoxicity at 2000 μg/ml or less concentration of OCE in RAW 264.7 cells. To evaluate the anti-inflammatory effects of OCE, we investigated expression levels of the pro-inflammatory cytokines such as TNF (Tumor Necrosis Factor)-α and IL (Interlukine)-6, and pro-inflammatory enzymes such as inducible nitric oxide synthase (iNOS) and cyclo-oxygenase-2 (COX-2) in LPS-induced RAW 264.7 cells. In addition, we examined whether OCE could inhibit translocation of NF-κB p65 into the nucleus in LPS induced RAW 264.7 cells. As results, we found that the mRNA and protein levels of TNF-α and IL-6 were decreased in LPS-induced RAW 264.7 cells after treatment with OCE in a dose-dependent manner. In addition, we confirmed that 2000 ug/ml concentration of OCE inhibited translocation of NF-κB p65 by immunnostaining and Western blot analysis, and decreased the protein expression levels of iNOS and COX-2. Accordingly, we suppose that the OCE has the anti-inflammatory effect through down-regulation of TNF-α, IL-6, iNOS, and COX-2 related to NF-κB p65 inflammatory signaling pathway.

Key words: *Oxya chinensis sinuosa*, Lipopolysaccharide, RAW 264.7 cells, Pro-inflammatory cytokine, NF-κB p65

PI28

Molecular cloning and expression profile of a vitellogenin of mason bee, *Osmia cornifrons*

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Osmia cornifrons plays a major role in the pollination of orchards, but basic information on vitellogenin and the oocyte development is limited. To better understand vitellogenin in hymenopteran insects, we cloned a cDNA encoding vitellogenin from the mason bee *O. cornifrons*. *O. cornifrons* vitellogenin cDNA contains 5,477 bp with an open reading frame of 1,783 amino acid residues, and has a predicted molecular mass of approximately 200.21 kDa and a pI of 6.55. *O. cornifrons* vitellogenin possesses four consensus (RXXR/S) cleavage sites and has conserved DGXR and GL/ICG motifs in the C-terminus. The deduced amino acid sequence of the *O. cornifrons* vitellogenin cDNA showed a 66% identity with *Megachile rotundata*, 53% to *Apis mellifera*, 51% to *Bombus ignitus*, and 42%-30% with other hymenopteran insect vitellogenins. Phylogenetic analysis showed that *O. cornifrons* vitellogenin clustered with vitellogenins from Megachildae, Apidae, Vespidae, and Formicidae species but not with those from Pteromalidae, Aphelinidae or Ichneumonidae species. The expression profile of *O. cornifrons* vitellogenin mRNA during development revealed that *O. cornifrons* vitellogenin was first detected in the pupal stage and was continuously detected during the adult stage. Interestingly, *O. cornifrons* vitellogenin mRNA expression was low in mid-diapause, then gradually increased beginning on day 3 of the newly emerged adult stage, and subsequently declined. These results suggest that the expression level of *O. cornifrons* vitellogenin mRNA is stage-specific.

Key words: Cloning, Expression profile, Mason bee, *Osmia cornifrons*, Vitellogenin

PI29

Genomic sequence and description of *Flavobacterium enshiense* type strain DK69^T and comparison of several genomes of *Flavobacterium* type strains

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Flavobacterium enshiense DK69^T (=CCTCC AB 2011144^T=KCTC 23775^T) was isolated from Bafeng Pharmaceutical Company in the city of Enshi, Hubei Province, China. Based on 16S rRNA gene sequences phylogenetic analysis which belongs to the genus *Flavobacterium* of the family Flavobacteriaceae. Strain DK69^T is a novel species of the genus *Flavobacterium*, for which propose the name *Flavobacterium enshiense* sp. nov. and DK69^T as the type strain. The draft genome sequence and annotation of *F. enshiense* DK69^T was hereby presented. An organism is represented by 1 chromosome with approximately 3.4 Mb of the genome, and formed a separate lineage supported by a high bootstrap value with *F. cauense* R2A-7^T and *F. suncheonense* GH29-5^T. The strain *F. enshiense* DK69^T DNA G+C content is 37.7%, that a value within the genus *Flavobacterium* reported range, and a lot of common proteins prove close relations between strain DK69^T with the genus *Flavobacterium*. On the basis of phenotypic, phylogenetic and genomic analyses, we proved that strain DK69 T was exist in the genus Flavobacterium stable.

Key words: *Flavobacterium enshiense* DK69^T, *Flavobacterium*, Genomic analyse, Phylogenetic analyse

PI30

Identification of plant compounds that disrupt the insect juvenile hormone receptor complex

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Insects impact human health through vector-borne diseases and cause major economic loses through damaging crops and stored agricultural products. Insect-specific growth regulators represent attractive control agents because of their safety to the environment and humans. We identified plant compounds that serve as juvenile hormone antagonists (PJHANs). Using the yeast two hybrid system transformed with the mosquito JH receptor as a reporter system, we demonstrate that PJHANs affect the JH receptor, methoprene-tolerant (MET), by disrupting its complex with CYCLE or FISC, formation of which is required for mediating JH action. We isolated five diterpene secondary metabolites with JH antagonist activity from two plants: *Lindera erythrocarpa* and *Solidago serotina*. They are effective in causing mortality of mosquito larvae at relatively low LD_{50} values. Topical application of two diterpenes caused reduction in the expression of Met target genes and retardation of follicle development in mosquito ovaries. Hence, the newly discovered PJHANs may lead to development of a new class of safe and effective pesticides.

Key words: Juvenile hormone, Receptor, Endocrine disruptor

PI31

Production of porcine parvovirus virus-like particles using the baculovirus expression system

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Porcine parvovirus (PPV), a member of the genus Parvovirus, family *Parvoviridae*, is a significant causative agent in porcine reproductive failure, causing serious economic losses in the swine industry. We have studied usefulness of recombinant porcine parvovirus (PPV) virus-like particles(VLPs) as an efficient recombinant vaccine. PPV is a non-enveloped virus and its capsid is assembled from three viral proteins (VP1, VP2, and VP3). The major capsid protein, VP2 is the main target protein for neutralizing antibodies in PPV. When VP2 is expressed in large amounts, it assembled into virus-like particles (VLPs) similar in size and morphology to the original virions. In this study, we generated the recombinant *Bombyx mori* nucleopolyhedrovirus (BmNPV) to express the VP2 protein. Expression of the VP2 protein was analyzed by SDS-PAGE and Western blot. The recombinant VP2 protein of approximately 64 kDa was detected by both analyses. The formation of VLP by recombinant VP2 was confirmed through transmission electron microscopy examination. The purified VP2 protein assembled into spherical particles with diameters ranging from 20 to 22 nm.

Key words: Baculovirus expression system, BmNPV, Porcine parvovirus, Virus-like particle

PI32

An analysis of insects appear in the press middle school textbooks

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We investigated insect and spider species appear in the press nine science textbooks of middle school. There were a total of 47 species, ten orders, three classes: as by a publishing company, 17 species, six orders, two classes in Dusandonga; ten species five orders, two classes in Kumsung; 11 species, eight orders, two classes in Donghwasa; eight species, six orders, two classes in Jihaksa; five species, five orders, two classes in DiDimdol: as by category for classifications, six species, two orders in Arachnida; 42 species, eight orders in Insecta. The appearing frequency for species levels are sequently counted as Apis mellifera (7 times), Allomyrina dichotoma (4 times), Oxya japonica japonica (3 times).

Key words: Middle school textbooks, Insects, Spider, Korea

PI33

Extent and divergence of heteroplasmy of the DNA barcoding region in *Anapodisma miramae* (Orthoptera: Acrididae)

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A partial sequence of the mitochondrial cytochrome oxidase subunit I (COI) gene is widely used as a molecular marker for species identification in animals, also termed a DNA barcode. However, the presence of more than one sequence type in a single individual, also known as heteroplasmy, is one of the shortcomings of barcode identification. In this study, we examined the extent and divergence of COI heteroplasmy, including nuclear-encoded mitochondrial pseudogenes (NUMTs), at the genomic-DNA level from 13 insect species including orthopteran *Anapodisma miramae*, and a long fragment of mitochondrial DNA and cDNA from *A. miramae* as templates. When multiple numbers of clones originated from genomic DNA were sequenced, heteroplasmy was prevalent in all species and NUMTs were observed in five species. Long fragment DNA (~13.5 kb) also is a source of heteroplasmic amplification, but the divergent haplotypes and NUMTs obtained from genomic DNA were not detected in *A. miramae*. On the other hand, cDNA was relatively heteroplasmy-free. Consistently, one dominant haplotype was always obtained from the genomic DNA-origin clones in all species and also from the long fragment- and cDNA-origin clones in the two tested individuals of *A. miramae*. Furthermore, the dominant haplotype was identical in sequence, regardless of the DNA source in *A. miramae*. Thus, one possible solution to avoid the barcoding problem in relationship to heteroplasmy could be the acquisition of multiple numbers of barcoding sequences to determine a dominant haplotype that can be assigned as barcoding sequence for a given species.

Key words: DNA barcoding, COI, Mitochondrial DNA, Orthoptera, *Anapodisma miramae*, NUMTs, Heteroplasmy, Pseudo-sequence

PI34

Complete mitochondrial genome of the *Camponotus atrox* (Hymenoptera: Formicidae): a novel tRNA rearrangement

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Mitochondrial genomes (mitogenomes) of Hymenoptera have shown both rapidly evolving and more slowly evolving groups, the aspect of which makes the hymenopteran phylogeny complicate to resolve. Further, those of Hymenoptera represent one of the most notable groups, with an exceptional and abundant gene rearrangement. In this study, we sequenced the complete mitogenome of *Camponotus atrox* that is distributed only in Korea. The genus known as carpenter ants, contains comparatively large body-sized species and is the most species-rich (more than 1,000 species). In this study, the complete mitogenome of *C. atrox* was sequenced and it was compared to other hymenopteran sequences, particularly to those of ants in terms of genome organization, arrangement, and sequence composition. Amplification of two long overlapping fragments subsequent to amplification of two short fragments was performed to sequence the whole genome by shotgun sequencing approach. The mitogenome of *C. atrox* was 16,540 bp in size, contained typical sets of mitochondrial genes (13 PCGs, 22 tRNAs, two rRNAs, and one the A+T-rich region). The *C. atrox* A+T-rich region was the longest in the sequenced ants as 1,402 bp and is comprised of an identical tandem repeat consisting of six duplicated 100-bp copies and one 96-bp copy, lacking four bp of the end portion of the 100 bp copy. The nucleotide composition of *C. atrox* mitogenome was also biased toward A/T content at 78.8%, as is the case with other ants. We found that the *C. atrox* mitogenome has a unique gene arrangement that has never been found in Hymenoptera including ants. Detailed comparison will be made on the poster presentation.

PI35

Complete mitochondrial genomes of the caddisflies (Trichoptera): an implication for lepidopteran phylogeny

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The sister relationships between Trichoptera and Lepidoptera have often been supported in a diverse study, but all mitochondrial genomes (mitogenomes) based phylogenetic studies for intraordinal relationships of Lepidoptera have utilized alternative outgroups mainly due to unavailability of trichopterans until recently. Therefore, the effect of alternatives or Trichoptera as outgroups on the intraordinal relationships of Lepidoptera remained unknown. In this study, we sequenced three complete mitogenomes of Trichoptera belonging to two suborders and characterized the genomic features of Trichoptera and tested the effect of Trichoptera as outgroup to the lepidopteran phylogeny. The 15,208 ~ 15,285-bp long caddisfly mitogenomes harbor gene content typical of the animal mitogenomes (13 protein-coding genes, two rRNAs, and 22 tRNAs and one non-coding A+T-rich region). The orientation and gene order of the three species belonging to the suborder Integripalpia was identical to that of the most common type that has been hypothesized as ancestral for insects, but *Cheumatopsyche brevilineata* belonging to another suborder Annulipalpia has rearranged QIM, all with forward direction between the A+T-rich region and ND2, instead of the ancestral IQM, with Q inverted. Further, the annulipalpian species had a typical start codon ATG, instead of CGA that are commonly found in other trichopteran species and majority of Lepidoptera. A high A+T composition (77.4% - 79.3%), dominant usage of TAA stop codon and conserved ATACTAA motif between $tRNA^{Ser(UCN)}$ and ND1 was similar to lepidopteran genomes, but also have a quite different features. Phylogenetic analysis with different outgroups (Diptera, Hymenoptera, and Orthoptera, Coeloptera, and Trichoptera) and 115 lepidopteran mitogenomes has shown insensitivity either with Trichoptera, Diptera, or Coleoptera, but artificial grouping and lowered nodal support with Hymenoptera and Orthoptera. The Trichoptera-based consensus topology were: (((((((Bombycoidea +

Noctuoidea) + Pyraloidea) + Papilionoidea) + Cossoidea) + Tortricoidea) + Yponomeutoidea) + Hepialoidea).

Key words: Mitochondrial DNA, Mitogenome, Trichoptera, Outgroup, Lepidopteran phylogeny

PI36

Effects of gamma radiation on different developmental stages of the oriental tobacco budworm, *Helicoverpa assulta* (Lepidoptera: Noctuidae)

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Ionizing radiation is increasingly used as an alternative to methyl bromide for post-harvest crop fumigation. In this study, we studied the effects of gamma irradiation on *Helicoverpa assulta* (Lepidoptera: Noctuidae) at different stages of development to determine the minimal dose for the prevention of normal emergence of adults. We selected five doses of gamma rays (100, 200, 300, 400, and 500 Gy) based on preliminary experiment and irradiated eggs, larvae, pupae, and adults. A dose of 100 Gy irradiated to eggs allowed 21.83% of larvae to pupate, but these all died during the pupal stage. A dose of 100 Gy to last-instar larvae caused larval or pupal death, or the emergence of abnormal adults; no normal adults developed. Irradiation of pupae with doses of 300 Gy and above resulted either in their death or emergence of abnormal adults; however, after 100 or 200 Gy, normal adults emerged and F_1 eggs were produced, but no eggs hatched. Following irradiation of adults, eggs were produced at all doses, although the numbers were significantly decreased compared to untreated controls ($P < 0.05$; 69.45 ~ 125.50 vs. 475.05 eggs per female); however, none of the eggs hatched. As prevention of normal emergence is a key outcome for measuring the effectiveness of radiation, then the 100 Gy dose was effective for irradiation of eggs and larvae, and 300 Gy for pupae.

Key words: Oriental tobacco budworm, *Helicoverpa assulta*, Noctuidae, Gamma irradiation, Quarantine

PI37

Mitochondrial DNA variations in Korean *Apis cerana* (Hymenoptera: Apidae) and development of another potential marker

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The geographic relationships and biogeography of *Apis cerana* have been studied extensively, but Korean populations have not been investigated thoroughly. Thus, 118 samples from nine Korean localities were sequenced for the typical non-coding region (termed NC2) of *A. cerana* mitochondrial DNA (mtDNA), along with 66 samples from seven Asian localities (China, Vietnam, and Thailand). Furthermore, a non-coding region, located between mitochondrial (mt) $tRNA^{Met}$ and $tRNA^{Gln}$ (termed NC1), was selected from the *A. cerana* mt genome sequence as an additional marker. Ten haplotypes were found from NC2: four undiscovered haplotypes were found in Korea and China, respectively, whereas previously reported haplotypes were found in Vietnam (Japan1) and Thailand (Japan1 and IndiaB4). A phylogenetic analysis confirmed that Korean *A. cerana* belonged to the Mainland Asian group. The most widespread haplotype in Mainland Asia was Japan1, and it was also found dominantly in samples from other countries including Korea, accounting for 71%. Such dominance of Japan1 suggests extensive gene flow onto Mainland Asia mediated by Japan1. The newly developed ~231-bp long NC1 region provided nine haplotypes with twice the number of variable positions, compared to those in NC2 (six in NC1 vs. three in NC2). An NC1-based phylogenetic analysis revealed the presence of two phylogenetic groups in Korea, without any region-based clustering. These results suggest an introduction of *A. cerana* from two different sources and a randomized admixture of the sources.

Key words: Asian cavity-nesting honey bee, *Apis cerana*, Non-coding sequence

[†]These authors contributed equally to this paper.

PI38

Mitochondrial DNA sequence variation of the spotted-wing drosophila, *Drosophila suzukii* (Diptera: Drosophilidae)

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The spotted-wing drosophila (SWD), *Drosophila suzukii* (Diptera: Drosophilidae), was originally observed in a few Asian countries, but is now found in North America and Europe. Genetic information on geographic variation and relationship may broaden our understanding of origin and migration. As a first step, a portion of mitochondrial COI gene was sequenced to understand genetic relationship and diversity in Korea. Sequencing of 176 individuals collected from 12 Korean localities and each one locality from China and USA provide a total of 80 haplotypes, with the maximum sequence divergence of 1.52%, suggesting high haplotype diversity, with moderate sequence divergence. Population genetically, two foreign populations were significantly differentiated from Korean populations ($p < 0.05$), but overall no Korean populations completely isolated from others, indicating that *D. suzukii* can be characterized as the species with the high rate of per generation female migration and low level of geographic separation among localities. Phylogenetically, no discernable haplotype group was found and similar pattern also was detected when GenBank-registered *D. suzukii* haplotypes from China, Japan, USA, Italy, Spain, and Portugal (94 sequences) were analyzed together with the present result, indicating phylogenetic homogeneity of worldwide *D. suzukii* and commixture of haplotypes from different countries.

Key words: *Drosophila suzukii*, mitochondrial COI, population genetics

[†]These authors contributed equally to this presentation.

PI39

A novel, highly effective and environmentally friendly recombinant baculovirus insecticide, NeuroBactrus

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A novel recombinant baculovirus, NeuroBactrus, was constructed to develop an improved baculovirus insecticide with additional beneficial properties, such as a higher insecticidal activity and improved recovery, compared to wild-type baculovirus. For the construction of the NeuroBactrus, the *Bacillus thuringiensis cry1-5* crystal protein gene was introduced into the *Autographa californica* nucleopolyhedrovirus (AcMNPV) genome by fusion of *polyhedrin-cry1-5-polyhedrin* under the control of the *polyhedrin* promoter. In the opposite direction, an insect-specific neurotoxin gene, *AaIT*, from *Androctonus australis* was introduced under the control of an early promoter from *Cotesia plutellae* bracovirus by fusion of a partial fragment of *orf603*. The Polyhedrin-Cry1-5-Polyhedrin fusion protein expressed by the NeuroBactrus was not only occluded into the polyhedra, but it was also activated by treatment with trypsin, resulting in an approximately 65-kDa active toxin. In addition, qrt-PCR revealed that the neurotoxin was expressed from the early phase of infection. The NeuroBactrus showed a high level of insecticidal activity against *Plutella xylostella* larvae and a significant reduction in the median lethal time (LT_{50}) against *Spodoptera exigua* larvae compared to those of wild-type AcMNPV. Re-recombinant mutants derived from

NeuroBactrus in which *AaIT* and/or *cry1-5* were deleted were generated by serial passages *in vitro*. Expression of the foreign proteins (Bt toxin and AaIT) was continuously reduced during the serial passage of the NeuroBactrus. Moreover, polyhedra collected from *S. exigua* larvae infected with the serially passed NeuroBactrus showed insecticidal activity similar to that of wild-type AcMNPV. These results suggested that the NeuroBactrus could be recovered to wild-type AcMNPV through serial passaging.

Key words: Recombinant baculovirus, Insecticide, *Bacillus thuringiensis*, *AaIT*, Recovery to wild-type

PI40

Autographa californica multiple nucleopolyhedrovirus *ac78*, a core gene that is essential for BV production and general occlusion body formation

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ORF78 (*ac78*) of *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) is a baculovirus core gene of unknown function. To determine the role of *ac78* in baculovirus life cycle, an *ac78*-deleted mutant AcMNPV, Ac78KO, was constructed. Quantitative PCR analysis revealed that *ac78* is a late gene in the viral life cycle. After transfection into *Spodoptera frugiperda* cells, Ac78KO produced a single-cell infection phenotype indicating that no infectious budded viruses (BVs) were produced. The defection in BV production was also confirmed by both viral titration and Western blot. However, viral DNA replication is unaffected. Analysis of BV and occlusion derived virus (ODV) revealed that AC78 is associated with both forms of the virions and is a structural protein located to viral envelope. Electron microscopy showed that *ac78* also plays an important role in embedding of ODV into occlusion body. This study therefore demonstrates that AC78 is a late virion associated protein and is essential for the viral life cycle.

Key words: AcMNPV, *ac78*, BV production, ODV embedding

PI41

Construction & characterization of novel *Bacillus thuringiensis* cry1-type genes with improved insecticidal activities

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Crystals of proteinaceous insecticidal proteins, Cry proteins, produced by *Bacillus thuringiensis* (Bt) have been generally used used to control insect pests. In this study, through the 3D structure prediction and accompanying mutagenesis study for the Mod-Cry1Ac, 7 and 16 amino acid residues from domain I and II, respectively, responsible for its insecticidal activity against larvae of *Plutella xylostell*a, *Spodoptera exigua* and *Ostrinia furnacalis* were identified. To construct novel *cry* genes with enhanced insecticidal activity, we randomly mutated these 24 amino acid sequences by *in vitro* muti site-directed mutagenesis, resulting in totally 34 mutant *cry* genes. For further characterization, these mutant *cry* genes were expressed as a fusion protein with polyhedrin using baculovirus expression system. SDS-PAGE analysis of the recombinant polyhedra revealed that expressed Cry proteins was occluded into polyhedra and activated stably to 65 kDa by trypsin. When the insecticidal activities of these mutant Cry proteins against to larvae of *P. xylostella*, *S. exigua*, and *O. furnacalis* were assayed, they showed higher or similar insecticidal activity compared to those of Cry1Ac and Cry1C. Especially, among them Mutant-N16 showed the highest insecticidal activity against to both of *P. xylostella, S. exigua* and *Ostrinia furnacalis*. Therefore, Mutant-N16 is estimated to have the potential for the efficacious bioagent.

Key words: *Bacillus thuringiensis*, Transgenic plants, *cry* genes, Mutagenesis

PI42

Novel high-throughput baculovirus expression vector based on *Bombyx mori* nucleopolyhedrovirus

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The baculovirus expression system is one of the most popular methods used for the production of recombinant proteins but has several complex steps which have proved inherently difficult to meet a multi-parellel process. We have developed a novel recombinant bacmid, bEasyBm that enabling easy and fast generation of pure recombinant virus without any purification step. In the bEasyBm, *att*R recombination sites were introduced to facilitate the generation of recombinant viral genome by *in vitro* transposition. Moreover, extracellular RNase gene from *bacillus amyloliquefaciens*, barnase, was expressed under the control of *Cotesia plutellae* bracovirus early promoter. Therefore, only when the barnase gene was replaced to gene of interest, the bEasyBm could replicate in host insect cells. When the bEasyBm was transposed with pDualBac-EGFP and pDualBac-LUC respectively, there were no non-recombinant backgrounds were detected from unpurified BmEasy-EGFP or BmEasy-LUC stocks. In addition, the resulting recombinant virus, BmEasy-EGFP, showed comparable level of EGFP expression efficiency with the plaque-purified recombinant virus, BmEGFP, which was constructed using bBmGOZA system. Based on these results, high-throughput condition for generation of multiple recombinant viruses in a time was established.

Key words: High-throughput, Baculovirus expression system, bEasyBm, Barnase, *in vitro* transposition

PI43

The *Autographa californica* multiple nucleopolyhedrovirus ORF11 is essential for BV production and ODV envelopment

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ORF11 (*ac11*) of *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) is a highly conserved gene of unknown function. To determine the role of *ac11* in baculovirus life cycle, an *ac11*-knockout mutant AcMNPV, Ac11KO, was constructed. qPCR analysis revealed that *ac11* is an early gene in the life cycle. After transfection into *Spodoptera frugiperda* cells, Ac11KO produced a single cell infection phenotype indicating that no infectious budded viruses (BVs) were produced. The defection in BV production was confirmed by both viral titration and Western blot. However, viral DNA replication is unaffected. Electron microscopy showed that *ac11* is required for nucleocapsids envelopment to form ODV and their subsequent embedding into OB. This study therefore demonstrates that *ac11* is an early gene which is essential for the viral life cycle.

Key words: AcMNPV, *ac11*, BV production, Nucleocapsids envelopment

PI44

High GC genome analysis of *Cellulosimicrobium* sp. HY-13 using single molecule real time sequencing (SMRT)

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Symbiotic microorganism isolated from earthworm, *Cellulosimicrobium* sp. HY-13 produced hemicellulolytic enzymes. In this study, The whole genome sequencing of earthworm-symbiotic bacteria *Cellulosimicrobium* sp. HY-13 and analysis of the genome information for the expression of cellulase/hemicellulase enzyme groups. The enzyme set is known to provide useful tools for the solutions of bio-recalcitrance problems in biomass study. Whole genome sequence of *Cellulosimicrobium* sp. HY-13 was analyzed by NGS approach with SMRT and de novo assembly. The genome of the strain HY-13 showed 4.6Mbp size with 74.24% GC content, and it contained 3,813 proteins. The whole genome sequencing of the strain HY-13 was the first case in *Cellulosimicrobium* spp.. Genetic database was established after gene annotation process and the DB was implemented in G-browser format in server PC. New genes coding 11 cellulose-degrading enzymes and 20 hemicellulose-degrading enzymes were identified. Some error were corrected and de-bugged based on the whole genome sequence (2 amino acids in previous work).

Key words: Symbiotic microorganism, *Cellulosimicrobium* sp., Genome sequencing, SMRT, Error collection

PI45

Apolipophorin III from honeybees (*Apis cerana*) exhibits antibacterial activity

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Apolipophorin III (apoLp-III) is involved in lipid transport and innate immunity in insects. In this study, an apoLp-III protein that exhibits antibacterial activity was identified in honeybees (*Apis cerana*). *A. cerana apoLp-III* cDNA encodes a 193 amino acid sequence that shares high identity with other members of the hymenopteran insect apoLp-III family. *A. cerana* apoLp-III is expressed constitutively in the fat body, epidermis, and venom gland and is detected as a 23-kDa protein. *A. cerana* apoLp-III expression is induced in the fat body after injection with *Escherichia coli*, *Bacillus thuringiensis*, or *Beauveria bassiana*. However, recombinant *A. cerana* apoLp-III (expressed in baculovirus-infected insect cells) binds directly to *E. coli* and *B. thuringiensis* but not to *B. bassiana*. Consistent with these findings, *A. cerana* apoLp-III exhibited antibacterial activity against both Gram-negative and Gram-positive bacteria. These results provide insight into the role of *A. cerana* apoLp-III during the innate immune response following bacterial infection.

Key words: *Apis cerana*, Honeybee, Apolipophorin III, Innate immunity, Antibacterial activity

PI46

Bumblebee (*Bombus ignitus*) inhibitor cysteine knot peptide acts as an antifungal peptide and insecticidal venom toxin

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Inhibitor cysteine knot (ICK) peptides exhibit multifunctional roles, such as ion channel blocking, insecticidal, and antimicrobial activities. In this study, a bee (*Bombus ignitus*) ICK peptide (BiICK) that acts as an antifungal peptide and as an insecticidal venom toxin was identified. BiICK contains an ICK fold that is expressed in the epidermis, fat body, or venom gland and is present as a 6.7-kDa peptide in bee venom. Recombinant BiICK peptide (expressed in baculovirus-infected insect cells) bound directly to *Beauveria bassiana* and *Fusarium graminearum,* but not to *Escherichia coli* or *Bacillus thuringiensis*. Consistent with these findings, BiICK showed antifungal activity, indicating that BiICK acts as an antifungal peptide. Additionally, we show that BiICK has insecticidal activity with recombinant BiICK. These findings demonstrate the antifungal activity and insecticidal activity of BiICK peptide.

Key words: *Bombus ignites*, Bumblebee, Inhibitor cysteine knot fold, Venom, Antifungal peptide, Insecticidal toxin

PI47

Biological potential of the human BMP7 expressed in silkworm

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Bone morphogenetic protein-7 (BMP-7) is important for bone tissue repair and embryonic development. The goal of this research is to characterize of biological activity of full-length precursor and mature human BMP7 expressed in silkworm. We examined the production and purification of recombinant hBMP7 (rhBMP7) in silkworm expression system (BEVS). The rhBMP7s showed the 53 kDa (full-length sequences) and 20 kDa band (only mature sequences) on CBB staining and Western blot and were glycosylated and susceptible to PNGaseF and Endoglycosidase H. The rhBMP7s induced alkaline phosphatase activity in preosteoblastic C2C12 cells. In this study, our findings provide a possibility for the production of biologically active rhBMP7 in silkworm system.

Key words: BMP7, Silkworm, Glycosylation, Alkaline phosphatase

PI48

Antifungal activity of propolis extracts against skin flora

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The purpose of the study is to evaluated the antifungal activity of propolis extracts from Korea against skin flora. The skin flora used for experiment were four fungi such as *Epidermophyton floccosum*, *Trichophyton tonsurans*, *T. mentagrophytes*, and *T. rubrum*. In this study, two methods, agar diffusion and broth microdilution were determinated antifungal activity of propolis. Minmun inhibitory concentration (MIC) values of 80% ethanol extracts on *E.floccosum*, *Trichophyton tonsurans*, *T. mentagrophytes*, and *T. rubrum* were 0.25%, 0.13%, 0.25% and 0.15% respectively. Also minimun bacterial concentration values were 0.5%, 0.25%, 1.0% and 0.5%. It is conclude that the 80% ethanol extract of propolis from Korea could be applicable to cosmetics and medical agents as a natural preservatives effective in antifungal activity against skin flora.

Key Words: Propolis, Antifungal activity, Skin flora

PI49

Effect of cosmetic included purified bee venom (*Apis mellifera* L.) on the improvement of skin wrinkle

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In the last few decades, there has been a substantial increase in the population of aged people. Aging skin is a common concern for many people these days. The purpose of this study was to evaluate the efficacy of serum containing purified bee venom in reducing wrinkles. Bee venom serum was applied to the face after toner of 22 subjects twice a day for 12 weeks. The changes of the skin wrinkles were analyzed with the visual grade of wrinkle and the image of the skin replica. Compared to before and after treatment, the visual grade of wrinkle was improved 11.83% after 12 weeks. The wrinkle squares and depths were significantly reduced after 12 weeks ($p < 0.05$). Also, the wrinkle counts were significantly reduced after 12 weeks ($p < 0.5$). Adverse reactions of subjects were not observed during the course of the study. These observations indicate that the treatment of purified bee venom seems to be effective in improving wrinkles and can be usefully tho the anti-aging cosmetic industry.

Key Words: *Apis mellifera*, Purified Bee Venom, Wrinkle, Skin

PI50

PCR based determination of rice DNA in artifical honey

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Adulteration of honeys is often based on artificially added cheap rice syrup. By the help of polymerase chain reaction (PCR), rice syrup added to honey can be detected indirectly by amplifying a specific rice DNA sequence coming from the added rice syrup. The PCR method described here might serve as support to mass spectrometry methods that are used for detection of honey adulterations with sugars from C_3 plants.

Key Words: Honey, Rice, PCR

PI51

Tyrosinase inhibitory activity of darae (*Actinidia arguta*) pollen

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Bee pollen is the pollen ball that has been packed by worker honeybees into pellets. Bee bread is the bee pollen with added honey and bee secretions and stored in brood cells. The incredible nutritional and medicinal value of bee pollen has known for thousands of years. In our present study, effect of pollen on melanogenesis was investigated by assessing tyrosinase, a key enzyme in melanogenesis. In addition, extraction condition was optimized for maximum efficacy. The total extract of pollen significantly inhibited tyrosinase activity (30% inhibition at 100 μg/ml). Further investigation using the fractions of pollen extract showed that the inhibitory activity of EtOAc-soluble fraction was the most potent (96% inhibition at 100 μg/ml), which was followed by *n*-BuOH and CH_2Cl_2-soluble fractions. Next the effect of extraction condition such as extraction solvent, extraction time and extraction temperature on tyrosinase inhibition was investigated. Our study showed that extraction solvent greatly affected tyrosinase inhibition. The optimized extraction condition for maximum tyrosinase inhibition was also suggested using response surface methodology. These results will provide useful information about optimized extraction condition for the development of darae pollen as a cosmetic.

Key Words: *Actinidia arguta* Pollen, Melanogenesis, Tyrosinase

PI52

Antimicrobial activity of the synthetic peptide Periplanetasin-1 from cockroach *Periplaneta americana*

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The antimicrobial peptides (AMPs) play a pivotal role in meditating resistance to microbial infection, and this species is increasingly used as a reliable source of new AMPs. However, transcriptome for this species is not available and a more rational selection of AMPs is necessary. First, total RNA was isolated from the whole body of adult non-infected and *E. coli*-infected *Periplaneta americana* strains. Using an Illumina Hiseq sequencer, we generated a total of 85,984 pooled contigs and singletons with and without *E. coli* infection, respectively. Then, we performed *in silico* analysis of the *Periplaneta americana* transcriptome using bioinformatics tools for screening putative AMPs. Based on evidence of length, charge, isoelectric point, *in vitro* and *in vivo* aggregation and presence of stretches, together with no similarity to human and absence of protease cleavage site, AMP candidates were selected and tested their antimicrobial activities. Among them, a synthetic peptide, named Periplanetasin-1, was tested its antimicrobial activity using radial diffusion assay. The result showed that Periplanetasin-1 had antibacterial activities against Gram positive and negative bacteria strains and it also evidenced with no hemolytic activity. In conclusion, our results suggested that Periplanetasin-1 derived from *Periplaneta americana* could be useful for developing peptide antibiotics agent.

Key words: Antimicrobial peptide, RNA sequencing, Periplanetasin-1, Hemolytic activity, *Periplaneta americana*

PI53

Scolopendin 2, a cationic antimicrobial peptide from centipede, and its membrane-active mechanism

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Scolopendin 2 is a 16-mer peptide (AGLQFPVGRIGRLLRK) derived from the centipede *Scolopendra subspinipes mutilans*. We observed that this peptide exhibited antimicrobial activity in a salt-dependent manner against various fungal and bacterial pathogens and showed no hemolytic effect in the range of 1.6 μM to 100 μM. Circular dichroism analysis showed that the peptide has an α-helical properties. Furthermore, we determined the mechanism(s) of action using flow cytometry and by investigating the release of intracellular potassium. The results showed that the peptide permeabilized the membranes of *Escherichia coli* O157 and *Candida albicans*, resulting in loss of intracellular potassium ions. Additionally, bis-(1,3-dibutylbarbituric acid) trimethine oxonol and 3,3′-dipropylthiacarbocyanine iodide assays showed that the peptide caused membrane depolarization. Using giant unilamellar vesicles encapsulating calcein and large unilamellar vesicles containing fluorescein isothiocyanate-dextran, which were similar in composition to typical *E. coli* O157 and *C. albicans* membranes, we demonstrated that scolopendin 2 disrupts membranes, resulting in a pore size between 4.8 nm and 5.0 nm. Thus, we have demonstrated that a cationic antimicrobial peptide, scolopendin 2, exerts its broad-spectrum antimicrobial effects by forming pores in the cell membrane.

Key words: Scolopendin 2, *Scolopendra subspinipes mutilans*, Antimicrobial peptide, Membrane damage

PI54

Molecular cloning and effects of TmCactin gene silencing on *Tenebrio* larval mortality

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Innate immune system is very important to protect host itself from pathogenic microorganism infection in insect. Cactin, cactus-interacting protein was for the first time identified in Drosophila and was discovered to be involved in dorsal-ventral patterning and intracellular toll signaling cascade. In the present study, we have identified and functionally characterized *Tenebrio Cactin* (*TmCactin*) in the beetle, *Tenebrio molitor* by RNASeq/EST. Analysis of RNA interference indicates that TmCactin plays an important role in Gram-negative and -positive bacteria infection, not fungal infection in *T. molitor* larvae.

Key words: Toll signaling, Cactin, *Tenebrio molitor*, Microbial infection, RNA interference, AMP

PI55

Identification and characterization of TmToll-like receptor 7 (TmTLR7) from the mealworm, *Tenebrio molitor*

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Toll signaling cascade has been well studied especially in *Drosophila* and beetle model. Recently, the role of extracellular spätzle-Toll signaling cascade was identified using *Tenebrio molitor* model. However, there is no information for *Tenebrio* toll-like receptor (TLR) genes. Here, we have screened and so far identified seven TLR genes from RNAseq and expressed sequence taq (EST) generated from *T. molitor*. *Tenebrio Toll-like receptor 7* (*TmTLR7*) gene was cloned and partially characterized. The results show that TmTLR7 contains 3,939 bp of ORF encoding 1,311 amino acid residues, 847 bp of 5'-UTR and 231 bp of 3'-UTR except poly-A tail. Domain analysis shows that the TmTLR7 includes one signal peptide region, seven leucine rich repeat region, one transmembrane domain and one TIR domain. Developmental expression patterns shows that *TmTLR7* mRNA was highly expressed on late instar larvae. Tissue specific expression patterns indicates that *TmTLR7* transcripts were highly expressed in Malpighian tubules at late instar larvae and integument at 5-day old adult. *TmTLR7* was strongly induced at 6 hrs post infection of *Escherichia coli* and *Staphylococcus aureus*, and at 9hrs post infection of *Candida albicans* and *Listeria monocytogenes*.

Key words: Toll-like receptor, TLR7, *Tenebrio molitor*, Microbial infection, Induction patterns

PI56

Functional characterization of Tm14-3-3ζ on autophagy signaling in *Tenebrio molitor*

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14-3-3 is a family whose members are highly conserved eukaryotic proteins that play pivotal roles in the regulation of cell survival, apoptosis, and signal transduction. In this study, two isoforms of the *Tenebrio* 14-3-3 proteins, Tm14-3-3ε and Tm14-3-3ζ, were identified and their functions in countering pathogenic infections were investigated. A peptide-based polyclonal antibody was generated for determination of subcellular localization of Tm14-3-3ζ. Tm14-3-3ζ is localized in the membranes of midgut epithelial cells, nuclei of the fat body and cytosol of hemocytes but little or no in Malpighian tubules. A confocal microscopic analysis, furthermore, revealed that Tm14-3-3ζ protein and the signals for LysoTracker as an autolysosome signal were not merged. During a critical window of larval to pupal transition, expression levels of *Tm14-3-3ζ* were inversely correlated to the acidification levels of lysosomes. Injection of C-2 Ceramide revealed a time-dependent increase in the transcripts of *TmATG8* whereas it decreases in the expression level of *Tm14-3-3ζ* transcripts in the first hour. Depletion of Tm14-3-3ζ triggers the conversion of TmAtg8-I to TmAtg8-II (active form) as determined by Western blot analysis with TmAtg8 polyclonal antibody. Our results suggest that Tm14-3-3ζ protein has negative regulatory roles in autophagy.

Key words: *Tenebrio molitor*, 14-3-3ζ, Autophagy, Atg8

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