2014 Inrernational Symposium and Annual Meeting of THE KSABC

June 19 >> 21, 2014 Haeundae Grand Hotel, Busan, Korea

▣ Program

PL	Plenary Lectures		
AL	Award Lectures		
IS	International Symposia	IS1	Biochemistry · Molecular Biology
		IS2	Natural Products
		IS3	Environmental Science
		IS4	Food Science · Microbiology
S	Symposia	S1	Biochemistry · Molecular Biology
		S2	Natural Products
		S3	Environmental Science
		S4	Food Science · Microbiology
		S5	Biologics
		S6	Next-generation Biogreen21 : CNPM
		S7	Bio/Molecular Informatics Center
YS	Young Scientists Presentation	YS1	Biochemistry · Molecular Biology
		YS2	Natural Products
		YS3	Environmental Science · Food Science
W	Workshop		
P	Poster Session	PBM	Biochemistry · Molecular Biology
		PNP	Natural Products
		PES	Pesticide · Environmental Science
		PFM	Food Science · Microbiology

▣ Schedule

June 19 (Thu)						
Time	Grand Ballroom (2F)			Nam-won (2F)	Choong-won (2F)	Private room (22F)
	Hall A	Hall B	Hall C			
09:00-09:30	Registration					
09:30-11:40	YS1	YS2	YS3	-	S6	S7
11:40-13:00	Lunch / Workshop					
13:00-13:20	Opening Ceremony (Hall A)					
13:20-14:05	PL-1 (Hall A)					
14:05-14:20	Break					
14:20-16:00	IS1	IS2	IS3	S5		
16:00-17:00	Poster Session Ⅰ (Convention Hall, 2F)					
17:00-17:50	Workshop (Hall B)					
18:00-20:00	Welcome Reception (Hall A+C, Emerald Hall)					

June 20 (Fri)				
Time	Grand Ballroom (2F)			Choong-won (2F)
	Hall A	Hall B	Hall C	
09:00-10:00	Registration			
10:00-11:00	IS1	IS2	IS4	S6
11:00-11:45	PL-2 (Hall A)			
11:45-13:00	Lunch / Workshop			
13:00-14:00	S1	S2	S3 / S4	
14:00-15:00	Poster Session Ⅱ (Convention Hall, 2F)			
15:00-16:00	AL (Hall A)			
16:00-16:30	General Assembly Members Meeting & Closing Ceremony (Hall A)			

June 21 (Sat)	
9:00	Research Institute Tour

☞ Hall A

Date	Lectures	Time	Speaker	Affiliation
June 19 (Thu)	YS1-1~8	09:30-11:40	Young Scientists Presentation	
	PL-1	13:20-14:05	Yasuyuki Arakane	Chonnam National University
	IS1-1	14:20-14:50	Bharat Bhusan Patnaik	Central Silk Board, India
	IS1-2	14:50-15:20	Hari B. Krishnan	USDA-ARS, University of Missouri, USA
	IS1-3	15:20-15:50	Kohei Oda	Kyoto Institute of Technology, Japan
June 20 (Fri)	IS1-4	10:00-10:30	Young Dae Yun	Konkuk University
	IS1-5	10:30-11:00	Keiko Taguchi	Tohoku University, Japan
	PL-2	11:00-11:45	Sunil Kochhar	Nestlé Reserach Center, Switzerland
	S1-1	13:00-13:30	Pil Joong Chung	Seoul National University
	S1-2	13:30-14:00	Mi Young Noh	Chonnam National University
	S1-3	14:00-14:30	Yong Hun Jo	Chonnam National University
	AL-1	15:00-15:20	Yoongho Lim	Konkuk University
	AL-2	15:20-15:40	Hoi-Seon Lee	Chonbuk National University
	AL-3	15:40-16:00	Min-Gi Kim	Chonbuk National University

☞ Hall B

Date	Lectures	Time	Speaker	Affiliation
June 19 (Thu)	YS2-1~8	09:30-11:40	Young Scientists Presentation	
	IS2-1	14:20-14:50	Irawan WijayaKusuma	Mulawarman University, Indonesia
	IS2-2	14:50-15:20	Nanju Alice Lee	University of New South Wales, Australia
	IS2-3	15:20-15:50	Yonghwan Kim	Sookmyung Women's University
	W	17:00-17:50	David Park	Dr. David English Lyceum
June 20 (Fri)	IS2-4	10:00-10:30	Maitree Suttajit	University of Phayao, Thailand
	IS2-5	10:30-11:00	Yongsoo Choi	Korea Institute of Science and Technology
	S2-1	13:00-13:30	Hee-Sung Chae	Dongguk University
	S2-2	13:30-14:00	Jin-Gyeong Cho	Kyung Hee University

☞ Hall C

Date	Lectures	Time	Speaker	Affiliation
June 19 (Thu)	YS3-1~7	09:30-11:40	Young Scientists Presentation	
	IS3-1	14:20-14:50	Jin-Cheol Kim	Korea Research Institute of Chemical Technology
	IS3-2	14:50-15:20	Choong-Min Ryu	Korea Research Institute of Bioscience & Biotechnology
	IS3-3	15:20-15:50	Tsunaki Asano	Tokyo Metropolitan University, Japan
June 20 (Fri)	IS4-1	10:00-10:30	Jeyakumar Henry	Singapore Institute or Clinical Sciences, Singapore
	IS4-2	10:30-11:00	Eunju Park	Kyungnam University
	S3-1	13:00-13:30	Jeong-Heui Choi	Chonnam National University
	S4-1	13:30-14:00	Jin Hyo Kim	National Academy of Agricultural Science. RDA

☞ Nam-won

Date	Lectures	Time	Speaker	Affiliation
June 19 (Thu)	S5-1	14:20-14:45	권형주	한림대학교
	S5-2	14:45-15:10	박해준	씨젠
	S5-3	15:10-15:35	임형권	목암연구소
	S5-4	15:35-16:00	정문섭	VGX Int

☞ Choong-won : 해당 기관에서 자체 운영 (최종 초록집에 수록 예정)

☞ Private room : 해당 기관에서 자체 운영 (최종 초록집에 수록 예정)

Plenary Lectures

PL-1

Differentiation and Ultrastructure of Rigid Cuticle of a Beetle

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The success of insects in colonizing a wide array of terrestrial and aquatic habitats and evolving a flight mechanism is due in large part to the development more than 300 million years ago of a light-weight organically hardened exoskeleton. This extracellular material serves as both a skin and skeleton, acting as armor but sufficiently lightweight to permit flight.

Insect exoskeletons are composed of cuticle, a biomaterial primarily formed from proteins and the polysaccharide chitin. Despite this limited compositional palette, cuticle has remarkably diverse properties, ranging from soft and flexible to hard and rigid. Chitin fibers are embedded in a matrix of protein molecules, which are cross-linked by catechol derivatives, forming a dual-network composite material. In a process called sclerotization, cuticular proteins (CPs) are cross-linked by quinones derived from the oxidation of catechols, and by formation of bonds between tyrosine side chains, resulting in hardening of the exoskeleton. Some CPs contain a conserved amino acid sequence known as the R&R motif, which may function as a chitin-binding region, coordinating interactions between the chitin fibers and protein network. The factors that lead to synthesis and assembly of cuticular regions with differing mechanical properties are not well understood.

In this study, we investigated development and differentiation of rigid cuticle using the red flour beetle, *Tribolium castaneum* adult, as a model insect. *Tribolium* as a beetle is superior model for studying rigid cuticle formation because they have a highly modified (sclerotized and pigment) forewing (elytron) which can be separated from other tissues easily and cleanly. We analyzed ultrastructure of elytral cuticle during development (from 3 d-old pupae to 3 d-old adults) by transmission electron microscopy (TEM). In 3 d-old pupae, pupal cuticle separated from the epidermal cells (apolysis), and the outermost envelop of adult cuticle was being formed. Protein-rich epicuticle and procuticle composed numbers of horizontal laminae and vertical canals were formed at 4 and 5 d-old pupal stages. After adult eclosion, additional thick horizontal laminae were evident and apical membrane of the epidermal cells became undulae like-structure at 1 d-old adult, and then final three layers with no horizontal laminae were formed by 3 days after adult molting.

We further analyzed protein localization of several CPs that are highly abundant into the rigid cuticle of a beetle. These results will contribute understanding cuticle formation and differentiation in insect during post-embryonic development.

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

PL-2

Interactions of human diet (case study, Chocolate) and its influence on metabolism deciphered by metabolomics

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The current revolution in nutrigenomics sciences will at term offers new opportunities for preventive medicine, prognostic strategies and personalized healthcare and nutritional management solutions. The rise in multifactorial disorders (including inflammatory bowel disease), the lack of understanding on the molecular processes at play and the needs for disease prediction in asymptomatic conditions are some of the many questions that system biology approaches are foreseen to address. By opening a direct biochemical window into the metabolome, metabolomics is a unique science perfectly suited for the identification of biomarkers capable of providing better understanding of these complex metabolic phenomena.

In the present study, we investigated how chocolate, one of the foods with the greatest appeal to the general population influences our metabolic system and its positive effect on the moods.. The attractive tastes and textures of chocolate and chocolate products delight the senses and make it a well-known comfort food. Cocoa seeds undergo natural fermentation before they are turned into a key ingredient for chocolate making namely cocoa fat and cocoa powder. The latter is indeed rich source of peptides and flavonoids, and produces the delectable taste and aroma of chocolate after roasting. In spite of a large body of literature on the cocoa flavor, there are very few studies devoted to the role of proteins/peptides in the flavor development of cocoa or chocolate. Cocoa storage proteins, which makes up to 10-15 % (w/w) dry weight, is made up of four predominant proteins of apparent molecular weight 14.5-, 31- and 47-kDa and 21-kDa representing 95 % (w/w) of total protein. We developed an in-vitro fermentation process of cocoa beans, mimicking the natural fermentation and identified a number short-chain peptides originating from storage proteins that are the key cocoa/chocolate flavor precursors. The presentation will cover the characterization of proteins and peptides and flavor precursors. Additionally, results from a recent study employing metabolomics approach to study the possible metabolic signatures linked to the regular intake of dark chocolate in healthy subjects will be presented. In summary, metabolic profiles of plasma and urine samples when combined with multivariate statistics show discrimination of subjects according to their chocolate liking as given by the scoring to the questionnaire on chocolate consumption. The class separation using plasma metabolic profiles was present even from samples collected before the chocolate intake, supporting most likely the occurrence of metabolic imprint or memory independent of the chocolate intake. Results indicate that subjects who do not like chocolate harbor statistically different lipoprotein profile in the postprandial phase.

In conclusion, through the rigorous characterization of interactions between the diet and the metabolism, metabolomics is envisioned to provide new ventures for modulating the human health.

Award Lectures

AL-1

Plant Derived Polyphenols

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Polyphenols are ubiquitously found from plant kingdom as secondary metabolites. They can be classified based on their carbon skeletons: phenolic acids (C6-C1), hydroxycinnamates (C6-C3), stilbenoids (C6-C2-C6), flavonoids (C6-C3-C6), and polymers including glycosylated polyphenols. As expected from their naming, the hydroxyl groups can be substituted with various functional groups which result in diverse changes of their biological activities. Before a couple of decades most researchers were not concerned with them due to their weak biological activities. Recently, however, we are concerned about care (disease prevention) than cure (treatment of diseases), so that the low toxicities of plant-derived polyphenols stand out. One of them, resveratrol is found from grapes including wines, which has been known to lengthen the span of life of *Caenorhabditis elegans*. Its methoxylated derivatives were prepared and it was found that the position of the methoxy group causes the cell morphology change dramatically. Likewise, the number of methoxy groups in benzoflavanones determines the cell cycle arrest pattern at G1 or G2/M phase. Therefore, a rationale can be deduced: "Small structural differences cause large biological activity changes!". To prove this rationale, several hundreds of polyphenol derivatives were designed and synthesized, and their biological activities were tested. The quantitative relationships between the physicochemical properties of polyphenols and their biological activities were investigated and their molecular mechanisms were elucidated. The findings obtained from the research of the plant-derived polyphenols could be used for developing therapeutic or preventive agents.

AL-2

Mite Indicator: Color Alterationand Changes in Acaricidal Potency by Introducing Functional Radicals and Bio-Functional Compounds against *Dermatophagoides* spp. and *Tyrophagus putrescentiae*

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The most serious pests are the American house dust mite, *Dermatophagoides farinae*, and the European house dust mite, *D.pteronyssinus*, because of their ubiquitous occurrence in the home. In addition, they impacted on human health. House dust mites are the most important source of indoor allergens that cause allergic diseases such as asthma, and atopic dermatitis. The stored food mite, *Tyrophagus putrescentiae* (Astigmata: Acaridae), is the dominant mite species found in stored products, such as cheese, ham containing high fat and protein, and different kinds of nuts. Although the stored food mites live on the external surface of stored food products, they cause serious economic losses because they penetratethe surface. *T. putrescentiae* have been also reported as etiological agents of several allergic symptoms among agro-industrialists and farmers. House dust and stored food mites are increasingly associated with changes in the residential environment of humans, such as fitted carpets, more tightly sealed windows, and poor ventilation that allow their proliferation. Populations of house dust and stored food mites are controlled by applying synthetic acaricides, such as avermectines, benzyl benzoate, and benzene hexachloride. However, continued use of synthetic acaricideshas occasionally resulted in the appearance of resistance, human health concerns, and undesirable effects on non-target organisms. These problems have aroused interest in the development of alternatives to control these mites, particularly those with contact and fumigant action, which allow applications in indoor living environments.

My lab has been working on screening of more than 1,000 species of medicinal plants, diverse bio-functional compounds isolated from medicinal plants, and structurally related analogues, such as acids, aldehydes, ketones, phenols, and quinones, against *D.farinae*, *D.pteronyssinus,* and *T.putrescentiae*. However, although many studies have been studied, the secondary infection, which is attributed to murdered mites, has been gradually increased. To solve serious problems, the study on development of mite indicator has been first attempted in my lab. Using medicinal plants, which contained many biological activities, we could isolate bio-functional compounds as mite indicators against house dust and stored food mites.

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AL-3

Development of Bio-functional Materials Isolated from Natural Products for Management of Hygienic Insects

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The essential oil and methanol extract of 10 medicinal plants as the bio-functional materials were prepared and tested for their mosquito larvicidal and acaricidal activities. According to preliminary results, the methanol extract of *Tabebuia avellanedae* barks and *Cynanchum paniculatum* root oil showed the strong biological activities against three mosquito larva and domestic mites, respectively. Identification of the active constituents from *T. avellanedae* and *C. paniculatum* was conducted using silica gel column chromatography and high performance liquid chromatography. The structures of active constituents were tested by EI/MS, ^{1}H-NMR, ^{13}C-NMR, DEPT-NMR, COSY-NMR, and HMQC-NMR spectrum, and identified as 2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthalenedione and 2'-hydroxy-5'-methoxyacetophenone, respectively.

Mosquito larvicidal activities of 2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthalenedione and its structurally related derivatives were examined against the fourth instar larvae of *Aedes aegypti*, *Culex pipiens pallens*, and *Ochlerotatus togoi*. On the basis of the 50% lethal concentration (LC_{50}) values against *C. pipiens pallens* larvae, the most toxic compound was 2,3-dichloro-5,8-dihydroxy-1,4-naphthalenedione (0.71 mg/l), followed by 2-bromo-1,4-naphthalenedione (0.98 mg/l), 2,3-dibromo-1,4-naphthalenedione (1.14 mg/l), 1,4-naphthalenedione (1.26 mg/l), 5-hydroxy-1,4-naphthalenedione (1.41 mg/l), 1,2-naphrhalenedione (1.43 mg/l), 1,4-naphthalenediol (3.20 mg/l), 5,8-dihydroxy-1,4-naphthalenedione (3.29 mg/l), 2,3-dichloro-1,4-naphthalenedione (4.56 mg/l), 5-hydroxy-2-methyl-1,4-naphthalenedione (5.37 mg/l), 2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthalenedione (8.30 mg/l), 2-methyl-1,4-naphthalenedione and 2-hydroxy-1,4-naphthalenedione (10.10 mg/l). Similar results against *A. aegypti* and *O. togoi* larvae were observed for 2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthalenedione and its derivatives.

Acaricidal activities of 2'-hydroxy-5'-methoxyacetophenone and its structurally related derivatives against house dust (*Dermatophagoides farinae* and *D. pteronyssinus*) and stored food mites (*Tyrophagus putrescentiae*) were examined using impregnated fumigant bioassay and compared with that of the widely used synthetic acaricide, benzyl benzoate and DEET. On the basis of the LD_{50} values against *D. farinae*, 3'-methoxyacetophenone (0.41 μg/cm^2) was 89.9 times more toxic than DEET (36.87 μg/cm^2), followed by 4'-methoxyacetophenone (0.52 μg/cm^2), 2'-methoxyacetophenone 0.75 μg/cm^2), 2'-hydroxy-5'-methoxyacetophenone (1.03 μg/cm^2), 2'-hydroxy-4'-methoxyacetophenone (1.29 μg/cm^2), acetophenone (1.48 μg/cm^2), 2'-hydroxyacetophenone (1.74 μg/cm^2), 2',5'-dihydroxyacetophenone (1.87 μg/cm^2), 2',4'-dihydroxyacetophenone (2.10 μg/cm^2), and benzyl benzoate (9.92 μg/cm^2). In regard to structure-activity relationships between acaricidal activities and functional radicals (hydroxyl and methoxy groups) on the acetophenone skeleton, a monomethoxy group (2'-, 3'-, and 4'-methoxyacetophenone) on the acetophenone skeleton was more toxic than were the other groups (2',4'- and 2',5'-dimethoxyacetophenone, 2'- and 4'-hydroxyacetophenone). These results indicated that acaricidal activity against three mite

species changed with the introduction of functional radicals (hydroxyl and methoxy groups) onto the acetophenone skeleton.

Accordingly, these results suggest that bio-functional materials isolated from natural products and their derivatives could be useful as the agent for management of hygienic insects.

International Symposia

IS1-1

Functional gene annotation through the development of post-transcriptional silencing mechanism in the coleopteran beetle, *Tenebrio molitor* – Advances and strategies towards pest control

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Post-transcriptional gene silencing (RNAi) is a functional genomics tool that involve the degradation of target mRNA, and is mediated by small interfering RNAs (siRNAs). It has now been thought of as an economical and ecologically sound alternative for pest control and consequently crop improvement. In insects, many targeted studies show that double-strand RNA-mediated silencing of essential genes is an efficient strategy to annotate the functions in innate immunity and development. The responses to RNAi in insect vary from minor effects such as reduction in fitness, cessation in feeding and disruption of functional rhythm to major phenotypic defects manifested in developmental arrest, incomplete metamorphosis to increased mortality against microbial infections. We have been characterizing candidate genes for functions in innate immunity and development, after selecting target genes from cDNA library and transcriptome sequencing of *T. molitor* larvae. Although the analysis at the sequence and structure level has been worthwhile to identify the similarity or diversity of these genes in comparison with their orthologs, the development of RNAi pathway in the insect was crucial for functional gene annotation. It was a challenge to demonstrate the transcriptional silencing process in *T. molitor* attributed to the gene-specific efficiency of knockdown. We have been working on the role of the Toll ligand, spatzle in activation of the downstream Toll pathway and role in melanization of the larvae after gene silencing. It was also very important to note that the silencing of the intracellular cytoplasmic adaptor protein MyD88 is responsible in conferring resistance against Gram-positive and fungal infections. Silencing of NF-κB factor, Relish has shed focus on the antimicrobial peptide genes that can be regulated by the Imd pathway. We have shown that silencing of PGRP-LE leads to an increased susceptibility against an intracellular DAP-type Gram-positive pathogen, *Listeria monocytogenes* but not to Gram-negative *E. coli*. The hemolymph exchangeable protein, apolipophorin-III from *T. molitor* leads to phenotype defects and other abnormalities in the adult after its post-transcriptional silencing in the larval and pupal stages. The results, although seems very opportunistic for application in insect control, are further evaluated for its impacts on marker genes that directly regulate the developmental phenotype in beetles. The goal is to provide the latest advances in the application of RNAi technology towards gene function annotation in the model insect, *T. molitor*.

Keywords: *Tenebrio molitor*, RNA interference, functional genomics, pest control

IS1-2

Nodulation Outer Proteins (Nops) and Soybean-*Sinorhizobium fredii* Interactions

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Soybean has the ability to enter into symbiotic association with certain soil-dwelling rhizobia resulting in the formation of nitrogen-fixing nodules. In addition to the traditional slow-growing soybean symbiont, *Bradyrhizobium* spp., this legume can be nodulated by fast-growing strains of *Sinorhizobium fredii*. Our laboratory is utilizing two strains of *S. fredii*, USDA191 and USDA257, as model systems for the study of extracellular proteins involved in the communication between rhizobia and soybeans. Both strains are capable of nodulating soybean cultivar "Peking", but only USDA191 can nodulate agronomically improved soybean cultivars. The inability of USDA257 to nodulate improved cultivars is governed by a locus that encodes components of a specialized transport system, the type-three protein secretion system (T3SS). Initially isolated from animal and bacterial pathogens, the T3SS is utilized to the deliver effector proteins directly into host cells. USDA257 secretes, through T3SS, several nodulation outer proteins (Nops) when grown in presence of *nod* gene inducing flavonoids. Negation of Nops secretion can reduce, enhance, or have no effect on the number of nodules formed depending upon the host involved. Expression of *nod/nop* genes in *S. fredii* is regulated by flavonoids released by soybean roots in conjunction with the positive activator NodD, which is a member of the LysR-type transcriptional regulator (LTTR) family of proteins. Recent studies have demonstrated that in addition to the positive control provided by NodD, negative regulation of the *nod/nop* genes in *S. fredii* by NolR, a global regulatory factor, also occurs.

IS1-3

New Families of Carboxyl Peptidases: Serine-carboxyl peptidases and Glutamic peptidases

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Right now peptidases (proteinase) are classified into 7 groups based on their catalytic residues (1). Serine-peptidase, Cysteine-peptidase, Metallo-peptidase, Aspartic-peptidase, Threonine-peptidase, Glutamic- peptidase and asparagine-peptidase. Fortunately we succeeded in finding two new families of peptidases, serine-carboxyl peptidase (sedolisins) and glutamic peptidase(eqolisins) in 2001 and 2004, respectively.

Serine-carboxyl peptidase (sedolisins): The first enzyme of this family is sedolisin from *Pseudomonas* sp. The three dimensional structure with inhibitor was solved at high resolution in 2001 (2). The catalytic residues were composed of Ser287, Glu80, and Asp84 residues. Other important amino acid residue was Asp170, which was involved in the formation of oxianion hole. Similar peptidase is widely distributed in nature from Archaea to animals including human beings. One of these enzymes is related to a human fatal hereditable disease, Batten disease.Common features of these enzymes are 1) optimum pH is acidic, 2) the catalytic residues are composed of Ser, Glu, and Asp. Sedolisin family (Sedolisins) was assigned to be a sub-family of serine-peptidase.

Glutamic peptidase (Eqolisin):The first enzyme to be identified as glutamic peptidase is *Scytalido*-carboxypeptidase B from *Scytalidiumlignicolum* ATTC 24568. The structure was unique because it wascomposed of two beta-sheets, which was the first such structure in the peptidase field (3). The catalytic residues were composed of Glu136 and Gln53. The distribution of this family was limited to plant pathogenic fungi. One such enzyme was isolated from a fungal infection in an HIV-infectedpatient.Common features of this family are 1) optimum pH is acidic, 2) the catalytic residues are composed of Glu136 and Gln53. This family was assigned as the 6th family of peptidase as described above.

In this presentation, the background of these findings and their application aspects will be introduced(4).

Keywords:catalytic mechanism, glutamic peptidase, pepstatin, serine-carboxyl peptidase, structure, substrate specificity

References:

1) MEROPS-the peptidase database (http://merops.sanger.ac.uk/)
2) Alexander Wlodawer, Mi Li, Zbigniew Dauter, Alla Gustchina, Kenichi Uchida, Hiroshi Oyama, Ben M. Dunn and Kohei Oda.: Carboxyl proteinase from *Pseudomonas* defines a novel family of subtilisin-like enzymes. ***Nature Structural Biology***, **8**, 442-446 (2001)

3) Masao Fujinaga, Maia M. Cherney, Hiroshi Oyama, Kohei Oda and Michael N.G.James.: The molecular structure and catalytic mechanism of a novel carboxyl peptidase from *Scytalidium lignicolum*. ***Proc. Natl. Acad. Scie***., **101**, 3364-3369 (2004)

4) Kohei Oda.: New families of carboxyl peptidases: serine-carboxyl peptidases and glutamic peptidases, ***J. Biochem***., **151**, 13-25 (2012)

IS1-4

In situ quantitative imaging of cellular lipids using molecular sensors

Young-Dae Yoon

Konkuk University

Membrane lipids are dynamic molecules that play important roles in cell signalling and regulation, but an in situ imaging method for quantitatively tracking lipids in living cells is lacking at present. Here, we report a new chemical method of quantitative lipid imaging using sensors engineered by labelling proteins with an environmentally sensitive fluorophore. A prototype sensor for phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P2)-a key signalling lipid in diverse cellular processes-was generated by covalently attaching a single 2-dimethylamino-6-acyl-naphthalene group to the N-terminal α-helix of the engineered epsin1 ENTH domain, a protein that selectively binds PtdIns(4,5)P2. The sensor allows robust and sensitive in situ quantitative imaging in mammalian cells, providing new insight into the spatiotemporal dynamics and fluctuation of this key signalling lipid. Application of the sensor to immune cells reveals the presence of a local threshold PtdIns(4,5)P2 concentration required for triggering phagocytosis. This sensor strategy is generally applicable to in situ quantification of other cellular lipids.

IS1-5

Molecular Mechanisms and Functions of Keap1-Nrf2 system

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Department of Medical Biochemistry, Graduate School of Medicine, Tohoku University, Japan

Nrf2 is a transcription factor that regulates gene expression of detoxifying and antioxidative enzymes. Keap1 serves as a substrate adaptor for Cullin3-based ubiquitin E3 ligase and promotes proteasomal degradation of Nrf2. Oxidative and electrophilic stimuli attack reactive cysteine residues of Keap1 to attenuate the degradation of Nrf2. Nrf2 activation is not essential for survival, but plays an important role in cytoprotection. Interestingly, sustained activation of Nrf2 is found in various human cancers. Disrupted interaction of Keap1 and Nrf2 cannot degrade Nrf2 any more to accumulate it in the nuclues. Cancer cells utilize Nrf2 for not only chemoprotection but also cellular proliferation. In *Keap1* knockout mouse, constitutively activated Nrf2 proliferates keratinocytes and causes abnormal hyperkeratosis in forestomach and esophagus. However, there is no apparent abnormality in the liver, which is mostly consisting of hepatocytes. As hepatocytes are quiescent cells, we hypothesized that Nrf2 exerts a pathological function under the proliferating conditions. We focused on *Pten*, a tumor suppressor gene, whose deletion leads to Akt activation. The liver-specific *Pten* and *Keap1* double knockout mouse displayed robustly elevated expression of Nrf2 target genes and increased accumulation of Nrf2 in the nucleus. Intriguingly, Akt phosphorylation resulting from the *Pten* deletion was enhanced in the double knockout mice. These results indicate that presence of reciprocal activation of Nrf2 and Akt signaling in the liver. Here, we report that Nrf2 activation is regulated by Keap1 in the cytoplasm and subsequently by Gsk3 in the nucleus.

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IS2-1

Search for Potent Bioactive Compounds from Ethnobotanically-Selected Plants in Indonesia

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ABSTRACT

Plants having long history in traditional health care application represent an understandable choice for scientifically investigation. It is fascinating to determine whether the traditional uses are supported by actual biological effects or merely based on folklore. In the present study, plants currently used by several indigenous tribes as folk medicine in East Kalimantan, Indonesia were investigated their biological activities. The selected plants were extracted with methanol or ethanol in room temperature. The alcoholic extracts obtained were subjected to antimicrobial, antioxidant, xanthine oxidase and acute toxicity. Results of the bioassays displayed potential activity *Sonneratia caseolaris* and *Eleutherine americana*. Bioassay-guided isolations of the active compounds from the two plants were reported here.

Key words: antifungal activity, bioassays, dermatophyte, East Kalimantan, phytochemical study, plant extracts.

INTRODUCTION

Tropical forest of Indonesia covers 110 million hectares occupied by about 80% of world medicinal plants. It is estimated that in the Indonesian tropical forest, more than 28.000 plant species exist and 1.000 species of them have been known and already used for medicinal purposes (Pramono, 2002). In general, investigation into the efficacy of Indonesian medicinal plants is still limited on folklore information of the utilization of the plants by local people. East Kalimantan is an island in Indonesia having a huge tropical forest along with Sumatera and Papua. The plant biodiversity exist in the island promises high potential of biological activities. In the course of our search into natural antifungal agents, the present paper reports the potential of some plants selected on the basis of ethnobotanical information in East Kalimantan, Indonesia to possess antimicrobial, antioxidant and

xanthine oxidase inhibitory activities. Our exploration into medicinal plants used by the Tunjung tribe in West Kutai, East Kalimantan, Indonesia showed that about 72 plant species, mostly collected from forest, have been used by the people as traditional health care. Application of the plants by the local people including for treating skin infection, diabetic, health supplement, liver diseases and so on. Based on the screening, we reported here the biological activities and the isolated active compounds from two potent plants, *Sonneratia caseolaris* and *Eleutherine americana*.

Sonneratia caseolaris

In East Kalimantan, Indonesia, the leaves and the fruits powder of *Sonneratia caseolaris* are used for skin treating talcum powder by the Paser tribe. Extracts of this plant are traditionally used as an astringent and antiseptic, in sprains and swellings, and in arresting hemorrhage. Our study was undertaken to determine the antioxidant and antimicrobial activities and pyhytochemicals of *S. caseolaris* growing in East Kalimantan. The antioxidant activity was evaluated with a DPPH radical scavenging assays. The antimicrobial activity was tested against *Salmonella typhi*, *Bacillus cereus*, *Staphylococcus aureus*, *Aspergillus niger*, *Trichophyton mentagrophytes* and *Candida albicans*. The antioxidant activity of the aqueous and ethanolic extract (up to 94%) was similar to that of ascorbic acid (96%) used as a positive control. The antimicrobial assays informed that only ethanolic extracts possessed moderate to strong activity against *T. mentagrophytes*, *B. cereus* and *S. typhi*, in comparison to those of miconazole and terramycin, indicating the potential of the respective extracts to prevent or control the pathogenic microbial infection. Bioassay-guided isolation yielded luteolin-7-*O*-glucoside from the plant. The results bioactivity assays and phytochemical analysis show that *S. caseolaris* is potentially a rich source of biologically active compounds and are worthy of further study.

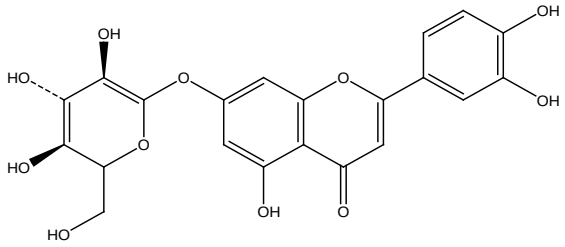

Fig 1. Chemical structure of luteolin-7-*O*-glucoside from *S. caseolaris*.

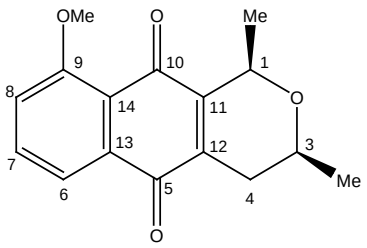

Fig 2. Chemical structure of eleutherine from *E. americana*.

Eleutherine americana

Bawang tiwai (*Eleutherine americana* L. Merr.) is a small plant from Iridaceae, a botanical family that comprises 90 genera and about 1200 species (Schultes and Rafauf 1990). In Indonesia, the rhizome of this plant has long been used as a medicinal plant to cure coronary disorder, hepatitis and genital diseases. The rhizome of this plant has long been used as a folk remedy for coronary disorders. Bioassay–guided fractionation of *n*-hexane soluble fraction in the methanolic extract of the bulb of the plant led to the isolation of an active compound. Identification of the compound done by EI-MS, ^{1}H, ^{13}C and two-dimensional NMR analyses elucidated the compound as eleutherin. The compound showed potent antibacterial, antifungal and melanin inhibitory activity, in vitro, with relatively low concentration in comparison to standard compounds.

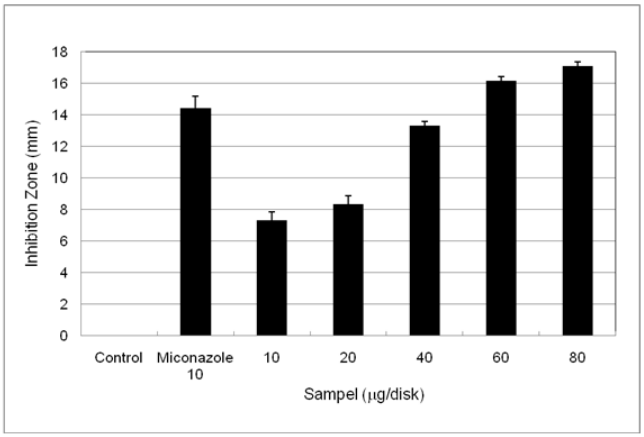

Fig 3. Antidermatophyte of eleutherin against *T. mentagrophytes*.

AKNOWLEDGMENT

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IS2-2

Food Allergens: Current Analytical Status, Challenges and Future Needs

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Foods are consumed for their nutritional values, but occasionally, they cause adverse reactions to a small population of consumers. Adverse reactions to foods are called food sensitisation, which can be divided into two general categories based on immunological responses. Those reactions involving immune responses is termed food allergy, which is mediated by immunoglobulin E (IgE). Those which do not involved immune responses are generally termed food intolerance.

Food allergy is no longer an individual's health conditions, but is becoming a global public health issue. Prevalence of food allergy has risen significantly and in some cases, such as peanut allergy, has doubled in the two decades. Prevalence of food allergy is higher in children than in adults. Approximately 1 in 16 children and 1 in 50 adults suffer from at least one form of food allergy. Despite the research efforts to develop cure for food allergy, the most effective medical treatment is still total avoidance of offending foods. Such treatment is not ideal as it could lead to malnutrition of certain micronutrients.

For allergy sufferers, making food choice is not anenjoyable experience, as for healthy individuals, but it is a burden that they and people associated with them have to live with. Accidental assumption of problem foods could lead to unpleasant allergic reactions on skin, gastrointestinal and respiratory systems, and in worst cases could develop into life-threatening anaphylactic reactions. To enable allergy sufferers to make informed choice and avoid offending foods in pre-packaged products, several regulatory agencies around the world adopted the CODEX guideline of mandatory labelling of major food allergens. The mandatory labelling of food allergens has been implemented in Australia, New Zealand, the United State, Canada, European Union, Japan and Korea. More recently, Hong Kong, China and Malaysia joined this initiative. The allergens to be labelled include the 8 major allergens, namely peanut, tree nuts, cow's milk, eggs, crustaceans, fish, soybean and cereals (gluten), and country-specific allergens such as seasame seeds, mustard seeds, celery, lupin Buckwheat, mollusks and sulfur dioxide/ sulfites. The list of food allergens for mandatory labelling no doubt will be growing in number as new allergensare discovered and characterised.

It is sometimes unavoidable during the food processing that allergic ingredients of one food product are included advertently in another non-allergenic food product. This is called cross contact and the allergen residue then becomes a hidden or undeclared food allergen in the food product because the food manufacturer fails to declare its presence. Presence of such undeclared food allergens in food products poses significant health risks to allergy sufferers. For food manufacturers, accurate labelling of food allergens on their products is a great burden. Increasing risks of undeclared food allergens may be posed by food products imported from countries which lack food allergen labelling policy.

Accurate detection of food allergen residues in pre-packaged food productsis therefore critical for effective risk management of food allergens in manufacturing premises. Except for sulphur dioxide/sulphite, allergens present in the regulated food ingredients are protein in nature. Not all of the proteins in these ingredients are allergenic, but several allergenic proteins are usually present in each ingredient. Analytical techniques suitable for food allergen detection can be divided into three categories based on their analytical principles. Currently, immunoassays for the most food allergens under regulations are commercially available and are the most accessible technique for food analytical services and food manufacturers. These assays are developed based on antibodies raised against either specific allergens or protein mixtures of allergic ingredients. The second category is DNA-based assays where DNA markers of allergenic proteins in food products are targeted. They are also available commercially for several major food allergens, however, are not used as widely as immunoassays for real world applications. More recently, mass spectrometry has been emerged as a technique that could circumvent the limitations of both antibody-based and DNA-based assays, which also allow for multi-allergen detection.

This paper will present food allergen detection for risk management; challenges of food allergen detection, overview of various techniques suitable for food allergen detection, development of immunoassays with a case study on peanut allergens and effects of food processing of food allergen detectability.

IS2-3

Understanding the mode of action of genotoxic agents from natural products using a human disease model

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A majority of cytotoxic chemotherapy agents have been identified from natural products. Among the agents are mitomycinC(MMC), which induces interstrand crosslink DNA damage, and camptothecin(CPT), a topoisomerase I inhibitor, both of which showed remarkable antitumor activities and thus have been used for cancer chemotherapy. Understanding the mode of action of those cytotoxic agents is critical not only for improving chemotherapy effects, but also for reducing the side effects including chemoresistancy. In this study we employed human disease model, Fanconi anemia, to investigate how human cells response upon treatment of MMC and CPT. Fanconi anemia is a rare recessive disorder characterized by genome instability, congenital malformations, progressive bone marrow failure and predisposition to hematologic malignancies and solid tumors. At the cellular level, hypersensitivity to DNA interstrand crosslinks is the defining feature in Fanconi anemia. Mutations in fourteen distinct Fanconi anemia genes have been shown to interfere with the DNA-replication-dependent repair of lesions involving crosslinked DNA at stalled replication forks. We found biallelic SLX4 mutations in two individuals with typical clinical features of Fanconi anemia and showed that the cellular defects in these individuals' cells are complemented by wildtype SLX4, demonstrating that biallelic mutations in SLX4 (renamed here as FANCP) cause a new subtype of Fanconi anemia, Fanconi anemia-P. SLX4 interacts with multiple nucleases including XPF-ERCC1, MUS81-EME1 and SLX1 and is implicated in repairing DNA damage induced by MMC and CPT, and in Holliday junction resolution. In an attempt to clarify the requirement for the specific nucleases in various functions of SLX4/FANCP, we complemented a SLX4 null human fibroblast cell line with SLX4/FANCP mutants that specifically lack the interaction with each of the nucleases. We showed that the SLX4-dependent XPF-ERCC1 activity is essential for ICL repair but is dispensable for repairing TOP1 inhibitor-induced DNA lesions. Conversely, MUS81-SLX4 interaction is critical for resistance to TOP1 inhibitors but is less important for ICL repair. Mutation of SLX4 that abrogates interaction with SLX1 results in partial resistance to both cross-linking agents and TOP1 inhibitors. These results demonstrate that SLX4 modulates multiple DNA repair pathways by regulating appropriate nucleases.

IS2-4

Status, progress, and future direction of natural products chemistry research in Thailand

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Thailand is well-known for its richness of medicinal herbs, plant-based natural products. Thai natives have used local plants as their foods and traditional medicine in their daily life and activities. However, chemistry of natural products in Thailand started by 1950 in Chulalongkorn University (CU) and at the beginning it was slowly growing and reported by chemists and pharmacists. At the present chemistry research of natural products has been focused in all Thai universities and institutions, mainly in Mahidol Univerisity, Chulabhorn Research Institute (CRI), CU and Chiang Mai University. Most of the research field comprises of active ingredients of compounds isolated from medicinal herbs, agricultural products, local plants and marine products and microorganisms. A significant number of their chemical structures have been elucidated and many of which have been patented. Several novel chemical structures and biological activities of natural compounds have been reported. The research in the field of natural products is to search for plants and active chemicals for further development into new drugs used in various diseases. Since natural products offer a diversity and complexity of structure, research aims into the chemistry of natural products and chemotaxonomy of plants for new value-added products, with useful medicinal and chemical properties. The main activities in the most laboratories of natural products have focused on plant constituents which can be employed as precursors in the production of pharmaceuticals. Recently, CRI scientists have become interested in research in marine organisms for novel bioactive compounds with potential medicinal applications. However, total syntheses of biologically interesting natural products are also being extensively investigated by Thai chemists. A large amount of the biologically active compounds such as antioxidants, anti-inflammatory agents, cytotoxicants have been studied for their pharmacological effects and safety uses. Some examples of complete profile of active natural compounds will be presented and discussed at the KSABC Symposium.

Keywords: Thai medicinal herbs, plant-based natural products, active ingredients, Thai universities

IS2-5

LC-MS based high throughput screening of natural products: one step screening without fractionization

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High throughput screening (HTS) was first introduced in 1970s and has been practically used in most big pharmaceuticals since 1990s because of the need to screen millions of library compounds for drug discovery. Most conventional HTS methods based on a spectrometric detection, however, have a few drawbacks upon applying for screening complex natural products which require repeating subfractionization steps to find bioactive compounds and sometimes have a detection interference due to self-absorbance or emission of natural products, themselves.

A mass spectrometric screening method, including affinity based LCMS technique can be practically used to find bioactive compounds in crude natural products without fractionization and directly or indirectly provide information about chemical I.D of the bioactive compounds simultaneously. In this presentation, a high throughput ultrafiltration LC-MS technique will be introduced to screen natural products for bioactive compounds targeted to various macromolecular proteins and enzymes. In addition, development and suitability of a cell based anti-inflammation screening method using 2D LCMS method will be presented and show the screening capability in natural product.

IS3-1

Development of a new biocontrol agent using the combination of *Bacillus velezensis* G341 and *Lysinibacillus sphaericus* TC1 for the control of tomato wilt caused by *Ralstonia solanacearum*

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During our study on the development of microbial pesticides for the control of bacterial wilt, we found that the mixture of *Bacillus velezensis* G341 and *Lysinibacillus sphaericus* TC1 effectively suppressed the development of tomato bacterial wilt caused by *Ralstonia solanacearum*. In *in vitro* test, the fermentation broth of strain G341 effectively inhibited growth of *R. solanacearum.* This effect was attributed to the production of diffusible antibacterial compounds. The diffusible antibiotics were isolated and identified *via* mass spectrometry as difficidin and oxydifficidin. Both Gram-positive and Gram-negative bacteria tested were sensitive to two compounds, with *R. solanacearum* being the most sensitive one with minimum inhibitory concentration of 6.25-50 mg/l, respectively. In addition, bioassay in sealed dishes revealed that the strain G341 significantly inhibited the cell growth of *R. solanacearum*. The antibacterial volatile compounds were identified by gas-chromatography mass spectrometry. The detected volatile compounds included dimethyldisulfide, 1-butanol and 3-hydroxy-2-butanone(acetoin). In comparison, *L. sphaericus* TC1 did not produce any agar diffusible antibacterial substance, but did volatile compounds such as tetrachloriethylene, 3-methyl-1-butanol and methylpyrazine. When the two strains were co-incubated in fermentation broth, the antibacterial activity of the fermentation broth was stronger than that of G341 alone. In *in vivo* test, the combination of fermentation broths of the two strains showed synergistic effect. The new biocontrol agent also effectively controlled the development of tomato in various fields. The results suggested that the new biocontrol agent can be used as a potential tool to control bacterial wilt disease in tomato and other crops as an eco-friendly manner.

IS3-2

The scent of love and hate: Induced resistance by gaseous compounds

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Plant-microbe interaction is like a never-ending story referred to as “arms racing”. Microbes have been developed to overcome plant defense mechanisms. By contrast, plants have evolved general and specific defense mechanisms to protect themselves from diverse enemies, including pathogens and even herbivores. To maintain fitness in the presence of enemies, plant defense mechanisms are aimed at inducing systemic resistance: in response to the attack of pathogens or herbivores, plants initiate extensive changes in gene expression to activate “systemic acquired resistance” against pathogens and “indirect defense” against herbivores. Systemic acquired resistance (SAR) is a plant self-defense mechanism against a broad range of pathogens and insect pests. First, I will present that that bacterial volatile elicit plant basal immunity called as “induced systemic resistance”. In Arabidopsis and tobacco, bacterial volatiles stimulated systemic defenses against necrotorphic and biotrophic pathogens, which was confirmed by the detection of the systemic expression of pathogenesis-related genes in response to ethylene and cytokinin-signaling pathway activation. Further investigation revealed that specific volatiles such as 2,3-butanediol and acetoin produced from rhizoshere bacteria help host plant secrete certain root exudates for inhibiting deleterious bacteria but for increase the population of 2,3-butanediol producing bacteria resulting to recruit 2,3-butanediol producers around roots. At the same time, the 2,3-butanediol is capable to augment plant systemic defense against leaf pathogens. Secondly, among chemical SAR triggers, plant and bacterial volatiles are promising candidates due to high effectiveness and the fact of being cheap chemicals with relatively low concentrations compared to agrochemicals. However, before large scale application in agriculture to manage diseases, high evaporation rates after application, plant growth alteration, and inconsistent effectiveness need to be considered as major pitfall. In this study, we provide a new evidence of volatile organic compound (VOC)-mediated SAR against both a bacterial angular leaf spot pathogen *Pseudomonas syringae* pv. *lachrymans* and a sucking insect aphid *Myzus persicae* in the open field of cucumber and against *Xanthomonas axonopodis* pv. vesicatoria and *Cucumber mosaic virus* in pepper without changing plant growth until harvesting. Unexpectedly, the drench of two VOCs, 3-pentanol and 2-butanone to seedlings caused significant increase of numbers of ladybird beetle that is known as a natural enemy of aphid in cucumber plants and reduced disease severity of *X. asonopodis* pv. vesicatoria and CMV in pepper as well as increase of fruit yield. The defense-related genes was induced in volatile treated plants indicating to trigger oxylipin pathway responding in the emission of green leaf volatile that recruit the natural enemy in cucumber and to elicit salicylic acid pathway. Our results provide new evidence that bacterial volatile-mediated induction of plant immune responses are more common than expected and new resources to prevent plant diseases and insect damage even in the open field.

IS3-3

Construction of insect cuticle through oxidation reactions of phenolic compounds by laccase system: its physiological significance and application to industrial usage

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Insect cuticle is a non-cellular matrix that is composed of chitin and chitin binding proteins. Normally the new cuticle being constructed beneath the old cuticle is soft and colorless, but is hardened and pigmented in stage dependent manner. Here we focus on the molecular mechanism that is involved in cuticle hardening and coloration process, especially regulation of an enzyme that catalyzes oxidation reaction of phenolic substances. This enzyme, laccase, is a key enzyme in this process, and it has been shown that the insect cuticle cannot be constructed properly without this enzyme. In the newly formed cuticle, laccase activity is very weak or negligible, but is activated after ecdysis. Here the possible mechanisms on regulation of laccase activity will be discussed on the analyses of the silkworm, *Bombyx mori* and the fruitfly, *Drosophila mekanogaster*. It will be also mentioned about future application of this system to make new biomaterials.

IS4-1

The importance of glycemic index in the management of diabetes and obesity

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Diet plays an important role in our lives.It plays an even more significant role in diabetes and obesity. There has been a dramatic increase in our understanding of how major chronic diseases such as diabetes, hypertension, cardiovascular disease and cancer are all associated with diet. The science of nutrition is the confluence of two major interests in our society-food and health. Growing demand for products and food ingredients that help reduce the risk of developing chronic diseases are sometimes known as ‘functional foods’. Food Chemists have typically categorised dietary carbohydrates into simple sugars and complex carbohydrates on the basis of their degree of polymerisation. This form of classification of Carbohydrates is a well-established concept in Food Science. However, the effect of Carbohydrate on health may be better categorised according to their physiological effects, notably their ability to raise blood glucose. The blood glucose response varies substantially among different carbohydrate containing foods and cannot be predicted by their gross chemical composition alone. This concept is now defined as the Glycaemic Index (GI). Using clinical studies, epidemiological observations and intervention trials, the presentation will highlight how foods can improve glucose control, increase satiety and minimise the risk of adipose tissue accretion, reduce blood pressure, Cholesterol and reduce oxidative damage. The recognition that the consumption of certain foods can elicit similar health benefits as those from drug interventions, presents a challenge on how to translate nutritional studies into practical advice to the wider community.

IS4-2

Antioxidant and Antigenotoxic Activity of Commonly Consumed Fruits and Vegetables in Korea

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Epidemiological studies have shown that fruits and vegetables are widely considered as essential components of a healthy diet and increasing their consumption is a practical approach for chronic diseases prevention, such as cancer, coronary heart disease, and diabetes. Fruits and vegetables contain large amount of natural antioxidant, such as vitamin C, E, flavonoids, and phenolic acids, which might confer these health protective benefits by interfering passively with oxidative damage to DNA, lipids and proteins. The objective of this study was to determine the total phenolic content (applying the Folin assay) and to assess the antioxidant capacities using DPPH radical scavenging activity and oxygen radical absorbance capacity (ORAC), and cellular antioxidant activity (CAA) of 19 fruits and 40 vegetables commonly consumed in Korea, which were selected on the basis of annual consumption per capita data. Also, the antigenotoxic activity was measured using single cell gel electrophoresis (comet) assay. The individual contributions to the antioxidant activity of the fruits and vegetables in the Korean diet were calculated. Orange, tangerine, strawberry, grape, plum and Japanese apricot showed higher total polyphenol contentand antioxidant activities among fruits, whilstgarlic, lotus root, Chinese chive, perilla leaf, mugwort, and onion showed higher antioxidant activities among vegetables. Tangerines and onionswere found to be the largest contributors of phenolics and ORAC values to the Korean diet. As fruits and vegetables are rich sources of diverse antioxidants, efforts to promote consumption of a variety of fruits and vegetables should be continued for public health benefits.

Symposia

S1-1

Epigenetic regulation of gene expression in plant seedling development

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We have previously isolated a rice gene encoding a histone deacetylase, *OsHDAC1*, and observed that its transgenic overexpression increases seedling root growth. To identify the transcriptional repression events that occur as a result of *OsHDAC1* overexpression (*OsHDAC1*^{OE}), a global profiling of root-expressed genes was performed on *OsHDAC1*^{OE} or HDAC inhibitor-treated non-transgenic (NT) roots, in comparison with untreated NT roots. We selected 39 genes that are induced and repressed in HDAC inhibitor-treated NT and *OsHDAC1*^{OE} roots, compared with NT roots, respectively. Interestingly, *OsNAC6*, a member of the NAM-ATAF-CUC (NAC) family, was identified as a key component of the OsHDAC1 regulon, and was found to be epigenetically repressed by *OsHDAC1* overexpression. The root phenotype of *OsNAC6* knock-out seedlings was observed to be similar to that of the *OsHDAC1*^{OE} seedlings. Conversely, the root phenotype of the *OsNAC6* overexpressors was similar to that of the *OsHDAC1* knock-out seedlings. These observations indicate that OsHDAC1 negatively regulates the *OsNAC6* gene that primarily mediates the alteration in the root growth of the *OsHDAC1*^{OE} seedlings. Chromatin immunoprecipitation assays of the *OsNAC6* promoter region using antibodies specific to acetylated histones H3 and H4 revealed that OsHDAC1 epigenetically represses the expression of *OsNAC6* by deacetylating K9, K14 and K18 on H3 and K5, K12 and K16 on H4.We had also reported on the organellar localization of three rice HDACs, OsSIR2b, OsHDAC6, and OsHDAC10. The 35S:*OsSIR2b-GFP* and 35S:*OsHDAC10-GFP* constructs were introduced into tobacco BY2 cells. Co-localization analysis of the green fluorescent protein and MitoTracker fluorescent signals in the transformed BY2 cells indicated that OsSIR2b and OsHDAC10 are localized in the mitochondria. Transgenic Arabidopsis lines harboring 35S:*OsHDAC6-GFP* and 35S:*OsHDAC10-GFP* constructs were similarly analyzed, revealing that OsHDAC6-GFP is localized exclusively in chloroplasts, whereas OsHDAC10-GFP is localized in both mitochondria and chloroplasts. The localization of HDACs in the chloroplasts and mitochondria implies their roles in central metabolic pathways including photosynthesis.

Recently, epigenetics has been extended toMicroRNAs (miRNAs).They are a class of non-coding small RNAs ranging in size from 21 to 24 nucleotides (nt) found in plants, animals and other eukaryotes, which function in transcriptional and post-transcriptional regulation of target genes expression via target mRNA degradation or translational repression. Expression of many plant miRNAs is responsive to environmental stimuli but none has yet been associated with light. Arabidopsis *miR163* is 24 nucleotides in length and targets genes encoding several S-adenosylmethionine-dependent carboxyl methyltransferase (SAMT) family members. We had confirmed that *miR163* is light-inducible and predominantly expressed in roots during seedling etiolation as well as seed germination. Whereas under the same condition its target *PXMT1*, a target of miR163 and a

methyltransferase that methylates 1,7-paraxanthine, was found not only to be expressed in roots at the same developmental stages but also to be down regulated by light. The repression of *PXMT1* by light was abolished in a *miR163* knockout mutant; however, the repression was restored to wild-type levels in complementation line expressing *miR163* gene in *miR163* mutant background. Analysis of *miR163* mutant and transgenic plants reveals that *PXMT1* mRNA levels were inversecorrelated by changes in *miR163* levels. Finally, we found the function of *miR163* at the early stage of light response is to promote seed germination through targeting of PXMT1. We found that *miR163* and its target *PXMT1* were predominantly expressed in radicle during seed germination and the expression pattern of the two genes are inversely correlated. Moreover, compared with wild-type, *miR163* mutant and *PXMT1*-overexpression line showed delayed radicle emergence rate during seed germination under continuous light. This *miR163* mutant phenotype can be complemented by expression of 35S: pri-*miR163*. Together, our results indicate that light-inducible *miR163* targets *PXMT1* mRNA to promote seed germination in Arabidopsis.

S1-2

Chitinase 7 (TcCHT7) is required for cuticle lamina organization and chitin deposition in *Tribolium*

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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 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). The CHTs belonging to different groups have distinctly different developmental patterns of expression and tissue specificity, suggestive of distinct biological functions.

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. 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 form the plasma membrane of epidermal cells by proteolysis. Chitin seems to accumulate within the assembly zone of the elytral and body wall cuticle in ds*TcCHT7*-treated animals. Transmission electron microscopy revealed that down-regulation of *TcCHT7* transcripts resulted in disorganization of chitin laminar and vertical canals in the procuticle. These results suggest that TcCHT7 may 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

S1-3

RNAseq and expressed sequence tag based identification of autophagy-related genes and expression analysis of TmTOR gene 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. Autophagy manifests itself as macro-, micro- and chaperone-mediated autophagy (CMA). Microautophagy is known to activate by the digestion of cytosolic proteins using autophagic tube in mammalian or vacuolar membrane in plant or fungal systems. In CMA cytosolic proteins gets recognized by chaperone-co-chaperone complex, that subsequently interacts with lysosome-associated membrane protein (LAMP) type 2A, eventually digesting the cytosolic proteins. Macroautophagy, in more general terminology is called autophagy, and is a well-known digestion process that includes target molecules for recognition, phagophore formation to transfer membrane particles from mitochondria, golgi or endoplasmic reticulum, autophagosome formation by initiation, nucleation and elongation steps, and autolysosome formation. Autophagy signals can be induced by extra- or intracellular stressors and signals such as starvation, growth factor deprivation, ER stress, and pathogen infection. It is well known that the autophagy signal components are well conserved. In this study, we highlight a comprehensive set of *T. molitor* autophagy-related genes using transcriptome sequencing and EST analysis that may operate at different levels in the autophagic process. We analyze the autophagy-related genes from *T. molitor* by a holistic comparison with *D. melanogaster* and *H. sapiens* autophagy genes. Transcript analysis of TmTOR and Western blot analysis of TmAtg8 (marker protein for autophagy), revealed that autophagy was induced during pupation, but the expression level of TmTOR is decreased. However, with *Listeria monocytogenes* challenge in *T. molitor*, autophagy was induced but, TmTOR was not decreased. We speculate that TmTOR may negatively regulate developmental autophagy, but not pathogen induced autophagy.

Key words: Autophagy signaling, EST, RNA-seq, *Tenebrio molitor*

S2-1

Gamma-mangostin, a novel c-Met inhibitor, suppresses cell proliferation of gastric cancer

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Among many cancer therapeutic targets, c-Met has recently given particular attention. This kinase play a critical role in cell proliferation and the survival of several human cancers. Thus, we developed γ-mangostin as a novel c-Met inhibitor and investigated its anti-cancer effects on human gastric cancer. γ-mangostin inhibited the phosphorylation of c-Met and its constitutive downstream effectors such as cyclin D1, c-Myc, CD44, STAT3, AKT, and ERK. γ-mangostin was found to anti-cancer agent on gastric cancer cells, especially cell lines that overexpressed c-Met. Apoptosis induced by γ-mangostin was accompanied by increased levels of cleaved caspase-3 and PARP. These findings indicate that γ-mangostin may exert anti-cancer effects by directly affecting cancer cell growth or survival via the c-Met pathway. We therefore suggest that γ-mangostin is a novel therapeutic candidate effective against gastric cancers that overexpress c-Met.

S2-2

Biological active materials from the roots of *Oryza sativa* L.

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Rice (*Oryza sativa* spp *japonica*) is one of the most popular staple crops, and is widely cultivated for human consumption. Each year, 4 megatons of rice are produced in Korea, which subsequently results in the production of 600,000 tons of rice roots. The utilization of rice roots provide additional benefits to farmers, as until now the roots have been used only as agricultural by-product waste for composting. Some secondary metabolites from rice roots can be used as consumable products in pharmaceutical applications as well as in cosmetics. Since rice plants grow in water-logged conditions during a majority of their life cycle, the roots produce and exude several metabolites that show growth inhibitory activity against neighboring plants, including weed species. Furthermore, rice is reported to exude indoles, diterpenes, and phenolic acids with phytoalexin properties. However, the metabolites produced by rice roots have been studied very little. Therefore this study was initiated to identify other active metabolites of rice root. The roots of *O. sativa* were extracted with 80% aqueous MeOH at room temperature. And the concentrated extract was partitioned with EtOAc, *n*-BuOH and H_2O, respectively. The repeated silica gel, octadecyl silica gel (ODS), Sephadex LH-20 column chromatographies for EtOAc and *n*-BuOH fractions, led to isolation of six new compounds along with 19 known ones, and the chemical structures were determined based on spectroscopic data analyses. These compounds were isolated for the first time from the *O. sativa* root. Moreover, the isolated metabolites were evaluated for their biological activities, such as their anti-inflammatory, tyrosinase inhibition, and anti-melanogenic effects. Among compounds, diterpenoid, oryzalexin A inhibited LPS-stimulated NO production in RAW264.7 macrophages with IC_{50} value of 15.0 μM. Phenyl compounds, *trans-p*-coumaric acid methyl ester and *N*-(trans-cinnamoyl)tryptamine strongly inhibited the production of extracellular melanin (IC_{50} 17.7 and 19.6 μM, respectively).

S3-1

Analyses and Decreasing Patterns of Veterinary Antianxiety Medications in Soils

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An ultrasonic-assisted extraction method was developed to detect 16 antianxiety medications in soil samples using liquid chromatography–high resolution mass spectrometry (LC-HRMS), Orbitrap mass spectrometer. The determination method resulted in satisfactory sensitivity, linearity, recovery, repeatability, and within-laboratory reproducibility. Acepromazine, azaperone, and xylazine were incubated in control, amended, and sterilized soils. The amendment with powdered blood meal affected the relatively fast dissipations of acepromazine, azaperone, and xylazine in the soils. Dissipation kinetics of acepromazine were consistent with bi-phasic kinetics (first-order multi compartment) and the other couples were fit to single first-order kinetics. A hydroxylated acepromazine was identified from soil samples using Orbitrap mass spectrometry. According to sorption batch experiments, the adsorption of acepromazine and azaperone was greatly high, whereas that of xylazine was relatively low. Xylazine was persistent in the incubated soils, and acepromazine demonstrated fast initial dissipation; hence, xylazine could have a potential harmful effect on the environment.

S4-1

Preliminary survey on the worrisome organic contaminants in Korean sundried salt

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Although sundried salt has been classified to food material since 2008, the safety guideline is not broadly considered on number of organic pollutants. In here, we present the preliminary screening result of 24 persistance organic pollutants (POPs), 16 polycyclic aromatic hydrocarbons (PAHs), 256 pesticides, 3 alkylbenzene and 6 phthalates in Korean sundried salt. POPs and pesticides were not detected on the considered safety guideline level. On the other hands, some samples were detected BTEX, PAHs and phthalates on the environmental range. Consequently, BTEX, PAHs and phthalates could be monitored the residual concentration and established their safety guideline for sundried salt.

S5-1~4

Speaker :

Lectures	Speaker	Affiliation
S5-1	권형주	한림대학교
S5-2	박해준	씨젠
S5-3	임형권	목암연구소
S5-4	정문섭	VGX Int

Abstract : 추후 안내 예정