

**2019**  
**International Symposium**  
**and Annual Meeting**  
**of the **KSABC****



**Plenary**  
**Lecture**



**PL-1**

## **Determination of toxicodynamic differences of sodium channel isoforms to pyrethroids in juvenile and adult rat brain tissue microtransplanted into *Xenopus* oocytes**

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Microtransplantation of mammalian neurolemma is a tool to examine the endogenous structure and function of transmitter receptors and ion channels associated with the central nervous system. Microtransplanted neurolemma can originate from a variety of sources, possess ion channels and receptors in their native configuration, and are applicable to examine diseases associated with different channelopathies. In these experiments, we examined the age-related differences in VSSC expression and concentration-dependent responses to pyrethroids in juvenile and adult rat brain tissue microtransplanted in *Xenopus* oocytes. Automated western blotting results indicate that adult neurolemma exhibited 2.5-fold higher level of expression of VSSCs compared with juvenile neurolemma when normalized to the housekeeping protein  $\beta$ -tubulin. The predominate isoform expressed in both tissues was  $\text{Na}_v1.2$  with both showing a significant difference from zero. Adult neurolemma, however, expressed 2.8-fold more  $\text{Na}_v1.2$  than juvenile and also express  $\text{Na}_v1.6$  at a higher level (2.2-fold). In addition, neurolemma tissue microtransplanted into *Xenopus* oocytes showed reconstituted native ion currents in the plasma membrane of oocytes that was sensitive to TTX and abolished by choline ion replacement, functionally demonstrating the presence of VSSC. Increasing concentrations of permethrin and deltamethrin exhibited concentration-dependent increases in TTX-sensitive current from both adult and juvenile tissues. Concentration-dependent response curves were analyzed using the equivalence test and the slopes of the curves were different ( $p < 0.05$ ). VSSCs associated with juvenile neurolemma were up to 2.5X more sensitive to deltamethrin than VSSCs in adult neurolemma. In contrast, VSSCs from juvenile neurolemma were less sensitive than adult VSSCs at lower concentrations (0.6-0.8X) and more sensitive at higher concentrations (up to 2.4X). However, because the expected brain concentrations in humans following realistic exposure levels are approximately 21- (deltamethrin) to 333- (permethrin) times below the threshold for response in rat neurolemma, age-related differences, if any, are not likely to be toxicologically relevant.

**2019**  
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**Special**  
**Lecture**



**SL-1**

## **Policy of Research Grant Support by Division of Life Sciences NRF**

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In order to support basic research innovation for the next five years, the 4th basic research promotion comprehensive draft plan was established by Ministry of Science and Technology (MOST). Comprehensive plans are based on the following 10 key ideas. ① Provide a balanced support to all competent researchers. ② Support creative ideas freely. ③ Challenge the world's best research. ④ The long-term perspective rather than the immediate results. ⑤ Balanced support to ensure that there are no areas to be neglected. ⑥ Prepare conditions for young researchers, who will be the future leaders, to grow. ⑦ Create an environment that can focus on research. ⑧ To spread a mature research culture based on trust. ⑨ Strengthen communication with researchers and citizens. ⑩ Strive to contribute basic research to the world. Detailed strategies for basic research innovation will be discussed.

I appreciate attendance of interested researchers and would like to hold a public hearing.

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# International Symposia

**IS1**

Biochemistry · Molecular Biology

**IS2**

Environmental Sciences

**IS3**

Natural Products · Bioactive Materials · Biomedical Sciences

**IS4**

Food Sciences

**IS5**

Applied Microbiology

**IS1-1**

## **Improving end-use quality and immunogenic potential of wheat flour**

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Wheat is a major food crop grown throughout the world that is used in a wide range of different food products because of the unique viscoelastic properties of the flour. These properties are conferred by the gluten proteins, a complex group of proteins that are unusual in that they contain large regions of repetitive sequences with high proportions of glutamine and proline. The gluten proteins are divided into gliadins and glutenins that confer extensibility and elasticity, respectively, to wheat flour dough. The monomeric gliadins consist of alpha, gamma, delta and omega types while the glutenins form large insoluble polymers composed of high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS) that are linked by disulfide bonds. Recent data from genome sequencing studies revealed 26 alpha, 11 gamma, two delta, five omega gliadin and 10 LMW-GS genes that encode full-length proteins in the reference wheat Chinese Spring in addition to the four HMW-GS genes already characterized. Ultimately, the composition of the gluten proteins in the flour, determined by both the genetics of the plant and the growth environment, is critical for end-use quality. Many gluten proteins also trigger a number of important human health conditions, including celiac disease, IgE-mediated food allergies and non-celiac wheat sensitivities.

A better understanding of the relationships between specific gluten proteins and their contributions to human health conditions and end-use functional properties is important for efforts to develop wheat that will be either less likely to trigger immunogenic responses or might be better tolerated by patients with celiac disease and food allergies. Towards this end, we have suppressed the expression of genes encoding three different groups of highly immunogenic proteins in a commercial U.S. wheat cultivar using RNA interference. Genes targeted include the omega-5 gliadins, the major sensitizing allergens in the serious wheat allergy wheat-dependent exercise-induced anaphylaxis (WDEIA), and the omega-1,2 gliadins and alpha gliadins that contain important epitopes involved in celiac disease. Effects of genetic modifications on flour protein composition, end-use properties and allergenic potential will be discussed. The results suggest that biotechnology approaches can be used to improve the healthfulness of wheat, while maintaining or even improving its end-use qualities.

IS1-2

## Direct role of MUTE in orchestrating stomatal differentiation

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Plant stomata are cellular valves composed of paired guard cells that impact plant growth, sustenance, and atmospheric environment. For the differentiation of a functional stoma, a single symmetric division of an immediate precursor is absolutely essential. We have revealed the comprehensive inventories of transcriptional changes driven by the Arabidopsis bHLH protein MUTE, a potent inducer of stomatal differentiation. MUTE switches the gene expression program initiated by its sister bHLH, SPEECHLESS, thereby 'locking in' the differentiation program while taking over the shared targets. MUTE directly induces a suite of cell-cycle genes and their direct transcriptional repressors. The architecture of the gene regulatory network initiated by MUTE represents an Incoherent Type 1 Feed-Forward Loop, which yields a robust, sharp pulse of output response. Our mathematical modelling and a series of experimental perturbations support a notion that MUTE orchestrates the transcriptional cascade leading to a tightly-restricted, robust pulse of cell-cycle gene expression, thereby ensuring the single cell division to make stomata. Currently, we are further characterizing the promising MUTE target genes aiming to elucidate the molecular mechanism of how MUTE terminates self-renewing cell state and specifies cell fate in response to endogenous signaling.

**IS1-3**

## **In vivo Imaging of Reactive Oxygen Species (ROS) Using a New Ratiometric ROS Bio-reporter for Studying the Function of ROS**

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Reactive oxygen species (ROS) are spontaneously generated during plant growth and when plants experience various stresses. Although accumulation of ROS can be toxic to plants, it also acts as signaling molecules, which are closely associated with adapting to stresses. Tight regulation of ROS homeostasis is required to adapt to stress and survive. However, *in vivo* spatiotemporal information of ROS dynamics is still largely undefined, in part due to the limited range of available ROS bioreporters. In order to understand the dynamics of ROS changes and their biological function in adapting to stresses, a quantitative ROS transcription-based bioreporter was developed using a promoter fusion stratagem. This bioreporter uses a ROS-responsive promoter from *ZAT12* to drive Green Fluorescent Protein (GFP) expression and to compare the resulting expression to a constitutively expressed Red Fluorescent Protein (mCherry). The ROS-bioreporter, ZAT12p-ROS, was used to assess ROS response to oxidative stress (H<sub>2</sub>O<sub>2</sub>), salt stress (100 mM NaCl), and pathogen related elicitor flg22. The ZAT12p-ROS bioreporter showed increases in the ratio values of GFP to mCherry signals within 10 to 30 min post stress treatments, consistent with stress-induced ROS accumulation in the hypocotyl and root. Such stress-associated ROS signals correlated with the induction of abiotic/biotic stress responsive markers such as *RbohD*, *ZAT12*, *SOS2*, and *PR5* suggesting the ZAT12p-ROS provides a robust indicator of increased ROS, which is related to stress responses. Based upon the temporal response patterns and magnitude of signal increases, the ZAT12p-ROS bioreporter appears to be suitable for cellular mapping of ROS changes in response to abiotic and biotic stresses.



IS2-1

## Target Identification of Bioactive Molecules

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Molecular target identification is pivotal in nutraceutical and pharmacological research, and drug and pesticide discovery. It involves identification and characterization of a specific molecular target to which a bioactive compound binds, such as an enzyme or receptor. Agrochemicals are used to increase crop production and food quality. Nutraceuticals are products derived from food sources with extra health benefits, in addition to the basic nutritional value found in foods. Agricultural and therapeutic research synergistically shares the same goal – human health, which is accomplished from one health approach covering from healthy environment, to safe and nutritious foods, to healthy lifestyles and advanced medicines. Since the establishment in 1960, the Korean Society for Applied Biological Chemistry (KSABC) has become a representative forum of the Korean applied biological chemistry and the related industry. KSABC is also a “network hub” to link biochemistry, molecular biology, environmental sciences, natural product, bioactive materials, biotechnology, food sciences, applied microbiology, and agricultural biologicals and related areas.

Three cases will be presented to indicate how molecular targets have been identified. First, molecular screening revealed that the C-glycosylflavone isoorientin inhibits glycogen synthase kinase-3 $\beta$  (GSK-3 $\beta$ ). GSK-3 $\beta$  is a key enzyme catalyzing hyperphosphorylation of tau protein. To our surprise, isoorientin specifically inhibits GSK-3 $\beta$  via a substrate competitive mode, rather than the common ATP competitive mode. It effectively attenuates GSK-3 $\beta$ -catalyzed tau hyperphosphorylation and is neuroprotective against amyloid-induced neurotoxicity in human SH-SY5Y cells. It is known that hyperphosphorylation of tau proteins in neurons plays a pivotal role in Alzheimer’s disease (AD) pathology. AD is the most frequent neurodegenerative disorder in the aged population, which cannot be prevented, cured or even slowed.

The second is to use quantitative proteomics to identify tentative molecular targets. 24-Methylenecycloartanyl ferulate (24-mCAF) isolated from rice bran oil was found to be cytotoxic to A549 cells that are a non-small cell lung cancer (NSCLC) cell line. An iTRAQ-based quantitative proteomics analysis suggested that 24-mCAF inhibits cell proliferation and activates cell death and apoptosis. 24-mCAF induces up-regulation of Myb binding protein 1A (MYBBP1A), a tumor suppressor that halts cancer progression. In vitro enzymatic assays confirmed that 24-mCAF inhibits the activity of AKT and Aurora B kinase, two Ser/Thr kinases involved in MYBBP1A regulation and that may represent important targets in NSCLC. Lung cancer is the second most prevalent cancer. NSCLC is the most common type of lung cancer. The low efficacy in current chemotherapies impels to find new alternatives to prevent or treat NSCLC.

Finally, a potential target was first hinted by acute toxicity tests. Linalool, estragole, and methyl eugenol are the active chemicals in many essential oils, particularly basil oils. Basil oil and the aforementioned monoterpenoids have been widely used for health benefits as well for pest insect control. However, the molecular target of those chemical constituents is not well-understood. It is well-known that the  $\gamma$ -aminobutyric acid type A receptors (GABA<sub>A</sub>R) and nicotinic acetylcholine receptor (nAChR) are primary molecular targets of synthetic insecticides used in the market today. We studied the electrophysiological effects of linalool, estragole, methyl eugenol, and citronellal on GABA<sub>A</sub>R and nAChR to further understand their versatility as traditional medicines and as insecticides. Our results revealed that linalool inhibits both GABA<sub>A</sub>R and nAChR, which may explain its insecticidal activity. Linalool is a concentration-dependent, non-competitive inhibitor on the GABA<sub>A</sub>R as evidenced by negligible effects of linalool on the EC<sub>50</sub> values of GABA for the rat  $\alpha$ 1 $\beta$ 3 $\gamma$ 2L GABA<sub>A</sub>R. The half maximal inhibitory concentration (IC<sub>50</sub>) of linalool on the GABA<sub>A</sub>R was approximately 3.2 mM. As multiple monoterpenoids are present in the same essential oil, it is likely that linalool synergistically interacts with methyl eugenol (and other monoterpenoids) as both a GABA<sub>A</sub>R agonist and positive allosteric modulator, thus putatively explaining for its sedative and anticonvulsant effects.

**IS2-2**

## **Heavy Metal Contamination in Soil-Water Plant System and Their Human Health Risk Assessment in Peri-Urban Areas of Bangladesh**

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Metal contamination of agroecosystem has become a serious environmental problem due to uncontrolled long-term disposal of untreated industrial effluents containing toxic metals and indiscriminate use of metal-containing fertilizers in agricultural fields. As a consequence, heavy metal contamination of soils by the polluted river water is posing an increasing threat to surface water irrigation, which causes toxic effect on soils and crops ultimately entering foods and also creates environmental problem affecting agricultural production, food quality and human health. The aim of this present study was designed to explore the intensity of heavy metal contamination in soils and crops at Turag, Balu, Khiru and Bangshi river sites irrigated with industrially contaminated river water at farmers' field during dry season toward food safety for human consumption. Sixty water samples of each river were collected randomly to measure pH, EC, TDS and metal status (Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn) in order to categorize their suitability for irrigation usage. Again, sixty soil samples were collected to determine soil texture, pH, EC, OC and metal ions such as Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn. Plant samples were also collected from the adjacent crop fields irrigated with the contaminated river waters and were analyzed to determine the levels of metal ions like Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn. pH values of four river water samples were slightly acidic to alkaline in nature. The contaminated four river water samples were under low to medium salinity (C1, EC<250  $\mu\text{S cm}^{-1}$  & C2, EC=250-750  $\mu\text{S cm}^{-1}$ ) and alkalinity (S1, SAR<10) hazards expressing as C1S1 and C2S1. As regards to the measured TDS values, river water samples under test were rated as fresh water (TDS<1,000  $\text{mg L}^{-1}$ ) in quality. The levels of Mn and Cr ions in four rivers under study exceeded the safe limit of WHO/FAO standard and were considered as contaminants for irrigation usage. The order of heavy metals magnitude for four rivers was as: Fe>Mn>Cr>Pb>Zn>Cu>Ni for Turag river, Fe>Mn>Pb>Cr>Zn>Cu>Ni for Balu river, Fe>Pb>Mn>Zn>Cr>Cu>Ni for Khiru river and Fe>Mn>Cr>Pb>Cu>Zn>Ni for Bangshi river. In order to better understanding the relationships between soil characteristics and heavy metal mobility, the obtained results showed the strong influence of pH, EC and soil texture on retention and mobility of heavy metals. On the basis of heavy metal levels, soil irrigated with the contaminated river water under investigation was found in the order of Fe>Mn>Zn>Cu>Cr>Pb>Ni for Turag; Fe>Mn>Zn>Cr>Cu>Pb>Ni for Balu; Fe>Mn>Zn>Cr>Pb>Cu>Ni for Khiru and Fe>Mn>Zn>Cr>Pb>Cu>Ni for Bangshi. Among the identified metals, the accumulations of Cr, Mn and Pb ions were detected in the contaminated soils irrigated with river waters and these metal ions were treated as soil contaminants due to long-term irrigation usage. Geoaccumulation index (Igeo) values of four river sites indicated polluted to moderately polluted soil in terms of heavy metals while pollution load index (PLI) indicated soil environment of those river sites with progressive deterioration. The calculated enrichment factor (EFc) values of four river water sites exhibited the enrichment of Cr and Pb in soils. The sequence of soil pollution level of the studied four river areas were Bangshi>Turag>Balu>Khiru. Multivariate analysis revealed the correlation of chemical parameters of soil and water samples loading by the contaminated river water irrigation. The bioaccumulations of Pb, Ni and Cr in leafy vegetables viz., water spinach, red amaranth, indian spinach and stem amaranth were recorded in three rivers sites and bioaccumulations of Pb and Cr in rice grain and straw were also found in four river areas for long-term irrigated sites. Heavy metal contamination of vegetables, rice grain and straw grown around four river sites would pose a great health risk to the local population through dietary intake for long-term consumption. Considering the scenario of total target hazard quotient (TTHQ), health risks associated with the consumption of vegetables in three rivers sites as TTHQ values were in the order of Turag>Balu>Bangshi river while the TTHQ of rice grain in Khiru river site was 1.23 indicating potential health risks. From the research findings, it is concluded that the contamination of soil, water and plant systems caused by heavy metals under consideration should not be ignored in the irrigated sites of four rivers under study toward food safety and health risks.

## IS2-3

## Discovery of Secondary Metabolites with Nematicidal Activity from Higher Fungi against Root-knot Nematodes

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Root-knot nematodes (RKN; *Meloidogyne* spp.) are one of the most economically damaging group of plant-parasitic nematodes worldwide. RKN is responsible for a large part of global damage to reach \$100 billion caused annually by nematodes. In order to discover new nematicidal metabolites and develop new microbial bionematicides using higher fungi, we examined nematicidal activities more than 4500 fungal culture filtrates against juveniles of *Meloidogyne incognita*, which is a causal agent of root knot disease on various crops. Through the screening, we found that the culture filtrates of two fungal strains such as *Aspergillus niger* F22 and *Xylaria grammica* KCTC 13121BP were active to *M. incognita* with marked mortality of second-stage juveniles and inhibition of egg hatching. The nematicidal metabolite from the culture filtrate of *A. niger* F22 was identified as oxalic acid by organic acid analysis and gas chromatography-mass spectroscopy (GC-MS). Exposure to 2 mmol/L oxalic acid resulted in 100% juvenile mortality at 1 day after treatment and suppressed egg hatching by 95.6% at 7 days after treatment. Oxalic acid showed similar nematicidal activity against *M. hapla*, but was not highly toxic to *Bursaphelenchus xylophilus*. A new microbial nematicide named “Nemafree” using *A. niger* F22 was commercialized from March 2017. On the other hand, bioassay-guided fractionation from the culture filtrate of an endolichenic fungus *X. grammica* EL000614 and instrumental analyses led to grammicin being identified as the nematicidal metabolite. Grammicin showed strong second-stage juvenile killing and egg-hatching inhibitory effects, with a 50% effective concentration at 72 h ( $EC_{50/72\text{ h}}$ ) of 15.9  $\mu\text{g/mL}$  and a 50% effective concentration at 14 days ( $EC_{50/14\text{ days}}$ ) of 5.87  $\mu\text{g/mL}$ , respectively. In pot and field experiments, a wettable powder-type formulation and fermentation broth filtrate of *X. grammica* KCTC 13121BP effectively suppressed the development of RKN disease on tomato and melon plants. Additionally, the optimization of the fermentation condition for grammicin production by *X. grammica* EL000614 was carried out. The maximum amount of grammicin in a minimal medium-based optimum medium in a flask was 5.75 g/L after 3 weeks fermentation, which is approximately 4 times higher level than potato dextrose broth (PDB) medium culture. The optimum culture (OC) filtrate also showed 4 times stronger nematicidal activity against 2<sup>nd</sup> juveniles of *M. incognita* and *M. arenaria* than PDB culture filtrate. The results suggest that *X. grammica* and grammicin may have potential applications for control of RKN disease of various crops. Further research on the development of fermentation and formulation processes, and evaluation of disease control efficacy against various nematode diseases are necessary for the development of new bionematicides.

**IS3-1**

## **Mechanisms mediating anti-inflammatory effects of dietary bioactive compounds in obesity and insulin resistance**

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Obesity is a complex disease that is associated with the expansion of white adipose tissue, creating a system in which the body progressively transitions into an inflammatory state. Obesity also leads to several metabolic disorders including diabetes and cardiovascular diseases, all of which are also characterized by chronic low-grade inflammation. As a result, bioactive compounds with anti-inflammatory properties have gained significant attention as anti-obesity targets. Some of these dietary components also increase brown adipose tissue activity and/or browning of white adipose tissue.

Our lab has extensively studied the mechanistic basis for anti-inflammatory effects of various dietary and plant bioactive compounds including omega 3 polyunsaturated fatty acids, tart cherry anthocyanins, tocotrienols, and botanicals in white and brown adipose tissues using diet-induced obese mice. We previously reported that mice fed high fat diets supplemented with the omega-3 polyunsaturated fatty acid, eicosapentaenoic acid, (HF-EPA) exhibited reduced adipose and systemic inflammation, improved glucose clearance and insulin resistance, and reversed fatty liver, compared to mice fed high fat diets without EPA (HF). Tocotrienols and tart cherry anthocyanins also exhibited potent anti-inflammatory effects in white adipose tissue.

Mechanisms mediating the beneficial effects of omega 3 fatty acids in both white and brown adipose tissue included decreased expression of adipogenic, lipogenic and inflammatory genes and increased lipid oxidation, leading to adipocyte hypotrophy and lower macrophage infiltration in adipose tissue in mice fed high fat diets supplemented with EPA compared to non-supplemented mice. We have also demonstrated that EPA increased oxygen consumption and the thermogenic protein, uncoupling protein 1 (UCP1) and upregulated expression of other master regulators of thermogenesis in brown adipose tissue. Unexpectedly, using UCP1-deficient mice, we found that these protective effects of EPA were independent of UCP1.

To further dissect mechanisms of EPA effects in obesity, we used RNA and small RNA sequencing to profile differential gene and miRNA expression in white and brown fat. We identified several gene-miRNAs pairs that may mediate metabolic effects of EPA in adipose tissue. In white adipose tissue, several of these pairs mapped to pro-inflammatory pathways such as NF- $\kappa$ B; while in brown adipose tissue, pathways related to thermogenesis such as peroxisome proliferator-activated receptor (PPAR) signaling were most prominent. Thus, the depth of transcriptomic and miRNA profiling revealed novel mRNA-miRNA interaction networks in both white and brown adipose tissues that may mediate effects of EPA on energy homeostasis.

In conclusion, natural compounds such as omega 3 fatty acids are important regulators of energy metabolism. Moreover, global genomic studies are important in identifying novel pathways regulated by these compounds that may lead to novel targets to prevent/treat metabolic complications associated with obesity.

IS3-2

## Cannabis in California, USA

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As of January 2019 in the United States, adult-use cannabis is legal in 10 states and medical cannabis is legal in 33 states, the District of Columbia, and four out of five U.S. territories.

On November 8, 2016, California voters approved Proposition 64, thus legalizing the adult usage, possession, and cultivation of cannabis. California had already legalized the use of medical marijuana 20 years ago, by passing Proposition 215.

In response to the legalization, three government agencies (CDFA, CDPH, BCC) have established a licensing structure and framework for regulating adult use and medical cannabis activities.

This presentation will introduce each aspect of the cannabis business infrastructure, including but not limited to their main activities, how they relate to one another, and the appropriate regulations. An introduction to the cannabis plant, major cannabinoids and their medical effects, and the endocannabinoid system will also be discussed.

Because the cannabis plant is still listed as a Schedule I substance of the Controlled Substances Act established by the federal government, research pertaining to cannabis has been strictly limited. Although there have been many reports of the medical effects of cannabis or cannabinoids that are extracted from the cannabis plant, there is currently insufficient data to support the safety and validity of these effects.

Adult use cannabis were legalized for the past two years from 2017 to 2018 in California under emergency regulations. Beginning in 2019, fully adapted regulations are in effect for the entire cannabis market. This is just the beginning for cannabis in the state of California. With the advance of more research and experiments on cannabis, the demand of cannabis-related products could skyrocket not only for patients, but also for adult use consumers in the near future.

**IS3-3**

## **Optimization of compound mixtures via ex vivo fluorescence monitoring for improved cellular delivery and effects**

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It is crucial to understand how natural compound mixtures work together in cells and in vivo to improve their clinical efficacy and therapeutic effects. In this presentation, synergistic effects of compound mixtures were examined in terms of intracellular uptake and bio-distribution using curcumin and umbelliferone derived from *Angelica (A.) gigas*. Localization of these natural compounds was easily monitored by fluorescence imaging ex vivo in the presence of different types of compounds. After administration of natural compound mixtures intravenously, fluorescent intensities in cells and tissues were visualized using in vivo imaging systems. Bioactivities in vitro e.g. antioxidant activities and cellular reactive oxygen species levels were examined in the presence of other compounds for macrophages. To elucidate molecular mechanism of compound mixtures, inhibition of the efflux pump P-glycoprotein (P-gp) and stability of natural compounds were quantitatively analyzed in vitro. Taken together, compound mixtures were optimized for enhanced intracellular localization and enzymatic stability, which might lead to improved bioactivity of natural compounds for therapeutic applications.

**IS4-1**

## The dangers of hidden fat: bringing intra-pancreatic fat deposition to the fore

**Max S. Petrov\***

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It is well established that obesity (defined as excess fat mass) increases risks for several diseases, including but not limited to diabetes mellitus, cardiovascular diseases, and cancer. Emerging evidence indicates that, over and above fat mass per se, the pattern of fat distribution has a profound influence on systemic metabolism and health outcomes. Variations in the capacity of different depots to store and release fatty acids and to produce adipokines are important determinants of fat distribution and its metabolic consequences. Depot differences in cellular composition and physiology, including innervation and blood flow, likely influence their phenotypic properties. Several studies showed that adipocytes from different anatomical sites are intrinsically different as a result of genetic or developmental events. This presentation will put a spotlight on the phenotypic characteristics of fat deposition in the pancreas and mechanisms that link its depot-specific biology to metabolic disorders.

**IS4-2**

## **Safety Issues of Food and Agricultural Products in Kazakhstan**

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Agriculture is an important sector of Kazakhstan's economy. The area of agricultural land of the country is 76.5 thousand hectares, including arable land — 24.1 million hectares, pastures — 46.5 thousand hectares. In the North, climatic conditions favor for the cultivation of spring wheat, oats, barley and other crops, as well as allow the development of vegetable growing, melons and cultivate a number of industrial crops — sunflower, flax, tobacco. In the South of the Republic, in the foothills and river valleys, where a lot of heat, with artificial irrigation give high yields of cotton, sugar beet, yellow tobacco, rice, fruit orchards and vineyards. Natural conditions of Kazakhstan, their diversity cause significant potential opportunities for the development of livestock. The Republic traditionally engaged in sheep, horse, camel, cattle breeding. Kazakhstan is among the top ten world exporters of wheat (sixth place in 2011-2012). The country has fairly good land, but its territories suffer from environmental obstacles such as lack of water and harsh climatic conditions. In grain-growing areas are cultivated mainly strong and hard wheat varieties with a high content of gluten, such wheat is in great demand on the world market. Since the mid-2000s, Kazakhstan has been a net importer of agri-food products.

The implementation of the concept of economic development involves ensuring food security. In 2017, exports of the food industry of Kazakhstan by 44% consisted of processed products, the remaining 56% accounted for raw materials (wheat, barley, etc.). As for imports of food products, the ratio of processed and raw materials was 70% to 30%. Thus, ensuring food and food security of the state is a strategic task for the Government of Kazakhstan. The problem of food security includes political, social, technical, economic, medical and other aspects.

The country's land resources was in critical condition. Fertile arable land was in depletion. There is a desertification of pastures. Air pollution, especially in large industrial centres, has reached alarming levels. These include the zone of ecological disaster, which are the Aral and Semipalatinsk regions, where the destruction of natural ecological systems, degradation of flora and fauna. It should also be noted that Kazakhstan is classified as a country with a large water deficit. But despite this, our natural reservoirs are heavily polluted by enterprises of mining, metallurgical and chemical industries, municipal services of cities and pose a real environmental threat. Also the intensification of agricultural production, the desire to get more out of a unit of acreage make to active use of agrochemicals, genetically modified plants, hormones and growth stimulants. And their use increases the risk of poor quality and dangerous food products, from which in our time of rapid logistics can suffer whole Nations.

On the territory of Kazakhstan until the 90-ies pesticides were used in almost the entire area. Pesticides with POPS properties have never been produced in Kazakhstan. They are not currently imported or exported, nor are they planned to be used, imported or exported in the future. However, large amounts of POPS previously produced and used in the former USSR have been accumulated in most of Kazakhstan.

However, up to the 90s DDT was used in Kazakhstan in veterinary medicine. Small residues of DDT are still found in soil, plants and water, air in the work area and food.



Priority issues related to chemicals in Kazakhstan include:

- air pollution (SO<sub>2</sub>, NO<sub>x</sub>, CO, dust, O<sub>3</sub>, PAH),
- presence of hazardous chemicals in agricultural food products (pesticides, nitrates)
- pollution of drinking water (heavy metals, oil products),
- processing/destruction of hazardous waste (radioactive waste, obsolete pesticides, ash and other),
- occupational health in agriculture (pesticides, fertilizers),
- pollution of inland waters and waterways (PAHs, phenols, heavy metals, pesticides, Unions),
- pollution of groundwater (pesticides, petroleum products, PCBs, heavy metals),
- soil pollution (oil products, pesticides, heavy metals).

In 2018, 58855 objects producing and selling food products were under control (2017. – 57435), of which 7553 or 12.8% were examined. Above the national average of surveys was in Aktobe – 32.9%, Pavlodar – 26.1%, North Kazakhstan – 23.0%, Karaganda – 20.1%, Almaty – 14.3% and Shymkent – 15.5% regions.

6558 food objects or 86.8% were examined with the use of laboratory methods. The number of objects that do not meet the requirements of sanitary regulations – 252 or 3.3 %.

In 2018 territorial authorities public health rejected and removed from realization 1077956,77 kg (2017. – 297383,23 kg) of food and raw materials, of which produced in RK – 798230,09 kg or 74,1% (2017. – 82588,27 kg) imported from other countries – 279726,7 kg or 25.9% (2017. – 214795 kg).

In comparison with 2017, the consumer market of Kazakhstan received 3.6 times more of substandard and unsafe food products for the health of the population. In 2018, compared with 2017, there was an increase in controlled safety indicators that do not meet the requirements of sanitary rules in the controlled epidemiologically important food facilities. In the Republic, the number of outbreaks of acute intestinal infections and food poisoning has increased dramatically, by 1.5 times compared to 2017.

The consumer protection Committee of the Ministry of national economy of the Republic of Kazakhstan at the end of January 2019 stated that 35.8% of fish products and 27.1% of meat products, as well as 22.7% of bakery products sold in Kazakhstan, do not meet the safety and quality requirements. During the year, with monthly monitoring at the stage of implementation, more than 29 thousand samples of food were selected and examined. According to the Committee, 86% does not meet standards of food production are accounted for by imports. The products of domestic manufacturers that do not meet the requirements amounted to 14%.

The main segment of unsafe and substandard food products falls on the share of imported producers: from the Russian Federation – 52%, Kyrgyzstan – 4%, the Republic of Belarus – 3%, Ukraine – 2.5%, China, Turkey, Latvia, USA, Moldova, Georgia, France, Uzbekistan and other countries – up to 1%. 17.5 tons of dangerous food products were removed from the sale.

One of the key conditions for the participation of the exporting country in international food trade is the country's ability to ensure the quality and safety of its products.

**IS4-3**

## **Functional thermotolerant yeasts isolated in Vietnam for ethanol and fruit wine production**

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In Vietnam, ethanol is concerned in many different products in terms of fermentation technology including alcoholic drinks and bio-fuel. Due to the challenges of increasing global temperature and benefits associated with processing at high temperature, using thermotolerant yeasts for ethanol fermentation is a potential orientation in tropical countries as Vietnam. In our recent studies, the diversity of thermotolerant yeast isolates performing high capacities and stability for the controlled processing of alcoholic winemaking and ethanol production from the cheap and available raw materials in region has been addressed and achieved.

A total of 712 yeast isolates was purified from many different kinds of raw material sources in the Mekong Delta, Vietnam; such as ripe fruits, flowers of fruit-tree, cocoa, fermented products, alcoholic fermentation starters, sugarcane, molasses, sawdust, agricultural by-products and soil samples. All of these yeast isolates could grow well at 37°C and there were about 80%, 45% and 10% of yeasts could grow at 40°C, 43°C and 45°C, respectively. More than 80% of yeasts were able to grow in the medium containing 9% (v/v) of ethanol and it was decreased to about 40% of yeasts growing in the medium supplemented 12% (v/v) of ethanol. Due to the thermotolerance and ethanol fermentation capacity at high temperature, 88 yeast isolates were selected and characterized. The predominantly abundant identified species include *Candida tropicalis*, *Saccharomyces cerevisiae*, *Pichia kudriavzevii*, and *Candida glabrata*. The database of these selected yeasts has been systemized and recorded including information on morphological, physiological and biochemical characteristics; thermotolerant ability; ethanol tolerance; fermentation capacity and assimilation ability of carbon sources; DNA sequence results and names of identified species.

With the aim to pave the way for the application of useful thermotolerant ethanologenic yeasts towards the industrial fermentation technology, the technical feasibilities for the ethanol production and the winemaking by using the selected thermotolerant yeasts have also been investigated. The fermentation capacity of the tested yeasts was monitored and compared at different temperatures (37°C, 40°C, 43°C). Then the selected yeasts were further examined for the optimum fermentation conditions at 37°C in a factorial design with three factors at three levels, including yeast inoculation level, initial sugar concentration and fermentation time. For the ethanol fermentation, sugar-cane juice and molasses were employed as raw materials. The highest ethanol concentration could be found at about 10.5% (v/v) and up to 5.5% (v/v) during the fermentation at 37°C and 40°C, respectively. For wine manufacture, the different kinds of fruits were employed as raw materials such as: pineapple, watermelon, dragon fruit, guava, and three-leaved wild vine. The highest ethanol concentration of the final wine product could reach about 12% (v/v) and up to 7% (v/v) during the fermentation at 37°C and 40°C, respectively.

The research findings on the diversified collection of thermotolerant ethanologenic yeasts isolated from Vietnam and the processing feasibility indicate the promising application of such newly functional yeasts for the controlled ethanol production at high temperatures from agricultural by-products and the winemaking manufacture from different available fruit resources in region. The further advanced study on the expression levels of the selected genes and the metabolic pathways will also be studied to explore the regulation of these genes to get maximum benefits of the superior thermotolerant yeasts for the high-temperature ethanol production.

IS5-1

## Anaerobic ammonium oxidizing (anammox) bacteria – from ecophysiology to practical application –

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Anaerobic ammonium oxidation (anammox) is a microbial process where  $\text{NH}_4^+$  is anaerobically oxidized to  $\text{N}_2$  gas with  $\text{NO}_2^-$  as an electron acceptor. This novel pathway of nitrogen cycle was predicted, but not experimentally proven. Undiscovered anammox bacteria were finally discovered in early 1990s. Since then extensive studies have been conducted to reveal their physiology, biochemistry, and ecology, and it is now known that anammox bacteria are ubiquitously detected from anoxic natural and man-made ecosystems and key players in the nitrogen cycle. For example, anammox process significantly contributed to nitrogen loss; *e.g.*, the anammox process accounted for nearly 100% of total nitrogen loss in anoxic water column of the Namibian oxygen minimum zone (OMZ) and up to 79% of total nitrogen loss in the marine sediments. However, our understanding is largely limited due to a lack of pure culture of anammox bacteria.

The nitrogen removal process using this newly discovered anammox bacteria has been recognized as a promising cost-effective and low energy alternative to the conventional nitrification-denitrification processes due to a significant reduction of aeration and alkalinity for nitrification and organic carbon for denitrification. To our best knowledge, there are probably more than 114 reported full-scale anammox treatment plants around the world. Anammox-based process is, however, not yet recognized as popular nitrogen removal process like the conventional nitrification-denitrification processes. There are still some challenges in practical application of anammox-based treatment process at full-scale, *e.g.*, longer start-up period, limited application to mainstream municipal wastewater and poor effluent water quality.

In this presentation, current our knowledge of anammox bacterial ecophysiology and engineering application of the anammox process for wastewater treatment will be presented.

**IS5-2**

## **Functional Banking and Characterizing Human Gut Microbiome**

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Since a USA National Institutes of Health launched the Human Microbiome Project (HMP) in 2007, the human microbiome has been gaining increasing attention in the world. Furthermore, in addition that human gut microbiome has been known to play an important role in a biological metabolism and digestive capacity, recent studies revealed that it is implicated in a variety of diseases such as inflammatory bowel diseases, colorectal cancer, and interestingly even neuronal disorders. The human microbiome, therefore, has been considered as a forgotten organ.

In November 2016 Biological Resource Center in KRIBB initiated the Korean Microbiome Project like HMP called Korean Gut Microbiome Bank (KGMB) to investigate healthy Korean gut microbial flora, which can be used as reference for the study of microbiome related diseases for Korean. Interestingly, unlike other country national microbiome projects which was performed only by culture-independent methods, culture-dependent methods producing real microbiome resources have been applied to the KGMB because the need of the real microbiome resources has increased recently in the microbiome fields in which the culture-independent methods were mostly done. This talk will introduce and discuss the KGMB. Furthermore, the characterization of human microbiome involved in several disease including colorectal cancer will be also discussed.

**IS5-3**

## Regulation of Plant and Human Immunity by Interaction of Salicylates with Their Target Proteins

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Salicylic acid (SA) plays a key roles in plants, including plant development, stomatal closure, thermogenesis, and response to abiotic and biotic stresses. Its role in plant immunity is the most extensively studied, but it is still only partially understood. In plants, more than two dozens of SA-binding proteins (SABPs) are identified based on the classical biochemical approaches and genome-wide high-throughput screens. Interestingly some of these proteins exhibited high affinity against SA, while the others showed low affinity, *in vitro*. Presence of SABPs exhibiting a wide range of affinities for SA may provide great flexibility and multiple mechanisms through which SA can act. Importantly, similar screening of human proteome identified several target proteins of SA and its natural and synthetic derivatives (Salicylates). Many of these human proteins, like their plant counterparts, are associated with immunity or disease development. High Mobility Group Box protein (HMGB) and Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH) were identified as SABPs and play important roles in disease responses in both plants and humans.

**IS5-4**

## **Development of techniques for practical applications of multifunctional microorganism in agriculture**

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The complete genome sequence of *Bacillus velezensis* GH1-13 revealed that it possesses a single 4,071,980-bp circular chromosome with 46.2% GC-content, and we also identified a unique plasmid of 71,628-bp with 31.7% GC-content. The genome was found to contain various enzyme-encoding operons, including indole-3-acetic acid (IAA) biosynthesis proteins, 2,3-butanediol dehydrogenase, various non-ribosomal peptide synthetases, and several polyketide synthases. The lipopeptides, including bacillomycin, fengycin and surfactin, were detected by mass spectrometry and NMR. The optimal carbon and nitrogen sources for mass cultivation were determined by glucose and soy bean flour, respectively. The results showed that  $7.5 \times 10^9$  cells/mL,  $6.8 \times 10^9$  endospore cells/mL and sporulation yield of 90% after 30 h cultivation in 500 L submerged fermenter at 37°C, pH 7.0. Cells and cell-free supernatant of GH1-13 strain exhibited the potent antifungal activity against phytopathogenic fungi. It was also confirmed that indole-3-acetic acid (IAA) production of GH1-13 strain was greatly increased by addition of 0.3% tryptophan. The microbial formulations of GH1-13 strain were performed into liquid, powder, granule and pellet types. Plant growth promoting of four formulation types were examined to pepper and tomato plants, and biocontrol effect of liquid and powder types was also tested against *Colletotrichum gloeosporioides* causing pepper anthracnose. Microbial agents of GH1-13 were applied in pepper and potato plant, and resulted in productivity enhancement of 27% and 18%, respectively.

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# Symposia

**S1**

Biochemistry · Molecular Biology

**S2**

Environmental Sciences

**S3**

Natural Products · Bioactive Materials · Biomedical Sciences

**S4**

Food Sciences

**S5**

Applied Microbiology

**S6**

Agro-Bio Genome Editing

**S7**

Forensic Science

**S8**

Pesticide Positive List System



**S1-1**

## **Highly specific affinity tag based protein purification using 2B8 tag and 2B8 antibody**

**Kyung-Ha Lee, Seong Hee Bhoo\***

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Protein purification is an indispensable procedure in most of biology, biochemistry and molecular biology fields. Purification of target proteins with high quality and purity is generally difficult. Several tag systems have been established for this purpose of protein purification. Small peptide epitope tag and their affinity purification systems are excellent for protein purification because they have high affinity and specificity for acquiring the target proteins. Therefore an excellent tag should have high affinity and specificity for better purification. However, not all peptide based epitope tag systems meet these requirements. For example, the purification His6-tagged proteins using zinc ion chelate affinity resin often results in the non-specific co-purification of zinc ion binding proteins in the starting total proteins. Furthermore we often meet non-specific binding of monoclonal antibodies to endogenous proteins in certain cell types, even when using the most popular commercial tag systems such as Flag tag and Myc tag systems. In this case of monoclonal antibody based protein purification, antibodies with high affinity and specificity are critical requirement for the best protein purification. Most of antibodies have  $K_D$  values in the low micromolar ( $10^{-6}$ ) to nanomolar ( $10^{-9}$ ) range. Even commercial tags such as HA, flag and myc, in which these tag are known as high affinities, have  $K_D$  values of 4.5 nM, 6.5 nM and 80 nM respectively. These tags are commonly used worldwide for protein purification but not enough for the best protein purification. Here we developed a novel affinity tag based protein purification designated 2B8 tag system. 2B8 tag is consisted of 9 amino acids (RDPLPFFPP) which has an extremely low  $K_D$  value of picomolar ( $10^{-12}$ ) range for 2B8 antibody. Since lower  $K_D$  value means higher affinity, 2B8 tag system will be an outstanding protein purification system even more 2B8 tag is a highly specific to the antibody.



## S1-2

## Molecular and Functional Study of Insect Odorant Receptor

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The olfactory nervous system recognizes and distinguishes many different chemicals in the general living environment. Insects have evolved a group of odorant-gated ion channels composed of highly-developed olfactory receptors capable of distinguishing and distinguishing between various chemicals with symbolic or evasive specificities. Recently, aphid genomes related to olfaction, including olfactory receptors and proteins, have been identified and olfactory receptors have been reported that are differentially differentiated from *Drosophila*. The genome of the olfactory receptor has a very conservative sequence and a systematic signaling system. A representative receptor, odorant-gated ion channels comprised of a highly conserved co-receptor (Orco) has a homotetramer channel structure with four subunits arranged symmetrically around the central hole. It has a very similar structure to the 7-transmembrane receptor present in the human body and has a very similar structural form and gating mechanism to receptors of neurotransmitters.

In this study, whole cell voltage clamp recording was performed with cell expression system of OR85b gene, which is a subtype of olfactory neuro-receptor isolated from *Drosophila*. After the successful expression of this receptor, microbial culture extract of microorganism, a harmful insect inducer, was used to investigate whether olfactory receptor activity was regulated. The activity of the receptor was confirmed in the recording media diluted 10,000 times with the microbial culture extract.

Therefore, it is possible to identify attractant or repellent substance using the olfactory receptor activity regulating system of insects. Through this study, MZ01 shows the attracting phenomenon by activating insect receptor OR85b, The results of the scientific analysis of the performance of the extracts are presented.

**S1-3**

## **Elucidating the shared and unique responses of *Arabidopsis thaliana* to multiple environmental stresses using meta-analysis approach**

**Ashutosh Singh\***

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### **Background**

Abiotic stress severely affects both physical and biochemical properties of plant cells, which then eventually alter survival and productivity. Abiotic stresses are one of the major causes of meagre plant growth and reduced crop yields globally. In most of the plant species, >50% growth reduction was observed due to abiotic stress. Since, the stress adaptation mechanisms are largely unknown, elucidating these tolerance mechanisms is essential to accelerate plant adaptability to natural field conditions in order to enhance their growth and yield. A huge amount of transcriptomic data is available for plants exposed to various abiotic stresses. Comparison of the transcriptomic data of plants exposed to individual and combined stresses may explain the molecular mechanisms behind the cross-talk between stresses. Additionally, comparison of molecular profiles of an organism under different stresses would make it possible to identify the conserved stress mechanisms. Gene co-expression networks study is becoming increasingly popular as one of the approaches to identify sets of interacting genes.

*Arabidopsis thaliana* is a well-studied model plant organism with extensive biological knowledge base and resources including complete genome sequence and the highest number of microarray studies. In the present study, a comprehensive meta-analysis using RankProd method was performed on *A. thaliana* microarray-based transcriptomic dataset for drought and cold stress. Further, co-expression network analysis was done using WGCNA R package.

### **Results**

From 29 series, 6350 and 7210 differentially expressed probes, mapped to 6120 and 7079 gene models in *A. thaliana* were identified with PFP (percentage of false prediction)  $\leq 0.01$ , under drought and cold stress respectively. Here, we report, 41% and 31% genes unique to drought and cold stress respectively. DEGs common to both the stresses were found to be 2890. Most of the genes showed conserved expression pattern (72% or 2083) with 1084 up-regulated and 999 down-regulated in both drought and cold stresses. Hence, molecular profiles of common DEGs suggests that common molecular pathways are altered in a similar manner in response to both the stresses. Further, gene ontology profiles of DEGs in cold and drought stresses were statistically compared to assess the biological similarity and differences between the two DEGs list. We were able to identify 142 common significant gene ontology terms such as, 'photosynthesis', 'respiratory burst', 'response to hormone', 'signal transduction', 'metabolic process', 'response to water deprivation' etc.

The co-expression network analysis clustered the degs of drought and cold stress into 21 and 16 modules respectively. The module preservation statistical analysis showed that eight cold modules have well-defined drought counterparts (summary Z-score >10) and two cold modules: Pink and Purple have moderate preservation ( $10 < Z\text{-score} > 5$  and higher

preservation median rank). The preserved modules genes were found to associate with biological processes: photosynthesis, stress acclimation process, response to abiotic stress and metabolic process, which generally altered during stress. The differential consensus module eigengene network analysis also advocated the similar findings and also suggested existence of a relationship among photosynthesis and defense response as well as relation between abiotic stress responsive and translation related genes under abiotic stress condition.

### **Conclusion**

This analysis was able to identify DEGs which include- DEGs already reported by individual studies and additionally, new DEGs which were overpass by individual studies. Thus, this approach magnifies the strength and sensitivity in the identification of vital stress response genes which may be overlooked by individual studies.

The comparative analysis of differential expression analysis and gene ontology enrichment of the two stresses revealed the existence of shared and unique components between cold and drought stress. It was found that several transcription factor families common in both the stresses regulates several common stress-responsive genes adhering to ABA-dependent pathway. The shared stress-responsive genes were found to be involved in ROS scavenging, stomatal movement etc. This helps the plant to reclaim the homeostatic state which was disturbed under the influence of both the stresses.

Gene co-expression network analysis also supported the findings of meta-expression analysis by revealing the existence of highly inter-correlated stress-specific and consensus modules with specific profiles of expression under drought and cold stress respectively. Altogether, the result from our study gives information about the common and unique biological and molecular behavior of the plant in response to various abiotic stresses which can be utilized for multiple stress response engineering.

**S2-1**

## **Hormetic effect of veterinary antibiotics on the enzyme activity of soil planted with *Pisum sativum* L.**

**Reep Pandi Tasho, Jae Young Cho\***

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Microbial communities are important for the ecosystem, whether for direct interactions with the plants or for nutrient and organic matter recycling. In this study, the hormetic effect of veterinary antibiotics- *kanamycin* (KA), *sulfamethazine* (SA), and *tetracycline* (TC) on soil microbial biomass and enzyme activity was examined. Soil microbial biomass and enzyme activity (dehydrogenase, urease, and alkaline phosphatase) were assessed under two different experimental conditions: A) Planted with *Pisum sativum* L. B) Unplanted. The role of pea plants in alleviating antibiotic toxicity was evident from the study. KA and TC antibiotics strongly adsorbed to soil components and were less bioavailable than SA. As a result, the test parameters were susceptible to increasing concentrations of SA. Soil microbial biomass exhibited a biphasic response for KA and TC antibiotics. However, the increase (+16%) was not enough to be classified as hormesis. Soil enzyme activity, on the other hand, depicted a dose-dependent hormetic response for KA and TC antibiotics. This study shows that nominal concentrations of KA and TC antibiotics, in presence of pea plants, increase the tolerance or adaptability to veterinary antibiotics by significantly enhancing the function of soil enzymes rather than increasing microbial biomass.

S2-2

## Methane cycling under nitrogen-limited conditions: diazotrophy as a life-strategy of methanogens and methanotrophs?

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Wetlands are the single largest source of atmospheric methane. The large uncertainties in the predictions of atmospheric methane are partly due to lack of understanding of the regulating factors of the underlying microbial processes. Net methane emission is the balance of methane production by archaeal methanogens and the consumption by aerobic and anaerobic bacteria and archaea, all affected by the availability of nitrogen. However, so far studies focused on nitrogen excess in fertilization studies. We investigated the performance of methane cycling microbes under nitrogen-limiting conditions with a special focus on N<sub>2</sub> fixation. To create nitrogen limitation we used a planted (*Glyceria maxima*) microcosms system with separated root- and bulk soil compartments filled with sediment from an agricultural ditch. Actively N<sub>2</sub> fixing methanogens and methanotrophs were screened in situ as well as in vitro by mRNA based assessment of nifH genes as well as by using stable isotopes in combination with fluorescent in situ hybridization and Nano scale secondary ion-beam mass spectrometry (NanoSIMS) of extracted cells. Methane emission was significantly lower in the absence of fertilizer. Both, growth and activity of methanogens and methanotrophs were reduced by limited by nitrogen availability. NanoSIMS of individual cells indicated that methanogens as well as methanotrophs are actively fixing N<sub>2</sub> but at a very low level. Our results suggest that nitrogen availability is a strong regulating factor of methane emission from agricultural as well as natural wetlands and should be incorporated in methane emission models.

**S2-3**

## **Biochar as Catalytic Materials for Biorefinery Applications**

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Biochar is a carbon-rich material, made by pyrolysis of biomass feedstocks. While biochar is widely used for removal of contaminants from soil and water, its applications beyond the soil and water remediation are relatively rarely studied. In this presentation, it will be introduced and discussed how to make biochar that can be used as heterogeneous catalysts and their applications to biorefinery, including biodiesel production, syngas production, tar removal, etc.

## S3-1

## Imaging of $\beta$ -amyloid plaques by near-infrared fluorescent probe in Alzheimer's disease

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Amyloid depositions in brain are the main hallmarks of Alzheimer's disease (AD). Since they are believed to precede clinical symptoms by several years, imaging of such fibrillary aggregates offers an opportunity of the early detection of AD and will be useful for identifying new therapeutic drugs and monitoring the treatment results as a surrogate marker. In this context, radionuclide or NIRF imaging has been proposed as a promising and noninvasive method to visualize  $\beta$ -amyloid (A $\beta$ ) plaques *in vivo* because of its acceptable depth of penetration and minimal degree of tissue damage. Here, we describe the synthesis and characterization of the fluorescent curcumin derivative **5b**, which readily penetrates the intact blood-brain barrier (BBB) and binds to A $\beta$  plaques. Using NIRF imaging, we demonstrated the specific interaction of **5b** with A $\beta$  plaques in 5X FAD transgenic mice *in vivo*, as confirmed by the dissection analysis of brain slices. Semi-quantitative analysis revealed that an intense fluorescence signal was detected in the brain, and the significant binding of **5b** was observed for 5X FAD transgenic mice aged 15 months. Thus, the curcumin derivative **5b** is a promising noninvasive NIRF imaging probe to visualize amyloid plaques and evaluate the effects of potential AD drugs *in vivo*.

**S3-2**

## **Total Synthesis of Antibiotic Disciformycin A and B<sup>1</sup>**

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The development of ring closing alkyne metathesis (RCAM) followed by stereoselective reduction of the resulting alkyne has rendered opportunities to access *Z*- or *E*-alkenes.<sup>2</sup> Beyond the selective formation of (*Z*)- or (*E*)-disubstituted alkenes, we recently demonstrated that a macrolide having a trisubstituted alkene with well-defined stereochemistry can be prepared by a RCAM/*trans*-selective hydrostannation sequence. This approach is complementary to ring closing alkene metathesis (RCM) since (stereoselective) formation of trisubstituted alkenes by RCM is problematic. Testing this method in the total synthesis of a complex natural product is desirable to broaden the generality of the strategy. Disciformycins A and B, isolated from cultures of *Pyxidicoccus fallax* by the Müller group in 2014,<sup>3</sup> were chosen as our targets as they exhibit considerable antibacterial activity against Gram-positive bacteria. This presentation will describe details of unforeseen synthetic challenges and our endeavors to resolve these problems met along the way.

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S3-3

## Relation of environmental variation to skin biophysiological parameters of Korean females

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Skin is the most organs on the surface of the body and is the organ where the phenotype of aging appears. Skin aging is an endogenous cause that progresses with time and exogenous causes caused by the external environment. Aging due to UV exposure is one of the causes of extrinsic aging, and UV directly or indirectly affects cells to promote senescence. It produces ROS (Reactive oxygen species) in the cell during the influence of UV. ROS affects DNA breakdown, oxidation of proteins and lipids in the cell, and carbonylated protein produced by protein oxidation in the stratum corneum of the skin is under investigation for skin aging.

The purpose of this study was to investigate the correlation between carbonylated protein (ROS) induced by oxidative stress induced by ROS and wrinkle improvement and elasticity improvement. In the in vitro test, the skin improvement test with the antioxidant-proven raw materials showed that the reduction of carbonylated protein, the improvement of skin wrinkles and the improvement of skin elasticity were found to be related to each other. This study confirmed the possibility of applying the carbonylated protein assay to the skin aging test.

**S4-1**

## **Studies on volatile analysis of various foods by different extraction techniques**

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Several existing analytical methods are available for the analysis of volatile compounds detected from various foods. Gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) methods have employed several volatile extraction techniques including distillation, solvent extraction, purge-trap or dynamic headspace techniques. Especially, headspace techniques such as stir bar sorptive extraction (HS-SBSE) and solid phase microextraction (SPME), dynamic headspace (DHS) used mainly these days regarding a simple, rapid, and solvent free technique for the volatile extraction. Until the present study, HS-SBSE has shown to provide the higher recoveries than HS-SPME and has improved the analytical performance in terms of the elimination of interfering substances and low concentration capacity. In recent years, SPME Arrow technology has been developed as a new tool of the SPME system and it combines advantages of the SPME and the HS-SBSE. In spite of the use in larger sorbent phase volumes and longer diameter and length of the fiber, a thermal desorption unit is not required for the SPME Arrow. However, very little information on volatile analysis in various food samples using HS-SBSE and HS-SPME-Arrow is available today.

In the present study, volatile compounds from various foods were analyzed by using HS-SBSE-TDU-GC-MS and HS-SPME-Arrow-GC-MS equipped with a 60 m×0.25 mm i.d. (df=0.25 μm) DB-WAX bonded-phase fused-silica capillary column. HS-SBSE and HS-SPME-Arrow methods were optimized for detecting volatile compounds by following extraction conditions; adsorbent phase, extraction temperature, extraction time, and NaCl concentration, sample preparation before extraction. These newly introduced techniques were compared for the utility with SPME method and showed the better sensitivity and reproducibly than HS-SPME. These results support the use of HS-SBSE and SPME Arrow for volatile analysis from foods.

## S4-2

## Anti-obesity effect of green bean fermented by lactic acid bacteria

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The aim of this study was to ensure that materials with high quality bio-antiobesity can be developed by fermenting coffee green beans with various physiological activity, using lactic acid bacteria. In this study on the evaluation of the anti-obesity, using the *R. toruloides* model, the extract from fermented green beans showed increase of physiological activity materials and neutral lipid. Also, on 3T3-L1 preadipocyte experiment, inhibition on the lipid differentiation and accumulation was found. These results suggest that coffee green bean by lactobacillus-mediated fermentation and with the increase of these compounds may be increased anti-obesity effect.

**S4-3**

## **Database on flavonoids and phenolic acids in Korean agro-food resources using mass technology**

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Flavonoids and phenolic acids are the most important group in secondary plant metabolites and generally produced as defense mechanisms against pathogens and disease organisms. These compounds have been reported to play an important role in the preventive aspects in human disease. Therefore, we have developed a comprehensive database (DB) on flavonoids and phenolic acids contained in the main foods consumed by the diet. In order to construct DB from agro-foods, the LC-MS condition for the flavonoids and phenolic acid derivatives were optimized and the identification of these structures was completed through interpretation of library and positive mass fragmentation. Based on mass technology (QToF-MS), flavonoid (1,950 pages, 268 agro-foods) and phenolic acid (1,130 pages, 308 agro-foods) DB was composed of their contents, chemical library and chromatogram/mass spectrometric data including 1,684 flavonoids and 1,163 phenolic acids, respectively. Furthermore, by using this information, the functional ingredient search and characterized maps were available for user through building web service system (<http://koreanfood.rda.go.kr>). Our research can be used as the basic data for the study and application of functional ingredients to contribute to food industry development, understanding of metabolic pathways, and discovery of biological activity as a national food functional DB.

S5-1

## Unraveling the DNA damage response in the radiation-resistant fungus, *Cryptococcus neoformans*

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Although there are no natural environments under intense radiation, some living organisms have been found to show high radiation resistance. The basidiomycetous fungus *Cryptococcus neoformans* has been known to be highly radiation resistant and has been often found in fatal radioactive environments. To elucidate the mechanisms underlying the radiation-resistance phenotype of *C. neoformans*, we identified genes affected by  $\gamma$ -radiation through genome-wide transcriptome analysis and characterized their functions. We found that genes involved in DNA damage repair systems were upregulated whereas genes involved in the ergosterol biosynthesis were downregulated post  $\gamma$ -radiation exposure. Most importantly, we discovered a number of novel *C. neoformans* genes, the expression of which was modulated by  $\gamma$ -radiation exposure, and their deletion rendered cells susceptible to  $\gamma$ -radiation exposure, as well as DNA damage insults. Among these genes, we found that a unique transcription factor containing the basic leucine zipper domain, named Bdr1 (a bZIP TF for DNA damage response 1), served as a regulator of the  $\gamma$ -radiation resistance of *C. neoformans* by controlling expression of DNA repair genes, and its expression was regulated by the evolutionarily conserved DNA damage response protein kinase Rad53. Furthermore, we demonstrated that Rad53 was required for DNA damage response and was phosphorylated by both PI3K-like kinases Tel1 and Mec1 in response to DNA damage. Transcriptome analysis revealed that expression levels of genes involved in the DNA repair and DNA replication were controlled by Rad53 in response to  $\gamma$ -radiation. Moreover, Chk1, which is another effector kinase like Rad53 in the budding yeast, exhibit redundant and distinct roles in the DNA damage stress. Taken together, the current transcriptome and functional analyses could shed light on understanding  $\gamma$ -radiation response of *C. neoformans*.

**S5-2**

## **Regulation of fungal development and pathogenesis through histone acetylation/deacetylation in the rice blast fungus**

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Fungal pathogens have huge impact on health and economic wellbeing of human by causing life-threatening mycoses in immune-compromised patients or by destroying crop plants. A key determinant of fungal pathogenesis is their ability to undergo developmental changes in response to host or environmental factors. Here we set out to investigate contribution of one of the most important epigenetic modifications, histone acetylation/deacetylation to this morphogenetic process, using a model plant pathogenic fungus, *Magnaporthe oryzae*. For functional and comparative analysis of histone modifications, a web-based database (dbHiMo) was constructed first to archive and analyze histone modifying enzymes from eukaryotic species whose genome sequences are available. Based on the database entries, we carried out functional analysis of genes encoding histone acetyltransferases (HAT) and deacetylases (HDAC). Gene deletion or silencing approaches showed that disruption of histone acetylation status generally leads to defect in vegetative growth and asexual sporulation. RNA-seq analysis on one of the HAT mutant ( $\Delta$ *Mosas3*) indicated that such growth defect is related to down-regulation of a whole array of genes involved in nitrogen and carbon metabolisms. Differentially expressed genes in the mutant showed significant overlap with genes that are differentially expressed in  $\Delta$ *Mort109* mutant, suggesting link between DNA damage response/repair and metabolic repression in epigenetic level. For HDAC, our data suggest that Class I HDACs have great impact on fungal biology. In particular, we revealed that *MoHOS2* is required for asexual reproduction through stage-specific regulation of some of the conidiogenesis-related genes. Interestingly, in  $\Delta$ *Mohos2*, it appears that reduced pathogenicity is attributed to mis-regulation of genes encoding effector proteins. Furthermore, we showed that lack of deacetylase activity alone is able to recapitulate most of phenotypic defect in  $\Delta$ *Mohos2*. Taken together, our systematic analyses provide not only insights into implication of histone acetylation/deacetylation in fungal development and pathogenesis but also novel targets for new agrochemicals against fungal pathogens of plants.

## S5-3

## Therapeutic effect of extracellular matrix degradation enzyme delivered by combination engineered bacteria with anticancer drugs

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To overcome limitations associated with bacteria-mediated cancer therapy, the engineered bacteria that encoded therapeutic drug have been reported. Several attenuated bacteria such as *Salmonella typhimurium* could target tumor tissue and regress the tumor mass. However, engineered bacteria have some hurdle to apply the clinical applications. We designed the combination therapy with bacteria and anti-cancer drugs. Extracellular matrix (ECM) of tumor tissue is one of targets for tumor therapy since the growth of tumor cells and tissues depends on its formation. Hyaluronic acid (HA) is major polysaccharide component of ECM and increases the interstitial fluid pressure (IFP) within the tumor tissue by absorbing significant amounts of water, resulting in an inhibition of anti-cancer drugs diffusion to tumor tissue. In this study, we assessed a possibility that ECM breakdown would increase the activity of tumor therapeutic drugs. We constructed plasmid encoded highly expressing hyaluronidase (HAase) under the controlled constitutive promoter originated from *Staphylococcus aureus*. The transformed attenuated *S. typhimurium*  $\Delta$ ppGpp (SL-HAase) exhibited the enzyme activity that degraded HA in agar plates. The bacterial lysates showed enhanced enzyme activity over 16-fold compared to control bacteria. In order to measure in vivo degradation of HA in tumor tissue, we injected SL-HAase into 4T1 and PC3 xenograft models intravenously resulting in observation of significant decrease of ECM in both 4T1 and PC3 tumor tissue after 5 days compare to control group. To assess the combination therapy of an engineered bacteria with a chemotherapeutic drug, we injected ECM degrading bacteria and then doxorubicin every 2 days (a half of dose, 5 mg/Kg) in 4T1 and PC3 xenograft models, resulted in inhibition of tumor growth compared to control group. The ability of ECM degrading bacteria to enhance chemotherapy efficacy is likely due to increased drug perfusion by reduced IFP.

**S6-1**

## **Trait modifications using a protoplast CRISPR/Cas9 system in horticultural crops: review and progress**

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Crop improvement through various breeding tools has contributed to sustainable agriculture, and been practiced to enhance yield and quality of biomaterials for human beings. Conventional breeding technologies, have been greatly used to enhance crop production. Recently, a targeted genome editing (TGE) using engineered nucleases such as clustered regularly interspaced short palindromic repeat (CRISPR) nucleases has been applied to improve the traits of plants successfully. TGE has emerged as a novel plant-breeding tool that represents an alternative approach to classical breeding, but with higher mutagenic efficiency. The CRISPR/Cas9 system was originally developed based on a defense system used by prokaryotes against foreign pathogens. The CRISPR/Cas9 system consists of RNA-guided engineered nucleases (RGNs) that recognize their cognate nucleotide sequences in target genes through complementary base pairing. CRISPR/Cas9-mediated genome editing through a direct delivery of pre-complexed purified CAS9 protein along with in vitro-transcribed sgRNA can be achieved by adopting a protoplast culture, with advantages of no requirement of codon optimization or expression promoter, and DNA-free editing for bypassing GMO regulations. Research groups have recently reported target-directed genome editing using protoplast transformation and whole-plant regeneration technology in some horticultural crops species including lettuce, tobacco, apple and potato. In a continuation of previously established protocol for gene knock-out in petunia protoplasts via Cas9-RNP delivery, we have successfully performed site-specific mutagenesis in petunia to modify flower color modifications. From the recent results, it was found that orthologous or paralogous genes, which occur widely in plant speciation, should be investigated when the CRISPR-Cas system is applied to target-specific gene editing to achieve proper target mutagenesis in plants.



## S6-2

## Optimization of meristem maturation and shoot growth in tomato

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Sympodial growth and flowering time are two major traits for high yield tomato, regulated by molecular maturation of shoot apical meristem. Here we show that tomato productivity can be fine-tuned and optimized by exploiting mutations controlling flowering time pathway and shoot growth pathway. First, we show the mutants showing relatively weak late flowering, among selected flowering time mutants, produced more yield compared with the determinate tomato. A new *sp* weak mutant allele, *sp-5732*, from 242 Core Collection produced more sympodial shoots and improved tomato fruit yield up to 42% in the field. Second, we discovered IL3-2-2, overexpressing tomato florigen, could optimize crop productivity between leaf and inflorescence production among the variants bearing successively two or three leaves sympodial index of indeterminate tomato. Third, we also bred compact tomato varieties using *sler* and *sod* mutants, which allow tomato cultivation under restricted space conditions such as growth chamber in building. Altogether, these variants support new resources for tomato breeding and the function of genes could be used as multiple genetic targets to overcome background issue on breeding process with advances in biotechnology.

**S6-3**

## **Identification of Genes Involved in Trichome Development and Insect Resistance in Tomato**

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Trichomes are specialized epidermal structures that protect plants from abiotic and biotic stresses. In depth knowledge of the molecular mechanisms that control trichome development in *Arabidopsis*, which produces unicellular nonglandular trichomes, has provided significant insight into the genetic basis of variation in trichome habit. Solanaceous plants including tomato produce several different types of multicellular nonglandular and glandular trichomes on aerial tissues. In contrast to our understanding of unicellular nonglandular trichomes, much less is known about the development and ecological function of multicellular trichomes. Here, we report identification of several genes involved in trichome development and their roles in plant defense against insect attack in tomato.

S7-1

## Forensic Chemistry: Review of Status and Needs

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The term “forensic” is practically a synonym for “legal” or “related to courts”. Forensic Genetics & Chemistry Division in Supreme Prosecutors' Office of Korea is divided into two main areas of scientific investigation: forensic chemistry and DNA & biology. Forensic chemistry deals with application of chemical principles for the solution of problems that may arise in conjunction with serving justice. It embraces wide and diversified fields, which include not only forensic toxicology but also the analysis of any material that may have presence in the course of legal proceedings. The Forensic Chemistry section conducts analysis on varieties of physical evidences relating to criminal cases such as illegal medicines, poisons, adulterated foods, narcotics and hallucinogenics as well as harmful chemicals in various biological samples and seized materials. Our section has maintained ISO/IEC 17025 accreditation which is the most important international standard for testing and calibration laboratories. In addition, the lab conducts research on noble analytical techniques for new psychoactive substances, drug impurity profiling as well as other crime scene evidences to cope with the rapid changing criminal environment. In this presentation, a brief summary of current status of forensic chemistry as well as recent legal challenges for handling crime scene evidences in the course of scientific investigation will be discussed.

**S7-2**

## **Identification of phytolaccosides in the biological samples from pokeweed intoxication patients using liquid chromatography-tandem mass spectrometry**

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Many plants in the *Phytolaccaceae* are distributed throughout tropical and subtropical area. About 35 species was included in genus *Phytolacca*, and they are common in America, South-Africa and South-Asia. Among the *Phytolacca*-species, *P. americana* L. (American pokeweed) and *P. esculenta* VAN HOUTTE (Chinese pokeweed) are two representative species. While *P. americana* is native to the temperate regions of North America and it propagated over the whole world, *P. esculenta* is native to China and its occurrence has been reported from many European country.

Pokeweed have toxic compounds in fruits, roots, leaves and stems, and it was reported that the toxic substances were the most abundant in roots and the amount of toxic compounds were not different between the two species. Pokeweed are toxic to cattle, horses and man. It was reported that their toxicity in cow included severe gastro-intestinal irritations, spams, purging, convulsions and death by paralysis of the respiratory organs, and abdominal pain, nausea, vomit, diarrhea, visual and auditory hallucination, and mental change were reported from the patients who had eaten pokeweed roots raw.

Pokeweed produces various types of chemical compounds. From the roots of *P. americana*, four types of lectins and lignanes were isolated from seeds, and betalain pigments from berries. The toxic compounds of pokeweed are triterpenoid saponins. As saponins, phytolaccoside A, B, D, E, G were isolated from *P. americana*, and esculentoside H, J, L, K, M, I and N were isolated from *P. esculenta*. With saponins, their aglycon phytolaccagenin, phytolaccagenic acid, esculentic acid and jaligonic acid were also isolated from *P. americana* and *P. esculenta*.

Two persons took plant roots misidentification and transferred to emergency room with vomit and abdominal pain. Blood and gastric contents from irrigation were collected from the patient 1, and blood and urine were collected from the patient 2. The biological samples and the roots were analyzed with liquid chromatography-tandem mass spectrometry (LC-MS/MS) to identify toxic substances. From the blood, gastric contents, and the ingested roots, esculentoside A, B, C, and H were identified, and very small amounts of esculentoside A, B, H were identified from urine. The applied analytical method was validated for the parameters such as linearity, limit of detection, precision, accuracy, matrix effect, recovery, and process efficiency.

S7-3

## Military forensic science and Applied Biological Chemistry

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Scientific Investigation Laboratory (SIL) of Criminal Investigation Command (CIC), Ministry of National Defense (MND) in Korea pursues substantial truth through scientific experiments on military-related crimes and accidents and provides educational supports regarding scientific investigation. As the only forensic examination agency in the Korean military, SIL is internationally accredited as forensic institution from KOLAS (Korea Laboratories Accreditation Scheme) and As methods of committing crime are becoming more diverse, we are making efforts to develop new examination methods.

The forensic examination of natural science is the process that reveal the cause of death and accident and the offender by scientific experiments and thinking for the fields of chemistry, biology, physics, engineering and medical science and so on. Finding objective and scientific evidence in the crime scene is an important factor in making reasonable decisions under the judicial system. SIL of MND works for the examination and research of forensic science about the field of autopsy, chemistry, toxicology, DNA, document, fingerprint, image, digital, firearm, toolmark and criminal psychology in the area of military. Particularly, in the applied biological chemistry it is performed that the cause of death and drug-facilitated crime including sexual assault are revealed by the analysis of abuse drugs and toxic chemical and a offender is identified by DNA analysis. Also, the environmental pollutants such as petroleum, asbestos, lead, PAHs and so on were examined. If there is a difference from other forensic institutions, SIL is in charge of firearms/explosives analysis and DNA comparison test for the remains in the Korean War. Particularly, On March 2010, in the sinking of Korean navy ship, named as Cheonan, by the attack of North Korea, we participated in the part of forensic science with many authorized institutions and identified the high explosives by UPLC-Q/TOF. And also, on August 2015, in the DMZ landmine provocation 2 sergeants were amputated. Our team identified TNT (with other evidences as the fragments of landmine body) and verified the provocation by North Korea.

For the research, recently, the classification of petroleum or soil evidences were statistically interpreted by multivariate analysis such as principle component analysis, linear discriminant analysis and support vector machine. The comparison of IR frequencies between ab initio calculation and experiment for the understanding and member education was researched by the quantum chemistry. The formation of anion adduct and properties of explosives in gas-phase are studied.

SIL of MND are doing our best for defense security, safety of people and truth-seeking. When the professional scientists in various fields should assist and volunteer in the military forensic science, SIL will be built as a more specialized forensic institution.

**S7-4**

## **Quantitative Tandem Mass Spectrometry for Simultaneous Analysis of Pesticide Multiresidues in Human Serum and Urine**

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Determination of multiresidual pesticides in a biological sample is essential for an immediate decision and response related to various pesticide intoxication. Serum and urine samples are very useful to detect pesticides within 2-3 days after acute poisoning from victims or patients. In this study, a rapid and simultaneous analytical method for pesticide multiresidues (more than 300 analytes) in serum and urine was established using liquid and gas chromatography-triple quadrupole mass spectrometer (LC-MS/MS and GC-MS/MS). The positive/negative switching electrospray ionization (ESI) mode and scheduled multiple reaction monitoring (MRM) were used to perform high-throughput pesticide detection within 15 minutes. Tiny volumes of serum or urine (100  $\mu$ L) were used for sample preparation considering a realistic situation where a lot of urine cannot be collected from a pesticide poisoning victim or patient. For the optimization of sample preparation, three versions of QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) procedures were scaled-down and compared, and the procedure using magnesium sulfate and sodium chloride without non-buffer reagents as well as dSPE clean-up was selected. The limit of quantitation (LOQ) in this method was 10 ng/mL for most of the target pesticides, sufficiently low to detect pesticides in applications. To evaluate the reliability of the established method, validation such as linearity, accuracy/precision and recovery test were conducted and the results satisfied the reasonably established criteria. The established bioanalytical methods are sufficient for application to biomonitoring in agricultural exposures and applicable in the forensic and clinic.

S8-1

## A Key to the Success of PLS Implementation: Authorative Registration of Pesticides Using Minor Crops

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Rural Development Administration (RDA) has registered 314 pesticides by 2017, enabling them to use 1,223 pest control programs for farmers to provide safe agricultural products to the people and to provide suitable pesticides for farmers as a result of the authorative registration of pesticides since 1998. Authorative registration of pesticides using minor crops can be divided into three stages of projects before 2012, 2013~2017 and after 2018 project, and it is important to prepare for positive list system(PLS) of pesticides be implemented in 2019.

Minor crops introduced and cultivated by farmers for income increase are required to register pesticides due to the occurrence of pests in the crops two to three years after introduction. The registration tests of unregistered pesticides is performed by the Rural Development Administration (RDA) after 2~3 years.

It is difficult to satisfy the demand of various pesticides which can be used in the field by the method of registering one crop after one conventional crop test. In order to solve this problem, the RDA introduced a group registration system in 2017 and applied the results of one crop test to various crops.

In 2018, 248 activity and phytotoxic tests and 949 crop residue tests were conducted in the first year of large-scale authorative registration testing. As a result, 1,670 pesticides were newly registered and 4,441 pesticide safe use guidelines were established provisionally. Finally 7,018 pesticides which will be officially registered until 2021 were applied for use expansion registration with such provisional safe use guidelines.

In 2019, it will be to carry out 246 activity and phytotoxic tests and 880 crop residue tests to register 1,853 pesticides.

**S8-2**

## **Pesticide MRL Setting and PLS Enforcement in Korea**

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There are two types of pesticide MRLs setting in Korea. Domestic and Import tolerance (IT). Setting of domestic MRLs carried out when the standard is requested by the Rural Development Administration, and the MRLs of the imported agricultural products are established at the time of application for IT from the pesticide company or the food company. Both are recognized as MRLs in Korea and applied equally to domestic and imported agricultural products. To propose MRLs, the residual data of each field trials are evaluated according to CODEX guide line or EU evaluation manual. The OECD calculator value is proposed as a MRL and considered to be in harmony with the codex MRL if the submitted data is identical to the data reviewed by the codex. MRL of countries that have performed field trials or applied for IT, and MRL for major exporting countries also can be considered. If the proposed MRL is applied and there is no concern for the chronic risk assessment of Korean, the proposed MRL will be reviewed and confirmed at the Expert Review Meeting and the Food Hygiene Deliberation Committee.

In Korea, a new pesticide management system, Positive List System (PLS), has begun. The first stage was started on 31, December 2016 for tree nuts, oilseeds and tropical fruits, and the second stage was implemented on 1, January 2019 for other crops. The purpose of introducing PLS is to protect the health of the people by preventing misuse and abuse of pesticides. The introduction of PLS is expected to increase the public confidence in agricultural products and improve competitiveness of agricultural products produced in Korea.



S8-3

## Suggestion of Solutions to Problems Caused by Implementation of Positive List System

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The improved living standards of people render a quality life, and the social favorability of well-being and safe food for a good health is also increasing day by day. Many Pesticides applied to crops for controlling insect pests, phytopathogens, and weeds can remain in the final agricultural products. Accordingly, in order to strengthen the safety management of agricultural products and to secure international competitiveness, Korea government agencies have introduced the 'Positive List System (PLS)' for the domestic and overseas imported agri-food products from January 2019. PLS is a system that administers unregistered pesticides for which no MRL is set to specific crop, at a uniform level of 0.01 mg/kg.

In contrast, during implementation of PLS, various concerns are being raised by the agricultural producers because the unpredictable pesticides can remain in the crop irrespective of the intentional farming activities such as soil residue pesticides, residues in rotational crops and drift of applied pesticides. Therefore, for the successful implementation of PLS, a strategy to minimize the damage of producers due to unpredictable pesticide contamination is needed.

The pesticide residues in the soil, which can occupy the largest portion by unpredictable pesticides, may be taken up by crops. This may pose a major risk in respect to the safety of these products for human consumption and economical damage related to PLS for producers. Many reports have shown that significant amounts of pesticide residues in agricultural soils can be taken up by plant roots, and transferred to edible parts, such as leaves and/or fruits. In particular, because pesticides residues absorbed from soil are mostly retained in the roots of the plant, root and tuber crops may be the most susceptible to pesticide residue contamination.

Today, I would like to present the results of the actual experiment data on uptake to crops of pesticide residues in the soil that may be affected by the implementation of the PLS system.

**Keywords:** Crop uptake, Pesticide residue, PLS, Soil management criteria