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**Soo Yeon Choi, Jaemin Lee, Dong Gu Lee, Sanghyun Lee, Eun Ju Cho.** ***Acer okamotoanum* improves cognition and memory function in Ab25–35-induced Alzheimer’s mice model. (2017) Appl. Biol. Chem. 60(1): 1–9**

We studied the effect of ethyl acetate (EA) fraction from *Acer okamotoanum* on cognitive improvement and protective abilities in amyloid beta (Aβ)25–35 peptide-injected Alzheimer’s disease (AD) mice. EA was oral administration at 100 and 200 mg/kg/day during the 14 days. We studied the protective effect of EA against AD on the basis of behavioral tests including T-maze test, Novel object recognition test, and Morris water maze test. Control group injected with Aβ25–35 showed significant impairments in memory function. But the oral administration of EA (EA 100 and EA 200 groups) improved the cognition and memory function. In addition, EA against Aβ25–35 peptide has been shown to inhibit lipid peroxidation levels and nitric oxide production in tissues. Acetylcholinesterase (AChE) was elevated in the brain by Aβ25–35peptide, whereas administration of EA (EA 100 and EA 200 groups) significantly decreased AChE level. Our results indicated that EA improves learning and long-term memory against Aβ25–35 peptide–caused deficit through attenuation of oxidative stress.

**Hyeri Lee, Min Hee Hwang, Miju Cho, Dong Gun Lee, Eun Byeol Go, Young-Keun Cheong, Chon-Sik Kang, Nam Taek Lee, Namhyun Chung.** **Comparison of blood glucose levels and allergic responses on treatment with six wheat cultivars. (2017) Appl. Biol. Chem. 60(1): 11–16**

Allergies and indigestion problems have increased in our society with increasing wheat consumption. Among wheat proteins, omega-5 gliadins (ω5-gliadins) and high molecular weight (HMW)-glutenin are known to be some of the major factors responsible for an allergic response and indigestion. Certain low molecular weight (LMW)-glutenin subunits are also able to promote local inflammation, and share common epitopes with ω5-gliadins. It is known that different wheat cultivars have different combinations of HMW- and LMW-glutenins. We investigated the relationship between the composition of LMW-glutenins and the degree of allergic response or indigestion. The extent of allergic reaction and indigestion characteristics in response to six different wheat cultivars were compared. Patterns of the change of blood glucose level with time, which were employed to measure the indigestion, were quite different, depending on the type of wheat cultivars. The extent of allergic response, as measured with the histamine and IgE levels, was also quite different, depending on the type of wheat cultivars. In addition, the extents were not correlated with patterns of LMW-glutenin DNA and HMW/LMW glutenin protein of various wheat cultivars. The results from the present study suggest that some more other factors, together with factors studied above, need to be considered to better explain the physiological phenomena of variation in blood glucose level and allergic response.

**Jeongtaek Lim, Hong-Sik Hwang, Suyong Lee. Oil-structuring characterization of natural waxes in canola oil oleogels: rheological, thermal, and oxidative properties. (2017) Appl. Biol. Chem. 60(1): 17–22**

Natural waxes (candelilla, carnauba, and beeswax) were utilized as canola oil structurants to produce oleogels. Physicochemical properties of the oleogels were evaluated from textural, thermo-rheological, and oxidative points of view. The oleogels with candelilla wax exhibited the highest hardness, followed by carnauba and beeswax oleogels, while the most adhesive and cohesive properties were observed in the beeswax oleogel. The flow behaviors of the oleogels over temperature exhibited greater sensitivity of carnauba wax oleogels to temperature. The storage moduli of the oleogels were more temperature-dependent, causing the crossover of the storage and loss moduli during the temperature change. Highly linear correlations (*R*2 > 0.96) were observed in the log plots of solid fat content and rheological property. In addition, the lowest peroxide values were observed in the candelilla wax oleogels, followed by the carnauba and beeswax oleogels, demonstrating that oleogels with a harder texture exhibited greater resistance to oxidation during storage.

**Sun-Goo Hwang, Ju Hee Kim, Jun-Cheol Moon, Jin-Hyuk Kim, Cheol Seong Jang. Comparative analysis of chloroplast DNA sequences of *Codonopsis lanceolata* and *Platycodon grandiflorus* and application in development of molecular markers. (2017) Appl. Biol. Chem. 60(1): 23–31**

*Codonopsis lanceolata* and *Platycodon grandiflorus* (order Asterales) originate from East Asia. Despite the high commercial availability of *C. lanceolata* and *P. grandiflorus*, limited genetic research has been performed on these plants. We applied a targeting enrichment method to detect genetic diversity in *C. lanceolata* and *P. grandiflorus* and recovered their chloroplast genomes from total DNA sequence data. Chloroplast DNAs (cpDNAs) were 61,154 bp (*C. lanceolata*) and 81,214 bp (*P. grandiflorus*) in length. Sixteen simple sequence repeats and 15 long repeat sequences were determined, which are useful as potential markers in both plant species. We surveyed the phylogenetic relationships with increased resolution in 14 plant species, including other 8 species from the order Asterales and 4 from the order Apiales. In addition, we demonstrated the availability of recovered chloroplast genomes through cpDNA marker development to determine the authenticity of food fraud at the DNA level of plant species.

**Ki-Chang Lee, Yeung Seok Yoon, Fan-Zhu Li, Jong-Bang Eun. Effects of inlet air temperature and concentration of carrier agents on physicochemical properties, sensory evaluation of spray-dried mandarin (*Citrus unshiu*) beverage powder. (2017) Appl. Biol. Chem. 60(1): 33–40**

This study was to investigate the influence of spray drying conditions on the physicochemical characteristics and sensory evaluation of mandarin (*Citrus unshiu*) beverage powder. The results show that moisture content, color, pH, vitamin C, water solubility index and drying yield were significantly affected by the carrier agent concentrations and the inlet air temperatures. However, water activity and water absorption index were not significantly influenced by the spray drying conditions. Sensory evaluation results of taste, color and overall acceptability of mandarin beverage powders added with corn syrup were higher than those added with maltodextrin. As the result of process suitability for spray drying mandarin beverage by using different parameters, it is concluded that 35% corn syrup concentration and 135 °C inlet temperature were suitable to produce mandarin beverage powder with preferable taste and color.

**Murat Tunçtürk, Tamer Eryiğit, Ali Rahmi Kaya. Nutritional properties, minerals, and selected heavy metal contents in herby cheese plants of Lamiaceae. (2017) Appl. Biol. Chem. 60(1): 41–47**

Ten of the most popular and well-known edible plant species from the Lamiaceae which are used in the production of herby cheese were examined to evaluate their nutritional properties, mineral, and heavy metal content. This study has revealed significant variations in the contents of minerals among the studied plant species. The most of plant samples were rich in some of the vital minerals such as Fe, Cu, Ca, K, Mn, and Zn, which are known to be important in health maintenance. *Ziziphora capitata* plant materials had the highest concentration of toxic heavy metals, including chromium (1.72 mg kg−1), where *Mentha longifolia* had the highest cobalt (1.14 mg kg−1). The results of this study suggest that the use of these plant species in herby cheese will not contribute to heavy metal toxicity, but may be useful in treating micronutrient deficiency.

**Song-I Han, Jungwhoi Lee, Myeung Seung Kim, Sung Jin Chung, Jae-Hoon Kim. Molecular cloning and characterization of a flavonoid glucosyltransferase from Byungkyool (*Citrus platymamma* hort. ex Tanaka). (2017) Appl. Biol. Chem. 60(1): 49–55**

Uridine diphosphate glucosyltransferase (UGT) attaches glucoside to proteins, various flavonoids, and phenolic compounds. The modification of flavonoid affects its water solubility, stability, and bioavailability of flavonoids. In this study, citrus genomic sequence database was searched for UGTs of citrus, and a UDP-glucosyltransferase (*bGT173*) was isolated from Byungkyool (*Citrus platymamma* hort. ex Tanaka). The cloned cDNA gene was 1365 bp in length and encoded 456 amino acids. Phylogenetic analysis suggested that *bGT173* was a member of the flavonoid 3-*O*-glucosyltransferase group. mRNA expression of *bGT173* was higher in leaves compared to flowers, stems, and fruits. The recombinant protein of *bGT173*was expressed in *Escherichia coli*, and tested for its activity on seven flavonoids (apigenin, eriodictyol, hesperetin, kaempferol, luteolin, naringenin, and quercetin). Both kaempferol and quercetin were good substrates for *bGT173*, demonstrating that *bGT173* preferentially glucosylated the 3-hydroxyl group of flavonols. Furthermore, quercetin 3-*O*-glucoside produced in *E. coli* showed the same anti-migration activity on pancreatic cancer cells similar to the standard chemical, suggesting that *bGT173* is a good candidate for bioconversion of quercetin to quercetin 3-*O*-glucoside.

**Bit-na Yang, Eun-hye Choi, Soon-Mi Shim. Inhibitory activities of kaempferol against methylglyoxal formation, intermediate of advanced glycation end products. (2017) Appl. Biol. Chem. 60(1): 57–62**

Methylglyoxal (MGO), an intermediate of advanced glycation end products (AGEs), is generated by the maillard reaction between carbonyl groups in reduced sugars and amino groups. The aim of this study was to examine inhibitory activities of kaempferol against the formation of AGEs by trapping MGO. Quantification and identification of MGO and Kaempferol MGO adduct was analyzed by ultra performance liquid chromatography with a photodiode array detector and mass spectrometry, respectively. 26.1, 29.5, 29.3, and 31.2% of MGO remained after reacting with 0.1, 0.25, 0.5, and 1.0 mM of kaempferol at 24 h of incubation time, respectively. The mono- or di-MGO adducts of kaempferol were identified, and hydroxyl groups in the A-ring of kaempferol were found to be the major active sites for trapping MGO. Results from the current study propose that kaempferol could suppress the formation of AGEs by trapping its reactive intermediate, MGO.

**Eun-Ho Lee, Jun-Hyo Cho, Dong-Hee Kim, Shin-Hyub Hong, Na-Hyun Kim, Mi-Jeong Park, Eun-Jin Hong, Young-Je Cho. Anti-inflammatory activity of manassantin A from ultra-fine ground *Saururus chinensis* in lipopolysaccharide-stimulated RAW 264.7 cells. (2017) Appl. Biol. Chem. 60(1): 63–71**

As a research of inflammation inhibitory activity using a natural resource, the inflammation inhibitory activity by purified manassantin A from *Saururus chinensis* was experimented. In the result of MTT assay with manassantin A, cell viability decreased at concentration of 100 μM. LPS-treated RAW 264.7 cell group treated with 6.25–50 μM concentration of manassantin A showed approximately 4–55% NO expression compared to LPS non-treated group. Inflammation inhibitory activity and NO expression inhibition increased as RAW 264.7 cell treated with higher concentration of manassantin A. Expression inhibition of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) was also identified. An excellent prostaglandin E2 expression inhibition effect was identified with manassantin A at concentration of 6.25–25 μM. A high interleukin-1β, tumor necrosis factor-α, and IL-6 expression inhibition effect was manassantin A at concentration of 6.25–50 μM of 0–97, 6–32, and 22–66% was identified, respectively. A high interleukin-1β, tumor necrosis factor-α, and IL-6 expression inhibition effect was manassantin A at concentration of 6.25–50 μM of 0–97, 6–32, and 22–66% was identified, respectively. Expression inhibition effect was identified to be concentration dependent. Therefore, manassantin A is expected to show high inflammation inhibitory effect in RAW 264.7 induced from LPS by inhibiting iNOS and COX-2 protein expression along with cytokine expression inhibition.

**Mi-Jeong Yang, Hyun Woo Lee, Hoon Kim. Enhancement of thermostability of *Bacillus subtilis*endoglucanase by error-prone PCR and DNA shuffling. (2017) Appl. Biol. Chem. 60(1): 73–78**

The *Bacillus subtilis* endo-β-1,4-glucanase gene (*beg*), which encodes the enzyme BEG that comprises 499 amino acid residues, was mutated by error-prone polymerase chain reaction and DNA shuffling to make variants with improved functionalities. The mutated DNAs were transformed into *Escherichia* *coli* DH5α, and among the 1370 transformants, a positive clone 8-20 was obtained finally based on a halo assay and thermostability and alkaline tolerance analyses. The mutated enzyme BEG8-20 of clone 8-20 was changed at seven amino acid residues compared to the wild-type enzyme BEGwt: K45E, I102Y, M112V, D226Y, D295E, L423S, and D460G. The optimum temperature and pH of BEG8-20 were nearly the same as those of BEGwt. However, the thermostability of BEG8-20 was increased 1.53-fold (21.4 min vs. 14.0 min) based on the half-life of the residual activity at 70 °C. This increase in enzyme thermostability is advantageous for the hydrolysis of lignocellulosic materials to produce fermentable sugars. In the activity staining experiment, only a truncated smaller enzyme was observed for BEG8-20, whereas two forms, a matured (52 kDa) and a truncated smaller (34.5 kDa) enzyme, were observed for BEGwt owing to proteolytic internal cleavage at the linker region between the 297th and 356th amino acid residues. These results indicate that amino acid substitutions in the mutant enzyme have rendered the protein prone to cleavage at the C-terminal region.

**Ju Hee Kim, Jun-Cheol Moon, Tae Sun Kang, Kisung Kwon, Cheol Seong Jang. Development of cpDNA markers for discrimination between *Cynanchum wilfordii* and *Cynanchum auriculatum* and their application in commercial *C. wilfordii* food products. (2017) Appl. Biol. Chem. 60(1): 79–86**

*Cynanchum wilfordii* has been used as a health-enhancing food product in Korea. Owing to its morphological similarity to *C. wilfordii* and relative ease of cultivation, *C. auriculatum* has been illegally used as a substitute plant for *C. wilfordii*. In the present study, we developed markers to discriminate between *C. wilfordii* and *C. auriculatum*. Species-specific primer sets for *C. wilfordii* and *C. auriculatum* were designed based on the single nucleotide polymorphisms of the chloroplast *matK* genes for quantitative real-time PCR using SYBR green. The limit of detection of primer sets for each species was assessed by analyzing serially 1/10-diluted DNAs at concentrations of 0.001–100% (=10 ng) and binary mixtures of a flour matrix spiked with decreasing concentrations (103–1 mg/g) of non- and heated *C. wilfordii* and *C. auriculatum*, respectively. We found that species-specific primer sets indicated good amplification efficiency and correlation coefficients (*R*2) of the standard curves in the extracted DNA. The developed markers were successfully applied to 19 commercial *C. wilfordii* food products and could prove a useful tool for verifying the presence of *C. wilfordii* and *C. auriculatum* in commercial products.

**Dong Jin Lee, Young Man Yoon, Ik Won Choi, Ji Su Bae, Dong Cheol Seo. Effect of seasonal variations of organic loading rate and acid phase on methane yield of food waste leachate in South Korea. (2017) Appl. Biol. Chem. 60(1): 87–93**

The objective of this study was to determine the effect of seasonal variations of organic loading rate (OLR) and acidogenic phase on methane yield of food waste leachate (FWL) treated in biogas facility in South Korea. A biogas facility operating in G city was selected as the target for this study. Remarkable seasonal fluctuations in methane yield occurred in this facility repeatedly. Methane yield in the summer was significantly lower compared to that in other seasons. In order to determine the operation efficiency, precision investigation (methane yield, OLR, etc) was conducted from March 2014 to April 2015. Characteristic parameters and operating factors of a two-stage anaerobic digestion were analyzed to obtain volatile fatty acids (VFAs), chemical oxygen demand, nutrients, total nitrogen, and so on. Data comparison revealed that the monthly average values of OLR and VFAs tended to increase rapidly in the summer (up to 3.92 kgVS/m3 day and 9263 mg/L, respectively). In contrast, methane yield in the same season was at 0.28 Sm3CH4/kg VS, which was much lower than the average value (0.42 Sm3CH4/kg VS) of methane yield in other seasons. The decrease in methane yield ranged from 69.0 to 57.9% in the summer. These results suggested that methane yield might be influenced by the operating conditions with seasonal organic loading fluctuations. In other words, methane yield might be affected by a shock load of VFAs due to inapposite operation of acidogenic phase with easily degradable FWL, particularly in the summer. The results of this study will provide important information on how an ongoing biogas facility of FWL should be operated in the summer.

**Min Hee Hwang, Dong Gun Lee, Eun Byeol Go, Miju Cho, Young Sig Park, Namhyun Chung. Anti-diabetic effect of magnesium salt extracts from deep-sea water in C57BLKS/J-db/db mice. (2017) Appl. Biol. Chem. 60(1): 95–99**

Recently, the incidence of diabetes has increased steadily worldwide. Type 2 diabetes constitutes about 90% of all cases of diabetes, and it is associated with many complications. Currently, the drugs for therapy of type 2 diabetes are considerably limited owing to disadvantages such as side effects and high rate of secondary failure. To overcome these problems, it is necessary to develop effective therapeutic agents from safe natural products. Deep-sea water (DSW) is abundant in minerals such as potassium (K), calcium (Ca), and magnesium (Mg). Mg supplements are known for their usefulness in the treatment of type 2 diabetes. In this study, we examined the anti-diabetic effect of Mg salt extracts from DSW in a diabetic mouse model. We observed that the groups treated with Mg salt extract showed better response toward fasting blood glucose level and oral glucose tolerance test when compared to the positive control. Overall, the Mg salt extract improved the symptoms of impaired fasting glucose and glucose tolerance, suggesting that it can be used as an effective agent for the treatment of type 2 diabetes mellitus.

**Soojung Park, Kyochan Kim, Sang-Il Han, Eun Jung Kim, Yoon-E Choi. Organic solvent-free lipid extraction from wet *Aurantiochytrium* sp. biomass for co-production of biodiesel and value-added products. (2017) Appl. Biol. Chem. 60(2): 101–108**

Increasing demand for renewable energy has led to the production of biodiesel from microalgae. Microalgae have been regarded as one of the best feedstocks for biodiesel due to their high growth rate and lipid content as compared to other crops and plants. However, use of microalgae is still hindered by technical barriers and high processing costs. The economic viability and environmentally friendly aspects of biodiesel production will be significantly improved by co-producing value-added chemicals and extracting lipids directly from wet biomass without involvement of organic solvents, respectively. In this study, organic solvent-free lipid extraction from wet *Aurantiochytrium* sp. biomass was developed to co-produce biodiesel and value-added products such as docosahexaenoic acid. Organic solvent-free lipid extraction was performed by applying alkali and heat treatments to wet biomass. Key parameters for the extraction from *Aurantiochytrium* sp. were optimized to increase extraction yields. A ratio of 15 mg/mL of biomass to alkaline solution and an alkaline solution concentration of 1% w/w were determined to be the optimal conditions. Both heating temperature and treatment time were associated with positive effects on lipid recovery, up to a certain level. The maximum extraction yield (77.37% of total lipid) was obtained by heating at 150 °C for 30 min. Additionally, microwaves were employed to the extraction system and could further reduce the reaction time. Our study could be expanded to other types of microalgal biomass and will aid in establishing a protocol for organic solvent-free lipid extraction directly from wet biomass.

**Sunghun Cho, Jaemin Lee, Joyce P. Rodriguez, Buom-Yong Ryu, Chan Kyu Han, Sanghyun Lee. A new 5*H*-purin-6-amine from the leaves of *Sedum sarmentosum*. (2017) Appl. Biol. Chem. 60(2): 109–111**

Phytochemical constituents were isolated from *Sedum sarmentosum* leaves using open column chromatography and medium-pressure liquid chromatography. Their structures were identified as 2,4-pyrimidinedione (1), *N*-methylhydroxylamine (2), 5*H*-purin-6-amine (3), uridine (4), l-tyrosine (5), and l-prolyl-l-tyrosine (6) using mass spectrometry and 1H- and 13C-nuclear magnetic resonance spectroscopic analysis. Among them, compound 3 (5*H*-purin-6-amine) was isolated for the first time from a natural source.

**In Kyung Bae, Kyeongsoon Kim, Sung-Deuk Choi, Kyu-Sik Chang, Hoi-Seon Lee, Sung-Eun Lee. Mosquito larvicidal activities of naturally occurring compounds derived from *Piper* species. (2017) Appl. Biol. Chem. 60(2): 113–117**

Mosquitos transmit human diseases including malaria, dengue fever, yellow fever, and encephalitis. Methylenedioxy compounds are considered to be safe synergists that enhance the activity of active ingredients to control mosquito populations. Seven naturally occurring compounds from *Piper nigrum* and *P. longum* were used to determine their larvicidal activities against larvae of *Culex pipiens pallens*. Among the tested compounds, myristicin and dodecanol showed potent larvicidal activity. Several modes of larvicidal action have been suggested for natural compounds, and in this study, their larvicidal effects on the surface water tension were considered and determined using a tensiometer in order to understand how lowering water surface tension was associated with mortality. In conclusion, lowering water surface tension was related to the larvicidal activities of myristicin and dodecanol.

**Yong Dong Noh, Hye Jin Park, Kwon Rae Kim, Won Il Kim, Ki Youl Jung, Sung Un Kim, Vance N. Owens, Jin Seong Moon, Sung Wook Yun, Sang Yoon Kim, Chang Oh Hong. Contrasting effect of phosphate on phytoavailability of arsenic and cadmium in soils supporting medicinal plants. (2017) Appl. Biol. Chem. 60(2): 119–128**

Soil and plant samples were collected from 84 fields where medicinal plants were cultivatedto determine the effect of soil phosphate (P) on the concentration of plant-available arsenic (As) and cadmium (Cd) and on the uptake of these toxic elements by medicinal plants. Concentrations of total P and available P in soils affected the phytoavailability of As and Cd differentially. Plant-available As in the soil and its uptake in the plant increased with increasing concentration of plant-available P in the soil due to competition between arsenate and P for the adsorption site at the soil surface and an increase in soil pH caused by specific adsorption of P. In contrast, phytoavailability of Cd decreased with increasing concentration of available P in soil. This was mainly attributed to an increase in Cd adsorption caused by P-induced negative charge of soil.

**Haejo Yang, Youngjae Shin. Antioxidant compounds and activities of edible roses (*Rosa hybrida spp.*) from different cultivars grown in Korea. (2017) Appl. Biol. Chem. 60(2): 129–136**

Edible roses have been identified as a potential source of antioxidant compounds promoting human health. In order to assess this potential, nine cultivars of edible roses harvested in Jincheon, Chungbuk, were examined in this study. Extracts of flower petals of edible roses were prepared, and the constituent antioxidant compounds and their antioxidant activity were analyzed. Total anthocyanin concentrations and total flavonoid concentrations were significantly higher in the Mister Lincoln cultivar than those in others. Total phenolic compounds and total antioxidant activity in the cultivars Mister Lincoln and Orange Meillandina were significantly higher than those in other cultivars (*p* < 0.05). Total anthocyanin content was highly correlated with flavonoid content (*R* = 0.927), and the relationship between total phenolics and DPPH radical scavenging activity was also strongly correlated (*R* = 0.915). Overall, antioxidant compounds and antioxidant activity of edible roses were found to be greater than those of fruits and leafy vegetables. Thus, edible roses are a natural source of antioxidant compounds, and they are expected to have great potential for application in the production of functional foods and in the cosmetic industry.

**Jun-Hwan Park, Hoi-Seon Lee. Phototactic behavioral response of agricultural insects and stored-product insects to light-emitting diodes (LEDs). (2017) Appl. Biol. Chem. 60(2): 137–144**

Agricultural insects and stored-product insects are influenced by luminance intensities, exposure times, and wavelengths of light-emitting diodes (LEDs). Based on the phototactic behaviors of the agricultural insects, green or blue LEDs are most attractive for *Bemisia tabaci*, *Trialeurodes vaporariorum*, *Myzus persicae*, *Liriomyza trifolii*, *Spodoptera exigua*, and *Spodoptera litura*. Green LED attracts *Plutella xylostella* and *Frankliniella occidentalis*. Similarly, green or blue LEDs are more attractive to agricultural insects, such as *Liriomyza sativae*, *Sogatella furcifera*, and *Nilaparvata lugens*, than other wavelength LEDs. Concerning the phototactic behaviors of the stored-product insects, red LED is attractive for, in descending order *Tribolium castaneum*, *Sitophilus zeamais*, *Lasioderma serricorne*, and *Tyrophagus putrescentiae*. Blue LED captures most *Sitophilus oryzae* and *Sitotroga cerealella*. Red and blue LEDs are more attractive for stored-product insect pests rate than ultraviolet LED and green, yellow, white, and infrared LEDs. Based on the attraction rate of the stored-product insects on granary, red LED is most attractive for *S. cerealella* and *Plodia interpunctella*. These light sources are effective in controlling agricultural and stored-product insects. Applying LED technology for greenhouses and granaries along with conventional traps reduces crop loss due to moths, beetles, aphids, and weevils. LEDs have potential value in integrated pest management.

**Da-He Kim, Young-Ju Jeon, Myung-Jun Chung, Jae-Gu Seo, Young-Tae Ro. Complete sequence and gene analysis of a cryptic plasmid pLU4 in *Lactobacillus reuteri* strain LU4 (KCTC 12397BP). (2017) Appl. Biol. Chem. 60(2): 145–153**

A cryptic plasmid, pLU4, was isolated and sequenced from *Lactobacillus reuteri* strain LU4, a probiotic strain isolated from human feces in Korea. pLU4 was 33,411 bp in size with a G + C content of 40.1% and contained 41 putative open reading frames (ORFs) of more than 120 bp. Functions were assigned to 24 of these ORFS by a homologous sequence search and domain characterization, and the rest were annotated as conserved hypothetical proteins or hypothetical proteins, based on highly similar proteins found in other *Lactobacillus* species. A region encoding a cluster of conjugative transfer genes (*trs* or *tra*) in pLU4 showed high similarity and co-linearity with the *trs* region of plca36 and *tra* region of pLgLA39 from *L. casei*str. Zhang and *L. gasseri*, respectively. This is the first report of a *trs* cluster sequence from plasmids found in *L. reuteri* species. Comparative gene analysis revealed that pLU4 also encodes highly conserved genes for plasmid replication and stability found in several other *Lactobacillus* plasmids, indicating that plasmids from the genus *Lactobacillus* may contribute to horizontal gene transfer and adaptation to the environment. The findings of this study provide important information about these industrially relevant phenotypes and give insight into the structure, function, and evolution of large *Lactobacillus* conjugative plasmids.

**Jaemin Lee, Joyce P. Rodriguez, Norman G. Quilantang, Myoung-Hee Lee, Eun Ju Cho, Sonia D. Jacinto, Sanghyun Lee. Determination of flavonoids from *Perilla frutescens* var*. japonica* seeds and their inhibitory effect on aldose reductase. (2017) Appl. Biol. Chem. 60(2): 155–162**

*Perilla frutescens* var. *japonica* (PF) is an annual aromatic herb has been consumed as a food ingredient and medicinal crop in Asian countries. To evaluate the therapeutic efficacy of aldose reductase (AR) inhibition, we tested the PF seeds. The stepwise polarities of PF were tested for AR inhibition, and we determined the CH2Cl2 and EtOAc fractions to be good inhibitors (5.81 and 3.99 μg/mL, respectively). Compounds 1–3 were isolated from the CH2Cl2 and EtOAc fractions and identified as luteolin (1), apigenin (2), and diosmetin (3) by physicochemical and spectroscopic data. Among them, luteolin (1) and apigenin (2) had high AR inhibitory activity (1.89 and 4.18 μM). Deulsaem, a variety of PF, was determined to have the highest flavonoid content among ten PF seeds tested (2.10 mg/g). This study suggests that PF could be utilized as a natural source to treat diabetic complications.

**Shogo Sugai, Mayumi Ohnishi-Kameyama, Shinya Kodani. Isolation and identification of a new lasso peptide cattlecin from *Streptomyces cattleya* based on genome mining. (2017) Appl. Biol. Chem. 60(2): 163–167**

Lasso peptides are ribosomally synthesized and posttranslationally modified peptides with diverse biological functions. Recent genome mining has revealed that many species of actinomycetes possibly contain biosynthetic gene clusters of lasso peptides. With genome mining for lasso peptide biosynthesis, we screened several actinomycetes for lasso peptide production using high-performance liquid chromatography and electrospray ionization–mass spectrometry. Consequently, *Streptomyces cattleya* was identified as a producer of a new lasso peptide named cattlecin. Analysis of amino acid content on cattlecin indicated the presence of four moles each of Asp and His, three moles each of Gly and Tyr, and one mole of Ser. Tandem mass spectrometry (MS/MS) analysis of cattlecin revealed C-terminal sequence of WHHGWYGWWDD. The peptide sequence (SYHWGDYHDWHHGWYGWWDD) was the expected amino acid sequence of cattlecin based on genome mining. As a result of MS/MS analysis, the amine residue of the first Ser was proposed to form a macrolactam ring with the β-carboxyl residue of the ninth Asp. The biosynthetic gene cluster of cattlecin comprised four genes: *catA*, *catC*, *catB1*, and *catB2*, which is typical of a lasso peptide biosynthetic gene cluster in actinomycetes.

**Dung Tien Le, Nam Tuan Vu. Progress of loop-mediated isothermal amplification technique in molecular diagnosis of plant diseases. (2017) Appl. Biol. Chem. 60(2): 169–180**

Effective disease management of crops is crucial to sustain food security and safeguard potential losses in crop production that worth billions of dollars. The key to success in plant disease management is having the ability to detect the causal pathogen(s) early and accurately. Polymerase chain reaction (PCR) has been a gold standard in nucleic acid-based diagnostics. Apart from PCR, within the last decade, the development of a technique called “loop-mediated isothermal amplification” or LAMP has facilitated the development of hundreds of simple assays for plant disease diagnostics. There are now more than 200 LAMP publications per year, of which 20% identify plant disease pathogens. Among them, LAMP assays are available for pathogen detection of 50 plant viruses, 20 bacterial plant diseases, 7 fungal plant diseases and several phytoplasmas. Here, we provide a comprehensive analysis of all LAMP assays available for detecting plant diseases, including various detection chemistries used. We also discuss how to avoid pitfalls when developing LAMP assays. Finally, we offer perspectives of the applications of LAMP in plant disease management, addressing the questions as to which extent the assays are helpful and whether they should be used outside the laboratory. This review will be a “handbook” for researchers developing LAMP assays for plant disease diagnostics.

**Patiwit Loypimai, Anuchita Moongngarm, Pheeraya Chottanom. Extraction solvents affecting phytochemicals in food colorant prepared from purple glutinous rice bran. (2017) Appl. Biol. Chem. 60(2): 181–189**

This study investigated the effect of extraction solvents on the concentrations of bioactive compounds in the colorant obtained from purple rice bran. Ten different solvents including 100% water (W), 100% W–HCl 95%, ethanol (EOH), 95% EOH–HCl, 50% EOH, 50% EOH–HCl, 95% methanol (MeOH), 95% MeOH–HCl, 50% MeOH, and 50% MeOH–HCl were used to extract the rice bran. Results indicated that the solvents had a significant effect on the concentrations of the bioactive compounds and visual color in the colorant. The 50% EOH–HCl and 50% MeOH–HCl showed the highest levels of cyanidin-3-*O*-glucoside, cyanidin-3-*O*-rutinoside, delphinidin, cyanidin, pelargonidin, and malvidin, total anthocyanins, (+)-catechin, caffeic, *p*-coumaric, ferulic, and total phenolic acids, whereas the highest level of gallic acid was observed when extracted using 50% EOH and 50% MeOH. The highest concentrations of the tocols, γ-oryzanol, zeaxanthin, lutein, and β-carotene were observed in the colorant extracted by 95% EOH. This suggests that aqueous alcohol with a small amount of HCl (0.1 N) was an effective solvent for extraction of purple rice bran and preparation of colorant containing high important compounds. This solvent has potential as an alternative bio-solvent for green extraction of functional colorants.

**Leesun Kim, Jin-Woo Jeon, Ji-Young Son, Min-Kyu Park, Chul-Su Kim, Hwang-Ju Jeon, Tae-Hoon Nam, Kyeongnam Kim, Byung-Jun Park, Sung-Deuk Choi, Sung-Eun Lee. Concentration and distribution of polychlorinated biphenyls in rice paddy soils. (2017) Appl. Biol. Chem. 60(2): 191–196**

To monitor and evaluate the risk of polychlorinated biphenyls (PCBs) contamination in Pohang, Korea, the concentration and distribution of 29 PCBs in paddy soils were determined using high-resolution gas chromatography/high-resolution mass spectrometry. The overall concentrations of Σ29 PCBs in the paddy soils of the areas close to the heavily industrial city of Pohang (268–1833 pg g−1 dw) were higher than those in the paddies from Anseong (106.6–222.6 pg g−1 dw) in Korea. In Pohang, the major contributors to the Σ29 PCBs were the non-dioxin-like PCBs, including the PCBs 28, 52, 70, 101, 118, 138, 153, and 180, which correspond to 48–62% of the total PCBs. The toxic equivalency (TEQ) values obtained from the 12 dioxin-like PCBs from Pohang (0.03–1.03 pg TEQ g−1 dw) showed that PCB 126 contributed the highest toxicity, possibly posing a risk to the living organisms. The results of both principal component and cluster analysis based on the PCB homologue patterns demonstrated that each sampling site showed a similar PCBs contamination pattern, and Aroclor 1254, which is likely used by small and big steel factories, was identified as a major source of PCB contamination in Pohang.

**Se-Ah Choi, Ji Eun Lee, Min Ji Kyung, Ju Hee Youn, Jeong Bin Oh, Wan Kyunn Whang. Anti-diabetic functional food with wasted litchi seed and standard of quality control. (2017) Appl. Biol. Chem. 60(2): 197–204**

The prevalence of diabetes has become a huge health burden in parts of quality of life and economic cost for overcoming this chronic disease. We followed a bioactivity-guided isolation using α-glucosidase inhibitory assay, four major compounds were isolated, and their structures were elucidated using nuclear magnetic resonance, mass spectrometry, and ultra-performance liquid chromatography quadrupole time of flight mass spectrometry in litchi seed. As a result, the IC50 of α-glucosidase inhibitory assay of the crude extract, sugar-removed layer, pavetannin B2, procyanidin A2, and acarbose was 0.691 μg/mL, 3.686 μg/mL, 0.04 μM, 0.08 μM, and 55.845 μg/mL, respectively. With those compounds, we examined the protein tyrosine phosphatase 1B inhibitory activity. And the IC50 of pavetannin B2, procyanidin A2, and ursolic acid was 450.295, 338.257, and 19.686 μM, respectively. Contents analysis method for bioactive compounds, which can be used in manufacturing for extract preparations, was established. The findings of this study, litchi seed can be a cost-effective medicinal food in terms of recyclable resources in the litchi food industry and as a natural alternative medicine against type-2 diabetes.

**Min Sung Kim, So-Hyeon Baek, Sang Un Park, Kyung-Hoan Im, Jae Kwang Kim. Targeted metabolite profiling to evaluate unintended metabolic changes of genetic modification in resveratrol-enriched rice (*Oryza sativa* L.). (2017) Appl. Biol. Chem. 60(2): 205–214**

Resveratrol-enriched rice (RR) includes the stilbene synthase gene for resveratrol synthesis and the phosphinothricin-*N*-acetyltransferase gene for glufosinate tolerance. To investigate unintended effects resulting from RR’s genetically modified chemical composition, 56 polar and nonpolar secondary metabolites were analyzed with gas chromatography–mass spectrometry in RR and conventional non-transgenic rice. Rice was cultivated during two seasons along three representative climatic regions in the Republic of Korea. Principal components analysis was used to visualize chemical composition differences among rice samples. The results showed that chemical composition was more influenced by growing year and location than by whether or not the rice was transgenic. Pearson’s correlations and hierarchical clustering analysis also indicated no difference in the biochemical structures of RR versus non-transgenic rice. In addition, the glufosinate-ammonium treatment did not significantly change RR chemical composition.

**Sooim Shin, Moonsung Choi.** **Equilibrium study of copper absorption to different types of soft contact lens. (2017) Appl. Biol. Chem. 60(2): 215–219**

To measure binding affinity of copper, one of the heavy metals in particulate matter (PM) was applied to soft contact lenses made of two different materials because contact lenses are readily exposed to PM. Copper binding to ionized silicon hydrogel lens yielded an equilibrium association constant *K*a,eq value of 14.03 μM without color change of lens, compared to that of 19.16 μM for copper binding to de-ionized hydrogel lenses with color change of lens. The results indicated that the color change of lens is not consistent with the concentration of cooper deposition on lens, and copper bound relatively stronger in ionized silicon hydrogel lens than in de-ionized hydrogel lens. Therefore, the continuous exposure of contact lenses to high PM levels might lead to heavy metal deposition on the lens, which would be detrimental to ocular health.

**Yearam Jung, Soon Young Shin, Young Han Lee, Yoongho Lim.** **Flavones with inhibitory effects on glycogen synthase kinase 3β. (2017) Appl. Biol. Chem. 60(3): 227–232**

Because glycogen synthase kinase-3 (GSK-3) activity is linked to various human diseases, it has been targeted in new drug development. Flavonoids, including luteolin, apigenin, and quercetin, inhibit GSK-3β; however, the relationships between their structural properties and inhibitory effects are unclear. We measured the inhibitory effects of 34 flavonoid derivatives on GSK-3β and calculated hologram quantitative structure–activity relationships to provide information on pharmacophores for designing novel compounds with better activities. The in vitro binding effect of flavonoids was confirmed using Western blotting for myricetin, which showed the best inhibitory activity, and the binding mode between myricetin and GSK-3β was elucidated using in silico docking.

**Sang-Jin Park, Dong-Hwan Kim, Jeongha Yoo, Eun Young Hwang, Moon-Sik Shin, Nam-Taek Lee, Il-Rae Cho, Hee-Gun Kang, Young-Jin Kim, Sungman Park, Yoon-Won Kim.** **Detection of organophosphate bound butyrylcholinesterase using a monoclonal antibody. (2017) Appl. Biol. Chem. 60(3): 233–240**

In this study, a specific antibody against butyrylcholinesterase (BuChE) bound to organophosphate (OP) nerve agents was developed to be used in enzyme-linked immunosorbent assays (ELISA). OP nerve agents such as sarin, soman, and VX are known to act on the neuromuscular junctions and were synthesized at the Armed Force Chemical, Biological, and Radiological Defense Command in Republic of Korea and studied after confirming inactivation of BuChE. Each inactivated OP-BuChE (OP-iBuChE) was used as the antigen for developing monoclonal antibodies (mAbs) in mice for detection of OP bound BuChE. The fusion was performed after immunization and then hybridoma cells for the antibodies were generated. As a result, the partial specific antibody against soman-iBuChE was confirmed via ELISA. Thus, the antibody may recognize three-dimensional structure of OP-iBuChE that is changed upon soman and BuChE reaction, not an OP binding pocket of BuChE. Furthermore, this partial specific monoclonal antibody may be used in a competitive ELISA to detect the soman-iBuChE. Taken together, we suggest that the antibody could be applied to evaluate the soman presence in blood, serum, and urine.

**Dong-Keun Lee, Youn Shic Kim, Ju-Kon Kim.** **Determination of the optimal condition for ethylmethane sulfonate-mediated mutagenesis in a Korean commercial rice, *Japonica cv.* Dongjin. (2017) Appl. Biol. Chem. 60(3): 241–247**

Ethylmethane sulfonate (EMS) mutagenesis is a powerful hunting tool to seek novel players for improving agromonic traits. Together with rapid evolution of the next-generation sequencing techniques, the EMS mutagenesis has been revaluated for its utilization to breed crops in practical agriculture and to study functions of key players in valuable agronomic traits. In this study, we systematically investigated conditions for EMS mutagenesis in Dongjin (*Oryza sativa*, *Japonica*) rice plants to make a mutant population. Since the EMS mutagenesis depends on target tissue, EMS concentration and EMS exposure time, we fixed the EMS exposure time as 13 h and treated germinating seeds with various levels of EMS dosage (from 0.25 to 2% EMS concentration). EMS treatment clearly showed negative biological influences including low germination and abnormal seedling development of Dongjin rice plants. Based on the standard of about 50% lethal dose, 0.75 and 1% EMS dosage for 13 h was finally selected as the optimal conditions for EMS mutagenesis of Dongjin germinating seeds.

**Chang Ha Park, Hyun Ji Yeo, Nam Su Kim, Park Ye Eun, Sun-Ju Kim, Mariadhas Valan Arasu, Naif Abdullah Al-Dhabi, Soo-Yun Park, Jae Kwang Kim, Sang Un Park.** **Metabolic profiling of pale green and purple kohlrabi (*Brassica oleracea* var. *gongylodes*). (2017) Appl. Biol. Chem. 60(3): 249–257**

Kohlrabi (*Brassica oleracea* var. *gongylodes*) is a dietary *Brassica* vegetable with noted health-beneficial properties associated with its numerous metabolites. The aim of this study was to elucidate phenotypic variation between the two cultivars through comprehensive analysis of the relationship of their primary and secondary metabolites. High-performance liquid chromatography (HPLC) and gas chromatography time-of-flight mass spectrometry (GC-TOFMS) are considered useful tools for profiling primary and secondary metabolites. A total of 45 metabolites, including organic acids, amino acids, sugars, and an amine, were identified in pale green and purple kohlrabies using GC-TOFMS-based metabolic profiling. The resulting data sets were analyzed by principal component analysis to determine the overall variation, and the purple and pale green vegetables were separated by the score plots generated. Additionally, HPLC analysis of anthocyanins in both cultivars revealed that green kohlrabies did not contain any anthocyanidins, while 11 anthocyanins were quantified in the purple ones. Cyanidin was the dominant anthocyanin found in the purple cultivar, with cyanidin-3-(feruloyl)-diglucoside-5-glucoside being the major one. This study suggests that GC-TOFMS and HPLC are suitable tools to determine metabolic connection among various metabolites and describe phenotypic variation between green and purple kohlrabies.

**Ye-Jin Jeon, Sang-Guei Lee, Hoi-Seon Lee.** **Acaricidal and insecticidal activities of essential oils of *Cinnamomum zeylanicum* barks cultivated from France and India against *Dermatophagoides* spp., *Tyrophagus putrescentiae* and *Ricania* sp.. (2017) Appl. Biol. Chem. 60(3): 259–264**

The chemical composition of *Cinnamomum zeylanicum* bark oils cultivated from France and India was analyzed by GC–MS. The main components of *C. zeylanicum* oil were cinnamaldehyde (63.97 and 67.21%) and eugenol (6.84 and 19.79%) from France and India, respectively. Acaricidal and insecticidal activities of *C. zeylanicum* oils against *Dermatophagoides* sp, *T. putrescentiae* and *Ricania* sp. were evident. The LC50 values of *C. zeylanicum* oil were 123.77 and 93.06 mg/L in samples from France and India, respectively, against *Ricania* sp. adults in the spray bioassay. Using the leaf-dipping bioassay, the LC50values of the oil from France and India were 80.99 and 57.44 mg/L, respectively, against *Ricania* sp. nymphs. Acaricidal activity of the *C. zeylanicum* oil from India in the fabric disk bioassay (LD50, 0.64, 0.51 and 1.72 μg/cm3, respectively) was greater than France oil (LD50, 0.92, 0.81 and 1.82 μg/cm3, respectively). In the filter paper bioassay, india oil (LD50, 1.82, 1.55 and 3.08 μg/cm2, respectively) was more potent than France oil (LD50, 2.07, 1.94 and 6.20 μg/cm2, respectively) against *D. farinae*, *D. pteronyssinus* and *T. putrescentiae*. The results indicate that the essential oils of *C. zeylanicum* barks could be an effective natural acaricide and insecticide for controlling house dust mites, stored food mites and fruit pests.

**Sung Chul Kim, Se Jin Oh, Seung Min Oh, Sang Phil Lee, Jae E. Yang.** **In situ reclamation of closed coal mine waste in Korea using coal ash. (2017) Appl. Biol. Chem. 60(3): 265–272**

Adding coal ash to acid waste decreases its acidity and supplies essential nutrients to plants. The effects of coal ash on acid coal mine waste and acid mine drainage were investigated in the field. Treatments were mixtures of coal mine waste with coal ash (0, 20, and 40%). Addition of coal ash increased the pH of coal mine waste and leachate by 3.11 and 3.03 units, respectively. After stabilization, the concentrations in the leachate decreased from 4.65 to 0.44 mg L−1 for Fe and from 0.31 mg L−1 to “not detected” for Pb. The organic matter content and cation exchange capacity increased significantly (approximately twofold each), for coal mine waste with coal ash. Moreover, plant growth on coal mine waste that had been treated with coal ash was greatly stimulated. The application of coal ash offers an environmentally compatible, cost-effective way to remediate coal mine waste or leachate contaminated with heavy metals.

**So Hee Yoon, Min-Joo Kim, BoKyung Moon.** **Various biogenic amines in *Doenjang* and changes in concentration depending on boiling and roasting. (2017) Appl. Biol. Chem. 60(3): 273–279**

Biogenic amines are formed by microorganisms during fermentation. Major biogenic amines found in food are histamine, tryptamine, 2-phenylethylamine, putrescine, cadaverine, tyramine, spermidine, and spermine. *Doenjang* is a traditional fermented food made of soybean and is widely used for cooking of various foods in Korea. During fermentation, harmful substances such as biogenic amines could be produced in *Doenjang.* In this study, we examined the types and quantities of biogenic amines in commercial *Doenjang* and analyzed the destructive effects of cooking on biogenic amines in *Doenjang*. Biogenic amines were identified by high-performance liquid chromatography with a fluorescence detector (HPLC-FLD). The concentrations of biogenic amines in commercial *Doenjang* depended on the manufacturer and ranged from none detected to 415.08 mg/kg. Putrescine and tryptamine were the most abundant biogenic amines in *Doenjang* samples, whereas cadaverine was not detected in any commercial samples. For all cooking conditions, tryptamine, 2-phenylethylamine, putrescine, and tyramine were detected in *Doenjang*, and their concentrations decreased significantly after 10 min of roasting. The total concentration of biogenic amines in *Doenjang* soup was not changed significantly by boiling. Therefore, roasting, unlike boiling, can be considered more effective at reducing the amount of biogenic amines in *Doenjang*.

**Hye-yeon Kim, Se-hee Hwang, Jin-hee Lee.** **Effect of fermented vinegar on the reduction in trimethylamine in konjac glucomannan gel. (2017) Appl. Biol. Chem. 60(3): 281–285**

*Konjac glucomannan* (KGM) is one of the non-digestible dietary polysaccharides. Hydration of KGM at elevated temperature in the presence of calcium hydroxide affects the aggregation of KGM, making a konjac glucomannan gel formation that has a fish-like off odor. Trimethylamine (TMA) in konjac glucomannan gel has been investigated by solid-phase microextraction and analyzed by GC–MS. A fish-like off odor generated in the process of gelation has been remarkably reduced by treatment of the gel formation with fermented vinegar in which acetic acid concentration-treated group was over 1% (v/v), soaking time, for 10 min. Together with the instrumental analysis, a fish-like off odor generated from the same treatment was also analyzed by sensory evaluation. Acetic acid treatment at a concentration of over 1% (v/v) resulted in significant decrease in the fish-like off odor (*p* < 0.05) supporting the action mechanism of acetic acid as a proton donor for the reaction with TMA. Correlation between TMA content by GC–MS analysis and fish-like off odor by sensory evaluation was statistically significant by ANOVA, Duncan’s multiple range test, operated by SPSS program (*R*2 = 0.9848, *p* < 0.05).

**Abd Elaziz Sulieman Ahmed Ishag, Azhari Omer Abdelbagi, Ahmed Mohammed Ali Hammad, Elsiddig Ahmed Elmustafa Elsheikh, Osama Elgilani Elsaid, Jang-Hyun Hur.** **Biodegradation of endosulfan and pendimethalin by three strains of bacteria isolated from pesticides-polluted soils in the Sudan. (2017) Appl. Biol. Chem. 60(3): 287–297**

Biodegradation of endosulfan (*α* and *β*) and pendimethalin by *Bacillus safensis* strain FO-36bT, *Bacillus subtilis subsp. inaquosorum* strain KCTC 13429T and *Bacillus cereus* strain ATCC14579T isolated from pesticides-polluted soil was studied in mineral salt medium. Endosulfan and pendimethalin were incubated with the three bacterial strains with samples drawn at various intervals for GC analysis. Representative samples were subject to GC–MS analysis. The loss in the initial concentrations, 0.663 mM (*α* endosulfan), 0.319 mM (*β*endosulfan) and 1.423 mM (pendimethalin), was monitored and used to compute the half-lives following biphasic model. Removal percentage of endosulfan and pendimethalin in the media inoculated with the bacterial strains ranged from 24 to 95% (*α* endosulfan), 21–91% (β endosulfan) and 51–97% (pendimethalin), respectively. Despite the significant decrease in starting material in *B. safensis* cultures, no metabolites were detected, whereas two major metabolites of endosulfan, 1,2,3,4,7,7-hexachloro-5,6-dihydroxybicyclo{2.2.1}-2-heptene and 1,2,3,4,7,7-hexachloro-formaldehyde-6-methylbicyclo{2.2.1}-2-heptene, were detected in the *B. subtilis* cultures, and one metabolite of pendimethalin metabolite; *N*-(1-ethylpropyl)-3-methyl-2, 6-diaminobenzine, was detected in the *B. cereus* culture. Generally, the result indicates the potential capability of these microorganisms in complete mineralization of endosulfan and pendimethalin. Based on half-lives, the efficiency of bacterial strains can be ordered as follows: *B. subtilis* > *B. cereus* > *B. safensis* for endosulfan and *B. cereus* > *B. safensis* > *B. subtilis* for pendimethalin.

**Yang Qin, Soo-Yun Park, Seon-Woo Oh, Myung-Ho Lim, Kong-Sik Shin, Hyun-Suk Cho, Seong-Kon Lee, Hee-Jong Woo.** **Nutritional composition analysis for beta-carotene-enhanced transgenic soybeans (*Glycine max* L.). (2017) Appl. Biol. Chem. 60(3): 299–309**

Nutritional composition is important for assessing the safety of genetically modified (GM) crops for human consumption. Three beta-carotene-enhanced soybean lines were developed by introducing the *β*-conglycinin promoter::*Phytoene synthase*-*2A*-*Carotene desaturase*/t35S gene cassette into the genome of the commercial Kwangan (*Glycine max* L.) soybean variety. Transgenic soybeans were successfully detected on beta-carotene productions ranged from 170.47 to 213.58 µg/g. Comparative assessments of nutrition were conducted with 3 transgenic soybeans, their non-GM counterpart, and several commercial soybean varieties. Results indicated that most levels of proximate, fatty acids, amino acids, and vitamins showed non-significant differences between transgenic soybeans and their counterpart, and fit within the reference ranges established for other commercial soybeans and Organization for Economic Cooperation and Development Guidelines. However, significant differences on levels of crude fat, carbohydrate, *δ*-tocopherol, and oleic acid of transgenic soybeans comparing to those of non-transgenic counterpart Kwangan cannot eliminate the influences of transgene insertion. Alternations on compositions should be definite by further studies, such as transcriptome and metabolome profiling.

**So Wun Kim, Seo Hyun Lee, Cheol Woo Min, Ick Hyun Jo, Kyong Hwan Bang, Dong-Yun Hyun, Ganesh Kumar Agrawal, Randeep Rakwal, Sajad Majeed Zargar, Ravi Gupta, Sun Tae Kim.** **Ginseng (*Panax* sp.) proteomics: an update. (2017) Appl. Biol. Chem. 60(3): 311–320**

*Panax ginseng*, commonly known as ginseng, is a well-known medicinal plant that has been used as traditional medicine in China and Korea. Research in the past few decades supports the pharmacological effects of ginseng. For example, ginseng roots (extracts) exhibit multiple medicinal effects, such as anticancer, antiaging, and protection against circulatory shock, in humans. In this review, we summarize the progress made so far in the ginseng proteomics, starting from sample preparation to establishments of proteomes and databases. Both gel-based (1-DE and 2-DE in combination with LC–MS/MS) and gel-free proteomics technologies have been applied on wide range of samples, collected during different growth and developmental stages and under normal or adverse stress conditions. In particular, comparative proteome analysis has been carried out to investigate the protein profiles of Oriental, American and Indian ginsengs using majorly root and leaf tissues. Moreover, identification of stress-responsive proteins was a key focus that led to the detection of some of the common proteins such as heat shock protein (HSP), ATPase, enolase, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and ribonuclease/ginseng major protein (GMP). Acquired proteomics-based knowledge has been very fruitful in providing better insight into the ginseng biology, opening a door for comparative and translation research of other important medicinal plants. However, due to the fact that proteins undergo various post-transcriptional and post-translational modifications, obtained proteomics data do not always complement the transcriptomics data perfectly; therefore, future efforts would require the utilization of an integrated/holistic molecular-genetic (or omics) approach to explore the biology of this golden plant.

**Hyo-Eun Jang, Heejung Jung, Hyejung Mok.** **Ginseng (*Panax* sp.) Cleavable conjugation of CpG oligodeoxynucleotides onto microparticles for facile release and cytokine induction in macrophages. (2017) Appl. Biol. Chem. 60(3): 321–326**

The development of biomaterials for efficient intracellular delivery of the nucleic acid-based immune-stimulating molecule, CpG oligodeoxynucleotides (CpG), is crucial for their biological activity. In this study, we successfully fabricated polydopamine-coated porous poly(lactic-co-glycolic acid) microparticles (PPM) for the delivery of CpGs. After conjugation of CpGs to PPMs via cleavable disulfide linkages, CpGs were readily released from CpG-conjugated PPM (PPM-s–s-CpGs) in reductive conditions. Released CpGs exhibited significantly enhanced induction of two cytokines, TNF-α and IL-6, in RAW264.7 cells. GpC-conjugated PPMs showed negligible cytokine induction, whereas CpG-conjugated PPMs exhibited strong induction of the two cytokines in RAW264.7 cells. The PPM-s–s-CpGs can serve as immune-stimulating adjuvants to enhance the immune responses of vaccines.

**Yazan Ranneh, Faisal Ali, Abdah Md Akim, Hasiah Abd. Hamid, Huzwah Khazaai, Abdulmannan Fadel.** **Crosstalk between reactive oxygen species and pro-inflammatory markers in developing various chronic diseases: a review. (2017) Appl. Biol. Chem. 60(3): 327–338**

The inflammation process in the human body plays a central role in the pathogenesis of many chronic diseases. In addition, reactive oxygen species (ROS) exert potentially a decisive role in human body, particularly in physiological and pathological process. The chronic inflammation state could generate several types of diseases such as cancer, atherosclerosis, diabetes mellitus and arthritis, especially if it is concomitant with high levels of pro-inflammatory markers and ROS. The respiratory burst of inflammatory cells during inflammation increases the production and accumulation of ROS. However, ROS regulate various types of kinases and transcription factors such nuclear factor-kappa B which is related to the activation of pro-inflammatory genes. The exact crosstalk between pro-inflammatory markers and ROS in terms of pathogenesis and development of serious diseases is still ambitious. Many studies have been attempting to determine the mechanistic mutual relationship between ROS and pro-inflammatory markers. Therefore hereby, we review the hypothetical relationship between ROS and pro-inflammatory markers in which they have been proposed to initiate cancer, atherosclerosis, diabetes mellitus and arthritis.

**Prasad Andhare, Dweipayan Goswami, Cédric Delattre, Guillaume Pierre, Philippe Michaud, Hilor Pathak.** **Edifying the strategy for the finest extraction of succinoglycan from *Rhizobium radiobacter* strain CAS. (2017) Appl. Biol. Chem. 60(3): 339–348**

Succinoglycan is an industrially important exopolysaccharide (EPS) that is produced by certain bacteria. There are several procedures to extract this EPS, though the efficiency of all the available procedures is questionable and any improvement in the extraction efficient can greatly benefit the industry. Here we emphasize on optimization and development of new modus operandi to efficiently extract succinoglycan from liquid bacterial culture. Also, we studied the effect of different extraction methods on production, rheological and structural properties of succinoglycan. Eighteen different chemical and physical methods were tested for succinoglycan extraction from *Rhizobium radiobacter* CAS isolates with the principle of extracting EPS by precipitating it, where only eleven methods could precipitate the succinoglycan. Comparing the extraction yield of all methods, biopolymer extracted by acetone (3014 mg/L) was maximum followed by cetyl-trimethyl-ammonium-bromide (CTAB 2939 mg/L) and vacuum evaporation (2804 mg/L) methods. Upon comparison of rheological property of recovered succinoglycan, it was found that at shear rate 50 s−1 EPS recovered using acetone and CTAB methods tends to make the solution highly viscous with a viscosity of 150 and 146 mPa s, respectively. In agreement with these results, power law equation showed that EPS extracted by acetone and CTAB had high consistency index (*k*) and low flow behavior index (*η*). The current results showed that the physicochemical methods for EPS extraction significantly affect the structural composition of, though succinoglycan extracted using acetone and CTAB showed minimum structural abrasion.

**Sung Un Kim, Vance N. Owens, Sang Yoon Kim, Chang Oh Hong.** **Effect of different way of bottom ash and compost application on phytoextractability of cadmium in contaminated arable soil. (2017) Appl. Biol. Chem. 60(4): 353–362**

There have been few studies evaluating the effect of bottom ash (BA) on immobilization of heavy metals and reducing their phytoavailability. Further, work has not been conducted to evaluate the effect of BA along with mature animal manure compost (CP) on immobilization of cadmium (Cd) in soil and phytoavailability of this metal in contaminated soil. Therefore, this study was conducted to determine the effect of application of BA and CP on Cd phytoextractability. To elucidate the mechanism of Cd immobilization with BA and CP, soil was mixed without BA and CP, with BA only, with CP only, and with BA and CP together in the incubation. Bottom ash was applied at rates of 0 and 30 Mg/ha under different application rates of CP (0 and 30 Mg/ha) 2 weeks before sowing lettuce (*Lactuca sativa*). Our first experiment clearly demonstrated that reduced extractability of Cd with addition of BA, CP, and BA + CP was mainly the result of Cd adsorption by an increase in pH and negative charge of soil. Concentration of bioavailable Cd fraction (*F*1) effectively decreased with BA, CP, and BA + CP from 1.33 mg Cd/kg in control to 0.98, 0.29, and 0.26 mg Cd/kg, respectively. Applying BA and CP alone or together effectively reduced Cd uptake by lettuce. Concentration of Cd in lettuce decreased from 13.9 mg Cd/kg in control to 10.3 and 7.6 mg Cd/kg with application of BA and CP alone, respectively. However, applying BA with CP increased fresh lettuce yields more than BA applied alone. Therefore, combined application of BA and CP might be a good management practice in Cd contaminated arable soil from the view point of Cd phytoavailability and crop productivity.

**Jihyun Lee, Moon-Cheol Jeong, Kyung-Hyung Ku.** **Chemical, physical, and sensory properties of 1-MCP-treated Fuji apple (*Malus domestica* Borkh.) fruits after long-term cold storage. (2017) Appl. Biol. Chem. 60(4): 363–374**

The effects of 1-methylcyclopropene (1-MCP) on physicochemical properties and sensory characteristics of Fuji apple fruits stored at 2 °C for 9 months in air were investigated. The soluble solid content, titratable acidity (TA), sugars, sorbitol, organic acids, and phenolic compounds were evaluated in this study. We also determined volatile profiles using headspace solid-phase microextraction and gas chromatography–mass spectrometry. Instrumental textural properties and descriptive sensory analysis were also conducted in this study. The correlation coefficients between physicochemical and sensory properties were investigated. In comparison with the untreated apples, those treated with 1-MCP exhibited higher acidity (i.e., higher TA and organic acids), antioxidant total polyphenol content, sum of individual polyphenol content, force linear distance, lower volatile content, *b*\* (yellowness), and fructose and sucrose levels, after long-term cold storage. A significant difference was observed in sensory attributes such as red color of apple peel, textural properties (with peel and without peel), sweet taste, and sour taste between 1-MCP-treated and untreated apples. Sensory attributes related to textural properties (i.e., P\_Hardness and Crunchiness) showed positive correlation with instrumental textural properties (force peak and force linear distance) (*p* < 0.05). A significant positive correlation was observed between red color of apple peels (sensory attribute) and *a*\* (redness) (*p* < 0.05) and between sour taste and shikimic acid (*p* < 0.05).

**Supornpun Srimat, Kritsanee Iamjud, Peerapong Sangwanangkul, Sirikul Wasee, Kriengsak Thaipong.** **Antioxidant properties of selected Thai red-fleshed papaya genotypes during the external color break stage. (2017) Appl. Biol. Chem. 60(4): 375–384**

Papaya fruit during the external color break stage is a valuable raw material to make food, but its benefits for human health are still limited. Ten selected Thai red-fleshed papaya genotypes during the external color break stage were investigated for morphological, physicochemical, and antioxidant property traits. Most fruit traits evaluated in this study varied significantly among genotypes. The smallest fruit genotype, SNP-KD, had the highest antioxidant activity (4.41 ± 0.62 µmol ascorbic acid equivalents/g fresh weight [FW]), ascorbic acid (838.1 ± 90.2 mg/L), and total phenolic (547.0 ± 52.8 mg gallic acid equivalents/kg FW), and the reddest flesh genotype, KM4-13, contained the highest lycopene (87.5 ± 14.7 mg/kg FW). The correlations between ascorbic acid and total soluble solids and between lycopene and β-carotene were relatively high at *r* = 0.72 and 0.69, respectively, which indicates a high correlation was possible for both selections. Antioxidant activity was only strongly positively correlated with total phenolics (*r* = 0.78), which indicates that the total phenolics was an important contributor to antioxidant activity in papaya flesh, and it was feasible to use total phenolics to indirectly estimate antioxidant activity.

**Jonghwa Lee, Yongho Shin, Junghak Lee, Jiho Lee, Eunhye Kim, Jeong-Han Kim.** **Sensitivity enhancement using a microbore column and pulsed pressure injection in the simultaneous analysis of 356 pesticide multiresidues by gas chromatography–tandem mass spectrometry. (2017) Appl. Biol. Chem. 60(4): 385–390**

For more rapid and sensitive simultaneous analysis of a 356 pesticide multiresidues by gas chromatography–tandem mass spectrometry (GC–MS/MS), a microbore (20 m length × 0.18 mm i.d.) column, instead of the conventional narrow bore column (30 m length × 0.25 mm i.d.), and pulsed pressure injection (PPI) were evaluated in this study. An analysis time of 35.2 min with the narrow bore column was greatly reduced to 17.8 min with the microbore column. Retention times of all target compounds on the new microbore column were adjusted using the automated adjustment of retention time function. The microbore column gave significantly higher peak heights and narrower peak widths compared with the narrow bore column, resulting in enhanced sensitivity. The use of PPI at an optimized pressure of 250 kPa for the microbore column also increased peak heights and signal-to-noise (S/N) ratio by about 30%, indicating more than twofold–threefold enhancement of sensitivity compared with the narrow bore column without PPI. In the best case of the late eluting compound etofenprox, the S/N ratio significantly increased more than ninefold. In conclusion, compared to a narrow bore column, using a microbore analytical column with PPI function produced peak sensitivity enhancement and a shorter analysis time.

**Thi Minh Nguyet Nguyen, Maria Lomunova, Hee Soon Shin, Dong-Hwa Shon, Young Ho Kim, Inkyu Hwang.** **Anti-allergic effects of *Rosae multiflorae fructus* via inhibition of T cell proliferation and the mast cell function. (2017) Appl. Biol. Chem. 60(4): 391–402**

Anti-allergic effects of the hot water extract of *Rosae multiflorae fructus* (Rosae extract), which has long been used in oriental medicine for treatment of various diseases, were explored with a chicken ovalbumin (cOVA)-induced mouse model of food allergy. Compared to the sham mice to show severe allergic symptoms (i.e., anaphylaxis, diarrhea and decrease in the body temperature) following oral cOVA challenge, the Rosae extract-treated mice showed a marked improvement in those symptoms. Histology data demonstrated that Rosae extract treatment resulted in a amelioration in the intestinal inflammatory lesion and a reduction in the numbers of mast cells and eosinophils in the small intestine. Studies using DO11.10 TCR transgenic T cells indicated that Rosae extract had an activity to subdue the antigen-specific T cell activation/proliferation in vivo and thereby to lower the level of Th2 cytokine production by T cells during the antigen-specific immune response. Moreover, passive systemic anaphylaxis study showed that the extract also had an activity to inhibit the mast cells function in vivo, i.e., release of granules triggered by specific IgE-antigen interaction. Altogether, the results from this study not only imply a potential clinical application of Rosae extract in prevention and treatment of food allergy but also clearly elucidate the immunoregulatory mechanisms of Rosae extract underlying its anti-allergic effect.

**Won Park, Kwang-Soo Kim, Ji-Eun Lee, Young-Lok Cha, Youn-Ho Moon, Yeon-Sang Song, Eung-Gi Jeong, Sung-Ju Ahn, Suk-Whan Hong, Yong-Hwa Lee.** **Effect of different application levels of rapeseed meal on growth and yield components of rice. (2017) Appl. Biol. Chem. 60(4): 403–410**

Application of rapeseed meal as organic fertilizer improves soil environment and crop productivity by supplying the nutrients. This study aimed to investigate the optimal application levels of rapeseed meal for rice cultivation. Application of rapeseed meal increased the height of rice plants by about 5–10% compared to the control, and its application at a rate of 50, 100, 200, and 300 kg/0.1 ha increased the nitrogen content in rice leaves at 30 days after treatment by 107.3, 110.4, 114.7, and 114.7%, respectively, compared to the control. In treatments with a mixture of chemical fertilizer and rapeseed meal (50, 100, 200, and 300 kg/0.1 ha), the nitrogen content in rice leaves increased by 105.1–107.7% compared to that in the control; there was no significant difference between the treatment with chemical fertilizer only and that with the mixture of chemical fertilizer and rapeseed meal. The chemical properties of soil treated with rapeseed meal at 200 and 300 kg/0.1 ha or with the mixture of 200 kg/0.1 ha rapeseed meal and chemical fertilizer (1/2 level) showed that nitrate and ammonium increased in treated soils as compared to untreated soil. The yield and yield components were positively correlated with the application levels of rapeseed meal and the mixture of rapeseed meal and chemical fertilizer. The application of rapeseed meal at 100–200 kg/0.1 ha is considered optimal, and efficient application of nitrogen is achieved by mixing chemical fertilizer (11 kg/0.1 ha) with rapeseed meal (100–200 kg/0.1 ha).

**Khalil Akhtar, Syed Wadood Ali Shah, Assar Ali Shah, Muhammad Shoaib, Syed Kashif Haleem, Nighat Sultana.** **Pharmacological effect of *Rubus ulmifolius* Schott as antihyperglycemic and antihyperlipidemic on streptozotocin (STZ)-induced albino mice. (2017) Appl. Biol. Chem. 60(4): 411–418**

The aim of present study was to evaluate the antihyperglycemic and antihyperlipidemic effects of aerial parts of *Rubus ulmifolius* Schott on streptozotocin (STZ)-induced diabetic albino mice. A total of 48-, 60-day-old either sex (male and female) albino mice were treated with, normal control; 2% Tween-80 suspension (diabetic control); glibenclamide (500 μg/kg/orally); RU methanol extract (150 mg/kg/orally) (RUCrd1); RU methanol extract (300 mg/kg/orally) (RUCrd2); RU chloroform extract (150 mg/kg/orally) (RUC); RU ethyl acetate extract (150 mg/kg/orally) (RUE); and RU butanol extract (150 mg/kg/orally) (RUB) for a period of 15 days. Diabetes was induced in albino mice by single intraperitoneal injection of streptozotocin (50 mg/kg/b/w). After 15 days, group treated with glibenclamide, RUCrd1, RUCrd2, RUC, RUE and RUB exhibited a significant (*P* > 0.05) decrease in blood glucose level as compared to diabetic control groups. The total cholesterol, triglycerides and low-density lipoproteins as well as serum creatinine level, serum glutamate pyruvate transaminase, serum glutamate oxaloacetate transaminase and alkaline phosphatase were also significantly (*P* > 0.05) decreased in glibenclamide, RUCrd1, RUCrd2, RUC, RUE and RUB groups albino mice as compared to diabetic control. It was concluded that *Rubus ulmifolius* Schott extract has positive effect as hypoglycemic and antihyperlipidemic on diabetic albino mice.

**Ga-Eun Lee, Jinsun Kim, Cha-Gyun Shin.** **Antiviral activities of hydroxylated flavones on feline foamy viral proliferation. (2017) Appl. Biol. Chem. 60(4): 419–425**

Many hydroxylated flavones are reported to have antibacterial, anticancer, or antiviral activities. In this study, eleven hydroxylated flavones including 3,3′,4′,5,7-pentahydroxyflavone (quercetin), three polymethoxyflavones, two polyethoxyflavones, two polypropoxyflavones, one butoxyflavone, and two benzoxyflavones were tested for antiviral activity using feline foamy virus. Most of the compounds tested showed that they did not have significant cytotoxic effect on the crandell-Ress feline kidney cells. However, four compounds, including quercetin, 5,3′-dihydroxy-3,7,4′-trimethoxyflavone, 5-hydroxy-3,7,3′,4′-tetramethoxyflavone, and 3,5,7,3′,4′-pentahydroxyflavone, showed a strong inhibitory effect on feline foamy virus (FFV) proliferation, by reducing viral production to 7–24% of that in the un-treated control when they were added to the cells at a final concentration of 10 μM. These results were supported by western blot detecting viral protein in the infected cell lysate. In further analysis, quercetin was observed to have a direct inhibitory effect on reverse transcriptase and integrase in vitro, which can explain the mechanism by which quercetin inhibits FFV proliferation. These preliminary results suggest that hydroxylated flavones such as quercetin, 5,3′-dihydroxy-3,7,4′-trimethoxyflavone, 5-hydroxy-3,7,3′,4′-tetramethoxyflavone, and 3,5,7,3′,4′-pentahydroxyflavone have strong antiretroviral activities.

**Eun Bi Choi, Min Woo Lee, Jae Eun Park, Jun Young Lee, Chang Oh Hong, Sang Mong Lee, Young Gyun Kim, Keun Ki Kim.** **Photodynamic apoptosis and antioxidant activities *of Brassica***

***napus* extracts in U937 and SK-HEP-1 cells. (2017) Appl. Biol. Chem. 60(4): 427–435**

*Brassica napus* is the most common feedstock for biodiesel production, and its cultivation area has been rapidly increased. Thus, *B. napus* residues left in the field after harvest are valuable resources. However, there have been few studies on biologically active substances from *B. napus* plant. The objective of this study is to evaluate cytotoxicity/photodynamic activity and antioxidant activity of *B. napus* plant extracts. *B. napus* plants were sequentially extracted with organic solvents (hexane, chloroform, ethanol, and water) and then screened for antioxidant activity and cytotoxicity against leukemia U937 and human liver cancer SK-HEP-1 cells. Among the solvent extracts, the cytotoxicity was the highest when cells treated with chloroform extract and irradiated. Degree of apoptosis substantially increased in both cell types in concentrationdependent manner, and non-irradiated cells showed similar results as the control cells. For the highest concentrations

(100 lg/ml), toxicity effect in U937 and SK-HEP-1 cells was 94.62 ± 0.15% and 74.16 ± 1.54%, respectively. We observed the number of cells significantly decreased, and vesicles were floating in *B. napus* chloroform extract (BNCE) and light condition. BNCE induced DNA laddering pattern (between 300 and 1000 bp) and caspase-3/7 activation in both U937 and SK-HEP-1 cells. Total apoptotic U937 and SK-HEP-1 cells following BNCE 100 lg/ml and light treatment were significantly increased (92.62 ± 2.07% and 59.71 ± 4.38%, respectively) compared with control. Our results showed that U937 cells were more sensitive than SK-HEP-1 cells. For the antioxidant

activity, *B. napus* ethanol extract was the highest (IC50 = 0.52 mg/ml).

**Bijinu Balakrishnan, Si-Hyung Park, Hyung-Jin Kwon.** **Inactivation of the oxidase gene *mppG* results in the selective loss of orange azaphilone pigments in *Monascus purpureus*. (2017) Appl. Biol. Chem. 60(4): 437–446**

*Monascus* species are filamentous ascomycetes fungi and produce azaphilone (Az) pigment that is a well-known food colorant. Az is a class of fungal polyketides that bears a highly oxygenated pyranoquinone bicyclic core and is produced by a nonreducing fungal polyketide synthase with a reductive release domain (NR-fPKS-R). *MpPKS5* encodes an NR-fPKS-R for *Monascus* Az (MAz) and is clustered with four oxidoreductase genes including *mppG*; *mpp* designates *Monascus* pigment production. MAz pigments are classified as yellow and orange MAz, and their structures differ in two hydride reductions with yellow MAz as the reduced type. The biosynthesis of yellow MAz (monascin, **Y-1** and ankaflavin, **Y-2**) is completed by a reductive pathway involving a reductase gene *mppE*. This reductive pathway is diverged from a common MAz pathway involving two other reductase genes of *mppA* and *mppC*. This suggests that the biosynthesis of orange MAz (rubropunctatin, **O-1** and monascorubrin, **O-2**) is completed by an oxidative branch pathway and the cognate oxidative role of *mppG* is genetically characterized in the present study. A targeted gene inactivation mutant of Δ*mppG* displayed a severe impairment in the production of orange MAz with no significant alteration in the level of yellow MAz. The feeding experiment with **Y-1** in Δ*MpPKS5* indicated that **Y-1** could not be converted into **O-1**, which excludes the possibility that *mppG* mediates the conversion of yellow into orange MAz. This study supports the existence of divergent pathways in MAz biosynthesis and creates a recombinant strain for the selective production of yellow MAz.

**Joungsu Joo, Nam-Iee Oh, Nguyen Hoai Nguyen, Youn Hab Lee, Yeon-Ki Kim, Sang Ik Song, Jong-Joo Cheong.** **Intergenic transformation of *AtMYB44* confers drought stress tolerance in rice seedlings. (2017) Appl. Biol. Chem. 60(4): 447–455**

We transformed rice (*Oryza sativa* L. *Japonica* cv. Ilmi) calli with the *Arabidopsis*transcription factor gene *AtMYB44* using *Agrobacterium*-mediated transformation. The T-DNA construct to be transformed contained *tflA* cDNA (encoding a toxoflavin lyase) as a selectable marker. Since toxoflavin is a photosensitizing phytotoxin, transgenic plantlets were selected based on their capacity for root development on medium containing this toxin in the light. Homozygous lines were selected by determining the segregation patterns, expression levels, and copy numbers of *AtMYB44*. Intergenic genomic locations of the inserted T-DNA in the three transgenic lines were confirmed by adaptor-ligation polymerase chain reaction and analysis using FSTVAL (<http://bioinfo.mju.ac.kr/fstval/>), an open-access web tool used to localize the flanking sequences of the transgene. Drought tolerance of young seedlings of the transgenic lines was determined based on the recovery of wilted leaves by re-watering after 3 days of water deprivation in a 105-well (35*W* × 35*L* × 45*D* mm/per well) plate. The three transgenic lines showed average survival rates of 80.4, 93.5, and 72.6%, respectively, whereas wild-type plants failed to recover after re-watering. Thus, the transgenic rice plants exhibited significantly enhanced tolerance to drought stress, as was shown previously in *AtMYB44*-overexpressing transgenic *Arabidopsis* and soybean. These results suggest that AtMYB44 activates a drought tolerance mechanism that is conserved in both monocotyledonous and dicotyledonous plants.

**Ha Danh Duc.** **Degradation of chlorotoluenes by *Comamonas testosterone* KT5. (2017) Appl. Biol. Chem. 60(4): 457–465**

The isolated *Comamonas testosterone* KT5 utilized a broad range of toluene and chlorotoluenes, including 2-chlorotoluene, 3-chlorotoluene (3CT), 4-chlorotoluene, 2,3-dichlorotoluene, 2,4-dichlorotoluene, 2,6-dichlorotoluene and 3,4-dichlorotoluene (34DCT) as sources of carbon and energy. The strain was characterized its dissipation capability toward these compounds in both liquid culture and contaminated soil. In liquid cultures, KT5 utilized more than 90% of toluene, 3CT and 34DCT within 60 h at the initial concentration of 2 mM. Moreover, the strain showed a mineralization capacity of mixtures of toluene and chlorinated toluenes. Inoculation with the toluenes-degrading bacterial strain significantly enhanced degradation rates in soil. The dissipation rates of toluene, 3CT and 34DCT in non-sterile soil inoculated with bacteria were 97.8, 93.5 and 68.9% after 30 days, respectively. The biodegradation of toluene and chlorosubstituted toluenes in KT5 was occurred through the upper pathway to form benzoates and then ring fission via *ortho*-cleavage pathway. In addition, *C. testosterone* KT5 showed the mineralization capacities of benzoate and chlorinated benzoates with the rates comparably higher than the rates of toluenes. The multiple and efficient toluene degradation properties make this isolate a good candidate for bioremediation of environments contaminated with chlorosubstituted toluenes and benzoates.

**JoungDu Shin, Seung Gil Hong, SunIl Lee, SungChang Hong, JongSik Lee.** **Estimation of soil carbon sequestration and profit analysis on mitigation of CO2-eq. emission in cropland cooperated with compost and biochar. (2017) Appl. Biol. Chem. 60(4): 467–472**

Only a few have evaluated the mitigation of greenhouse emissions and profit analysis along with soil carbon sequestration for corn cultivation. This experiment was conducted to evaluate the carbon sequestration and mitigation of greenhouse gas emissions as well as their profit analysis with different composts mixed with biochar during corn cultivation. This experimental data provided the second year of corn cultivation. The soil type used was clay loam. The application amounts of synthetic fertilizer and biochar were 220–30–155 kg ha−1 (N–P–K) as the recommended amount after soil analysis and 2600 kg ha−1 based on 1.3% of soil bulk density. For the biannual experimental results, it appeared that carbon sequestration in cow manure cooperated with biochar was highest at 2.3 tons ha−1 and recovered from 67.3 to 78.5% with biochar application. Furthermore, mitigation of CO2-eq. emission as greenhouse gases was estimated to be at 7.3–8.4 MT ha−1, and its profit was evaluated from $7.2 to 8.4 as lowest, from $57.2 to 66.6 as medium and from $139.7 to 162.7 as highest per hectare regardless of organic compost types used. For agricultural practice in Korea, it is evaluated that the market price of CO2 in corn field cooperated with 2600 kg ha−1 of biochar application ranged from $57.2 to 162.7 per hectare in Korean Climate Exchange. For corn biomass, the treatment with biochar application did not significantly decrease compared with the only organic compost application. Based on these experimental results, it might be applied for carbon trading with clean development mechanism for agricultural practices.

**Hoon Choi, Byung-Ho Lee, Young-Sun Moon, Kyeongsoon Kim, Hoi-Seon Lee, Sung-Eun Lee.** **Antifungal and antiaflatoxigenic effects of a fumigant, ethanedinitrile, on *Aspergillus flavus*. (2017) Appl. Biol. Chem. 60(5): 473–476**

Antifungal effects of ethanedinitrile (EDN) and ethyl formate (EF) on *Aspergillus flavus* were investigated using radial growth bioassay. *A. flavus* was inoculated in the center of potato dextrose agar plate and treated with 1, 5, and 10 g/m3 of EDN, or 5, 35, and 70 g/m3 of EF. EDN strongly inhibited fungal growth. At 1 g/m3 of EDN, the fungal growth reduced by 22.2% by the final days of culture. The growth was completely inhibited by EDN at the concentration of 5 g/m3. Antiaflatoxigenic activity of both the fumigants was also assessed. Aflatoxin formation was determined using high-performance liquid chromatography with a fluorescence detector. *A. flavus* did not produce aflatoxin B1 and aflatoxin B2 at EDN concentrations >5 g. EF had no inhibitory effect on *A. flavus* growth and the formation of aflatoxin. These results suggest that EDN can be an alternative for currently used antifungal agents to control fungal and aflatoxin contamination of stored grains.

**Kyunghwa Han, Sangjae Han, Eunjin Kim, Heerae Cho, Youngho Seo, Hyubsung Lee, Junghun Ok, Mijin Seo, Kangho Jung, Yongseon Zhang.** **Salt removal of greenhouse soils using electrokinetic technology. (2017) Appl. Biol. Chem. 60(5): 477–485**

Excess nutrients are easily accumulated in greenhouse soils due to the interception of rainfall by plastic film and repeated over-application of compost and fertilizers. This study was conducted to evaluate the application of electrokinetic (E/K) technology for salt removal from soils with high electrical conductivity (EC) in greenhouses. Three types of soil in plastic film greenhouses were used: artificial soil (Site A), poorly drained soil (Site B), and well-drained soil (Site C). The salt-enriched surface soils were used to fill 37-cm-long-box-type E/K cells, and a constant voltage gradient was applied at a rate of 1 V cm−1 for 30 days. The decrease in soil EC was achieved with water content of greater than 30% for silt loam (Sites A and B) and 20% for sandy loam (Site C). The E/K technology decreased soil EC by more than 80%, with a greater reduction ratio for sandy loam than for silt loam. After 30 days of the E/K treatment, 98–99% of NO3-N and 95–99% of sodium were removed in all three sites under saturated condition, implying that nitrate and sodium ions had higher mobility than the other ions during the E/K process. The results obtained from the study suggested, therefore, that the E/K technology is highly efficient for wet and nitrate-enriched sandy loam soils, and the technology can be a feasible and environmentally sound practice for the removal of excessive nutrients in greenhouse soils without water pollution by nutrients such as nitrate as can be caused by flooding and repeated washing with water.

**Jaemin Lee, Joyce P. Rodriguez, Kang Hee Lee, Jun Yeon Park, Ki Sung Kang, Dae-Hyun Hahm, Chang Ki Huh, Sang Cheon Lee, Sanghyun Lee.** **Determination of flavonoids from *Cirsium japonicum*var. *maackii* and their inhibitory activities against aldose reductase. (2017) Appl. Biol. Chem. 60(5): 487–496**

The therapeutic activities of flavonoids from natural plant sources were investigated. The ethanol extracts from the aerial parts of *Cirsium japonicum* var*. maackii* (CJM) were tested for aldose reductase inhibition (ARI). Additionally, stepwise polarity fractions and flavonoids from CJM were evaluated for ARI. The ethyl acetate (EtOAc) fraction from CJM showed significant inhibitory effects. The compounds in the EtOAc fraction were identified as the flavonoids-cirsimaritin (**1**), hispidulin (**2**), and cirsimarin (**3**). Based on an ARI assay, the EtOAc fraction and hispidulin (**2**) exhibited good AR inhibitory activity (IC50 values of 0.21 μg/mL and 0.77 μM, respectively). An HPLC quantitative analysis of different parts of CJM showed that the aerial part collected in the spring season (CJL1) contains the highest total flavonoid content. These results serve as a basis for maximizing the flavonoid yield and for the efficient usage of various parts of CJM. Our results also suggest that CJM could be a useful ARI material for the treatment of various diabetic complications.

**Z. Khodabande, V. Jafarian, R. Sariri.** **Antioxidant activity of *Chelidonium majus* extract at phenological stages. (2017) Appl. Biol. Chem. 60(5): 497–503**

*Chelidonium majus*, from Papaveraceae family, is a rich source of different antioxidants with a range of medicinal activities including antispasmodic and diuretic properties. In this study, antioxidant potential of extracts from leaves during different phenological stages was measured by ferric-reducing power (FRAP) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity. Factors affecting antioxidant activity, i.e., total phenols, flavonoids, anthocyanin and carotenoids, were then investigated. Soluble sugar and total protein contents of samples were also determined. According to the results, maximum DPPH radical scavenging activity was 408/88 ± 24/83 g/g DW at growing stage, and the FRAP value reached maximum during fruiting stage (1.75 ± 0.04 mg/g FW). The leaves of flowering stage contained the most content of total phenol (17.8 ± 1.59 mg/g DW), flavonoid (69.7 ± 0.86 mg/g DW), anthocyanin (0.233 ± mg/g DW) and soluble sugar (0.338 ± 0.009 mg/g DW). However, the highest value for carotenoid (2.083 mg/g DW) and protein (0.27 ± 0.034 mg/g DW) was found at the vegetative stage.

**Haein Keum, Guyoung Kang, Namhyun Chung.** **Oxidation of pyrene using a hemoglobin-catalyzed biocatalytic reaction. (2017) Appl. Biol. Chem. 60(5): 505–508**

The efficiency to remove polycyclic aromatic hydrocarbons (PAHs) was determined using a hemoglobin-catalyzed biocatalytic reaction. The present study employed pyrene as a model of PAHs to study its oxidative removal in the presence of H2O2 and hemoglobin in mass ratio of 3:1. The extent of pyrene removal reached up to 91.1% in the presence of H2O2 and hemoglobin. However, the extent of pyrene removal was 21.3% in the presence of H2O2 only. The results indicate that pyrene removal might be due to pyrene oxidation by the biocatalytic reaction. Overall, this study demonstrated that hemoglobin-catalyzed biocatalytic reactions could remediate pyrene effectively.

**Inhwan Kim, Jihyun Lee.** **Comparison of different extraction solvents and sonication times for characterization of antioxidant activity and polyphenol composition in mulberry (*Morus alba* L.). (2017) Appl. Biol. Chem. 60(5): 509–517**

Various extraction solvents and sonication treatment times were compared to determine antioxidant activity and polyphenol composition in mulberry (*Morus alba* L.) fruits of the Gwasang No. 2 variety. Mulberry fruits were extracted with ethanol, methanol, acidic methanol (0.1% HCl), or acidic ethanol (0.1% HCl) with 0–60-min sonication to measure total phenolic content and antioxidant activity (DPPH radical scavenging activity). Moreover, a high-performance liquid chromatography method to analyze polyphenol compositions in mulberry extracts was developed and levels of common polyphenolic compounds in the different extracts were determined. The effects of different methanol ratios in acidic aqueous methanol (0–100%) on individual polyphenol contents in the extracts after 30 min of sonication were investigated. The total phenolic contents of the extracts using acidic methanol and sonicated for 60 min were significantly higher than phenolic contents upon extraction with other solvents and sonication times (*p* < 0.001). Interestingly, antioxidant activity of the extracts using acidic ethanol sonicated for 60 min was significantly higher than that of other extracts (*p* < 0.001). Cyanidin-3-glucoside and 3-rutinoside were the major polyphenol compounds in all mulberry extracts. Cyanidin-3-glucoside and 3-rutinoside concentrations were highest in the acidic methanol extract sonicated for 30 min (15,664 and 19,630 μg/g DW) (*p* < 0.001). The acidic methanol extract sonicated for 30 min contained significantly higher polyphenolic compound content than that of other extracts (*p* < 0.001). A decreased methanol ratio (0–80%) in acidic aqueous methanol resulted in chlorogenic acid overestimation in mulberry extracts. Thus, acidic 100% methanol with 30-min sonication is recommended for polyphenol analysis in mulberry.

**Joyce P. Rodriguez, Jaemin Lee, Jun Yeon Park, Ki Sung Kang, Dae-Hyun Hahm, Sang Cheon Lee, Sanghyun Lee.** **HPLC–UV analysis of sample preparation influence on flavonoid yield from *Cirsium japonicum* var. *maackii*. (2017) Appl. Biol. Chem. 60(5): 519–525**

This study was conducted to optimize the extraction conditions of flavonoids from *Cirsium japonicum* var. *maackii* (ICF-1). The effects of sample material ratio, solvent concentration, extraction time, solid-to-solvent ratio, and number of extractions on flavonoid extraction efficiency were analyzed. Three flavonoids were specifically investigated: cirsimarin (**1**), hispidulin (**2**), and cirsimaritin (**3**). In single-factor experiments, each variable had a significant effect on the determination of content of compounds **1**–**3**. The optimal conditions for extraction were found to be: mass, 15 g; ratio of spring and fall leaves, 4:1; extraction solvent, 70% ethanol; extraction time, 4 h; solid-to-solvent ratio, 1:20; and number of extractions, 1. The results of the study were used to maximize the potential of ICF-1 samples and optimize the efficiency of the extraction process.

**Nhan Nguyen Thi, Hae Seong Song, Eun-Ji Oh, Yeong-Geun Lee, Jung-Hwan Ko, Jeong Eun Kwon, Se-Chan Kang, Dae-Young Lee, In Ho Jung, Nam-In Baek.** **Phenylpropanoids from *Lilium* Asiatic hybrid flowers and their anti-inflammatory activities. (2017) Appl. Biol. Chem. 60(5): 527–533**

Three phenylpropanoids were isolated from the flowers of *Lilium* Asiatic hybrids through repeated silica gel or octadecyl silica gel column chromatographies. The chemical structures were determined to be 1-*O*-*trans*-caffeoyl-*β*-d-glucopyranoside (**1**), regaloside A (**2**), and regaloside B (**3**), based on spectroscopic data gathered from nuclear magnetic resonance (NMR) spectroscopy, electron ionization mass spectrometry (EI/MS), polarimetry, and infrared spectroscopy (IR) experiments. Compounds **1** and **2** showed significant DPPH radical scavenging activity of 60.1 and 58.0% at 160 ppm, respectively, compared with the 62.0% activity of the positive control, *α*-tocopherol. At a concentration of 50 μg/mL, compounds 1–3 inhibited the expression of iNOS to 4.1 ± 0.01, 70.3 ± 4.07, and 26.2 ± 0.63, respectively, and decreasing COX-2 expression to 67.8 ± 4.86, 131.6 ± 8.19, and 98.9 ± 4.99. Also, at the same concentration, compounds **1**–**3** decreased the ratio of p-p65/p-65 to 43.8 ± 1.67, 40.7 ± 1.30, and 43.2 ± 1.60, respectively, and the expression of VCAM-1 to 42.1 ± 2.31, 48.6 ± 2.65, and 33.8 ± 1.74, respectively.

**Jin-Sik Nam, Seo-Yeon Park, Hye-Lim Jang, Young Ha Rhee.** **Phenolic compounds in different parts of young *Annona muricata* cultivated in Korea and their antioxidant activity. (2017) Appl. Biol. Chem. 60(5): 535–543**

The antioxidant activities of 80% methanol and distilled water extracts of different parts (roots, twigs, and leaves) of young *Annona muricata* were estimated based on their total phenol and flavonoid content as well as in vitro 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activities, nitrite scavenging activity, Fe2+-chelating activity, ferric reducing antioxidant power (FRAP), and phenolic compound assays. The 80% methanol extracts showed a higher antioxidant effect than that of the water extracts. The 80% methanol root and leaf extracts showed higher total phenol (839.69 mg CAE/g) and flavonoid (168.52 mg RE/g) contents than those shown by the other extracts. In addition, the 80% methanol root extracts showed high DPPH (EC50 = 0.18 mg/mL) and ABTS (EC50 = 0.55 mg/mL) radical as well as nitrite (EC50 = 0.21 mg/mL) scavenging activities. The metal-chelating effect of the 80% methanol twig extract was the highest, but there were no significant differences among the 80% methanol extracts of the different parts. FRAP values of all extracts increased in a concentration-dependent manner, except for those of the distilled water leaf extract, while the 80% methanol root extracts showed the highest value. In addition, there was a strong positive correlation between the antioxidant activity and total phenol content (*P* < 0.01). *A. muricata* extracts were rich in various phytochemicals including rutin, epicatechin, ferulic acid, and *p*-coumaric acid. These findings indicate that *A. muricata* is a potentially useful source of substances with antioxidant effects.

**Hye Jun Choi, Soo Ji Kang, Kwang Won Hong.** **Comparison of NheA toxin production and doubling time between *Bacillus cereus* and *Bacillus thuringiensis*. (2017) Appl. Biol. Chem. 60(5): 545–551**

In this study, we compared the toxin gene expression, NheA toxin production, doubling time, and viable cell number for several strains of the food poisoning bacteria *Bacillus cereus* and the microbial pesticide *Bacillus thuringiensis*. The two *B. cereus* and six *B. thuringiensis* strains evaluated were confirmed to possess and transcribe the *nheABC*, *hblCDA*, and *cytK* genes using polymerase chain reaction (PCR) and reverse-transcription PCR. NheA toxin production was compared based on the absorbance at 414 nm using a Tecra BDE-VIA kit. The NheA-specific production (absorbance/viable cell number) values indicated that the two *B. thuringiensis* var. *kurstaki* isolates from microbial pesticide produced the highest amount of toxin (0.66–0.95) than other *B. thuringiensis* (0.14–0.45) and the *B. cereus* strains (0.19–0.31). However, the *B. thuringiensis* strains had longer doubling time (20–26 min) than the *B. cereus* strains (18–19 min). Interestingly, two *B. thuringiensis* var. *kurstaki* isolates produced the highest amount of NheA toxin, and their doubling times (20–22 min) were close to those of the *B. cereus*strains tested.

**Min Seo Kim, Ji Yeon Kim.** **Intestinal anti-inflammatory effects of cinnamon extracts in a co-culture model of intestinal epithelial Caco-2 cells and RAW264.7 macrophages. (2017) Appl. Biol. Chem. 60(5): 553–561**

Cinnamon is the bark of *Cinnamomum japonicum* SIEB, which possesses anti-microbial, anti-tyrosinase, and antioxidant activities. However, studies on the intestinal health benefits of cinnamon are scarce. Therefore, we were aimed at assessing the potential anti-inflammatory action of cinnamon extracts in a cellular model of intestinal inflammation. Cinnamon extracts were prepared using hot water extract and 70% ethanol extract. Prior to inducing inflammation, co-cultured Caco-2 cells with RAW264.7 cells were treated with the cinnamon extracts. Lipopolysaccharide (LPS) was treated to induce inflammation in RAW264.7 cells. Compared to LPS-treated groups, the cells treated with cinnamon extracts maintained high transepithelial electrical resistance and decreased tight junction permeability, similar to that shown by control Caco-2 cells. In addition, cinnamon extract-treated cells showed decreased levels of inflammatory cytokines, namely nitrite (NO), PGE2, interleukin (IL)-6, IL-8, and TNF-α. These results show that cinnamon may serve as a probable agent for the development of functional supplements for maintenance of gastrointestinal health.

**Hyerin Choi, Tae-Lim Kim, Man-Ho Cho, Seong-Hee Bhoo.** **Immuno-affinity purification of 2B8-tagged proteins. (2017) Appl. Biol. Chem. 60(5): 563–568**

Detection, purification and characterization of proteins are essential procedures in the field of biochemistry. Epitope tag systems are commonly used to characterize unknown proteins. The *Deinococcus radiodurans* bacterial phytochrome (DrBphP) protein has been used as an antigen to generate anti-DrBphP mouse monoclonal antibodies and to identify their specific epitopes. Among these antibodies, the 2B8 monoclonal antibody recognizes an epitope of 9 amino acids (RDPLPFFPP). The 2B8 epitope does not match the amino acid sequence for any known protein. On Western blot analysis, the 2B8 antibody showed strong and highly specific interactions with the 2B8 epitope. These results suggest that the 2B8 epitope-antibody is a useful epitope tag system for protein characterization. In addition, we generated a modified epitope (RDPLPAFPP) via point mutation in a previous study. This modified epitope showed significantly increased reactivity with the 2B8 antibody. In this study, we developed a protein purification system using the 2B8 epitope tag and antibody. 2B8 antibodies were bound to protein G-agarose beads as affinity ligands. Recombinant DrBphP proteins were then exposed to 2B8 antibody-bound protein G-agarose beads. Bound DrBphP proteins were then eluted by competition with the original or modified 2B8 epitope peptides. DrBphP proteins were successfully purified via an affinity chromatography system using a 2B8 original peptide and even better purified using the 2B8 modified peptide. These findings indicate that the 2B8 epitope tag system is a better tool for protein detection and purification.

**Kyongmi Chon, Hwan Lee, Hui Cheol Hwang, Jeongtaek Im, Kyung-Hun Park, Min Kyoung Paik, Yong-Soo Choi.** **The honey bee brood test under semi-field conditions for the assessment of positive reference chemicals in Korea. (2017) Appl. Biol. Chem. 60(5): 569–582**

Honey bee brood tests under semi-field conditions are higher-tier studies for investigating the potential impact of pesticides on brood development in honey bee colonies. Semi-field studies on the effects of pesticides on honey bees have been mainly carried out in the EU and USA, with only a relatively small number conducted in Korea and other Asian countries. Here, we report the first semi-field test in Korea, which was carried out from April to May 2016. The experiment included three treatment groups (control and two toxic reference chemicals), each with three replicate tunnels. The honey bee colonies were placed in tunnels covering 70 m2 and containing *Brassica napus.* Flight activity, mortality, the condition of the colonies, and brood development were assessed during the 28 days of testing period. The toxic reference treatments were 400 g dimethoate a.i./ha and 600 g diflubenzuron a.i./ha. Brood termination rates for marked eggs were 31.3% in the control group, 83.5 and 68.0% for dimethoate and diflubenzuron, respectively. These results confirm the sensitivity of the test method and indicate that these two chemicals could be used as appropriate toxic reference compounds in future semi-field tests in Korea.

**Shakirullah, Muhammad Subhan Qureshi, Sohail Akhtar, Rifat Ullah Khan. The effect of vitamin E and selenium on physiological, hormonal and antioxidant status of Damani and Balkhi sheep submitted to heat stress. (2017) Appl. Biol. Chem. 60(6): 585–590**

The present study was conducted to find the effect of vitamin E and selenium (Se) on physiological, hormonal and antioxidant status of Damani and Balkhi sheep under high ambient temperature. Forty Damani and Balkhi healthy multiparous, non-pregnant sheep having similar initial body weight were selected. The sheep in each breed were further equally divided into control and treated groups. The sheep were fed a dietary plan recommended by the National Research Council (Nutrient requirements of small ruminants: sheep, goats, cervids, and new world camelids, National Academies Press, Washington, DC, [2007](https://link.springer.com/article/10.1007/s13765-017-0313-9#CR4)). In addition, the sheep in the treated groups were supplemented with Se (0.3 mg) and vitamin E (50 mg) per kg of diet for 4 weeks. The results indicated that respiration and pulse rate decreased significantly (*P* < 0.05) on day 28 compared to the first day of the study in the treatment groups. The concentration of T3 and T4 was significantly (*P* < 0.01) high in Damani sheep compared to Balkhi. In addition, except follicle-stimulating hormone (FSH) and progesterone, T3 and T4 were significantly (*P* < 0.01) high in the treated sheep compared to the control. Malondialdehyde (MDA), cortisol and heat-shock protein (HSP-70) increased significantly (*P* < 0.05) in Balkhi sheep compared to Damani. In the treated sheep, MDA, cortisol and HSP-70 were significantly (*P* < 0.01) low, while superoxide dismutase (SOD) and glutathione peroxidase (GPx) were significantly (*P* < 0.01) high in the treatment sheep. It was concluded that vitamin E and Se at the present doses improved the physiological, hormonal and antioxidant status in Damani and Balkhi sheep. In addition, Damani sheep were more tolerant to heat stress than Balkhi sheep.

**Pham Anh Tuan, Chang Ha Park, Woo Tae Park, Yeon Bok Kim, Yong Joo Kim, Sun Ok Chung, Jae Kwang Kim, Sang Un Park. Expression levels of carotenoid biosynthetic genes and carotenoid production in the callus of *scutellaria baicalensis* exposed to white, blue, and red light-emitting diodes. (2017) Appl. Biol. Chem. 60(6): 591–596**

In the present study, efforts were made to determine effect of different light wavelengths on carotenoid biosynthesis in *Scutellaria baicalensis*, a promising traditional herbal medicine used in North America and Asia. The variation in the transcriptional level of carotenoid biosynthesis genes and carotenoid contents in the callus was analyzed after exposure to light from red (wavelength, 660 nm), blue (wavelength, 470 nm), or white (wavelength, 380 nm) light-emitting diodes. The transcription levels of phytoene synthase (*SbPSY*), ξ-carotene desaturase (*SbZDS*), β-ring carotene hydroxylase (*SbCHXB*), and zeaxanthin epoxidase (*SbZEP*) were upregulated on exposure to all three lights, with the greatest increase in expression observed upon exposure to blue light. In contrast, the transcriptional levels of two carotenoid cleavage dioxygenases (*SbCCD1* and *SbCCD4*) decreased under all three light treatments. Increased production of zeaxanthin, β-carotene, and 9-*cis*-β-carotene was observed on exposure to all 3 lights. The total carotenoid production rose by 27.35% under blue light treatment, while the white and red lights did not have any considerable effect. Our findings may establish new approaches for increasing carotenoid production in *S. baicalensis* as well as in other plants.

**Min Kyung Song, Su Jin Lee, Yoon Young Kang, Youngshim Lee, Hyejung Mok, Joong-Hoon Ahn. Biological synthesis and anti-inflammatory activity of arylalkylamine. (2017) Appl. Biol. Chem. 60(6): 597–602**

Hydroxycinnamic acid amides (HCAAs) are natural compounds with antifungal, anticancer, and anti-inflammatory activities. Extraction from plants and chemical synthesis have been the major approaches to obtain these compounds. We used a biological method to synthesize HCAA derivatives (arylalkylamines). Two genes, *SHT* encoding serotonin *N*-hydroxycinnamoyl transferase and 4CL encoding 4-coumaroyl-CoA ligase, were introduced into *Escherichia coli*. Using this *E. coli* transformant as a biocatalyst, 24 arylalkylamines were synthesized. The anti-inflammatory activities of five synthesized compounds, including *N*-*p*-coumaroyl phenethylamine, *N*-caffeoyl phenethylamine, *N*-*p*-coumaroyl 3-phenylpropylamine, *N*-*p*-coumaroyl 4-phenylbutylamine, and *N*-*p*-coumaroyl 4-methoxyphenethylamine, were measured. Among them, *N*-*p*-coumaroyl 4-phenylbutylamine showed the best anti-inflammatory activity.

**Ihsan Ullah, Muhammad Waqas, Muhammad Aaqil Khan, In-Jung Lee, Won-Chan Kim. Exogenous ascorbic acid mitigates flood stress damages of *Vigna angularis*. (2017) Appl. Biol. Chem. 60(6): 603–614**

Prolonged water stress adversely affects many aspects of plant physiology, resulting in severe damage to growth and productivity. In response to this and other environmental stresses, plants have evolved complex physiological and biochemical adaptations. To boost existing plant defense mechanisms, this study quantified the negative effects of waterlogging stress and how it may be mitigated by the addition of a natural protective agent. Adzuki beans (*Vigna angularis*) were grown in commercially available microbe-free soil and subjected to waterlogging stress for 2 weeks. Waterlogging significantly reduced all growth-related variables: shoot length, fresh and dry biomass, chlorophyll content in stressed versus unstressed plants. Waterlogging stress generated reactive oxygen species that heavily damaged plant tissues, causing electrolyte leakage and eliciting an antioxidative response. Specifically, stress-response phytohormone content altered, with a reduction in abscisic acid (ABA) and an increase in jasmonic acid (JA). Furthermore, antioxidants such as malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), polyphenol oxidase (PPO), and peroxidase (POD) were significantly enhanced in waterlogged plants versus non-waterlogged plants. Supplementation of exogenous ascorbic acid (ASC) at 3, 5, and 7 mM revealed that the lowest concentration further reduced ABA and increased JA, enhancing water evaporation rates to raise water-stress tolerance. Moreover, 3 mM ASC also led to lower MDA, CAT, SOD, PPO, POD, and ascorbate peroxidase concentrations in waterlogged plants than in waterlogged plant not treated with ASC. Thus, ASC at a concentration of 3 mM was the most successful in relieving effects of waterlogging stress on plants.

**Yeonjoong Yong, Hailemichael Tesso, Akalu Terfa, Aman Dekebo, Worku Dinku, Young Han Lee, Soon Young Shin, Yoongho Lim. Biological evaluation of the diterpenes from *Croton macrostachyus*. (2017) Appl. Biol. Chem. 60(6): 615–621**

To clarify whether the seeds of *Croton macrostachyus* can induce apoptosis, its methanolic extract was first subjected to a clonogenic survival assay, which measures long-term cytotoxic effects. Since it showed cytotoxicity on HCT116 human colon cancer cell lines, further separation was performed, and two single diterpene compounds were obtained. One of them was identified to be a novel compound, methyl 2-(furan-3-yl)-6a,10b-dimethyl-4-oxo-2,4,4a,5,6,6a,10a,10b-octahydro-1*H*-benzo[*f*]isochromene-7-carboxylate, based on the interpretation of the nuclear magnetic resonance spectroscopic and mass spectrometric data. Its treatment of HCT116 cells with 50 μg/mL triggered the cleavage of both initiator caspase-9 and effector caspase-7. Moreover, the cleavage of poly (ADP-ribose) polymerase, a substrate of caspase-7, increased after 24 h of treatment. These results demonstrate that this compound exerts antitumor activity by triggering caspase-mediated apoptotic cell death in HCT116 human colon cancer cells.

**Rupak Chakraborty, Donah Mary Macoy, Sang Yeol Lee, Woe-Yeon Kim, Min Gab Kim. Tunicamycin-induced endoplasmic reticulum stress suppresses plant immunity. (2017) Appl. Biol. Chem. 60(6): 623–630**

Most secretory and membrane proteins are properly folded in the endoplasmic reticulum (ER) before being transferred to their functional destinations. Physiological and pathological stresses induce unfolded and misfolded protein accumulation in the ER, termed as ER stress. Under ER stress, cells initiate a protective response to maintain cellular homeostasis, which is referred as unfolded protein responses. Although protein processing in the ER has been known to regulate cell lifespan and disease, few evidences that prove the role of ER stress in plant immunity have been reported. We investigated the interaction between ER stress and pathogenicity in Arabidopsis by utilizing the N-glycosylation inhibitor, tunicamycin (TM) as an ER stress inducer. TM induced the accumulation of PR1 (pathogenesis-related protein 1) and callose in plant leaves, which are markers for PAMP-triggered immunity (PTI) activation. However, TM pre-treatment increased susceptibility of Arabidopsis to all bacterial pathogens tested. Moreover, TM resulted in cell death of plant leaves with an additive effect to hypersensitive response by bacterial effector proteins, suggesting TM-induced cell death is independent of the effector-triggered immunity. These results imply that TM-induced ER stress weakens overall immune system of plant not a specific immune pathway, probably via disruption of post-translational modification of immune-related proteins in the ER and subsequent cell death by apoptosis or autophagy. This study provides proves for the distinct suppressive effect of ER stress on the plant immune system.

**Pinaki Hazra, Gargi Saha Kesh. Isolation and purification of phycocyanin from cyanobacteria of a mangrove forest. (2017) Appl. Biol. Chem. 60(6): 631–636**

A cyanobacterium is an ancient group of photosynthetic prokaryotes that are thought to be the first organisms to carry out oxygenic photosynthesis. Phycocyanin (PC) is used to capture light energy for photosynthesis, unique to cyanobacteria. Sundarbans, a mangrove forest, is situated in the delta of Ganges, Meghna and Brahmaputra rivers, India. It has been declared as world heritage centre for its biodiversity. We would be the first to report on the photosynthetic pigment, phycocyanin of any strain of cyanobacteria isolated from a mangrove forest, Sundarbans. We have isolated the photosynthetic pigment from a cyanobacterial strain, AP24. Sequential precipitation of crude extract was done by the use of ammonium sulphate. Dialysis of the protein sample, anion exchange chromatography on a DEAE-cellulose DE 52 column and gel filtration chromatography with Sephadex G-100 column chromatography increased the purity ratio of phycocyanin. The purification was done both by DEAE-cellulose [purity ratio of the eluted cyanobacterial phycocyanin (C-PC) (A620/A280) increased to 4.31] and by Sephadex column. The molecular weight of purified C-PC was found to be 72 kDa, which include two subunits α (17 kDa) and β (19 kDa) in a dimer association (αβ)2. The purified C-PC shows antioxidant activity. It was observed that the rate of haemolysis is decreased with increasing concentration of C-PC.

**Atefeh Javani, Fatemeh Javadi-Zarnaghi, Mohammad Javad Rasaee. Development of a colorimetric nucleic acid-based lateral flow assay with non-biotinylated capture DNA. (2017) Appl. Biol. Chem. 60(6): 637–645**

In recent years, many nucleic acid-based lateral flow assays (NALFAs) have been developed for rapid and simple detection of various analytes including DNA sequences. In a NALFA, target molecules are applied within a small volume of a rehydrating buffer. The analyte flows laterally to reach the capture molecules at where it forms a colorimetric signal. Usually, in NALFAs, capture molecules are modified for maximized adsorption on the surface. In most cases, the modification is a biotin. The biotinylated capture DNA is held at capture line by interaction with streptavidin. However, there is a demand on methods that permit utilizing unmodified capture molecules and allow a cost-effective development process. Here, we report on a biotin- and streptavidin-free model NALFA. We also present a systematic investigation on the effect of various rehydrating buffers’ composition and concentration. In addition, the impacts of a protein blocker, detergents and chaotropic and kosmotropic agents on the intensity of the signal over background were analyzed. It has been demonstrated that simultaneous presence of sodium dodecyl sulfate and bovine serum albumin doubles the intensity of visible bands in the presented unmodified NALFA. Finally, this paper presents an optimized cost-effective model system that can be adapted for hybridization-based NALFAs.

**Won Je Lee, Jaeho Kim, Dongho Lee, Suk-Whan Hong, Hojoung Lee. *Arabidopsis UDP*-*glycosyltransferase 78D1-*overexpressing plants accumulate higher levels of kaempferol 3-O-β-d-glucopyranoside than wild-type plants. (2017) Appl. Biol. Chem. 60(6): 647–652**

Flavonols are a class of flavonoids that are found in most plants. Certain flavonols exhibit anticancer, antioxidant, and antimicrobial functions. An array of genes plays a role in the regulation of flavonoid biosynthetic pathways, including the MYB–bHLH (basic helix-loop-helix-WD40 transcription factor complex. Flavonoids often display altered bioactivities after being glycosylated by the action of glycosyltransferases. These enzymes catalyze the transfer of sugars from a donor to various acceptors. In this study, we generated several transgenic lines of *Arabidopsis* that overexpress *UDP*-*glycosyltransferase 78D1* (*UGT78D1*), which are hereafter referred to as *UGT78D1*-*OX*, to address three questions: (1) Can *UGT78D1*-*OX* seedlings accumulate more flavonols? (2) Can *UGT78D1*-*OX* seedlings accumulate more flavonols in the presence of sucrose? and (3) Will *UGT78D1*-*OX* be more sensitive to abiotic stresses? We observed that *UGT78D1*-*OX* seedlings accumulated specific types of kaempferol, while they had a decreased content of flavonols in the presence of sucrose. Contrary to our expectation, more anthocyanins accumulated in the *UGT78D1*-*OX* lines, although the expression of *production of anthocyanin pigment 1* was slightly reduced in *UGT78D1*-*OX* seedlings compared with that in wild-type seedlings. It appeared that the overexpression of *UGT78D1* did not interfere with abiotic stress tolerance in the mutant plants.

**Ho-Jeong Lim, Da-Som Kim, Jeong Hoon Pan, Suk-Bok Pae, Hoe-Sung Kim, Eui-Cheol Shin, Jae Kyeom Kim. Characterization of physicochemical and sensory attributes of a novel high-oleic peanut oil cultivar(*Arachis hypogaea*ssp.*Fastigiata*L.*)*. (2017) Appl. Biol. Chem. 60(6): 653–657**

Many efforts by peanut breeders and reseachers have been made to develop high-oleic peanut cultivars because of not limited to their storage stability but also health benefits by oleic acid. Here, we characterized physicochemical properties and sensory attributes of a novel high-oleic peanut oil cultivar (*K*-*Ol; Arachis hypogaea* ssp. *Fastigiata* L.); properties of this cultivar were comprehensively analyzed and compared with two conventional peanut cultivars, *Daekwang*, and *Jopyung*. As results, the *K*-*Ol* cultivar represented a significantly higher ratio of oleic acid and linoleic acid than conventional cultivars. Further, the *K*-*Ol* dramatically increased oxidative stability and oxidation induction time compared to conventional oils. However, we noted no significant difference in physical properties, and the *K*-*Ol* showed the lowest total tocopherol content and antioxidant capacity, suggesting that oxidative stability of the *K*-*Ol* is likely contributed by the high-oleic acid content. In sensory attributes analyses using electronic nose, overall flavor values were higher in the *K*-*Ol* except for the strength. The taste scores of *K*-*Ol*measured by electronic tongue were fairly distributed to all tastes compared to conventional cultivars. This study provides important characterization data of the peanut oil, from the high-oleic peanut cultivar; further investigations are warranted with regard to its potential health benefits as well as industrial applications of this stable edible oil.

**Dong Gun Lee, Mindong Lee, Hyeri Lee, Min Hee Hwang, Eun Byeol Go, Miju Cho, Young Sig Park, Namhyun Chung. Single and repeated oral dose toxicity tests of saline groundwater in ICR mice. (2017) Appl. Biol. Chem. 60(6): 659–665**

Saline groundwater (SGW) is the underground saline water obtained from basalt layer through which seawater has infiltrated. SGW contains more than 10,000 mg/L dissolved salt solid, the value of which is less than that of seawater. As part of its safety test, single and repeated oral dose toxicity tests were conducted with male and female ICR mice for 14 days. In single oral dose test with dosage of 10, 30, and 50 mL/kg, no gross changes in appearance or mortality were observed. In repeated oral dose test with dosage of 8, 14, and 20 mL/kg, no significant changes in mortality or weights of body and organ were observed. Additional analysis of serum biochemical parameter and histopathology also indicated no meaningful change during the tests. When taken all together, these results show that no toxicity of SGW could be found with single and repeated toxicity tests. However, for final conclusion of safety, further toxicity studies need to be performed with animal and human subjects.

**Sangjung Park, Adeel Farooq, Hyejun Jo, Jihye Kim, Mihee Yang, Youngho Ko, Sungmo Kang, Hyenmi Chung, Tatsuya Unno. Investigation of microbial communities in water dispensers. (2017) Appl. Biol. Chem. 60(6): 667–672**

Water dispensers remove disinfectant residues from tap water and thus are commonly present in Korean households; however, microbial contamination in water dispensers has recently become a major issue. To understand the occurrence of microbial contamination in water dispensers, we investigated microbial contamination in different dispenser types through heterotrophic plate count (HPC) and MiSeq-based microbial community analyses. Two newly purchased water dispensers were placed in a basement room and left for approximately 2 months; the HPC analysis indicated microbial contamination in the drinking water collected from these dispensers (160,000 and 48,000 CFU/mL, respectively). Taxonomic classification indicated that the basement dispensers were likely contaminated by freshwater bacteria, such as *Acidovorax* and *Methylotenera.* However, two dispensers located at the half landing and the first floor showed lower microbial contamination (110 and 78 CFU/mL, respectively). Furthermore, frequently used dispenser on the first floor showed higher HPCs on the faucet surface, which were classified as general oral bacteria like *Hyphobacterium.* We also observed that a deserted dispenser (6-year-old with no maintenance) placed on the half landing showed the least HPCs, although it seemed to have lost its filtration ability. Our results suggested that removal of disinfectant residues leads to an increase in the freshwater bacterial population in water tanks within dispensers, which could be avoided by frequent water circulation.

**Jae Ryung Hur, Eun Hea Jho. Effect of hemoglobin on the growth and Cd accumulation of pea plants (*Pisum sativum* L.). (2017) Appl. Biol. Chem. 60(6): 673–678**

This study was set to investigate the effect of the presence of hemoglobin (Hb) in cadmium (Cd)-contaminated soil on phytotoxicity and Cd accumulation. The effect of Hb on the Cd accumulation by *Pisum sativum* L. (pea) and seed germination and growth was studied using pot tests with the artificially Cd-contaminated soil. The results show that the externally applied Hb to Cd-contaminated soil samples did not promote Cd accumulation by *P. sativum*. However, the Fe accumulation was greater in the presence of Hb. The seed germination was not affected, but the adverse effects on the plant growth increased with increasing Hb/Cd molar ratio from 0 to 0.015. This can be attributed to toxic effects of the Fe added with the Hb application. The results suggest that the presence of Hb may have harmful effects on pea plants used in phytoremediation of Cd-contaminated soil due to toxic effects imposed by Fe.

**Hoon Choi, Jeong-Han Kim. Risk and exposure assessment for agricultural workers during treatment of cucumber with the fungicide fenarimol in greenhouses. (2018) Appl. Biol. Chem. 61(1): 1–6**

The exposure pattern and potential risk of fenarimol emulsifiable concentrate to agricultural workers were investigated during the preparation of the pesticide suspension and the application of the prepared suspension to the cucumber in a greenhouse environment. The dermal exposure to fenarimol was 0.17 ± 0.11 mg (0.001 ± 0.001% of prepared active ingredient) for mixing/loading and 0.22 ± 0.15 mg (0.003 ± 0.002% of applied active ingredient) for application, respectively. The most exposed part of body was the hand (100%) during mixing/loading, whereas the primary sites during application were the back and legs. In particular, 54.8% of dermal exposure occurred on the shins. The inhalation exposure to fenarimol was detected as 3.7 ± 1.0 μg for the applicator. In comparison with the exposure patterns to pesticides for agricultural workers in greenhouse reported in previous studies, lower dermal and inhalation exposures to fenarimol were observed during mixing/loading and application, respectively. The results of the risk assessment demonstrated that the possibility of risk to fenarimol exposure was lowest during mixing/loading and application in the greenhouse environment.

**Vinod Kumar, Rajat Kumar, Deepa Rawat, Manisha Nanda. Synergistic dynamics of light, photoperiod and chemical stimulants influences biomass and lipid productivity in *Chlorella singularis* (UUIND5) for biodiesel production. (2018) Appl. Biol. Chem. 61(1): 7–13**

Microalgae have emerged as a potential alternative for the production of many useful compounds like protein, carbohydrate and lipid. Lipid-rich microalgae are important and rich source for alternative energy production. In order to commercially utilize microalgae for energy production, the lipid productivity should be enhanced. Keeping in view the above-mentioned potentials of microalgae, in the present study, we have attempted to display the role of chemical stimulants and light in the growth and lipid production of the microalgae *Chlorella singularis*(UUIND5). During the present investigations, effect of varying photoperiods and different types of lights and chemical stimulants, viz. CaCl2 and kinetin on growth rate and lipid production, was studied. The maximum growth rate recorded was 166 ± 0.3 mg/L/d, when 0.80 g/l CaCl2and 0.5 mg/l kinetin were added to Bold’s basal medium. *C. singularis* was then cultivated in this medium for 14 days under sunlight +LED (10-h sunlight + 14-h LED light) at photoperiod 24-h light/0-h dark. The maximum lipid yield 30.2% of dry wt. was obtained  under sunlight +LED. Further, the gas chromatography analysis also showed the presence of fatty acid methyl esters (FAME). FAMEs profile was analyzed according to ASTM D6751 specification. Thus, it was concluded that sunlight +LED at 24-h light/0-h dark (100 μmol photons m−2 s−1) photoperiod with CaCl2 and kinetin is an effective strategy to boost lipid productivity in *C. singularis* (UUIND5).

**Jong-Hwan Kim, Sung-Gil Choi, Young Sang Kwon, Su-Myeong Hong, Jong-Su Seo. Development of cabbage reference material for multi-residue pesticide analysis. (2018) Appl. Biol. Chem. 61(1): 15–23**

Cabbage reference material for pesticide multi-residue analysis was developed in accordance with the ISO Guide 35, ISO Guide 13528 and European Union Reference Laboratories-Proficiency Test standard protocols. Ten pesticides (acetamiprid, azoxystrobin, boscalid, buprofezin, carbendazim, difenoconazole, ethofenprox, imidacloprid, pyraclostrobin and tebuconazole) detected at relatively high levels in agricultural products in Korea were selected for this study. The developed material was evaluated for homogeneity and stability according to the statistical assessment method specified by international standards. Analysis of variance was carried out to calculate the within-bottle standard variation (*s* wb) and the between-bottle standard variation (*s* bb). Values of *s* wb and *s* bb varied by less than 4.7% of assigned values. Homogeneity was also assessed using Cochrane testing of outliers. All pesticides in the material were uniformly distributed within or between all bottles. Stability tests were conducted at room temperature (20–30 °C) for 12 days, under cold conditions (4–8 °C) for 40 days, under freezing conditions (− 20 °C) for 70 days and under deep freezer conditions (− 80 °C) for 234 days. Stability was evaluated based on the ISO Guide 35 statistical model, and results showed no significant decrease in stability during storage for any pesticide under any condition. We therefore conclude that the cabbage material could be used for future proficiency tests and/or validation of pesticide residue analysis.

**Eun-Seo Lim. Preparation and functional properties of probiotic and oat-based synbiotic yogurts fermented with lactic acid bacteria. (2018) Appl. Biol. Chem. 61(1): 25–37**

The main purpose of the current study was to assess the physicochemical properties of the synbiotic yogurt fermented with oat slurry and probiotic strains and the antioxidative and antibacterial activities of the oat-based synbiotic yogurt. The viable cells of *Lactobacillus brevis*SBP49 and *Lactobacillus acidophilus* SBP55 reached 108 CFU/g or more in the probiotic and oat-based synbiotic yogurt, and the resistance to artificial digestive juices and the adherence to intestinal epithelial cells of these lactic acid bacteria were also very high in these yogurts. In addition, oat flour added for the manufacture of the synbiotic yogurt significantly promoted the production of antimicrobial substances by these probiotics, thereby increasing the antibacterial effect of the strains against pathogenic food poisoning bacteria including *Bacillus cereus*American Type Culture Collection (ATCC) 11778, *Escherichia coli* O157 ATCC 43889, *Listeria monocytogenes* Korean Collection for Type Cultures (KCTC) 3569, *Salmonella enteritidis*ATCC 13076, *Salmonella typhimurium* KCTC 2514, and *Staphylococcus aureus* ATCC 6538. Meanwhile, the antioxidative activity of the oat-based synbiotic yogurt was significantly higher than that of the probiotic yogurt and its activity may be due to free radical scavenging ability of phenolic compounds contained in oat slurry.

**Xiaoyong Chen, Jia-Le Song, Qiang Hu, Hongwei Wang, Xin Zhao, Huayi Suo. Positive enhancement of *Lactobacillus fermentum* HY01 on intestinal movements of mice having constipation. (2018) Appl. Biol. Chem. 61(1): 39–48**

*Lactobacilli* have been used to treat many gastrointestinal disorders. But the outcome of *Lactobacilli* are strain specific. The strain *Lactobacillus Fermentum*, HY01, (LF-HY01) has a good performance in the environment of gastrointestinal tract. In this study, the aim is to investigate the preventive effects of LF-HY01 against activated-carbon-induced constipation in mice. Mice are randomized into four groups. Normal group was fed a normal diet, model group also has the same with activated carbon treatment, and low and high concentration groups are treated with LF-HY01. We have determined many indexes such as body weight, water content in faeces, defecation conditions, the level of small intestinal villi damages and levels of various neurotransmitters in serum, including motilin (MTL), gastrin (GT), endothelin (ET), somatostatin (SST), acetylcholinesterase (AchE), substance P (SP), and vasoactive intestinal peptide (VIP). LF-HY01 has no significant difference in each group, but it can significantly improve water content of faeces, defecation time of first black stool and activated carbon propelling rate in small intestine as compared of model group. Furthermore, LF-HY01 can effectively prevent small intestinal villi damages, which is less than that of model group. Moreover, LF-HY01 has the consistency to increase the levels of MTL, GT, ET, AchE, SP and VIP, and LF-HY01 can also have the ability to reduce the level of SST. These results suggest that *Lactobacillus Fermentum*, HY01, has a great impact in enhancing intestinal peristalsis ability and has the ability to prevent from activated-carbon-induced constipation in mice.

**Sehun Choi, Han-Seok Seo, Kwang Rag Lee, Sunghee Lee, Jihyun Lee. Effect of cultivars and milling degrees on free and bound phenolic profiles and antioxidant activity of black rice. (2018) Appl. Biol. Chem. 61(1): 49–60**

Six black rice cultivars (Heukjinju, Sintoheugmi, Heukhyangchal 1, Bosukheukchal, Sinnongheukchal, and Josengheukchal) and varying milling degrees (step 0, 0%; step 1, 4.2%; and step 2, 10.5%, w/w) were used to evaluate the effects of cultivars and milling degrees of black rice (*Oryza sativa* L.) on the total phenolic contents (TPC), total flavonoid contents (TFC), antioxidant activity (2,2-diphenyl-1-picrylhydrazyl free radical assay), and phenolic composition in free and bound phenolic fractions. Unpolished (step 0) Sintoheugmi showed significantly higher TPC, TFC, antioxidant activity, phenolic acid levels, and anthocyanin levels than other unpolished cultivars (*p* < 0.05). As milling degree increased, TPC, TFC, antioxidant activity, phenolic acid levels, and anthocyanin levels decreased significantly (*p* < 0.05). TPC, TFC, and antioxidant activity were significantly higher in free phenolic fractions than bound phenolic fractions of black rice extracts, regardless of cultivars (*p* < 0.05). The major phenolic acid was ferulic acid, and the major anthocyanin found in free phenolic fractions in black rice samples was cyanidin-3-O-glucoside. The sum of individual phenolic acid levels (255.2 ± 0.0 μg/g) and the sum of anthocyanins levels (831.4 ± 0.3 μg/g) were significantly higher in Sintoheugmi black rice than in the other cultivars for step 0 (unpolished rice) (*p* < 0.05). For step 1 and step 2, Heukjinju black rice contained significantly higher sum of phenolic acid levels and sum of anthocyanin levels than the other cultivars (*p* < 0.05). For use as a better functional ingredient, it is, therefore, important to consider different milling degrees together with different black rice cultivars having the highest antioxidant component.

**Ah Young Lee, Myoung-Hee Lee, Sanghyun Lee, Eun Ju Cho. Alpha-linolenic acid regulates amyloid precursor protein processing by mitogen-activated protein kinase pathway and neuronal apoptosis in amyloid beta-induced SH-SY5Y neuronal cells. (2018) Appl. Biol. Chem. 61(1): 61–71**

Alpha-linolenic acid (ALA), which is an omega-3 fatty acid from plant oils, has been reported to have beneficial effects on human brain health. However, the protective effect of ALA and its mechanism of action against amyloid beta (Aβ)-mediated neurotoxicity, neuronal apoptosis and amyloid precursor protein (APP) processing are unclear. To investigate the neuroprotective effect of ALA, we treated Aβ25-35-induced SH-SY5Y cells with ALA (1, 2.5, 5 and 25 μg/mL). In our results, Aβ25-35-induced neuronal cell loss was observed, whereas ALA significantly increased the cell viability and decreased lactate dehydrogenase release. In addition, over-production of reactive oxygen species caused by Aβ25-35 was attenuated by treatment with ALA, and these inhibitory activities were mediated by regulation of the mitogen-activated protein kinase signaling pathway. Furthermore, our data shows that Aβ25-35 cause an increase in protein expression of APP-C-terminal fragment β, β-site APP-cleaving enzyme and presenilin-1 in SH-SY5Y cells, while ALA significantly down-regulated the expression of those amyloidogenic APP processing-related proteins. In addition, we confirmed that ALA enhanced α-secretase activity by up-regulating the protein levels of A distintegrin and metalloprotease 10 and tumor necrosis factor-α-converting enzyme, indicating that ALA could promote non-amyloidogenic signaling pathways. ALA also significantly attenuated Aβ25-35-induced neuronal apoptosis by up-regulation of the Bcl-2/Bax ratio. These findings suggest that ALA may be a beneficial agent for promoting prevention of Alzheimer’s disease.

**Han-bin Kim, Sooim Shin, Moonsung Choi. Thermodynamic analysis of MauG, a diheme oxygenase. (2018) Appl. Biol. Chem. 61(1): 73–78**

MauG is a unique *c*-type diheme oxygenase. One heme of MauG is five-coordinate and solvent accessible with His53 as axial ligand, while the other heme of MauG is six-coordinate with His205 and Tyr294. MauG catalyzes posttranslational modification including oxygen insertion, cross-linkage of two tryptophan and oxidation of quinol to quinone of precursor methylamine dehydrogenase (preMADH) to form mature tryptophan tryptophylquinone (TTQ) which is one of protein-derived cofactors. Long-range remote catalysis of substrate is possible without direct contact between hemes of MauG and its substrate, preMADH. Although catalytic properties and mechanisms of MauG have been well studied, temperature dependence of MauG has never been reported yet. Therefore, the objective of this study was to perform thermodynamic analysis of MauG. Δ*H*° of 87.6 ± 6.7 kJ mol−1 and Δ*S*° of 232 ± 15.6 J mol−1 K−1 were directly measured for oxidized MauG in this study. Those results provide fundamental information on controlling electron transfer rates for biosynthesis of TTQ in MADH and are used as a good thermodynamic example study for other diheme systems.

**Eun-Jin Lee, Gui-Ran Kim, Kashif Ameer, Hyun-Kyu Kyung, Joong-Ho Kwon. Application of electron beam irradiation for improving the microbial quality of processed laver products and luminescence detection of irradiated lavers. (2018) Appl. Biol. Chem. 61(1): 79–89**

The laver (*Porphyra* spp.) is normally processed in three kinds of products: dried laver (DL), roasted laver (RL), and seasoned roasted laver (SL). This work evaluated the effects of electron beam (E-beam) irradiation at different doses (0, 1, 4, 7, and 10 kGy) on microbiological and physicochemical qualities and detection characteristics of irradiated samples by luminescence analysis. E-beam irradiation resulted in dose-dependent microbial reductions, showing that 1 kGy destroyed initial coliforms (< 2.35 log CFU/g) to undetectable levels (< 10 CFU/g), while 7 kGy (approved dose for seaweed in Korea Food Code) reduced total aerobic bacteria (3.72–6.33 log CFU/g) and yeasts and molds (2.05–4.98 log CFU/g) by about 2 log cycles. Chlorophyll content remained unaffected in irradiated samples as compared to control; however, carotenoids content and Hunter’s *b* values (degree of yellowness) showed a tendency to decrease in a dose-dependent manner (*p* < 0.05). However, E-beam irradiation less than 7 kGy did not significantly affect sensory properties of the processed laver products. Irradiated laver products (DL, RL, and SL) could be screened and detected by analyzing photostimulated luminescence and thermoluminescence, respectively, from the non-irradiated ones. The overall results indicated that E-beam irradiation is effective for ensuring the improved microbial quality (< 4 log CFU/g) for the exporting processed laver products without apparent quality changes.

**Sehun Choi, Han-Seok Seo, Kwang Rag Lee, Sunghee Lee, Jihyun Lee. Effect of milling degrees on volatile profiles of raw and cooked black rice (*Oryza sativa* L. cv. Sintoheugmi). (2018) Appl. Biol. Chem. 61(1): 91–105**

Volatile compounds in raw and cooked black rice (cv. Sintoheugmi) samples with different degrees of milling (step 0, 0%; step 1, 4.2%; and step 2, 10.5%, w/w) were investigated by headspace solid-phase microextraction and gas chromatography–mass spectrometry. A total of 101 volatile compounds were found. Among them, 44 compounds found in raw black rice were absent in cooked black rice and 20 compounds were newly formed in cooked black rice. The 8 identified major odor-active volatile compounds in raw and cooked black rice included 3 phenols (guaiacol, 4-vinylphenol, and 2-methoxy-4-vinylphenol), 2 benzenes (benzaldehyde and *p*-xylene), 2 furans (2-butylfuran and 2-pentylfuran), and 1 terpene (calamenene). Additionally, fatty acid oxidation products such as hexanal, 2-nonenal, octanal, and 2-pentylfuran were found in raw and cooked black rice samples. The relative concentrations of these volatile compounds were significantly higher in step 0 than in step 2 of raw and cooked black rice (*p* < 0.05). Partially milled cooked black rice (i.e., step 1) contained ~ 80% guaiacol (a favorable unique black rice flavor) of unpolished rice (step 0), with similar levels of several lipid oxidation indicator volatile products (e.g., 2-nonenal and 2-pentyl furan) of fully milled rice (step 2). Thus, partially milled black rice should be consumed rather than fully milled black rice.

**Muhammad Najmus Saqib, Muhammad Subhan Qureshi, Rifat Ullah Khan.** **Changes in postpartum metabolites and resumption of ovarian cyclicity in primiparous and multiparous dairy cows. (2018) Appl. Biol. Chem. 61(1): 107–111**

The postpartum period in high-yielding dairy cows creates an enormous drain of nutrients in favor of milk yield which antagonizes the resumption of ovulatory cycles. Therefore, a study was undertaken to evaluate the association of changes in postpartum serum metabolites with resumption of ovarian cyclicity. A total of 24 clinically healthy, freshly parturated primiparous (P-1) and multiparous (P-2) Holstein Frisian cows was selected. Cows were further divided on the basis of body condition score (BCS) 1 and 2 having BCS 3 or above 3, respectively. Weekly blood samples were collected and serum glucose, cholesterol, triglycerides, progesterone, and cortisol concentrations were determined for a period of 7 weeks. The glucose concentration was significantly (*P* < 0.05) higher in cows in P-2 during week 7. Cows in parity 2 had significantly (*P* < 0.05) high cholesterol during week 6 and 7. The serum triglyceride concentration in multiparous animals having BCS-2 during week 6 was increased significantly (*P* < 0.05). Serum cortisol was significantly (*P* < 0.05) high in P-1 during week 1 and 2 and significantly (*P* < 0.05) high in P-2 during week 7. Serum progesterone was significantly (*P* < 0.05) higher in cows during week 7 in P-2 having BCS-2. The increased serum progesterone concentration during postpartum period was associated with decreased levels of serum cortisol and more availability of cholesterol and glucose. The multiparous cows maintained the postpartum blood metabolite concentration and showed better adaptability to reproductive cyclicity during the postpartum period as compared to primiparous cows.

**Amir Hossein Forghani, Abbas Almodares, Ali Akbar Ehsanpour.** **Potential objectives for gibberellic acid and paclobutrazol under salt stress in sweet sorghum (*Sorghum bicolor* [L.] Moench cv. Sofra). (2018) Appl. Biol. Chem. 61(1): 113–124**

The phytohormones are important in plant adaptation to abiotic and biotic stresses by facilitating a wide range of adaptive responses. Application of gibberellic acid (GA3) and paclobutrazol (PBZ) as GA3 inhibitors have been shown to affect salinity tolerance through modulating phytohormones. The aim of this study was to find out the potential objectives for GA3 and PBZ as affected by salinity through altering the phytohormones and biochemical parameters in sweet sorghum. Following seed germination, seedlings were cultured in Hoagland nutrient solution containing NaCl supplemented with GA3 and PBZ for 12 days. The results were analyzed by principal component analysis to identify the best target(s) for salinity, GA3, and PBZ in sweet sorghum. Paclobutrazol associated with salt improved root/shoot length, carotenoid, and total chlorophyll by modulating cytokinin (CK)/GA3, indole acetic acid (IAA)/GA3, and total polyamines/GA3 ratios. Gibberellic acid-treated plants not exposed to salinity treatments notably improved phytohormones content such as cytokinin, auxin, abscisic acid (ABA), and polyamines resulting in increased stem growth. Moreover, the main objectives of GA3 were ABA, spermidine, and ABA/GA3 ratio in response to salinity. Though GA3 and PBZ have different roles against salt stress, ABA/GA3 ratio was a similar target of GA3 and PBZ. This work suggests that altered levels of GA3 resulting from PBZ- and GA3-treated plants cause different allocation patterns in sweet sorghum by regulation of CK/GA3, IAA/GA3, and total polyamines/GA3 ratio. Also, accumulation chlorophyll pigments, carotenoids, and water soluble carbohydrates of sorghum plants under salinity regulated by total polyamines/GA3 and ABA/GA3 ratios positively correlated with PBZ application.

**Jiho Lee, Eunhye Kim, Yongho Shin, Jonghwa Lee, Junghak Lee, Wolfgang Maasfeld, Jeong-Han Kim.** **Validation protocol for whole-body dosimetry in an agricultural exposure study. (2018) Appl. Biol. Chem. 61(1): 125–130**

Agricultural workers exposed to pesticides can experience adverse health impacts depending on toxicity and exposure amount. Whole-body dosimetry (WBD) is the most reliable, practical, and realistic method for measuring exposure. Since validation of analytical and experimental methodologies is critical for quantitative determination of exposure, we conducted a validation procedure to design an essential protocol for WBD exposure studies. Using the fungicide kresoxim-methyl, matrix-matched standards were prepared with various matrices including outer cloth, inner cloth, washing solution for gloves and hands, gauze, and glass fiber filter (IOM sampler) to determine the instrumental limit of quantitation for high-performance liquid chromatography (HPLC) (2 ng) and liquid chromatography–tandem mass spectrometry (LC–MS/MS) (10 pg). Method limits of quantitation (MLOQ) were also set for HPLC (0.1 mg/L) and LC–MS/MS (0.005 mg/L). We observed good analysis repeatability (coefficient of variation < 6%), and the linearity of the calibration curves was reasonable (*r* 2 > 0.998) in the range of 0.001–10 mg/L in various matrices. Recovery tests were carried out at three levels of concentration (MLOQ, 10 MLOQ, and 100 MLOQ) and resulted in good recoveries (72.7–105.6%). We did not observe breakthrough of the compound in tests of holding capacity for glass fiber pesticide filters. The procedures established in the present study are applicable as an essential, comprehensive protocol for exposure assessment studies using WBD.

**Kyoung Bok Lee, Ye Jin Kim, Hyo Jin Kim, Jaehyuk Choi, Jae Kwang Kim.** **Phytochemical profiles of Brassicaceae vegetables and their multivariate characterization using chemometrics. (2018) Appl. Biol. Chem. 61(2): 131–144**

Twenty-eight metabolites were extracted from nine Brassicaceae of Korean origin (broccoli, Brussels sprouts, cabbage, Chinese cabbage, kale, kohlrabi, pak choi, radish sprouts, and red cabbage) and analyzed using gas chromatography–mass spectrometry and high-performance liquid chromatography. Principal components analysis (PCA), orthogonal projection to latent structure-discriminant analysis (OPLS-DA), Pearson’s correlation analysis, hierarchical clustering analysis (HCA), and batch learning self-organizing map analysis (BL-SOM) were used to visualize metabolite pattern differences among Brassicaceae samples. The PCA score plots from the metabolic data sets provided a clear distinction between *Brassica* species and radish sprouts (genus *Raphanus* L.). Additionally, *B. oleracea* L. varieties were differentiated from *B. rapa* L. varieties by PCA and OPLS-DA score plots. HCA and BL-SOM of these metabolites clustered metabolites that are metabolically related. This study demonstrates that plants’ characterization by multivariate statistical analysis using metabolic profiling allows distinguishing their phenotypes and identifying desired characteristics.

**Kyu-Won Hwang, Joon-Kwan Moon.** **Translocation of chlorpyrifos residue from soil to Korean cabbage. (2018) Appl. Biol. Chem. 61(2): 145–152**

The loss of residual chlorpyrifos in soil and the amount translocated to Korean cabbage were investigated in this study. Field trials with Korean cabbage were carried out in two greenhouses located in Yongin (Field 1) and Gwangju (Field 2). Soil and Korean cabbage samples were collected on different days following the treatment of soil with chlorpyrifos at two different rates. The initial amounts of residue in soil were 1.15 and 3.58 mg/kg, and these decreased to 0.22 and 0.49 mg/kg at 36 days after treatment (DAT) in Field 1. These values were 20.9 and 59.3 mg/kg, decreasing to 3.03 and 5.24 mg/kg at 43 DAT in Field 2, respectively. In Field 1, the half-life of chlorpyrifos was approximately 15.0 and 10.2 days in soil treated with 0.12 and 0.24 g a.i./m2, respectively. In Field 2, the half-life of chlorpyrifos was approximately 27.7 and 9.6 days following application of 0.36 and 0.72 g a.i./m2, respectively. When compared with the initial concentration in soil, the absorption ratio of chlorpyrifos residue to Korean cabbage was 0.93–6.01 and 0.57–2.61%, respectively. Therefore, safe management guidelines for chlorpyrifos in soil used to cultivate Korean cabbage may be suggested as 3.3 mg/kg regarding the maximum residue limit of chlorpyrifos on Korean cabbage (0.2 mg/kg).

**Keon Hee Kim, Youngdae Yoon, Woon-Young Hong, JaeBum Kim, Yung-Chul Cho, Soon-Jin Hwang.** **Application of metagenome analysis to characterize the molecular diversity and saxitoxin-producing potentials of a cyanobacterial community: a case study in the North Han River, Korea. (2018) Appl. Biol. Chem. 61(2): 153–161**

A wide variety of cyanobacterial species that inhabit freshwater systems are known to produce diverse toxins and off-flavor compounds during the development of environmentally harmful blooms. However, cyanobacterial community development and toxin production potential have not been well studied. In this study, we examined the taxonomic diversity and saxitoxin production potential of cyanobacteria in the water and sediments of a large river, the North Han River in South Korea, by metagenome analysis using next-generation sequencing (NGS) and molecular biological approaches, respectively. NGS revealed that the entire cyanobacterial community in the study area consisted of 39 genera and 47 species. The most abundant genera were *Microcystis, Anabaena, Cyanobium,* and *Synechococcus,* which accounted for more than 90% of the entire community. The saxitoxin production potential of the cyanobacterial community was assessed by detecting the *sxtA* and *sxtG* genes related to saxitoxin production. Eleven *sxtA* and 24 *sxtG* genes were identified through molecular cloning and sequencing. Phylogenic analysis revealed that three *sxtA* genes that grouped in one phylogenic branch with *Scytonema* sp. were distinctly separated from the *sxtA* genes of *Anabaena, Aphanizomenon, Lyngbya*, and *Cylindrospermopsis*. Sixteen of the detected *sxtG* genes were phylogenically similar to those of *Anabaena circinalis* (*Dolichospermum circinale*), *Aphanizomenon gracile,*and *Aphanizomenon flos*-*aquae*. Our study demonstrates the utility of the metagenomics approach for characterizing the natural community structure of cyanobacteria containing diverse and even rare species, and the evaluation of saxitoxin-producing potential in the cyanobacterial community.

**Noble K. Kurian, Sarita G. Bhat.** **Food, cosmetic and biological applications of characterized DOPA-melanin from *Vibrio alginolyticus*strain BTKKS3. (2018) Appl. Biol. Chem. 61(2): 163–171**

Melanins are one of the most common pigments produced in nature and distributed throughout the biological kingdom. *Vibrio alginolyticus* strain BTKKS3 produced DOPA-melanin was used in the study. BTKKS3 melanin inhibited biofilm formation by pathogenic bacteria and effectively decreased the activity of four inflammatory enzymes tested viz. cyclooxygenase, lipoxygenase, myeloperoxidase and nitric oxide synthase. Melanin proved to be less cytotoxic to mouse fibroblast cells with an IC50 value of 134.98 μg/mL. The sun protection factor value of commercial sunscreens was enhanced by 3.42 units by DOPA-melanin.

**Byung Kwon Jung, Sung-Jun Hong, Gun-Seok Park, Min-Chul Kim, Jae-Ho Shin.** **Isolation of *Burkholderia cepacia* JBK9 with plant growth-promoting activity while producing pyrrolnitrin antagonistic to plant fungal diseases. (2018) Appl. Biol. Chem. 61(2): 173–180**

*Burkholderia* species are widely distributed across wide ecological niches. Many genera of *Burkholderia* are known to be associated with plants and are involved in processes such as suppression of soil-borne pathogens, acceleration of plant growth and endophytic colonization. In the present study, a strain belonging to the *Burkholderia cepacia* complex, which was termed JBK9, was isolated. The strain JBK9 showed broad-spectrum antifungal activities against *Phytophthora capsici, Fusarium oxysporum*, and *Rhizoctonia solani*, which are representative phytopathogenic fungi, inhibiting their growth by 59.56, 51.92, and 34.22%, respectively. The strain produced an antifungal compound that was confirmed to be pyrrolnitrin by TLC, HPLC, and NMR analyses. Using an in vitro assay for plant root colonization, we observed that the population densities of *B. cepacia* JBK9 on the upper 1 cm of host plant roots were significantly different between *Burkholderia* species. The high motility of these strains is likely to have contributed to their efficient root colonization. The isolated strain was evaluated in vivo for its ability to control *Phytophthora* blight via a pot test. Compared with *Burkholderia* strains KCTC 2973 and ATCC 25416, *B. cepacia* JBK9 demonstrated a stronger antifungal activity against *P. capsici*. The strain *B. cepacia* JBK9 could be further developed as a biological control agent for pepper plants.

**Min-Ju Jo, Sooim Shin, Moonsung Choi.** **Intra-electron transfer of amicyanin from newly derived active site to redox potential tuned type 1 copper site. (2018) Appl. Biol. Chem. 61(2): 181–187**

Amicyanin, one of the type I copper proteins which has been used for the study, mediates the electron transfer reaction between methylamine dehydrogenase and cytochrome c-551i in *Paracoccus denitrificans* for energy production. The 6×Histidine-tag site which has been widely used in purification of a recombinant protein was introduced at the N-terminus of amicyanin to make the complex of 6×His-tagged plus cobalt functioning as a newly derived redox cofactor in amicyanin. In this study, Pro94 of amicyanin was substituted to Ala and Phe to tune up the midpoint potential (*E*m) value of amicyanin 100 mV more positive and then intra-electron transfer rates were measured to examine whether the *E*m value of the type 1 copper site in amicyanin affects intraprotein electron transfer or not. By the addition of H2O2, the Co2+-loaded 6×His-tagged site was activated, and then electron was transferred from Cu1+of type 1 copper site of amicyanin to Co3+ plus 6×His-tagged site. Electron transfer rates of cobalt loaded P94A and F amicyanin were much slower than that of native amicyanin. These results suggest that the communication between the newly protein-derived redox cofactor, 6×His-tagged site plus cobalt, and type 1 copper site is truly occurred and that the strength of electron transfer reaction between them is able to be controlled by an *E*m value.

**Hüseyin Bulut, Nalan Yıldırım Doğan.** **Determination by molecular methods of genetic and epigenetic changes caused by heavy metals released from thermal power plants. (2018) Appl. Biol. Chem. 61(2): 189–196**

The heavy metals are released into the environment due to the activities such as meeting the increasing demand for energy, industrial activities, and agricultural pesticides. In many studies, the heavy metals have been proven to have genotoxic effects. As a result of burning the lignite coal in thermal power plants, the heavy metals of Cr, Fe, Mn, Cu, Pb, Cd, Zn, and Ni are spread into the environment within the ashes. In the present study, the gene expression levels were examined on the corn and wheat plants added with 500-m interval around the thermic power plant. For this purpose, the genes from 14-3-3 protein family, expression level of which increases under abiotic stress conditions, were analyzed. For the expression levels of plants, the 2−ΔΔCt values were calculated and then compared to 2−ΔΔCt values of β-actin gene, that is, the housekeeping gene. The heavy metal content analyses of the samples were carried out using ICP-MS, and it was determined that there were many heavy metals at higher amounts within the structure of samples having low level of gene expression. It has been understood that heavy metal stress causes a difference in gene expression level. The change introduced by heavy metal stress into the gene expression occurs in concrete in the translation products. The level of stress-induced gene expression, which is caused by heavy metals in the environment surrounding the plant, has been successfully determined by RT-PCR.

**Jaison Jeevanandam, Yen San Chan, Yee Hung Ku.** **Aqueous *Eucalyptus globulus* leaf extract-mediated biosynthesis of MgO nanorods. (2018) Appl. Biol. Chem. 61(2): 197–208**

Plant-based biosynthesis is gaining attention in nanoparticle synthesis as an alternate to chemical and physical synthesis routes due to their non-toxic and environment friendly nature. Leaf extract-based biosynthesis further facilitates rapid synthesis of non-toxic biocompatible nanoparticle that possesses various applications in biomedical and pharmaceutical industry. Metal oxides, especially MgO nanoparticles, show tremendous applications in medical industry. Moreover, plant-based biosynthesized MgO nanoparticles showed improved biophysical and biochemical properties. In the current study, MgO nanorods (MgONRs) are synthesized using *Eucalyptus globulus* aqueous leaf extract. The results are highly significant as rod-shaped nanoparticles possess superior cellular penetration ability than other morphologies and can be valuable in medical applications. A preliminary experiment was performed to identify the required reaction time for nanorod formation using dynamic light scattering technique. Later, one-factor-at-a-time approach was followed to identify the effect of each process parameters on average particle size of MgONRs. The optimized parameters were used for the synthesis of smaller-sized MgONRs. Fourier Transform infrared spectroscopy analysis was conducted to identify and analyze the functional groups in the leaf extract and MgONRs. The functional groups from phytochemicals and their transformation from enol to keto-form were found to be responsible for nanoparticle formation. The transmission electron microscope analysis showed that the optimized parameters yield 6–8 nm width of stacked MgONRs. Thus, the present work demonstrated a simple and rapid biosynthesis route for MgO nanorod synthesis which can be beneficial in biosensing and therapeutic application.

**Hossein Azizi Toupkanloo, Zoha Rahmani.** **An in-depth study on noncovalent stacking interactions between DNA bases and aromatic drug fragments using DFT method and AIM analysis: conformers, binding energies, and charge transfer. (2018) Appl. Biol. Chem. 61(2): 209–226**

This work is aimed at providing physical insights about the *π*–*π* stacking interactions of some popular drug fragments (DF) including indole (I), benzothiophene (Bt), benzofuran (Bf) and guanine (G), adenine (A), A-thymine (AT), G-cytosine (GC) base pairs using density functional theory (DFT), the atoms in molecule (AIM) theory, and natural bond orbital (NBO) analysis. Several stable conformers of present molecules and complexes were optimized at the M062X/6-311++G(d,p) level of theory. The result shows that the IG1 (see the notation below) and IA6 have maximum interaction energy in all of the two G-based and A-based conformers; and order of the adsorption strength is IG1 > BtG6 > BfG1 for G-based complexes and IA6 > BtA6 > BfG6 for A-based complexes. For the base pair–drug fragment complexes, the order of interaction energy was found according to IAT4 > BtAT3 > BfAT4 and IGC3 > BtGC2 > BfGC2, for AT and GC base pairs, respectively. Furthermore, our results show that stacking interaction leads to an increase and decrease in hydrogen bond length that involved in the nucleic base–drug fragment interactions. DFT-calculated interaction energies for all present conformers were found to be in a good agreement with the bond critical points data from AIM analysis. In contrast, no reasonable linear correlation was observed between NBO analysis and stability of the all studied conformers. Finally, in order to verify the DFT and AIM results, docking calculations were performed using AutoDock software. According to the binding energy of drug–DNA from AutoDock calculations, the D2-Bt and D1-Bf are the most and the least stable structures, respectively.

**Hyejung Gu, In-Bong Song, Hye-Ju Han, Na-Young Lee, Ji-Yun Cha, Yeon-Kyong Son, Jungkee Kwon.** **Anti-inflammatory and immune-enhancing effects of enzyme-treated royal jelly. (2018) Appl. Biol. Chem. 61(2): 227–233**

Royal jelly is produced by honeybees and has been shown to be various pharmacologically active. Enzyme-treated royal jelly (ERJ) is an allergen-free form of royal jelly that has been converted to shorter easy-to-absorb chain monomers. In this study, we investigated the anti-inflammatory and immunomodulatory effects of ERJ on macrophages and mice. We found that ERJ altered macrophage proliferation and was protective against lipopolysaccharide (LPS)-induced stress. The mice, fed ERJ for 4 weeks and stimulated LPS, significantly reduced levels of tumor necrosis factor-alpha, interleukins-1, 6, 10, 12, and interferon gamma compared to control mice. ERJ significantly increased the proliferation of B-lymphocytes and T-lymphocytes, as well as the activity of natural killer cells in a dose-dependent manner. Therefore, our results indicate that ERJ has strong anti-inflammatory and immune-promoting activities and can be developed as a potential food material for prevention of inflammatory disease.

**In-Wook Hwang, Bo-Min Kim, Young-Chan Kim, Sang-Han Lee, Shin-Kyo Chung.** **Improvement in β-glucan extraction from *Ganoderma lucidum* with high-pressure steaming and enzymatic pre-treatment. (2018) Appl. Biol. Chem. 61(2): 235–242**

In this study, the high-pressure steaming and enzymatic pre-treatment (SET) was used to improve β-glucan extraction from *Ganoderma lucidum* (*G. lucidum*), an oriental medicinal mushroom. Response surface methodology and central composite design were used to determine the optimum pre-treatment conditions: high-pressure steaming, enzymatic hydrolysis, and Viscozyme L concentrations. The optimal conditions were 15.51 min for high-pressure steaming, 0.84 g/100 mL of Viscozyme L, and 4.16 h for hydrolysis. The predicted β-glucan content in *G. lucidum* extract at optimal conditions, approximately twofold (8.05 g/100 g) of the control treatment value, was consistent with the empirical value. The total sugar and protein contents through SET were higher than those values of the control treatment. The cell migration assay showed that SET-processed *G. lucidum* extracts significantly suppressed B16F10 murine melanoma cell growth. SET process using Viscozyme L could be utilized for β-glucan extraction from *G. lucidum* to develop the functional food.

**Young-Sun Moon, Hoi-Seon Lee, Sung-Eun Lee.** **Inhibitory effects of three monoterpenes from ginger essential oil on growth and aflatoxin production of *Aspergillus flavus* and their gene regulation in aflatoxin biosynthesis. (2018) Appl. Biol. Chem. 61(2): 243–250**

Ginger (*Zingiber officinale*) essential oil (ZOE) possesses strong antibacterial and antifungal activities. In this study, the antifungal activity of ZOE against *Aspergillus flavus* was investigated, and a chemical analysis was carried out to identify compounds that control fungal growth. A total of 37 compounds were identified by gas chromatographic analysis with a mass detector, and the antifungal and antiaflatoxigenic properties of three constituents, *γ*-terpinene, isoborneol, and citral, against *A. flavus* were tested. All compounds exhibited strong antifungal activity at 1000 μg/mL, and the antifungal activity of *γ*-terpinene and citral remained until treatment with tenfold diluted solution. The decrease in aflatoxin production by the three compounds was observed until treatment with 10 μg/mL. To evaluate their antiaflatoxigenic activity, RT-qPCR was used to compare the expression of 11 genes involved in aflatoxin biosynthesis by *A. flavus*. Among the three compounds, *γ*-terpinene and citral markedly reduced the expression of most of the tested genes but a different pattern of downregulation of the expression was observed. *γ*-Terpinene did not downregulate *aflR*, *aflS*, and *yap*, whereas citral did not alter the expression of *aflC* and *aflG*. Therefore, *γ*-terpinene and citral may have the potential to control *A. flavus* growth and aflatoxin production in agricultural products, including at the storage stage.

**Hye-Jeong Hwang, Inseong Choi, Yoon Young Kang, Hyejung Mok, Yoongho Lim, Woon-Seok Yeo.** **Analysis of the biodistribution of natural products in mice by using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. (2018) Appl. Biol. Chem. 61(2): 251–255**

Natural products originating from plants have various beneficial pharmacological effects, such as anticancer, antimicrobial, and anti-inflammatory activities, while being nontoxic. Therefore, tremendous efforts have been invested in understanding their bioactivities in the body to facilitate therapeutic target validation. However, such research is still challenging for certain natural products, such as flavonoids, which are rapidly metabolized in and eliminated from the human body. To investigate the bioactivities of such products, particularly in certain tissues, it is necessary to understand their biodistribution in vivo. In this respect, reliable analytical methods with simple and efficient procedures for the in vivo evaluation of natural small molecules are urgently required. In particular, mass spectrometry (MS) can be effectively used to analyze small molecules after tissue extraction, as MS has various advantages including accuracy, simplicity, and high sensitivity. Herein, we report the biodistribution of a natural small molecule by using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). After intravenously injecting gomisin H into a mouse as a model natural product, it was extracted from each organ and then analyzed by MALDI-TOF MS. The analysis showed that gomisin H accumulated mainly in the liver and relatively large amounts of the product existed in the kidney and brain compared to those in other tissues.

**Tong Li, Jo-Won Lee, Li Luo, Jongkee Kim, BoKyung Moon.** **Evaluation of the effects of different freezing and thawing methods on the quality preservation of *Pleurotus eryngii*. (2018) Appl. Biol. Chem. 61(3): 257–265**

The individually quick-frozen (IQF) method is used to produce high-quality frozen food by freezing an individual piece of food separately from the remaining food. In this study, the effect of various freezing and thawing combinations on the quality preservation of *Pleurotus eryngii*(king oyster mushroom) was investigated. For this purpose, diced and mini *P. eryngii*mushrooms were frozen by natural freezing (NF, − 20 °C) or IQF (− 62.5 °C and speed 8.23 m/s) methods and thawed using three thawing methods—flowing water thawing (FT, 4 °C), microwave thawing (MT, 620 W), or natural air convection thawing (NT, 20 ± 5 °C). Quality characteristics, such as thawing loss, texture, water holding capacity, color, microstructure, and sensory quality, were evaluated. MT showed the most rapid thawing time, followed by FT and NT for all the samples. The results showed that thawing curve, water holding capacity, and hardness of IQF samples were better than those of NF samples after NT, FT, and MT. Scanning electron microscopy analysis revealed that cell integrity of the IQF sample was better than that of the NF sample. MT is the fastest of the thawing methods, but thawing after NF yielded a sample closest to the fresh mushroom sample. Therefore, when combined with NT, IQF minimized the quality changes in frozen diced and mini *P. eryngii*mushrooms. This study showed that the IQF technology can be used as a new preservation and distribution method of mushroom.

**Jihyun Park, Soon Young Shin, Dongsoo Koh, Young Han Lee, Yoongho Lim.** **Relation between structures of naphthalenylchalcone derivatives and their cytotoxic effects on HCT116 human colon cancer cells. (2018) Appl. Biol. Chem. 61(3): 267–272**

To find potent chemotherapeutic agents, cytotoxic effects of 42 synthetic chalcone derivatives bearing naphthyl groups on HCT116 human colon cancer cell lines were tested using the clonogenic long-term survival assay. The relationships between their half-maximal cell growth inhibitory concentrations (GI50) and structural properties were obtained using comparative molecular field analysis and comparative molecular similarity indices analysis. The structural conditions that showed maximum cytotoxic effects on the colon cancer cells were determined. In addition, a derivative, (*E*)-1-(2-hydroxy-4,5-dimethoxyphenyl)-3-(naphthalen-1-yl)prop-2-en-1-one, showing the best GI50 value, was assessed for stimulating reactive oxygen species (ROS) production. While its treatment on non-tumorigenic epithelial MCF-12A cell line did not affect the intracellular ROS levels, its treatment on MDA-MB-231 human breast cancer cell line showed ROS accumulation. These findings demonstrate that naphthalenylchalcones can be developed as potent chemotherapeutic agents.

**Naila Chand, Shabana Naz, Ziaur Rehman, Rifat Ullah Khan.** **Blood biochemical profile of four fast-growing broiler strains under high ambient temperature. (2018) Appl. Biol. Chem. 61(3): 273–279**

The present study was carried out to evaluate the effect of optimum and high ambient temperatures on biochemical parameter of four broiler strains. Broiler chicks (*n* = 242) of four different commercial strains (Ross, Hubbard, Cobb and Arber Acer) on day 15 were divided into two groups: thermo-neutral zone (TN) group and high ambient temperature zone (HAT) group. Chicks in TN group were housed at constant room temperature (25 °C ± 2 C° and RH 65 ± 5%) while chicks in HAT group were kept under HAT of summer. Chicks in each group were further divided into four subgroups, i.e., TN-Ross, TN-Hubbard, TN-Cobb, TN-Arber Acer and HAT-Ross, HAT-Hubbard, HAT-Cobb, HAT-Arber Acer. Each subgroup was further subdivided into four replicates having ten chicks per replicate. Blood was collected on day 21 and 42. Mean serum aspartate aminotransferase (AST), alanine amino transaminase (ALT), alkaline phosphates (ALP), glucose, cholesterol, triglyceride, and low-density lipoprotein (LDL) were significantly higher (*P* < 0.05) in HAT group, while total serum protein and high-density lipoprotein (HDL) were significantly higher (*P* < 0.05) in TN group. In TN zone group, significantly (*P* < 0.05) lower AST, ALT, glucose, cholesterol, triglyceride, and LDL and significantly (*P* < 0.05) higher HDL and total protein were recorded for Cobb and Hubbard strains. In HAT zone group, significantly (*P* < 0.05) lower AST, ALT, glucose, cholesterol, triglyceride, and LDL and significantly (*P* < 0.05) higher HDL and total protein were recorded for Ross and Arber Acer strains. The findings of the present study suggested that Ross and Arber Acer strains were more tolerant to summer HAT of tropical areas than Cobb and Hubbard, while Cobb and Hubbard strains were more effective in TN environment.

**R. K. Ghosh, T. Kar, B. Dutta, A. Pathak, R. Rakshit, R. Basak, A. Das, K. Waheeda, P. Basak, M. Bhattacharyya.** **Aberration in the structural paradigm of lens protein α crystallin by UV-C irradiation. (2018) Appl. Biol. Chem. 61(3): 281–287**

The conformation of lens protein α crystallin was investigated using different spectroscopic techniques under normal and UV-C-irradiated condition. The structural elucidation of commercially available lens protein α crystallin under the effects of UV-C irradiation has never been reported earlier. To study the effects of irradiation on the lens protein, we used UV–visible spectroscopy, CD spectroscopy, and steady-state and time-resolved fluorescence measurements along with FTIR study, under increasing doses of UV-C irradiation. Using the secondary and tertiary structural changes as parameters for detecting conformational perturbation, we investigated the structural paradigm shift in the lens protein α crystallin. Increasing doses of UV-C radiation resulted in decreasing β sheet content of α crystallin from 30 to 10%. The fluorescence profile confirmed the formation of ROS species in the protein upon extensive exposure to UV-C irradiation. These results inferred UV-C irradiation may induce alteration of secondary structure of the lens protein leading to impaired biological functioning.

**E. Gomathi, B. Balraj, K. Kumaraguru.** **Electrochemical degradation of scarlet red dye from aqueous environment by titanium-based dimensionally stable anodes with SS electrodes. (2018) Appl. Biol. Chem. 61(3): 289–293**

Textile effluents are toxic and carcinogenic materials that exist in the aquatic environment. In this study, the degradation efficiency of commercially available scarlet red dye investigated on TSA-SS Electro Fenton process (EFP) was reported. It is of great interest in the field of environmental engineering to remove dyes from aquatic environment. The influence of operating parameters such as pH (2–9), current density (0.1–0.5 mA/cm2), concentration of dye (0.1–0.5 g/L), H2O2 (0.1–0.5 g/L) concentration and Fe2+ concentration (0.01–0.03 g/L) were analyzed by batch system. The optimum degradation conditions were determined as pH—3, current density—0.4 mA/cm2, concentration of dye—0.4 g/L, H2O2 concentration—0.5 g/L and Fe2+ concentration—0.025 g/L. These results indicated that the degradation efficiency of scarlet red dye by EFP depends on solution pH and Fenton reagent concentration and a low pH value was favorable for the dye degradation. It has been demonstrated that more than 94% dye removal was obtained at 50 min. Electro Fenton process was also investigated by cyclic voltammetry technologies.

**Hye Jeong Choo, Eun Ji Kim, So Yeon Kim, Youngshim Lee, Bong-Gyu Kim, Joong-Hoon Ahn.** **Microbial synthesis of hydroxytyrosol and hydroxysalidroside. (2018) Appl. Biol. Chem. 61(3): 295–301**

Plant-derived phenolic compounds, such as hydroxytyrosol and hydroxysalidroside, have a beneficial impact on human health owing to their antioxidant activity. In this study, we used *Escherichia coli* to synthesize hydroxytyrosol. Tyrosine decarboxylase from *Papaver somniferum*, tyrosine oxidase from *Micrococcus luteus*, and 4-hydroxyphenylacetate 3-monooxygenase from *E. coli* were transformed into the bacterial cell. The resulting transformant successfully synthesized hydroxytyrosol. Furthermore, we used the engineered *E. coli* strains to synthesize ~ 268.3 mg/L hydroxytyrosol. Three uridine diphosphate-dependent glycosyltransferases (UGTs), which were previously shown to convert tyrosol into salidroside, were tested to synthesize hydroxysalidroside, and one of UGTs was used to synthesize hydroxysalidroside from hydroxytyrosol. Finally, *E. coli* harboring this UGT converted approximately 50% of hydroxytyrosol into hydroxysalidroside.

**Youngshim Lee, Dongsoo Koh, Seunghyun Ahn, Young Han Lee, Soon Young Shin, Yoongho Lim.** **Clonogenic long-term survival assay of HCT 116 colorectal cancer cells after treatment with the synthesized diphenyl imidazoline derivatives. (2018) Appl. Biol. Chem. 61(3): 303–312**

Fourteen diphenyl imidazoline derivatives were designed, synthesized, and identified using NMR spectroscopy and high-resolution mass spectrometry. Their cytotoxicities in HCT 116 colorectal cancer cell lines were measured using a clonogenic long-term survival assay and the half-maximal cell growth inhibitory concentration (GI50) values were in the range 3.1–58.4 μM. As the anticancer effects of diphenyl imidazolines were reported to be caused by the inhibition of mouse double minute 2 homolog (MDM2), the inhibitory effects of the most potent derivative on MDM2 were assessed through Western blotting analysis. In silico docking experiments revealed the binding mode between this derivative and MDM2.

**Soowan Kim, Jun-Kyu Lee, Yoon-Jae Song, Se Chan Kang, Baeyoung Kim, I-Jin Choi, Doo-Hyung Lee.** **Evaluating natural compounds as potential insecticides against three economically important pests, *Bemisia tabaci* (Hemiptera: Aleyrodidae)*, Frankliniella occidentalis* (Thysanoptera: Thripidae), and *Myzus persicae* (Hemiptera: Aphididae), on greenhouse sweet peppers. (2018) Appl. Biol. Chem. 61(3): 313–323**

Sweet pepper (*Capsicum annuum* L.) is one of the major export crops in the Republic of Korea. Currently, synthetic insecticides are frequently used to control major greenhouse pests including *Bemisia tabaci* (Hemiptera: Aleyrodidae), *Myzus persicae* (Hemiptera: Aphididae), and *Frankliniella occidentalis* (Thysanoptera: Thripidae) in Korea. However, the repeated use of chemicals has caused insecticide resistance to be developed by pests. Therefore, there is a growing demand to develop biopesticides that have high insecticidal effects but little adverse impacts to crops and nontarget organisms. In this study, three natural compounds were investigated for insecticidal effects against three pests, *B. tabaci*, *M. persicae*, and *F. occidentalis*, and nontarget effects to a pollinator, *Bombus terrestris* (Hymenoptera: Apidae) in laboratory. The three natural compounds, named JP503, G.sol®, and NO40, were an extract from *Perilla frutescens* var. *crispa* with phytoncide essential oil from pine tree, a commercialized disinfectant solution, and a type of nitric oxide in aqueous solution. Among these compounds, JP503 showed high and acute insecticidal effects on all of the three pests causing 100% mortality in 3 h. In addition, this compound resulted in the same level of acute lethality to the pollinator. Moreover, JP503 caused significant leaf damage when applied to sweet pepper plants in greenhouse conditions. The results indicate that the candidate compound would have limited potential for wide application to cash crop such as sweet peppers. Therefore, it is recommended that JP503 be used only in a site-specific manner such as applications to trap crops, barrier crops, or wild hosts adjacent to cash crop fields.

**Eun-Jung Hwang, Yong-Suk Lee, Yong-Lark Choi.** **Cloning, purification, and characterization of the organic solvent tolerant *β*-glucosidase, *Oa*BGL84, from *Olleya aquimaris* DAU311. (2018) Appl. Biol. Chem. 61(3): 325–336**

A marine bacterium, *Olleya aquimaris* DAU311, was isolated from Goraebul beach in the Republic of Korea. This strain had *β*-glucosidase activity on Luria–Bertani esculin plates. The *β*-glucosidase, *oabgl84*, was isolated, cloned, and sequenced, based on fosmid library. The gene encoded novel *β*-glucosidase and consisted of an open reading frame of 2304 bp, which encodes 768 amino acids. The deduced amino acid sequence had 99% identity to *Olleya* sp. VCSM12, 84% identity to *Olleya marilimosa*, and 78% similarity to *Lacinutrix* sp. Hel\_I\_90. *Oa*BGL84 belongs to the glycoside hydrolase family 3, and it was visualized using SDS-PAGE, approximately 84 kDa. The optimal temperature and pH of *Oa*BGL84 were analyzed as 40 °C and 6.0, respectively, using *p*NPG as substrate. The *K*m and *V*max values for *Oa*BGL84 were 1.35 mM and 25.3 μM/s, respectively. Furthermore, *Oa*BGL84 activity was completely inhibited by Cu2+ and Hg2+ ions. *Oa*BGL84 demonstrated extraordinary stability until 50% (*v*/*v*) benzene, *n*-hexane, or toluene. These results indicate that *Oa*BGL84 is useful candidate to degrade cellulose or soy isoflavone in the organic solvents for various biotechnological applications.

**Carolina de Santana Souza, Thamara Figueiredo Procópio, Bernardo do Rego Belmonte, Patrícia Maria Guedes Paiva, Lidiane Pereira de Albuquerque, Emmanuel Viana Pontual, Thiago Henrique Napoleão.** **Effects of *Opuntia ficus*-*indica* lectin on feeding, survival, and gut enzymes of maize weevil, *Sitophilus zeamais*. (2018) Appl. Biol. Chem. 61(3): 337–343**

In this study, the effects of *Opuntia ficus*-*indica* lectin (OfiL) on the survival and nutritional parameters of *Sitophilus zeamais* (maize weevil) adults were evaluated. OfiL was incorporated into the artificial diets at concentrations of 15, 60, and 95 mg/g (mg of lectin per g of wheat flour). Mortality was evaluated after 7 and 15 days, and the amount of food ingested and the weight of the insects were determined on the 7th day. In addition, the in vitro effects of OfiL on the gut enzymes of the insect were investigated. The ingestion of OfiL did not show any significant difference in the mortality rates compared to control. The relative consumption rate was also similar to that of the control, and no deterrent effect was detected. However, the values of the relative biomass variation and the efficiency of ingested food conversion were negative in the treatments at 60 and 95 mg/g, showing that lectin ingestion resulted in weight loss. OfiL exhibited a stimulatory effect on the protease activity from *S. zeamais* gut extract, which may cause uncontrolled hydrolysis of proteins in the digestive tract. This lectin did not promote significant alteration in the amylase activity. In conclusion, OfiL was able to exert anti-nutritional effects without causing a deterrent effect.

**Ju Hee Kim, Joo Young Hong, Jun-Cheol Moon, Kisung Kwon, Cheol Seong Jang.** **Development of molecular markers for detecting almond, peanut, pine nut, and walnut in commercial food using quantitative real-time PCR. (2018) Appl. Biol. Chem. 61(3): 345–354**

Nuts have been used globally as health foods. However, because nuts cause allergies, people need to be careful when eating food. Mostly foods are labeled, but sometimes intentional or unintentional mixing might occur. In the present study, we report DNA based on marker for the detection of four nuts almond, peanut, pine nut, and walnut using quantitative real-time polymerase chain reaction (qRT-PCR). Species-specific primer sets for four species were designed based on the single-nucleotide polymorphisms and insertion/deletion of the chloroplast gene, *matK*. The sensitivity of primer sets for the four species studied was assessed by analyzing DNA dilutions at concentration of 0.001–10 ng and binary mixtures of 0.1–100% of heat-treated and non-heat-treated samples. The four primer sets developed in the present study indicated appropriate amplification efficiency and correlation coefficients of the standard curves. In addition, to verify the applicability of these molecular markers, we performed a qRT-PCR with 14 commercial products and successfully detected the *matK* genes in several commercial food products that were declared to contain nuts. Thus, markers developed could be useful tools for confirming the presence of the four nut species in commercial products.

**Xia Feng, Jing Zhang, Yu Qian, Ruokun Yi, Peng Sun, Jianfei Mu, Xin Zhao, Jia-Le Song.** **Preventative effects of *Lactobacillus plantarum* YS-3 on oxazolone-induced BALB/c colitis in mice. (2018) Appl. Biol. Chem. 61(3): 355–363**

In this study, the preventative effects of *Lactobacillus plantarum* YS-3 (LP-YS3) on colitis were studied using an in vitro animal experiment. Oxazolone was administered to BALB/c mice to induce colitis, and the preventive effects of LP-YS3 were determined using serum- and tissue-related indexes. The mice treated with LP-YS3 showed a significant decrease (*p*< 0.05) in disease activity index for colitis by inhibiting colon shortening and increasing colon weight/length ratio. The application of LP-YS3 resulted in a significant reduction in myeloperoxidase, nitric oxide, and malondialdehyde activities and a decrease in glutathione activity (*p*< 0.05) in mouse colon tissues. LP-YS3 also significantly increased serum interleukin-2 and reduced interleukin-10 cytokines levels in mice (*p*< 0.05). Reverse transcription-polymerase chain reaction and western blot assays showed that LP-YS3 application resulted in a significant increase in neuronal nitric oxide synthase, endothelial nitric oxide synthase, c-Kit, and stem cell factor expressions and a decrease in inducible nitric oxide synthase, interleukin-8, and C-X-C motif chemokine receptor 2 expressions in mouse colonic tissues (*p*< 0.05). These findings indicate that LP-YS3 imparts preventative effects on oxazolone-induced colitis in mice.

**Yunlong Lei, Peipei Zhao, Chenglei Li, Haixia Zhao, Zhi Shan, Qi Wu.** **Isolation, identification and characterization of a novel elastase from *Chryseobacterium indologenes*. (2018) Appl. Biol. Chem. 61(3): 365–372**

Elastase is a type of protease that specifically degrades elastin. It has broad application prospects in medicine, food industry, and daily-use chemical industry. In this study, we isolated a bacterial strain WZE87 with high elastin-hydrolysis activity, which was identified as *Chryseobacterium indologenes* based on morphology, physiological and biochemical characteristics, and 16S rDNA sequence analysis. The elastase produced by this strain was purified by three steps: ammonium sulfate precipitation, Q-Sepharose fast-flow anion-exchange chromatography, and Sephadex G-75 gel-filtration chromatography. The purified elastase was 2376.5 U/mg in activity (a 8.3-fold increase in specific activity), and the recovery was 5.8%. Its molecular mass was estimated to be 26 kDa by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. This enzyme was stable in the pH range of 5.0–10.5 at 37 °C. The optimal temperature and pH were 37 °C and 7.5, respectively. The activity of this elastase was found to decrease when the temperature was higher than 50 °C. The activity was also inhibited by Zn2+, Fe2+, Fe3+, and Mn2+ ions. The specific hydrolytic ability of this enzyme was similar to that of papain on substrates like gelatin, casein, soybean-isolated protein and bovine hemoglobin. However, this elastase preferentially hydrolyzed elastin in a protein mixture because of its specific adsorption. Considering its promising properties, this protease may be considered a potential candidate for applications in related industries.

**Taiyu Liu, Jianguo Zhang.** **High-level expression and characterization of *Aspergillus niger* ATCC 1015 xylanase B in *Komagataella phaffii*. (2018) Appl. Biol. Chem. 61(4): 373–381**

Owing to the safety issues in food and feed industry, the GH11 xylanase B gene from *Aspergillus niger* ATCC 1015 was cloned and expressed in *Komagataella phaffii*. The highest xylanase B activity of 1827.19 U/ml was obtained after optimization of temperature, pH, and methanol addition through flask cultivation. The optimal temperature and pH were 55 °C and 5.0, respectively, and the highest relative activity of xylanase B reached 133.20% with the addition of 10 mmol/l cupric ions. Thus, the high-level recombinant xylanase B obtained in this study could have potential applications in food and feed industry.

**Jun-Hwan Park, Hoi-Seon Lee.** **In vivo fungicidal properties of *Diospyros kaki*-isolated compound and its analogues. (2018) Appl. Biol. Chem. 61(4): 383–388**

Fungicidal effects of active component purified from *Diospyros kaki* roots and its analogues against *Erysiphe graminis*, *Botrytis cinerea*, *Pyricularia grisea*, *Puccinia recondite*, *Rhizoctonia solani*, and *Phytophthora infestans* were investigated using a whole-plant method. Active constituents isolated from the chloroform fraction of *D. kaki* roots were characterized as plumbagin, using various spectroscopic analyses. To establish the structure–activity relationships, the fungicidal effects of plumbagin and its structural analogues were bioassayed against phytopathogenic fungi. At 0.25, 0.125, and 0.0625 g/L, plumbagin had a greater fungicidal effect than *p*-naphthoquinone, juglone, lawsone, 2-methoxy-*p*-naphthoquinone, or menadione against *P. grisea*, *B. cinerea*, *R. solani,* and *E. graminis*. Our results suggest that the methyl and hydroxyl functional groups at the 5′- and 2′-positions of *p*-naphthoquinone play key roles in its fungicidal effect against six phytopathogenic fungi. Therefore, plumbagin and its analogues may be suitable as antifungal agents to control plant diseases.

**Ho-Sung Lee, Nam ji Kwon, Yongsoo Kim, Hunjoo Lee.** **Prediction of mycotoxin risks due to climate change in Korea. (2018) Appl. Biol. Chem. 61(4): 389–396**

Climate change has been considered as a main threat for food safety by influencing on crop production and food supply chain through the change in temperature and humidity. To prevent risks of mycotoxins from climate change, it is important to predict mycotoxin risks with statistical approaches and stepwise process to compile large volume of datasets, such as climate change, contamination level, and cultivation area in specific regions. This paper aims at prioritization of vulnerable mycotoxins related to climate change in Korea. In addition, this paper focuses on prioritization of vulnerable raw materials for specific mycotoxins and prediction of vulnerable regions for vulnerable raw materials in Korea. Among six target mycotoxins (deoxynivalenol, fumonisin (B1 and B2), ochratoxin A, patulin, total aflatoxin (B1, B2, G1, and G2), and zearalenone), ochratoxin A (OTA) and total aflatoxin (TA) were identified as specific vulnerable mycotoxins. In addition, 4 raw materials (chestnut, dried red pepper, perilla seed, and soy bean) were identified as vulnerable raw materials for OTA and TA and vulnerable regions were predicted to be moved to the northward areas in Korea. These results can be utilized to design long-term national sampling plan for mycotoxins in food considering climate change in Korea.

**Eun-Young Jeong, Myung-Ji Lee, Min-Seung Kang, Hoi-Seon Lee.** **Antimicrobial agents of 4-methoxysalicylaldehyde isolated from *Periploca sepium* oil against foodborne bacteria: structure–activity relationship. (2018) Appl. Biol. Chem. 61(4): 397–402**

This study was designed to evaluate the antimicrobial activities of 4-methoxysalicylaldehyde isolated from *Periploca sepium* and its derivatives against six foodborne bacteria (*Listeria monocytogenes*, *Salmonella typhimurium*, *Shigella flexneri*, *S. sonnei*, *Staphylococcus intermedius* and *S. aureus*). Essential oil extracted from *P. sepium* roots exhibits strong antimicrobial activity against foodborne bacteria. The antimicrobial compound of *P. sepium*isolated by chromatographic techniques was identified as 4-methoxysalicylaldehyde. To compare the antimicrobial activities of 4-methoxysalicylaldehyde and its derivatives (4-hydroxysalicylaldehyde, salicylaldehyde, 3-methoxysalicylaldehyde, 5-methoxysalicylaldehyde, 3-methylsalicylaldehyde, and 5-methylsalicylaldehyde), the MIC test was performed. These activities were exhibited by 4-methoxysalicylaldehyde (MIC 30.1–67.3 μg/mL) followed by 4-hydroxysalicylaldehyde (MIC 41.1–61.5 μg/mL) and 4-methoxysalicylaldehyde (MIC 41.3–92.1 μg/mL) against all tested microorganisms. The results indicate that 4-methoxysalicylaldehyde and its derivatives represent natural antimicrobial alternatives.

**Jun Yeong Kim, Yoon Young Kang, Eun Ji Kim, Joong-Hoon Ahn, Hyejung Mok.** **Effects of curcumin-/boron-based compound complexation on antioxidant and antiproliferation activity. (2018) Appl. Biol. Chem. 61(4): 403–408**

Simple and reproducible formulation strategies are needed to improve the bio-availability of curcumin. In this study, curcumin was successfully complexed with two boron-based compounds: 2-aminoethyl diphenyl borate (DPBA) and bortezomib (BTZ; Velcade®). In reverse-phase high-performance liquid chromatography, DPBA/curcumin complexes (DPBA/cur) showed delayed elution times compared to those of free curcumin. The UV–visible absorbance peak of DPBA/cur and BTZ and curcumin complexes (BTZ/cur) appeared redshifted. DPBA complexation has a negligible effect on the antioxidant and antiproliferation properties of curcumin for two types of cancer cells: MCF-7 and A549. Thus, curcumin complexation with boron-based compounds could be a method to enhance in vivo stability without loss of bioactivity (i.e., antioxidant and antiproliferation effects).

**Chung Eun Hwang, Md. Azizul Haque, Jin Hwan Lee, Yeong Hun Song, Hee Yul Lee, Su Cheol Kim, Kye Man Cho.** **Bioconversion of γ-aminobutyric acid and isoflavone contents during the fermentation of high-protein soy powder yogurt with *Lactobacillus brevis*. (2018) Appl. Biol. Chem. 61(4): 409–421**

This study evaluated the changes in γ-aminobutyric acid (GABA) and isoflavone aglycone contents from soy powder yogurt (SPY) due to sprouting of soybean (1 cm) and fermentation with *Lactobacillus brevis*. The levels of GABA and the aglycone form increased, and the glutamate decarboxylase and β-glucosidase activities increased; however, the isoflavone glycoside and malonylglycoside contents decreased after fermentation for 72 h. In particular, after 60 h, the SPY presented the highest GABA content (120.38 mg/100 mL). The highest daidzein (179.93 µg/g), glycitein (44.10 µg/g), and genistein (126.24 µg/g) contents were present after 72 h of fermentation. In addition, the 2,2-diphenyl-1-picrylhydrazyl, 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, and hydroxyl radical scavenging activities increased from 69.65, 97.94, and 70.90% during this fermentation, respectively. This result suggests that SPY may be used for the preparation of high-protein soybean with high GABA and isoflavone aglycone contents, which can then be used as a natural ingredient of functional foods.

**So-Young Park, In-Hwan Song, Young-Je Cho.** **Elicitor treatment potentiates the preventive effect of *Saururus chinensis* leaves on stress-induced gastritis. (2018) Appl. Biol. Chem. 61(4): 423–431**

In this study, gastritis inhibitory substances were ethanol-extracted from *Saururus chinensis*leaves as a part of ongoing research on natural bioactive substances. Comparing *S. chinensis*extracts with and without elicitor treatment showed that total phenolic compounds in the leaves increased with increasing elicitor treatment. The contents of avicularin, manassantin A, manassantin B, and saucerneol D in the leaf, known gastritis inhibitory compounds, increased as elicitor treatment increased. *S. chinensis* extracts were administered to mice in a single oral dose of 0.25–2 g/kg, resulting in no observable toxicity after 1 week. *S. chinensis* ethanol extracts were administered to mice at a dose of 500 mg/kg before induction of gastritis by water-immersion restraint method. Macroscopic gastric hemorrhage and microscopic gastric damage assessed with hemorrhage, edema, epithelial cell damage, inflammatory cell infiltration, and ulcer were reduced by *S. chinensis* ethanol extracts. The elicitor-treated group showed a greater inhibitory effect on macroscopic and microscopic gastric damage. Elicitor-treated *S. chinensis* extracts inhibited gastritis more than non-treated *S. chinensis* extracts did, most likely due to greater anti-inflammatory effects. These results indicate that elicitor-treated *S. chinensis* extracts could be effective to prevent gastritis and could be used as a medicinal material source.

**In-Bong Song, Hyejung Gu, Hye-Ju Han, Na-Young Lee, Ji-Yun Cha, Yeon-Kyong Son, Jungkee Kwon.** **Omega-7 inhibits inflammation and promotes collagen synthesis through SIRT1 activation. (2018) Appl. Biol. Chem. 61(4): 433–439**

Excessive accumulation of reactive oxygen species (ROS) during oxidative stress accelerates the skin aging process. ROS stimulate inflammatory processes in the skin, leading to activation of matrix metalloprotease-1 (MMP-1). Silent information regulator 1 (SIRT1) controls a broad range of cellular functions including the expression of MMP-1. Omega-7 fatty acids such as palmitoleic acid have many beneficial effects on health, including improvement in cardiovascular risk factors and increased insulin sensitivity. However, the effectiveness of omega-7 fatty acids (herein referred to as omega-7) related to skin aging, characterized by the degradation of collagen and loss of elasticity, remains unclear. We here investigated the effects of palmitoleic acid, a representative omega-7, on collagen regeneration through its ability to activate SIRT1 and inhibit MMP-1 in the presence of hydrogen peroxide (H2O2)-induced oxidative stress in human HaCaT cells. SIRT1 activation by omega-7 decreased signaling levels of nuclear transcription factor kappa B (NF-κB) and inflammatory cytokines. However, inhibition of SIRT1 by sirtinol counteracted the advantage effects of omega-7 in H2O2-treated HaCaT cells. In addition, omega-7 significantly counteracted the decrease in collagen abundance and loss of elasticity induced by H2O2. Consistent with this observation, omega-7 significantly decreased H2O2-induced upregulation of MMP-1 in HaCaT cells. Together, these studies suggest the potential efficacy of SIRT1 in collagen regeneration and indicate that omega-7 is a possible functional food to improve skin health for the prevention of aging.

**Eun Ha Lee, Kwang Hyun Cha, Trang Thi Vuong, Sang Min Kim, Cheol-Ho Pan.** **Comparison of static and dynamic in vitro digestion models to estimate the bioaccessibility of lutein in lutein-rich foods. (2018) Appl. Biol. Chem. 61(4): 441–447**

This study aimed to determine the bioaccessibility of lutein in lutein-rich food, using static and dynamic models of in vitro gastrointestinal digestion. Here, kale powder (KP) and lutein supplement (LS) were used as representative lutein-rich foods. The bioaccessibility of lutein from KP did not considerably differ between static (59.92%) and dynamic (56.08%) digestion. Bioaccessibility was consistently maintained at the same level during dynamic digestion. The amount of lutein released from the LS during dynamic digestion was five times higher than that released during static digestion (67.88 vs 12.34%). The results showed that (a) bioaccessibility of lutein was affected by various factors such as food source, solid:liquid ratio, and interaction with dietary components, and (b) dynamic digestion should be suitable for evaluating the bioaccessibility of lutein in high-fat foods.

**Da-Som Kim, Hoe Sung Kim, Seong Jun Hong, Jin-Ju Cho, Jookyeong Lee, Eui-Cheol Shin.** **Comparison of the retention rates of thiamin, riboflavin, and niacin between normal and high-oleic peanuts after roasting. (2018) Appl. Biol. Chem. 61(4): 449–458**

This study investigated the amounts of thiamin, riboflavin, and niacin in normal and high-oleic peanuts and compared the retention rates after roasting via HPLC analysis. Method validation showed a high linearity (*r*2 > 0.99), and the limits of detection and quantification were 0.001–0.038 and 0.002–0.115 µg/mL, respectively. Accuracy and precision were confirmed using standard reference materials. Thiamin content was not significantly different between the normal and high-oleic cultivars; however, it significantly decreased in the roasted peanut cultivars. Although there were no significant differences in riboflavin between the cultivars, a significantly increased amount of riboflavin was observed in the roasted peanuts, which confirms that riboflavin is highly stable to thermal treatment such as roasting. With only a small difference between the cultivars, niacin showed a decreased retention rate with roasting in normal cultivars, but a significantly increased retention rate with roasting in high-oleic cultivars. The amount of thiamin, riboflavin, and niacin present in peanuts and their retention rates after roasting showed variations among the cultivars. This study provides basic data on the water-soluble vitamins in raw and roasted peanuts.

**María de Lourdes García-Magaña, Julián González-Borrayo, Efigenia Montalvo-González, Enrique Rudiño-Piñera, Sonia G. Sáyago-Ayerdi, Jesús Aarón Salazar-Leyva.** **Isoelectric focusing, effect of reducing agents and inhibitors: partial characterization of proteases extracted from *Bromelia karatas*. (2018) Appl. Biol. Chem. 61(4): 459–467**

The aim of this research is the partial characterization of proteases extracted from *B. karatas*; the isolation and purification of proteases from *B. karatas* fruits were achieved using precipitation, separation by size exclusion chromatography and anion-exchange chromatography; molecular mass (MM) was determined, and the effect of inhibitors, reducing agents and heat on enzyme activity was analyzed. These proteases were compared with proteases from *Bromelia pinguin* (*B. pinguin*) and evaluated under similar conditions. The isolation procedure was adequate; only a few protein bands are present in sodium dodecyl sulfate polyacrylamide gel electrophoresis. Furthermore, zymogram analysis showed protein bands with enzyme activity. Inhibitors, reducing agents and heat were unable to inactivate the proteases extracted from *B. karatas* and *B. pinguin*. The semi-purified extracts are a set of proteases with a MM of 66 kDa, but different isoelectric points (3.5–6.5 for *B*. *karatas* and 5–9 for *B*. *pinguin*), which are found in quaternary structures with proteolytic activity. When denatured, they segment into fragments of approximately 20 and 10 kDa. The data indicate that these plants could be used as sources of proteases since they present good proteolytic activity (21.93 UT for proteases from *B. karatas* and 43.58 UT for proteases from *B. pinguin*) and that *B. Karatas* has potential applications comparable to *B. pinguin* in the food and health industries.

**Ji Eun Woo, Sang Yup Lee, Yu-Sin Jang.** **Effects of nutritional enrichment on acid production from degenerated (non-solventogenic) *Clostridium acetobutylicum* strain M5. (2018) Appl. Biol. Chem. 61(4): 469–472**

*Clostridium acetobutylicum* has been used as a microbial platform for the production of butanol, acetone, and butyrate from biomass. This study examined the effect of nutritional enrichment on the production of acetate and butyrate by *C. acetobutylicum* in culture, and tested whether this nutritional change could shift metabolic flux in these microbial cells. The degenerated (non-solventogenic) *C. acetobutylicum* M5 strain, which lacks the pSOL1 plasmid that contains genes responsible for solvent production, was cultured in the rich medium, *C. acetobutylicum* medium 1 (CAM1). As a control, M5 strain was also cultured in clostridial growth medium (CGM). Batch fermentation of M5 strain in CAM1 achieved a cell density of 23.7 (OD600), which was 2.55 times that obtained when these cells were cultured in CGM. Fermentation in CAM1 yielded volumetric acetate and butyrate productivities of 0.42 g/L/h and 1.06 g/L/h, respectively, which were 2.33 and 1.33 times the values obtained in CGM. Nutritional enrichment also increased the acetate-to-butyrate ratio, which was 0.39 g/g for M5 cells grown in CAM1 and 0.25 g/g for those grown in CGM. These findings demonstrate that the tested nutritional enrichment triggers a metabolic shift in the acid production of a degenerated *C. acetobutylicum* in culture.

**Chan Young Jeong, Won Je Lee, Hai An Truong, Cao Sơn Trịnh, Suk-Whan Hong, Hojoung Lee.** ***AtMybL-O* modulates abscisic acid biosynthesis to optimize plant growth and ABA signaling in response to drought stress. (2018) Appl. Biol. Chem. 61(4): 473–477**

To combat constant stress from the external environment throughout their life cycle, plants have evolved their own defense mechanisms. Through robust and complicated defense mechanisms, plants have increased their productivity and adaptability under harsh conditions. In this study, wedemonstrated the function of the *AtMybL-O* gene by using knockout (ko) mutants to expand the existing research field. The *atmybl-o ko* mutant seedlings grew similarly to the wild type (WT) in response to osmotic stress, while the *AtMybL-O* overexpression lines exhibit growth suppression in the same growth condition. Further, we attempted to understand the functional mechanism of *AtMybL-O* with respect to stress response toward drought stress. Firstly, we determined the changes in gene expression of the mutants in response to mannitol treatment and identified a strong increase in the expression of *COR15b*, *DREB1A*, and *NCED3*gene in the mutant. Finally, through abscisic acid (ABA) measurement experiments, we observed that the ABA content of mannitol-treated mutants was higher than that of the WT. Therefore, our results indicate that *AtMybL-O* modulates ABA biosynthesis and ABA signaling in response to drought stress.

**Myung-Ji Lee, Sung-Eun Lee, Min-Seung Kang, Bueyong Park, Sang-Guei Lee, Hoi-Seon Lee.** **Acaricidal and insecticidal properties of *Coriandrum sativum* oils and their major constituents extracted by three different methods against stored product pests. (2018) Appl. Biol. Chem. 61(5): 481–488**

Essential oils of *Coriandrum sativum* were extracted by three different methods, including steam distillation (SDE), solvent (SE) and supercritical fluid extraction (SFE), to determine their acaricidal and insecticidal properties against *Plodia interpunctella*, *Sitotroga cerealella*and *Tyrophagus putrescentiae*. The fumigant bioassay against *P. interpunctella*, *S. cerealella*and *T. putrescentiae* revealed the strongest activity (LD50 9.38, 18.76 and 4.19 μg/cm3) of oil obtained via SDE, followed by extraction via SE (LD50 > 75.20, 21.11, and > 75.20 μg/cm3) and SFE (LD50 > 75.20, 27.36, and > 75.20 μg/cm3). The contact bioassay against *T. putrescentiae*revealed the most potent activities of oil obtained via SDE (LD50 19.29 μg/cm2), followed by oil via SE and SFE. The chemical composition of *C. sativum* oils obtained by SDE, HE and SFE was analyzed by GC–MS. The *C. sativum* oil obtained by SDE contained linalool (66.80%) compared with oils obtained by SE and SFE (70.67–70.80%). However, camphor (6.46%) was detected in SDE but not in the other two extracts. Based on the LD50 values of six major compounds derived from the three *C. sativum* oils against *P. interpunctella*, *S. cerealella* and *T. putresceentiae*, camphor was considered the most active (2.32, 19.31 and 3.24 μg/cm3, respectively) insecticide. The three values were about real camphor concentration in the oil via SDE. These results indicate that camphor contributes to the acaricidal and insecticidal activities of oil extracted via SDE of *C. sativum* seeds.

**Fei Tian, So Young Woo, Sang Yoo Lee, Hyang Sook Chun.** ***p*-Cymene and its derivatives exhibit antiaflatoxigenic activities against *Aspergillus flavus* through multiple modes of action. (2018) Appl. Biol. Chem. 61(5): 489–497**

Three monoterpenes, 1-methyl-4-(1-methylethyl)-benzene, and its derivatives, carvacrol and thymol, were tested for their antifungal and antiaflatoxigenic activities against *Aspergillus flavus*, and their potential in vitro mechanisms were evaluated. The monoterpenes significantly inhibited mycelial growth, spore production, and aflatoxin production in a dose-dependent manner. Furthermore, their antifungal effects were related to the suppression of fungal development regulatory genes (*brlA*, *abaA*, and *wetA*) and inhibition of ergosterol synthesis. Additionally, the down-regulation of the relative expression of genes related to aflatoxin biosynthesis (*aflD*, *aflK*, *aflQ*, and *aflR*) revealed an antiaflatoxigenic mechanism of the monoterpenes. These observations suggest that the three monoterpenes exhibit antiaflatoxigenic activities through multiple modes of action and may be useful for controlling aflatoxin contamination in food.

**Jun An, Jun-Cheol Moon, Cheol Seong Jang.** **Markers for distinguishing *Orostachys* species by SYBR Green-based real-time PCR and verification of their application in commercial *O. japonica* food products. (2018) Appl. Biol. Chem. 61(5): 499–508**

Human consumption of plant functional foods has been rapidly increasing owing to the health benefits they provide. In particular, in Korea, the plant *Orostachys japonica* has attracted attention for its anticancer and other effects. Of the 12 established *Orostachys* species, only three (viz., *O. iwarenge*, *O. malacophyllus*, and *O. japonica*) have been allowed for use as foods in Korea. In this study, 12 species-specific primer sets based on single nucleotide polymorphisms of five chloroplast genes and one nuclear gene were developed to discriminate *Orostachys* species through quantitative real-time PCR (qPCR) analysis with SYBR Green staining. The efficiencies of the designed primer pairs in amplifying the target species ranged from 80 to 110%, with strong correlation coefficients (*R*2 > 0.99), whereas no clear correlation coefficient was evident for the non-target species. In order to verify the specificity of the 12 developed *Orostachys-*specific primers, binary mixtures of the DNAs (tenfold serially diluted samples) from the target species and each of the other non-target species were generated for qPCR analysis, with results suggesting that the primers could clearly discriminate at least 0.1% of *O. japonica* DNA (10 pg) in the mixtures. With regard to the feasibility of the developed qPCR system for detecting *Orostachys* species in *O. japonica* food products, *O. japonica* DNA was detected in all eight commercial products tested, with low Ct values (< 20), whereas none of the other *Orostachys* species DNAs were detected, confirming that the tested foods contained only *O. japonica*. Therefore, developed primers and qPCR conditions would be useful for verifying the authenticity of commercial *O. japonica* food products.

**Eui Yeong Kim, Young Kyu Hong, Chang Hoon Lee, Taek Keun Oh, Sung Chul Kim.** **Effect of organic compost manufactured with vegetable waste on nutrient supply and phytotoxicity. (2018) Appl. Biol. Chem. 61(5): 509–521**

The amount of vegetable waste (VW) has increased, and demand for good quality of organic soil amendment is high. For these reasons, successive composting technique was tried to examine the possibility of increasing nitrogen contents in the compost. Collected VW was initially composted after mixing with either sawdust (SD) or cocopeat (CP) at different ratios (30–50% of SD or CP). After finishing the first composting cycle, finished compost was mixed with fresh VW at various ratios (10–30% of VW) for the second cycle of composting. Temperature, pH, electrical conductivity (EC), organic matter (OM) content, and carbon/nitrogen ratio (C/N ratio) were monitored, and compost maturity, phytotoxicity, nutrient contents and heavy metal concentration of the final compost in the second cycle of composting were measured. Temperature profiles of the first and second composts showed typical composting processes, and temperature was increased up to the range of 55–68 °C in both the first and second compost during the thermophilic period. Other chemical properties such as pH (6.60–9.10), EC (1.36–2.86 dS m−1), and OM content (49.40–64.04%) were within the ranges of typical composts. The nitrogen content (1.76–2.28%) was increased when successive composting technique was adapted. After finishing the second composting, average nitrogen content was increased at the range of 9.4–32.4% compared to the first cycle of compost. The maturity test showed that all the composts satisfied criteria of maturity level and concentration of hazardous heavy metal was below the threshold value in Korea. In conclusion, VW could be recycled to make organic soil amendment and successive composting process is an efficient technique to increase the nitrogen contents in the compost.

**Tae Jin Kim, Young Jin Park, Sang Un Park, Sun-Hwa Ha, Jae Kwang Kim.** **Determination and quantification of arbutin in plants using stable isotope dilution liquid chromatography–mass spectrometry. (2018) Appl. Biol. Chem. 61(5): 523–530**

Arbutin is a very safe whitening agent for human skin. Since it is more expensive than other agents and has a challenging synthesis, novel methods to obtain this valuable agent are needed. In this study, we developed a precise and accurate method to detect and quantify arbutin using stable isotope dilution liquid chromatography–mass spectrometry (LC–MS). One challenge that needed to be overcome was the matrix effect occurring during the LC–MS analysis due to the analyte ionisation enhancement or suppression in the electrospray ionisation source by co-eluting compounds. Notably, arbutin had different matrix effects in the various sample matrices. A solution to this problem was the use of [*d*4]-arbutin as a stable isotope-labelled internal standard (SIL-IS), as it compensated the matrix effect of arbutin because it was affected by almost the same matrix effect. The validation of the developed method showed excellent linearity (*r*2 = 1.000), precision (relative standard deviation ≤ 2.5%), accuracy (recovery, 97.42–98.52%), limit of detection (0.03 μg/mL), and limit of quantification (0.1 μg/mL). Finally, the method of arbutin detection was applied to blueberry leaves to compare the precision and accuracy results obtained by performing stable isotope dilution using LC–MS and gas chromatography–mass spectrometry. The method was applied to strawberry leaves and pear peels, indicating that the SIL-IS method can be expected to find application in the arbutin analysis in other plants.

**Savita Rani, Rakhi Singh, Barjinder Pal Kaur, Ashutosh Upadhyay, Dinkar B. Kamble.** **Optimization and evaluation of multigrain gluten-enriched instant noodles. (2018) Appl. Biol. Chem. 61(5): 531–541**

Central composite design was employed to optimize the cooking, textural and overall acceptability score of the instant dried noodles prepared with multigrain flour and gluten incorporation. Sorghum flour (*X*1, 10–50%), soy flour (*X*2, 10–20%) and gluten (*X*3, 2–4%) were the independent variables investigated with respect to five response variables including cooking time (*Y*1), cooked weight (*Y*2), cooking loss (*Y*3), hardness (*Y*4) and overall acceptability (*Y*5). The optimum level was found to be 24.61% sorghum, 13.23% soy and 2.95% gluten resulting in cooking time = 9 ± 0.60 min, cooked weight = 17.30 ± 0.17 g, cooking loss = 11.46 ± 0.64 g/100 g, hardness = 36.65 ± 3.2 N with overall acceptability score of 7.3 ± 0.71, respectively. Optimized noodles showed higher ash (3.40 ± 0.11%), protein (16.63 ± 0.55%), fiber (4.78 ± 0.04%) as well as iron content (4.53 ± 0.02 mg/100 g) than the control (0.83 ± 0.02%, 13.13 ± 0.84%, 0.00 and 2.38 mg/100 g) and Maggie noodles (3.19 ± 0.01%, 10.53 ± 0.30%, 0.41 ± 0.50% and 0.22 ± 0.00 mg/100 g) made with refined wheat flour. Optimized noodles also revealed good total phenolic content (84.57 ± 1.42 mg GAE/100 g DW) and 1,1-diphenyl-2-picrylhydrazyl scavenging activity (19.64 ± 0.20%). Hence, optimized noodles have substantial potential as a protein–fiber-rich complementary food to improve the nutrient delivery of mid-day meal scheme and satisfying the protein requirement of primary class children (12 g/child/day) as laid down by MHRD (India) under the scheme.

**Yongho Shin, Jonghwa Lee, Jeong-Han Kim.** **A simultaneous multiresidue analysis for 203 pesticides in soybean using florisil solid-phase extraction and gas chromatography–tandem mass spectrometry. (2018) Appl. Biol. Chem. 61(5): 543–548**

A multiresidue analysis method for the simultaneous determination of 203 pesticides in soybean was developed using solid-phase extraction (SPE) and gas chromatography–tandem mass spectrometry (GC–MS/MS). Scheduled multiple reaction monitoring by GC–MS/MS was optimized in electron ionization mode. The target pesticides satisfied the method limit of quantitation below 0.01 mg/kg, and an excellent instrumental repeatability was obtained. The calibration curve correlation coefficients (*r*2) for 201 (99.0%) pesticides were ≥ 0.990. The Multiclass Pesticide Multiresidue Method (No. 2) of the Korea Food Code was scaled down and applied for efficient sample treatment. Soybean sample (10 g) was extracted with 20 mL acetonitrile. The sample was filtered, partitioned with sodium chloride, and centrifuged. The supernatant (8 mL) was subjected to cleanup with a florisil SPE cartridge (500 mg), and the final extract was subjected to GC–MS/MS analysis. To remove fat, further liquid–liquid partitioning with *n*-hexane/acetonitrile was conducted before the SPE cleanup, and this procedure was compared to the non-partitioning procedure. The percentage of pesticides satisfying the recovery range of 70–120% with a relative standard deviation ≤ 20% in the non-partitioning and partitioning procedures were 87.2% and 78.8% at 0.01 mg/kg and 88.2% and 76.8% at 0.05 mg/kg, respectively. The average matrix effect value (%) was 5.5%. This miniaturized analytical method can be successfully applied for rapid and sensitive monitoring of multiresidues in soybean and related agricultural products.

**Yu Song Kim, Hee Kyung Yu, Beom Zoo Lee, Kwang Won Hong. Effect of DNA extraction methods on the detection of porcine ingredients in halal cosmetics using real-time PCR. (2018) Appl. Biol. Chem. 61(5): 549–555**

In recent years, halal cosmetics have attracted considerable attention worldwide. We developed a real-time PCR assay based on the mitochondrial gene *ndh5* for rapid detection of porcine ingredients in halal cosmetic products. We also compared several DNA extraction methods for the most efficient approach in different types of cosmetics. Porcine template DNA was spiked into three types of cosmetics (liquid-type and powder-type mask packs, and cream) and extracted with five commercial DNA extraction kits and the CTAB method. The extraction efficiency of each method was evaluated by determining the detection limits of real-time PCR assay. The lowest detection limit of real-time PCR for each cosmetic product was as follows: 2.28 × 100 copies for liquid-type mask pack when the Power Prep™ DNA extraction kit and TIANamp Genomic DNA kit were used, 2.28 × 101 copies for powder-type mask pack when QIAamp DNA stool mini kit and the Power Prep™ DNA extraction kit were used, and 2.28 × 100 copies for cream when the Power Prep™ DNA extraction kit was used. The pig-specific real-time PCR assay facilitated the detection of trace amounts of the template DNA in cosmetics, and an appropriate DNA extraction method was used depending on the type of cosmetics.

**Dong Jin Lee, Ji Su Bae, Dong Cheol Seo.** **Potential of biogas production from swine manure in South Korea. (2018) Appl. Biol. Chem. 61(5): 557–565**

This study is to compare biogas potentials with the theoretical methane yields of swine manure from livestock farm (LF) and in situ biogasification facilities treating swine manure. In the case of LF, theoretical methane yield based on VS and CODcr by element analysis was 0.39 Sm3CH4/kg and 30.96 Sm3CH4/ton, respectively. For the in situ biogasification facilities, theoretical methane yield based on VS and CODcr by element analysis was 0.30 Sm3CH4/kg and 8.28 Sm3CH4/ton, respectively. Theoretical methane yields based on the weight of swine manure from LF were about three times higher than those from in situ facilities (ISF). As a result, when swine manure has reached the ISF, the decrement of about 24.5–73.3% in the methane yield could be seen due to the 3–6-month stationing of swine manure in the storage tank of LF. In order to improve the biogasification efficiency of swine manure, it is important to maintain high concentration of swine manure during the collection process from LF.

**Bohyun Yun, Younghoon Kim, Nguyen Bao Hung, Kyung-Hwan Oh, Won-Il Kim, Hyeonheui Ham, Hyun-Ju Kim, Kyoungyul Ryu, Se-Ri Kim.** **Microbiological quality and characteristics of isolated *Escherichia coli* in irrigation water used in Napa cabbage cultivation. (2018) Appl. Biol. Chem. 61(5): 567–574**

To ensure the safety of Kimchi, the safety of Napa cabbage is the most important. Contaminated irrigation water can be a major cause of pathogens during growth of Napa cabbage. The purpose of this study was to investigate the microbial quality of irrigation water used in the cultivation of Napa cabbage. A total of 111 samples including surface water (*n* = 75) and groundwater (*n* = 36) collected from four different regions in Korea where Napa cabbage is intensively cultivated were analyzed for a fecal indicator (*Escherichia coli*) Moreover, 164 *E. coli* isolates from irrigation water were investigated for pathogenic characteristics including antibiotic resistance, pathogenic genes, serotype, and toxicity using *Caenorhabditis elegans. E. coli* was detected in 96% of surface water samples and 25% of groundwater samples. The level of *E. coli* in surface water (0.2–3.2 log MPN/100 mL) was higher than that in groundwater (0–2.0 log MPN/100 mL). When the 164 *E. coli* isolates were investigated concerning antibiotic resistance, resistance rates were 11.0%, 2.4%, 3.0%, 1.8%, 2.4%, 4.3%, and 3.0% for ampicillin, ampicillin/sulbactam, cefazolin, cefoxitin, gentamicin, levofloxacin, and trimethoprim/sulfamethoxazole, respectively. In addition, 10 (6.1%) of the isolates were positive for the *eaeA* gene, indicative of enteropathogenic *E. coli*. Eight of these 10 isolates were obtained from the surface water of the mountainous region II and were toxic to *C. elegans*. The results indicate the need to manage the microbial risk of irrigation water to enhance the safety of cultivated Napa cabbage.

**Sajid Ali, Muhammad Aaqil Khan, Won-Chan Kim.** ***Pseudomonas veronii* KJ mitigates flood stress-associated damage in *Sesamum indicum* L.. (2018) Appl. Biol. Chem. 61(5): 575–585**

Physiological characteristics of terrestrial plants are severely affected by waterlogging stress, leading to low photochemical efficiency of leaves and retarded growth and development. Plant growth-promoting rhizobacteria contain the *acdS* gene, which encodes for the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase. ACC deaminase cleaves the substrate ACC to produce α-ketobutyrate and ammonia and mitigates the adverse effect of prolonged water stress. The aim of this study was to characterize ACC deaminase-producing rhizobacteria and evaluate their effects on sesame (*Sesamum indicum* L.) under waterlogging stress condition. The rhizobacterium *Pseudomonas* KJ was characterized on the basis of sequencing of the partial 1501 bp fragment of 16S rDNA amplicon and confirmed as *Pseudomonas veronii* KJ. ACC-supplemented minimal medium revealed the phenotypic identification of *acdS* gene. The nucleotide sequence (1001 bp) of ACC deaminase gene of *P. veronii* KJ was also confirmed. We used *P. veronii* KJ as a bioinoculant in waterlogging stress and monitored the growth and developmental characteristics of sesame plants, including leaf chlorophyll fluorescence signals, concentration of chlorophyll, root and shoot length, and fresh and dry biomass in stressed versus unstressed plants. Plants treated with *P. veronii* KJ significantly (*P* ≤ 0.05) mitigated the waterlogging stress-related damage. Thus, the rhizobacterium *Pseudomonas veronii* KJ may be considered as a commendable addition to the consortium of beneficial microbes for its ability to reduce waterlogging stress-related damage in sesame plants.

**Eun Ryeol Shin, Woong Jung, Mi Kyoung Kim, Youhoon Chong.** **Identification of (-)-epigallocatechin (EGC) as a methylglyoxal (MGO)-trapping agent and thereby as an inhibitor of advanced glycation end product (AGE) formation.(2018) Appl. Biol. Chem. 61(5): 587–591**

Non-enzymatic glycosylation of proteins results in the formation of advanced glycation end products (AGEs). AGE modification of proteins and thereby damages to cells and tissues have been confirmed to contribute to the pathophysiology of aging and long-term complications of various age-related diseases. Anti-AGEs therapy has thus received significant attention, and several flavonoids including quercetin (**1**) and (−)-epigallocatechin gallate (EGCG) (**2**) have shown anti-AGEs activity through trapping and inactivating methylglyoxal (MGO), the crucial intermediate of AGEs formation. However, in the field of MGO-scavenging activity, (−)-epigallocatechin (EGC) (**3**), one of the key flavonoids in green tea, has received less attention compared with other flavonoids. In this study, we have shown strong MGO-scavenging activity of EGC (**3**), and EGC (**3**) was found to be equipotent to previously identified MGO-scavengers such as quercetin (**1**) and EGCG (**2**).

**Soo Jin Kim, Il Lae Jung, Hye-Eun Lee, Ji-Hoon Lee.** **Abiotic stress and tissue-specific reference genes for quantitative reverse transcription PCR analysis in Korean native watermelons, *Citrullus lanatus* ‘Black-King’ and ‘Speed-Plus-Honey’.(2018) Appl. Biol. Chem. 61(5): 593–598**

A wide variety of research on watermelon has been conducted, and such studies have been motivated by the published genome sequence database of watermelon. Screening of proper reference genes is the primary step for normalization in gene expression analysis. Based on previous studies conducted on *Arabidopsis* and cucumber, we selected eight candidate reference genes of *ClACT*, *ClEF1α*, *ClGAPDH*, *ClIDH*, *ClLUG*, *ClPTB*, *ClUBC2*, and *Cl18SrRNA*, respectively, encoding β-Actin, elongation factor 1-α, glyceraldehyde-3-phosphate-dehydrogenase, NADP-isocitrate dehydrogenase, leunig, polypyrimidine tract-binding protein1, ubiquitin-conjugating enzyme E2, and 18S ribosomal RNA from watermelon (*Citrullus lanatus*). The expression levels of these eight genes were evaluated by RT-qPCR under plant hormone-treatment (100 μM ABA) and abiotic stresses such as drought, cold (4 °C), and high salt concentration (250 mM NaCl). The expression patterns of these eight genes were further compared across different types of watermelon tissues such as flower, leaf, tendril, stem, root, and whole seedling. Our results showed that expressions of *ClACT* and *ClEF1α*, respectively in the Korean native watermelon cultivars *Citrullus lanatus* ‘Black-King’ and ‘Speed-Plus-Honey’ were least affected by the environmental stresses regardless of tissue types. Here, we suggest two ideal reference genes for watermelon RT-qPCR-based gene expression study.

**Jun Yeong Kim, Jihyeon Song, Heejung Jung, Hyejung Mok.** **I-motif-coated exosomes as a pH-sensitive carrier for anticancer drugs.(2018) Appl. Biol. Chem. 61(6): 599–606**

Nature-derived exosomes have been noted as emerging carriers for anticancer drugs. In this study, as a proof-of-concept, the anticancer drug doxorubicin (Dox) was loaded onto i-motif-modified exosomes (Exo-i-motif) to deliver Dox to cancer cells efficiently. The double-stranded biotin-i-motif/flare (ds-i-motif-bio)s efficiently released Dox in an acidic pH-responsive manner within 1 h. Based on gel electrophoresis, it was clearly confirmed that ds-i-motif-bio successfully interacts with biotin-conjugated exosomes and streptavidin (strep) via the biotin–streptavidin interaction. The particle sizes were below 150 nm without aggregation after strep-mediated modification of ds-i-motif-bio on the surfaces of the exosomes. In addition, released Dox had intact bioactivity for anti-proliferation after immobilization onto the exosomes. This study could serve as a new concept of pH-responsive delivery systems of anticancer drug using nature-derived exosomes with i-motifs.

**Soon Young Shin, Junho Lee, Ha-Na Gil, You Jung Jung, Gyeong Lan Kim, Gil Hak Kang, Yoongho Lim.** ***Schisandra chinensis* inhibiting TGFβ-induced activation of hepatic stellate cells.(2018) Appl. Biol. Chem. 61(6): 607–616**

Hepatic fibrosis is one of the critical steps contained in the pathogenesis of liver cirrhosis. Excessive deposition of collagen contributes to the development of fibrosis in chronic liver injury. Activation of hepatic stellate cells (HSCs) plays an important role in fibrogenesis and is accountable for providing extracellular matrix components. The berry of *Schisandra chinensis*has been known to exert hepatoprotective properties. However, its effect on HSCs is not completely understood. Therefore, in this study, we investigated the inhibitory effect of its ethanolic extract (SBE) on hepatic fibrogenesis. We found that SBE treatment effectively reduced the serum levels of alanine aminotransferase and aspartate aminotransferase as well as collagen deposition in the hepatic parenchyma in a thioacetamide-induced hepatic fibrosis mouse model. Moreover, SBE inhibited transforming growth factor β (TGFβ)-induced mRNA expression of α-smooth muscle actin (αSMA) and collagen type 1 α1 (COL1A1) in HSCs, suggesting that SBE exerts anti-fibrotic activity by attenuating TGFβ-induced HSC activation. To identify the active components of SBE accountable for HSC inhibition, SBE was further partitioned based on the hydrophobicity of the solvents such as water, *n*-butanol, ethyl acetate, chloroform, and *n*-hexane. The *n*-hexane fraction was selected and further separated using analytical high-performance liquid chromatography. We found that six lignans contained in the *n*-hexane fraction strongly reduced TGFβ-induced expression of both αSMA and COL1A1 mRNA. These data suggest that at least six lignans contained in SBE have the strong potential to prevent TGFβ-induced HSC activation.

**Eun Hea Jho, Youngho Youn, Seong Ho Yun.** **Effect of CO2 exposure on the mobility of heavy metals in submerged soils.(2018) Appl. Biol. Chem. 61(6): 617–623**

Increasing atmospheric carbon dioxide (CO2) concentration can affect CO2 level in soil, and this, in turn, may cause changes in soil chemical properties. This study investigated the effect of CO2 exposure on pH and heavy metal mobility in submerged soils. Laboratory-scale batch tests were carried out using two soil samples with different initial pH conditions (A: 5.3; B: 6.3). The changes in the pH values of the soil solutions (i.e., water layer above soil) of the CO2-affected soil samples and controls with time were not significant (*p* value > 0.05) with the both soil samples, and this may be attributed to the formation of bicarbonate, which may provide a buffering capacity. The effect on heavy metal mobility was different in the soil samples A and B. With the soil sample A, the soil heavy metal concentrations were generally lower in the CO2-affected soil than in the controls. Accordingly, the soil solution heavy metal concentrations were changed. With the soil sample B, the soil heavy metal concentrations of the CO2-affected soil and control did not show a significant difference (*p* value > 0.05). This can be partially attributed to the dissolution of carbonates that generate bicarbonates, and this is supported by the lower soil Ca concentration in the CO2-affected soil. Overall, the results suggest that the elevated CO2 level in submerged soils may have different effects on the soil chemical properties, and this necessitates continuous research efforts in order to manage and conserve soil environment under conditions of increasing atmospheric CO2 concentration.

**Byung Yun Ha, Hae Rim Kim, Doe Hyun Kim, Je Wook Woo, Young Joon Jo, Soon Il Kwon.** **Growth effects of the application of new controlled-release fertilizers on *Phalaenopsis* spp..(2018) Appl. Biol. Chem. 61(6): 625–633**

To develop a controlled-release fertilizer (CRF) suitable for nutrient absorption characteristics of *Phalaenopsis*, four kinds of new controlled-release fertilizer (NCRF 1–4) with different dissolution rates were developed and studied to determine the concentration and amount suitable for growth of *Phalaenopsis*. To make NCRF, new acryl-based polymers were developed and used as fertilizer coating solutions. In addition, a fluidized bed coater for coating fertilizer was developed and used in this study. To test the growth of *Phalaenopsis*, 10-month-old *Phalaenopsis* seedlings were planted in plastic pots (diameter 10 cm) filled with 100% *Sphagnum* moss and cultivated for approximately 100 days from May 29, 2015, to September 11, 2015. NCRF 1, NCRF 2, and Osmocote, an imported fertilizer, consistently exhibited release patterns of fertilizer nutrients in a directly proportional form; however, NCRF 3 and NCRF 4 displayed a sigmoid-like tendency of fertilizer nutrient release with a slower initial dissolution rate. Furthermore, leaf length, leaf width, fresh weigh, and root weight of *Phalaenopsis* were the highest when growing in 1.5 g/pot of NCRF 3 fertilizer, and the pH and electrical conductivity (EC) of the soil were stable at this concentration of NCRF 3. Based on our results, we suggest that 1.5 g/pot of NCRF 3 fertilizer is the ideal concentration and fertilizer for growing *Phalaenopsis*.

**Wonkyun Choi, Min-A Seol, Beom-Ho Jo, Il Ryong Kim, Jung Ro Lee.** **Development and application of a novel multiplex PCR method for four living modified soybeans.(2018) Appl. Biol. Chem. 61(6): 635–641**

Since the early 1990s when the first commercialization of living modified organism (LMO), LMO has been developed to improve nutrient quality and productivity of crops. As the self-sufficiency rate of soybean has gradually decreased in South Korea, most of soybeans have been imported. The cultivation and trade of LM crops are regulated in many countries and authorizations for the use are mandatory in most. In South Korea, the cultivation of LM crop is not allowed and unintentional release of LMO into the natural environment is prohibited. In this study, we developed a novel multiplex PCR method for four LM soybean events (CV127, MON87705, FG72 and MON87701) which were approved recently in South Korea. Multiplex PCR primers were designed for PCR amplification of four LMO event-specific fragments, and we analyzed 41 environmental monitoring samples to confirm the efficiency of this method. These results indicated that the multiplex PCR detection method is sufficient for four LM soybeans found in the natural environment. Based on our finding, we suggest that the new technique may be useful as a lead tool for the development of a detection method for various LMO/GMOs.

**Md. Azizul Haque, Soo Young Hong, Chung Eun Hwang, Su Cheol Kim, Kye Man Cho.** **Cloning of an organophosphorus hydrolase (opdD) gene of *Lactobacillus sakei* WCP904 isolated from chlorpyrifos-impregnated *kimchi* and hydrolysis activities of its gene product for organophosphorus pesticides.(2018) Appl. Biol. Chem. 61(6): 643–651**

Chlorpyrifos (CP) residues are absorbed from soil and often found in Korean cabbages that are being used to make *kimchi*. *Lactobacillus sakei* WCP904, harboring the organophosphorus (OP) hydrolase gene *opd*D, was isolated from CP-impregnated *mulkimchi*. The cloned gene *opd*D from strain CP904 comprises 825 base-pair nucleotides that encode 274 amino acids. The recombinant *Escherichia coli* harboring the *opd*D gene depleted 73% of CP after 6 days in M9 medium. In fact, the OpdD protein is a novel member of the GHSQG family of esterolytic enzymes or lactic acid bacterial Opd groups. The molecular weight of the OpdD protein was estimated to be 31 kDa using SDS-PAGE. Broad-spectrum activities of the OpdD protein were obtained against OP insecticides containing both P–O and P–S bonds. The OpdD protein exhibits maximum activity at 30 °C with pH 6. No enzyme activities of the mutated OpdD (Ser116 **→**Ala116) protein toward *ρ*-nitrophenyl butyrate and CP substrates were observed. These results suggested that the strain WCP904 scavenges insecticide residues from *mulkimchi*vegetables, thus abolishing health hazards by secreting OP hydrolase during fermentation.

**Min-Seung Kang, Hoi-Seon Lee.** **Acaricidal and insecticidal responses of *Cinnamomum cassia* oils and main constituents.(2018) Appl. Biol. Chem. 61(6): 653–659**

Insecticidal and acaricidal responses of *Cinnamomum cassia* oils made by organic solvent (OS), steam distillation (SD), and supercritical fluid (SF) and their components were examined in two bioassays (contact and fumigant bioassays) against *Plodia interpunctella*, *Sitophilus oryzae*, *S. zeamais*, *Tyrophagus putrescentiae*, and *Sitotroga cerealella* adults. Using the contact or fumigant bioassay against *T. putrescentiae* adults, OS oil exhibited the strongest toxicities (50% lethal dose [LD50], 2.60 μg/cm2 and 1.34 μg/cm3), followed by SF and SD oils. Furthermore, using two bioassays, SD oil against *S. oryzae* and *S. zeamais* adults exhibited the strongest toxicities (LD50, 102.25 μg/cm2 and 68.62 μg/cm3, 102.03 μg/cm2 and 57.59 μg/cm3), followed by SF and OS oils. Using the fumigant bioassay against *S. cerealella*and *P. interpunctella* adults, OS oil exhibited the strongest toxicities (LD50, 1.17 μg/cm3 and 0.79 μg/cm3) followed by SF and SD oils. Cinnamaldehyde, cinnamyl acetate, and coumarin against *T. putrescentiae* adults showed no significant differences in the contact bioassay, but in the fumigant bioassay, cinnamaldehyde exhibited the highest toxicity (LD50, 0.91 μg/cm3) followed by cinnamyl acetate and coumarin. Against *S. oryzae*, *S. zeamais*, *S. cerealella,* and *P. interpunctella* adults, cinnamaldehyde using two bioassays exhibited the most potent toxicities (LD50, 108.81 μg/cm2 and 77.80 μg/cm3, 104.72 μg/cm2 and 36.48 μg/cm3, 0.57 μg/cm2 and 2.29 μg/cm3), followed by coumarin and cinnamyl acetate in order. The results showed that cinnamaldehyde and the *C. cassia* oils could be effective values in the management of stored product pests.

**Yangmin X. Kim, Tae Jin Kim, Yejin Lee, Seulbi Lee, Deogbae Lee, Taek-Keun Oh, Jwakyung Sung.** **Metabolite profiling and mineral nutrient analysis from the leaves and roots of bell pepper (*Capsicum annuum* L. var. *angulosum*) grown under macronutrient mineral deficiency.(2018) Appl. Biol. Chem. 61(6): 661–671**

We analyzed the contents of 38 primary metabolites and 9 minerals in the leaves and roots of bell pepper (*Capsicum annuum* L. var. *angulosum*) to study metabolic responses to deficiency in nitrogen, phosphorus, potassium, calcium, or magnesium. Induced deficiencies of individual cations reduced the abundance of the other cations in both leaves and roots. Each nutrient-deficient condition was clearly grouped by principal component analysis, which also showed that leaves under cation-deficiency treatments were separated from those under non-cation-deficiency treatments. This was consistent with that a single cation deficiency decreased the levels of the other cations in leaves. Specifically, N deficiency reduced amino acids and organic acids in both tissues. The common response to P-, K-, Ca- or Mg-deficient conditions showed significant increases in the levels of amino acids in both tissues and organic acids in the roots. In the leaves, P- or Mg-deficient conditions reduced organic acids. Soluble carbohydrates were significantly increased under N-, K-, Ca- or Mg-deficient conditions in the leaves, whereas in roots under K deficiency. Notably, the level of γ-aminobutyric acid, an amino acid that helps protect against biotic and abiotic stresses, was increased threefold in leaves under K-deficient conditions and sixfold in roots under P-, K-, Ca-, or Mg-deficient conditions. These findings provide additional information about variations in metabolite and mineral abundance in bell pepper leaves and roots in response to mineral shortage.

**Youngseok Ham, Tae-Jong Kim.** **Anthranilamide from *Streptomyces* spp. inhibited *Xanthomonas oryzae* biofilm formation without affecting cell growth.(2018) Appl. Biol. Chem. 61(6): 673–680**

*Xanthomonas oryzae* (*Xoo*) causes bacterial blight in rice, which reduces crop yield and leads to significant economic damage. *Xoo* exerts its pathogenicity by biofilm formation, interfering with sap flow in the xylem vessels. Inhibition of *Xoo* biofilm formation may therefore alleviate the symptoms of bacterial blight and restore rice yields. *Streptomyces* spp. are soil bacteria that produce various secondary metabolites. In the present study, 38,888 extracts derived from *Streptomyces* spp. were screened for their ability to inhibit *Xoo* biofilm formation; four extracts exhibited strong inhibitory activity. Separation and purification of the extracts from strains 0320 and 4359 suggested that anthranilamide was the chemical responsible for this effect. Anthranilamide was found to inhibit biofilm formation without affecting *Xoo* cell growth; it is, therefore, a good candidate chemical for the treatment of bacterial blight in rice as it will not give rise to resistant bacterial strains. The selected four *Streptomyces* strains were also good candidates for biological treatment of bacterial blight in rice.

**So-Hyun Joo, Young-Soo Keum.** **Oxidative metabolism of quinazoline insecticide fenazaquin by *Aspergillus niger*.(2018) Appl. Biol. Chem. 61(6): 681–687**

Fenazaquin (4-(2-(4-t-butylphenyl)ethoxy)quinazoline) is a quinazoline insecticide, which contains a rare pesticidal toxophore, quinazoline. Its metabolic fate in animals and plants was previously reported. However, the microbial metabolism of the compound has never been studied. Microbial transformation is an important research area for the investigation of environmental safety issues of pesticides. Aspergillus niger was selected as a model soil fungus since it is ubiquitous in agricultural soils, with extensive genetic studies undertaken. Fenazaquin was rapidly metabolized by A. niger (half-life, t1/2 = 0.6 day). 4-Hydroxyquinazoline and 4-t-butylphenethyl alcohol were identified as major metabolites from the cultures. Fenazaquin was also rapidly transformed into the same metabolites (t1/2 = 0.1–0.5 day) under chemical oxidation (m-chloroperoxybenzoic acid). Among the several metabolic inhibitors, flavin-dependent mono-oxygenase inhibitor, methimazole yielded no inhibitory activity (t1/2 = 1.6 day). Several cytochrome P450 inhibitors including piperonyl butoxide, ketoconazole, and myclobutanil were also tested. Piperonyl butoxide strongly reduced fenazaquin metabolism (t1/2 = 58.7 days). However, ketoconazole and myclobutanil showed no activity even at fungi-toxic concentrations (t1/2 = 1.2–4.3 days) with major metabolites similar to those of control experiments. The results suggest that oxidative metabolism of fenazaquin was catalyzed by specific cytochrome P450s, which are insensitive to azole fungicides. In addition, piperonyl butoxide was found to be one of the most promising synergists of pesticides, through cytochrome P450 inhibition.

**Jie Pu, Yuan Long, Jian Zhou, Yanqiang Zhan, Xiaoyong Qin.** **MiR-124 regulates apoptosis in hypoxia-induced human brain microvessel endothelial cells through targeting Bim.(2018) Appl. Biol. Chem. 61(6): 689–696**

Human brain microvessel endothelial cells (HBMECs) are crucial for brain vascular repair and maintenance. The high-expressed expressions of microRNA-124 (miR-124) in brain have been investigated and revealed in many researches. In this work, we aimed to investigate the role of miR-124 in apoptosis of HBMECs and the underlying mechanism. Here, we found the low-expressed miR-124 in hypoxia-induced HBMECs using qRT-PCR analysis. MiR-124 targeting 3′-untranslated region (3′-UTR) of Bim mRNA was predicted by Targetscan database. Importantly, the decreased miR-124 expression and increased Bim expression, an opposite trend, were obtained in hypoxia-induced HBMECs. The further confirmation of the correlation between miR-124 and Bim was conducted by miR-124 overexpression and dual luciferase reporter assays. The inhibitory role of miR-124 in Bim expression was evidenced by results obtained from miR-124 overexpression analysis. Luciferase reporter assay further proved that miR-124 directly targeted the two conserved seed sites in the Bim 3′-UTR. The inhibited apoptosis of HBMECs was observed under both miR-124 overexpression and Bim knockdown condition in flow cytometry analysis. Collectively, these findings outline that miR-124 regulates apoptosis in hypoxia-induced HBMECs through targeting Bim, providing a better understanding of the role of miR-124 in apoptosis of HBMECs.

**Soon Young Shin, Youngshim Lee, Jihyun Park, Doseok Hwang, Geunhyeong Jo, Ji Hye Lee, Dongsoo Koh, Yoongho Lim.** **Cell growth inhibitory effects of polyphenols with naphthalene skeleton against cisplatin-resistant ovarian cancer cells.(2018) Appl. Biol. Chem. 61(6): 697–701**

Cisplatin often shows the drug resistance which could limit the chemotherapeutic efficacy. Thus, it is necessary to develop anticancer agents against cisplatin-resistant cancer cells. To identify pharmacophores exhibiting the cell growth inhibitory effect against cisplatin-resistant A2780/Cis ovarian cancer cells, we prepared 35 synthetic polyphenols bearing naphthalene skeleton including naphthalenyl chalcones, naphthalenyl flavones, naphthalenyl flavanones, 4,5-dihydro-1*H*-pyrazol-3-yl)naphthalen-2-ols, naphthalen-1-yl-*N*-phenyl-4,5-dihydro-1*H*-pyrazole-1-carbothioamides, and 4,5-dihydro-1*H*-pyrazol-3-yl)naphthalen-1-ol. The correlation between their inhibitory effects and structural properties was evaluated using hologram quantitative structure activity relationship and comparative molecular field analysis. The pharmacophores derived here can lead us to design new polyphenols against the growth of cisplatin-resistant cells.

**Hynda K. Kleinman, Kyeongsoon Kim, Hunhee Kang.** **Matrigel uses in cell biology and for the identification of thymosin β4, a mediator of tissue regeneration.(2018) Appl. Biol. Chem. 61(6): 703–708**

The thin extracellular matrix that is found basally in epithelial and endothelial cells and around smooth muscle, peripheral nerves, and fat cells is known as the basement membrane. A murine tumor matrix extract, termed Matrigel, has provided an abundant source of basement membrane proteins (laminin, collagen IV, heparan sulfate, etc.). Matrigel gels at room temperature into a structure similar to the authentic matrix. Embryonic tissue explants, stem cells, and various cell types differentiate when cultured on Matrigel. Matrigel has been used in various in vitro assays for angiogenesis, cell invasion, spheroid formation, organoid formation from a single cell, etc. In vivo Matrigel improves/promotes tumor xenograft growth and is used to measure angiogenesis, improve heart and spinal cord repair, increase tissue transplant take, etc. Endothelial cells plated on top of Matrigel form capillary-like tubules. The gene for thymosin beta 4 was induced at 4 h after plating endothelial cells on Matrigel, and when the thymosin beta 4 protein was added exogenously to the culture, tubule formation was accelerated. Thymosin beta 4, a small 43 kDa protein present in all body fluids and cells, has multiple biological activities, including reducing inflammation, apoptosis, and cytotoxicity while increasing cell migration, stem cell recruitment and differentiation, and tissue repair. Thymosin beta 4 was subsequently found to promote angiogenesis in vivo and to improve dermal and ocular healing in experimental injury models. It has regenerative activity in animal models of traumatic brain injury, stroke, multiple sclerosis, heart attack, peripheral neuropathy, liver and kidney fibrosis, and hair growth. Clinical trials have demonstrated its efficacy for both stasis and pressure ulcers and for both dry eye and a rare ocular disease. This mini review will discuss the development of Matrigel and the discovery of thymosin beta 4 as a regenerative protein that is upregulated when endothelial cells are plated on Matrigel.

**Jerald Conrad Ibal, Byung Kwon Jung, Chang Eon Park, Jae-Ho Shin.** **Plant growth-promoting rhizobacteria used in South Korea.(2018) Appl. Biol. Chem. 61(6): 709–716**

Many bacteria found in the rhizosphere provide contribution for the host plant’s growth and protection that are known as plant growth-promoting rhizobacteria (PGPR). Plant–microbe interactions in the rhizosphere are important factors in determining the health of plants. Research for commercialization of these PGPR as an alternative to the use of chemical fertilizers for a more environmentally friendly treatment is continuously being improved. In this review, we discuss the essential traits that rhizobacteria must possess for them to be considered PGPR and report the bacterial species that exhibit these essential plant growth-promoting activities and which are approved for use by the South Korean regulations.