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**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.

**Boyoung Kim, Yoo Yeon Kim, Harry Jung, Hajin Nam, Jun Gyo Suh. Delayed onset of obesity and glucose tolerance in interleukin 18 deficient mice by single housed condition.(2019) Appl. Biol. Chem. 62: 1**

Interleukin 18 (IL18) is a kind of proinflammatory cytokine that belongs to the interleukin-1 family. IL18 is associated with obesity and type 2 diabetes. To discover whether body composition parameters in IL18 deficient mouse are altered in single-housed condition, body weight, glucose tolerance, lipid profiles, fat masses, and size of white adipocytes were examined. Mice were housed singly and were divided as follows: C57BL/6 J male (B6-M), IL18 deficient male (IL18-M), C57BL/6 J female (B6-F), IL18 deficient female (IL18-M). Body weight statistically significantly increased in IL18-M at 9 months (p < 0.05). Glucose tolerance occurred in IL18-M at 6 and 9 months. Total cholesterol and HDL cholesterol were statistically significantly increased in IL18-F compared with B6-F at 9 and 12 months, respectively (p < 0.05). Also, total cholesterol of IL18-M was statistically significantly increased compared with B6-F and IL18-F at 9 months (p < 0.05). The perirenal and inguinal fat masses were statistically significantly increased in IL18-M at 9 months (p < 0.05). In addition, the size of white adipocytes was increased in IL18-M at 9 months. In single-housed condition, onset of obesity and glucose tolerance were delayed by 3 months in IL18-M. Taken together, these results suggest that housing condition is a very important factor for weight gain and onset of glucose tolerance in IL18 deficient male mouse.

**Eun-Ju Kim, Seong-Eun Park, Seung-Ho Seo, Oh-Cheol Kweon, Hong-Seok Son. A GC–MS based metabolic profiling of fermented tomato by lactic acid bacteria.(2019) Appl. Biol. Chem. 62: 2**

A GC/MS-based metabolite profiling was performed to investigate metabolic differences of fermented tomatoes according to the inoculation of different LAB strains. PCA score plot derived from 2554 signal features of GC–MS data and PCA biplot derived from 18 identified metabolites showed clear separation into three groups. Citric acid and malic acid were found to affect groups clustered with *Lactobacillus fermentum* (*LF*), *Bifidobacterium longum* (*BL*), and *Pediococcus pentosaceus* (*PP*) whereas lactic acid, succinic acid, and fructose were related to *Lactobacillus plantarum* (*LP*) and *Leuconostoc mesenteroid* (*LM*) groups. Meanwhile, *Lactobacillus brevis* (*LB*) was associated with erythritol. Aminoacyl-tRNA biosynthesis and metabolism of cysteine and methionine were identified as metabolic pathways affected by the use of different LAB groups (*LF*, *BL*, and *PP* vs. *LB* groups). This study highlights the applicability of metabolic profiling for understanding fermentative characteristics of LAB strains.

**Jae Eun Ju, Mi-Sook Kim, Joo Hyun Kang, Ji Young Lee, Mi So Lee, Eun Ho Kim, Namhyun Chung, Youn Kyoung Jeong. Potential role of immunological factors in early diagnosis of cancer cachexia in C26 tumor-bearing mice.(2019) Appl. Biol. Chem. 62: 3**

Cachexia is a wasting syndrome associated with high mortality in cancer patients through inducing the failure of normal metabolism and reducing the efficacy of cancer treatment. Thus, it is critically important to diagnose cancer cachexia early. To provide background data for the diagnosis of cachexia, cancer cachectic factors were characterized in the present situation, including immunological cachectic changes during cachexia progression in a cancer cachexia mouse model. Major constitution of cachexia progression is known as the stages of pre-cachexia, cachexia, and refractory cachexia. In the pre-cachexia stage, the weights of immune-related organs, including the thymus and spleen were significantly. T cell populations in spleen were markedly reduced and cachectic cytokines consistently increased in a time-dependent manner. Immunosuppression by activation of cytotoxic T-lymphocyte-associated antigen 4 was induced earlier in CD4+ cells versus other T cell populations. Furthermore, monocyte chemoattractant protein 1 and interleukin-6 levels in the cachexia group were significantly increased at 3 days from C26 cell inoculation whereas significant carcass weight loss as a classical diagnostic marker occurred at 9 days from C26 cell inoculation. In conclusion, the initiation of cachectic immunological changes was observed prior to weight loss, during the pre-cachexia stage. Accordingly, these findings reveal that the monitoring of humoral and immunological factors may be more sensitive than weight loss for the initial diagnosis and treatment of cachexia.

**Fardin Mirahmadi, Maryam Mizani, Rahmat Sadeghi, Mohammad Hadi Givianrad. Chemical composition and thermal properties of *Pistacia atlantica* subsp. *Kurdica* gum.(2019) Appl. Biol. Chem. 62: 4**

*Pistacia atlantica* subsp. *Kurdica* (PAK) is distributed throughout the Zagros Mountains and is indigenous to Kurdistan province in western Iran. This study focused on the composition and thermal properties of gum extracted from female and male trees from six regions of Kurdistan province. Significant differences were detected in the total protein, total ash, total carbohydrate and monosaccharaide contents according to gender and geographic region, but no significant difference was found for moisture content. Analysis of the monosaccharide composition using HPLC showed the presence of arabinose, galactose, glucose, rhamnose and xylose. Significant differences were observed for the amino acid contents of the various PAK gum samples. The most abundant amino acids were glutamic acid, aspartic acid, serine, proline and histidine; however, the relative proportions of amino acids varied considerably between samples. The results indicate that the volatile components (VoC) were significantly different between samples according to gender and region, with the predominant VoC being α-Pinene. The results of thermogravimetric analysis showed that the onset of the initial and main decomposition of the samples was at 80 °C and above 240 °C, respectively. The differential scanning calorimetry results showed that nearly all gum samples included two glass transition temperatures and heat capacity values and that nearly all of the values for the female gum samples were lower than for the male samples.

**Mustika Sari, Yustine Chung, Felicia Agatha, Hyung Kwoun Kim. Evaluation of antioxidant and antimicrobial activity of phenolic lipids produced by the transesterification of 4-hydroxyphenylacetic acid and triglycerides.(2019) Appl. Biol. Chem. 62: 5**

Several phenolic compounds derived from plant biomass are attracting attention because they display high antioxidant activity. In this study, antioxidant activity was confirmed in 4-hydroxyphenylacetic acid (HPA), and transesterification reaction using *Candida antarctica* lipase B was performed to enhance the solubility of HPA. The HPA-diolein (HPA-DON) was synthesized from HPA and triolein, while the HPA-fish oil diglyceride (HPA-dFO) was synthesized from HPA and Menhaden fish oil. To increase the conversion yield, the enzyme reaction conditions of the substrate molar ratio, enzyme amount, and reaction time were optimized. After the reaction, HPA-DON and HPA-dFO were purely separated, using prep-LC. The activity assays using DPPH and ABTS radicals confirmed that HPA-DON and HPA-dFO have antioxidant activity. HPA-DON has high activity in non-polar solvents, while HPA-dFO has strong activity in both polar solvents and non-polar solvents. In addition, HPA-dFO has the growth inhibition activity for *Bacillus coagulans*, *Geobacillus stearothermophilus*, and *Alcaligenes faecalis* that cause food spoilage. Therefore, HPA-dFO is a new synthetic substance that has both antioxidant activity and antibacterial activity. The results indicate that these HPA-derivatives can be expected to be developed as important materials in the food and cosmetics industries.

**Kathyleen Nogrado, Seul Lee, Kyongmi Chon, Ji-Hoon Lee. Effect of transient exposure to carbaryl wettable powder on the gut microbial community of honey bees.(2019) Appl. Biol. Chem. 62: 6**

Bees are important pollinators in agriculture. The bee population has recently begun to decline possibly due to pesticides. The bee gut microbiota strongly influences the health of bees. The gut microbiota of bees is composed of distinct members belonging to selective taxa. Chemicals like pesticides can alter the gut microbiota. The present study investigated the effect of carbaryl pesticides on gut microbiota of honey bees, which had come in contact with rapeseed plants (*Brassica napus*) sprayed with carbaryl wettable powder during the honey bee brood test under semi-field condition. Molecular techniques (conventional and quantitative polymerase chain reaction (PCR), clone library method, and DNA sequencing) were employed to analyze changes in the microbial communities between the pesticide-exposed and unexposed bees. Phylogenetic analysis of 16S rRNA genes of the clones from both groups, showed differences in their respective compositions of core and non-core bacteria. Both groups contained carbohydrate-degrading bacteria such as *Gilliamella apicola* and *Lactobacillus*. However, the unexposed bees harbored *Alphaproteobacteria*, which were absent in the exposed bees. Microorganisms found in honey bee guts such as *Snodgrassella alvi* and *L*. *kullabergensis*, however, were observed only in the exposed bees, but not in the unexposed bees. The difference between the two groups was distinctly recognized when copy numbers of 16S rRNA genes were compared by quantitative PCR. Results showed that the average gene copy number for the unexposed bees was higher than that for the exposed bees. This may indicate the toxic effect of pesticides on bees and gut microbiota.

**Yeong-Geun Lee, Isabel Rodriguez, Youn Hee Nam, Jung Eun Gwag, Sang Ho Woo, Hyoung-Geun Kim, Jung-Hwan Ko, Bin Na Hong, Tong Ho Kang, Nam-In Baek. Recovery effect of lignans and fermented extracts from *Forsythia koreana* flowers on pancreatic islets damaged by alloxan in zebrafish (*Danio rerio*).(2019) Appl. Biol. Chem. 62: 7**

Repeated column separation yielded four enterolactone type lignans from *Forsythia koreana* flowers (FKF), whose chemical structures were identified using several spectral technics. FKF MeOH extract (FKFM) and four lignans significantly recovered aloxan induced pancreatic islet in zebrafish. Especially, aglycones, **1** and **3**, exhibited relatively higher activity than the lignan glycosides, **2** and **4**. Therefore, FKFM was fermented using a *Microbacterium esteraromaticum*, BGP1, to yield the fermented FKFM (FKFM-BGP1). FKFM and FKFM-BGP1 were extracted using *n*-butanol to give *n*-BuOH fraction of each, FKFM-nB and FKFM-BGP1-nB, respectively. FKFM-BGP1-nB showed higher activity than FKFM-nB, as well the content of the aglycones, **1** and **3**, in FKFM-BGP1-nB, 2.42 ± 0.01% and 1.15 ± 0.01%, was revealed to be much higher than that in FKFM-nB, 0.01 ± 0.01% and 0.01 ± 0.01%, respectively. In conclusion, the lignan aglycones **1** and **3** as well FKFM-BGP1-nB from *F. koreana* flowers were proved to be potential anti-diabetic agents. Furthermore, we suggest that antidiabetic efficacy of FKFM-BGP1-nB might be related to lignan aglycones **1** and **3**.

**Sung Un Kim, Chuanpit Ruangcharus, Sandeep Kumar, Hyun Ho Lee, Hye Jin Park, Eun Sang Jung, Chang Oh Hong. Nitrous oxide emission from upland soil amended with different animal manures.(2019) Appl. Biol. Chem. 62: 8**

The nitrous oxide (N2O) emission of from arable soil following the application of manure is expected to vary by different animal manure types used. This study was conducted to determine the relationship between the type of animal manure used to amend soil and the amount of N2O emitted during the cultivation of sweet potato (*Ipomoea batatas*). An additional objective was to study the characteristics of nitrogen (N) and carbon (C) in different animal manures. Composted manures from chickens, cows, and pigs were applied to the soil at rates of 0, 10, and 20 Mg ha−1, respectively. The availability and concentration of N and C varied by manure type. The concentration of NH4+ was greater in pig manure (4638 mg kg−1) than in chicken (551 mg kg−1) and cow manure (147 mg kg−1). The mean cumulative N2O emission rate across soil application rates was also the highest with pig manure (11.9 kg ha−1 year−1), followed by chicken and cow manure, with emission rates of 10.8 and 10.1 kg ha−1 year−1, respectively. The majority of N2O measured during the sweet-potato-growing season was produced from aerobic nitrification. Dissolved organic carbon (DOC) concentrations in animal manures did not affect cumulative N2O emission rates, and no significant relationship was observed throughout the growing season between the concentration of DOC in soil and daily N2O emission. Cumulative N2O emission rates depended on the type of animal manure and might be governed by NH4+ concentration, rather than by total N concentration in animal manure type.

**Brandon Tonnis, Ming Li Wang, Shyam Tallury, Viktor Tishchenko, H. Thomas Stalker. Identification of a mutant from *Arachis veigae* with enhanced seed oleic and very long-chain fatty acid content.(2019) Appl. Biol. Chem. 62: 9**

High oleate is an important seed quality trait frequently incorporated in peanut varieties. Crop wild relatives (CWR) are potentially useful genetic resources for cultivar improvement through genetic introgression; but for wild peanut species, many chemical or nutritional traits are not well characterized. A mutant from *Arachis veigae* S. H. Santana & Valls (2*n* = 2*x* = 20), with increased oleic and very long chain (*C* ≥ 22) fatty acid content was identified from screening 209 accessions of 45 species using gas chromatography (GC). The *A. veigae* (formerly *A. sylvestris*) accession, VVeSv 8373 (PI 688970) contained 55.5% oleic acid in seeds, significantly higher than the average (18.3%) of other accessions within the same species and also significantly higher than the average (37.0%) of all wild peanut accessions evaluated. A C37T substitution was identified by sequencing the coding region of *FAD2H*, resulting in the nonsense mutation of Q13\* (a premature stop codon). This functional mutation may significantly reduce the fatty acid desaturase (FAD) activity and result in the enhanced oleate level. *Arachis veigae* also contained a high percentage of very long-chain (*C* ≥ 22) fatty acids, and their variation identified in this study is also discussed and compared with other species. The mutant with such an altered fatty acid composition may be useful for potentially improving seed or food nutrition quality.

**Jun An, Jun-Cheol Moon, Ju Hee Kim, Geum Sol Kim, Cheol Seong Jang. Development of DNA-based species-specific real-time PCR markers for four berry fruits and their application in commercial berry fruit foods.(2019) Appl. Biol. Chem. 62: 10**

Berry fruits have attracted attention because of their purported benefits for aging, cardiovascular disease, and cancer. Therefore, highly priced berry fruits might be targets for food adulteration and fraud. In this study, eight species-specific primer sets based on the single nucleotide polymorphism of the chloroplast genomes of four berry fruits (aronia, blackberry, cranberry, and strawberry) were developed for quantitative real-time PCR (qPCR) analysis by SYBR Green staining with the aim of preventing berry fruit food fraud. The developed primer pairs exhibited high efficiencies ranging from 88 to 110% with strong correlation coefficients (R2 > 0.99) for the amplification of each target species. However, no clear correlation coefficients were evident for non-target species. To evaluate the practicality of the developed primers, 18 commercial berry fruit products were verified by qPCR analysis. The developed primer pairs were amplified to a low Ct value (range 16.1–23.3) for the target species and proved capable of detecting target species in berry fruit commercial foods. Therefore, the developed qPCR-based species-specific markers could be suitable for the prevention of berry fruit food fraud and to verify marker reliability.

**Jung-Hwa Kwon, Hyun-Ji Oh, Dong-Sung Lee, Seo-Ji In, Kyeong-Hwa Seo, Jae-Woo Jung, Byeong-Ju Cha, Dae Young Lee, Nam-In Baek. Pharmacological activity and quantitative analysis of flavonoids isolated from the flowers of *Begonia semperflorens* Link et Otto.(2019) Appl. Biol. Chem. 62: 11**

*Begonia semperflorens* Link et Otto has been broadly raised up for ornamental purpose as well comestible blossom. As the reproductive structures of phanerogams, flowers contain various secondary metabolites and have many biological activities. Accordingly, we began the contrivance for isolation and analysis of flavonoids contained in *B. semperflorens* flowers. MeOH extraction of *B. semperflorens* followed solvent fractionation was prosecuted. Column chromatography of non-polar fraction gave four flavonoids using several resins. Identification of the flavonols were established as quercetin (**1**), kaempferol (**2**), astragalin (**3**), and isoquercetin (**4**) by interpreting a variety of spectral information. Quercetin (**1**) and kaempferol (**2**) inhibited NO production and protected against *t*-BHP-induced oxidative stress. Kaempferol (**2**) also protected cell death of glutamate-treated HT22. Quantitative analysis of flavonoid content in *B. semperflorens* flowers was also performed using HPLC experiment.

**Tao Zhang, Bencheng Zhao, Qiuyun Chen, Xiaoming Peng, Dongya Yang, Fengxian Qiu. Layered double hydroxide functionalized biomass carbon fiber for highly efficient and recyclable fluoride adsorption.(2019) Appl. Biol. Chem. 62: 12**

The removing of fluoride from water is highly desired from the viewpoint of environmental protection and sustainable development due to the adverse impacts on human and ecosystem. In this study, the hierarchical porous layered double hydroxide (LDH)/biomass carbon fiber (BCF) has been successfully fabricated by the combined sol–gel, carbonization and hydrothermal processes using sustainable bamboo fibers as raw material based on the assembly the LDH nanosheets on BCF surfaces. Structural characterization indicates that the LDH nanosheets were attached to the BCF surface via in situ crystal growth. N2 sorption measurements show that the LDH/BCF has relatively uniform accessible mesochannel size of 3.56 nm, and the surface area is as high as 39.89 m2/g. The resulting LDH/BCF exhibit a noticeable enhanced adsorption capacity for fluoride removal compared to that of Al2O3/BCF, accompanied by 15.21 mg/g of the adsorption capacity. The presence of the high negative charge anions had negligible influence on fluoride adsorption. Importantly, recovery adsorption capacity for fluoride was obtained for LDH/BCF for 5 consecutive cycles without a significant decrease in its adsorption properties. Therefore, the current research can offer a green approach to fabricate LDH/BCF with hierarchical structures for efficient removing fluoride from water, and the fabricated LDH/BCF will be an excellent candidate for pollution control based on the synergistic effects of BCF and LDH, high adsorption properties with good reusability.

**Hyeon Hwa Nam, Li Nan, Jin Cheon Park, Byung Kil Choo. Geraniin ameliorate experimental acute reflux esophagitis via NF-κB regulated anti-inflammatory activities in rats.(2019) Appl. Biol. Chem. 62: 13**

Repeated reflux of gastric acid and stomach contents into the esophagus leads to esophagus damage, including inflammation, ulcer, and hemorrhage in the epithelium. In this study, we aimed to demonstrate the ameliorating effects of geraniin, a phytochemical in the geraniums, on esophagus damage in an acute reflux esophagitis (RE) rat model. The inflammatory effects of geraniinwas measured by nitric oxide (NO) production and pro-inflammatory protein levels in lipopolysaccharide (LPS)-induced RAW 264.7 cells. To evaluate the protective effects of geraniin on damaged esophagus tissue in RE rats, the rats were divided into the following groups: normal control; RE-induced control; RE rats pretreated with geraniin 15 and 30 mg/kg body weight; and RE rats pretreated with ranitidine 30 mg/kg body weight as a positive control. The lesion area of esophagus was determined by the Image J program, and histological changes were examined by hematoxylin and eosin staining of rat esophageal tissue. The expression of pro-inflammatory proteins, cytokines, and tight junction proteins involved in esophagus damages was determined using western blotting of esophageal tissue. Geraniin revealed that anti-inflammatory effects against LPS-induced cells by significantly decreasing NO production and iNOS proteins level. Additionally, the results showed that improvement effects of geraniin on esophagus damages in RE induced rats. The expression of inflammatory proteins involved in nuclear factor NF-kB signaling pathways significantly decreased and tight junction protein (claudin-4 and claudin-5) was increased in esophageal tissue. We found the potential of geraniin as source of replacement therapy products source for inflammatory and reflux esophagitis disease.

**Yeong-Geun Lee, Dong-Geol Lee, Jung Eun Gwag, Misun Kim, Minji Kim, Hyoung-Geun Kim, Jung-Hwan Ko, Hyeonju Yeo, Seunghyun Kang, Nam-In Baek. A 1,1′-biuracil from *Epidermidibacterium keratini* EPI-7 shows anti-aging effects on human dermal fibroblasts.(2019) Appl. Biol. Chem. 62: 14**

Our previous study we isolated novel bacterial stain, *Epidermidibacterium keratini*, called EPI-7T from skin samples. Repeated column separation yielded one new pyrimidine compound, 1,1′-biuracil, from EPI-7T culture solutions grown in R2A medium. Its chemical structure was determined based on spectroscopic data, IR, FAB/MS, and NMR. And 1,1′-biuracil and EPI-7T culture solutions showed regulating effects of anti-aging associated mRNA expressions in UV-irradiated fibroblasts without toxicity in Hs68 cells. These results demonstrates the cosmetic potential of 1,1′-biuracil and EPI-7T as anti-aging agents.

**Kyong-Hee Nam, Do Young Kim, Hye Jin Kim, In-Soon Pack, Hye Jeong Kim, Young Soo Chung, Soo Young Kim, Chang-Gi Kim. Global metabolite profiling based on GC–MS and LC–MS/MS analyses in ABF3-overexpressing soybean with enhanced drought tolerance.(2019) Appl. Biol. Chem. 62: 15**

Abscisic acid (ABA) is a phytohormone that plays an important role in the adaptive responses to abiotic stresses. We examined the metabolic changes in transgenic soybean that over-expressed *Arabidopsis* ABA responsive element-binding factor 3 (*ABF3*), which participates in drought tolerance. Transgenic and non-transgenic plants were exposed to a water deficit, and their metabolic differences were verified by untargeted GC‒MS and LC‒MS/MS analyses. A total of 64 and 476 primary and secondary metabolites from leaf extracts were identified based on GC‒MS and LC‒MS/MS platforms, respectively. Principal component analysis derived from both GC‒MS and LC‒MS/MS data showed a clearly greater separation in the metabolite profiles among three different degrees of drought stress. However, no discrimination of metabolites between transgenic and non-transgenic plants was apparent. Furthermore, except for some free amino acids, quantitative differences in relative levels of those metabolites were less than 50% between genotypes. These results suggest that, during periods of drought, overexpression of *ABF3* in transgenic soybean might result in a negligible variance in primary and secondary metabolism when compared with its non-transgenic counterpart.

**Nho-Eul Song, Jun-Young Lee, Yun-Yeol Lee, Jong-Dae Park, Hae Won Jang. Comparison of headspace–SPME and SPME-Arrow–GC–MS methods for the determination of volatile compounds in Korean salt–fermented fish sauce.(2019) Appl. Biol. Chem. 62: 16**

A new solid phase microextraction (SPME)-Arrow method was evaluated for the analysis of volatile compounds in *kanari*-*aekjeot*, a Korean traditional salt–fermented sand lance sauce, and compared it to the standard headspace–SPME method. Factors observed to affect the extraction, including the fiber used, extraction temperature, extraction time, and NaCl concentration were carefully optimized. The Carboxen/Polydimethylsiloxane fiber exhibited the highest extraction efficiency for both analytical methods and was selected for further optimization of the extraction. The major volatile compounds extracted using both methods were 3-methyl butanoic acid, butanoic acid, acetic acid, 2,6-dimethylpyrazine, and benzaldehyde. The relative concentration (mg/L) of 3-methyl butanoic acid was 1.4-fold higher when using SPME. However, the SPME-Arrow method was more effective at extracting aromatic compounds including alcohol, aldehydes, and pyrazine. In particular, 3-methyl-1-butanol, 2-furanmethanol, and phenylethyl alcohol could only be detected using SPME-Arrow due to its larger sorbent volume. Thus, SPME-Arrow was evaluated as being more suitable for the extraction of pyrazines in sand lance fish sauce and might be useful for determining a broader range of volatile compounds in complex fermented foods.

**Jinhee Kim, Soon Yil Soh, Haejin Bae, Sang-Yong Nam. Antioxidant and phenolic contents in potatoes (*Solanum tuberosum* L.) and micropropagated potatoes.(2019) Appl. Biol. Chem. 62: 17**

This work investigated the extraction efficacy of phenolic acids on the potato and its byproducts. Also, the compositions of bioactive compounds and antioxidants were evaluated in various parts of the potato, such as the tuber, microtuber, peel, and flesh. The chemical constituents were quantified by HPLC analysis, and the highest levels of phenolics (88.99 mg/L) were obtained in acetone extracts from a micropropagated potato. The micropropagated potato demonstrated that notable phenolic compounds were mainly a bound form of phenolic acids including caffeic acid and vanillic acid. The micropropagated extracts using acetone showed the higher radical scavenging activity, 94.3% and 95.5% at 5 mg/mL in 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azinobis-(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS·+), respectively. In addition, the same extracts showed the highest (85.61%) β-carotene bleaching inhibition activity. A positive relationship existed between DPPH and either ABTS·+ (*r* = 0.58, *p* < 0.05), β-carotene bleaching (*r* = 0.65, *p* < 0.05), or total phenolics (*r* = 0.63, *p* < 0.05). However, ABTS·+ did not show a significant correlation between both total phenolics and β-carotene bleaching. The effective phenolic compounds contributing to antioxidant activity were caffeic acid and vanillic acid, which could be extracted in high amounts by acetone from potato peels and micropropagated potatoes.

**Leesun Kim, Hwang-Ju Jeon, Yong-Chan Kim, Seong-Hyun Yang, Hoon Choi, Tae-Oh Kim, Sung-Eun Lee. Monitoring polycyclic aromatic hydrocarbon concentrations and distributions in rice paddy soils from Gyeonggi-do, Ulsan, and Pohang.(2019) Appl. Biol. Chem. 62: 18**

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous pollutants that are released by incomplete combustion of carbon-containing materials. The top soils of rice paddies were collected from Gyeonggi-do (18 sites), Ulsan (20 sites), and Pohang (19 sites) in Korea to assess the spatial distribution and potential sources of PAHs. The total concentrations of 15 PAHs in the soils were 19.53–672.93, 125.01–3106.27, and 51.94–8106.21 mg/kg in Gyeonggi province, Ulsan, and Pohang, respectively. The concentration of 7 key carcinogenic PAHs were followed the order: Pohang (38.54–4826.63 ng/g) > Ulsan (28.54–1561.39 ng/g) > Gyeonggi province (19.53–206.51 ng/g). Three-ring PAHs were predominant in the soils from Gyeonggi-do while 3–5 ring compounds were abundant in the agricultural soils from the two industrial regions (Ulsan and Pohang). The PAH isomeric diagnostic ratios indicated that PAH contamination in the two cities mainly originated from pyrogenic sources. The principal component analysis indicated that pyrogenic coal burning and residential biomass combustion were major contributors to the soil contamination in the two cities. The transportation of PAHs through the air from industrial complexes and high volume of traffic may influence the PAHs distribution in the soils of the two cities in Korea.

**Sanghyun Han, Eun Kyung Choi, Woojun Park, Chongku Yi, Namhyun Chung. Effectiveness of expanded clay as a bacteria carrier for self-healing concrete.(2019) Appl. Biol. Chem. 62: 19**

Cracking of concrete over time, is a natural phenomenon. Longer service life of concrete structures is desirable. Self-healing concrete using bacteria, which could form CaCO3 crystals for crack sealing, has promised benefits to reduce cost for concrete maintenance, because cracks could be autonomously repaired without human intervention. However, because of harsh concrete internal environment render the effectiveness depending on the bacteria viability within concrete. In this study, expanded clay (EC) was used as a carrier, to protect bacteria (*Lysinibacillus boronitolerans* YS11) from the harsh environment during the process. Existence of bacteria inside EC was observed using electron microscopy. When exposed to bacterial solution of 1.0 × 109 cells/mL, bacterial density within EC reached approximately 0.82 × 107 cells/g of dry EC. Extent of bacterial viability within EC, submerged to solution containing 1.0 × 108 cells/mL, was 53.6% of free bacteria solution containing 1.0 × 107 cells/mL, as measured with fluorescein diacetate assay. When rate of calcium carbonate formation was measured with Ca2+ disappearance, rates were comparable between bacteria within EC (submerged to bacterial solution containing 1.0 × 108 cells/mL) and free bacteria (1.0 × 107 cells/mL). This finding indicates that bacteria with EC is very active for generation of CaCO3 within EC. All experimental results suggest that EC may be an adequate bacteria carrier for self-healing concrete.

**Gayoung Seo, Changlim Hyun, Seungin Choi, Young Mee Kim, Moonjae Cho. The wound healing effect of four types of beta-glucan.(2019) Appl. Biol. Chem. 62: 20**

Beta-glucans, which existed in the cell walls of cereals, bacteria, and fungi, comprise a group of β-d-glucose polysaccharides. We investigated the effects of four kinds of beta-glucan, that are derived from barley, yeast, mushroom, and euglena on wound healing. The migration and viability of keratinocyte or fibroblast were analyzed using the in vitro scratch wound healing assay, invasion assay, MTT assay, and in vivo assay. All the beta-glucans had a significant effect on keratinocyte migration at 20 μM and showed no toxicity on dermal fibroblast. Moreover, treatment of keratinocytes with the beta-glucan derived from the mushroom (*Schizophyllum commune*) promoted in vivo wound closure. The Integrin/FAK/Src pathway is known to affect cell migration by forming lamellipodia. Beta-glucan from *S. commune* activates the Integrin/FAK/Src signaling pathway in a time-dependent. Reactive oxygen species are associated with fibroblast differentiation to contract dermal layer and synthesize collagens. We found that fibroblast was activated by increasing NOX4 expression. We propose that beta-glucan derived from mushroom is capable of promoting keratinocyte migration via the induction of FAK/Src phosphorylation there by accelerating wound closure and activating dermal fibroblast differentiation through NADPH oxidase for matrix remodeling.

**Ali Osman Erdoğdular, Dilek Kılıç Apar. Bioremoval of reactive dye Remazol Navy by kefir grains.(2019) Appl. Biol. Chem. 62: 22**

Potential use of living and non-living kefir grains (small, gelatinous white/yellow irregularly shaped masses consist of live bacteria and yeasts) on removal of reactive dye Remazol Navy RGB from aqueous solutions were investigated. Experiments were carried out under different process conditions in order to optimize and model the bioremoval processes. At all conditions the living kefir grains exhibited higher dye removal efficiencies than the non-living grains. In 180 min, 96.3% and 79.4% dye removal was obtained with living and non-leaving kefir grains respectively, at pH 2, 25 °C for 100 mg/L initial dye concentration by using 2.4 g/L kefir grain. Maximum adsorption capacities by living and inactivated kefir grains were obtained at 400 mg/L initial dye concentration as 134.59 and 56.92 mg/g respectively. Consecutive batch studies show that the living kefir grains could be reused over at least 5 cycles with high dye removal efficiency without any nutrition supplement. The biosorption kinetics both for living and non-living kefir grains were best described with pseudo-first-order kinetic model. On the other hand the biosorption equilibrium for living and non-living kefir grains were better defined by Temkin and Langmuir isotherm models respectively. Results suggest that the kefir grains could be used efficiently, eco-friendly and economically for removal of dyes from aqueous solutions.

**Seung-A Baek, Soon Kil Ahn, Kil Won Kim, Jaehyuk Choi, Jinho Kim, Jaegyoon Ahn, Sun-Hwa Ha, Sang Un Park, Jae Kwang Kim. Metabolic profiling reveals glucose and fructose accumulation in *gcr1* knock-out mutant of Arabidopsis.(2019) Appl. Biol. Chem. 62: 23**

The ligands and functions of GCR1, the putative G-protein coupled receptor gene of *Arabidopsis thaliana*, and its role in metabolism are not well studied. Herein, we determined the contents of different pigments, glucosinolates, and lipophilic and hydrophilic compounds in *gcr1* knock-out mutant and wild-type plants to investigate the roles of GCR1. Overall, 68 and 58 metabolites were detected using high performance liquid chromatography, gas chromatography–quadrupole mass spectrometry, and gas chromatography–time-of-flight mass spectrometry in 10-day-old seedlings and 24-day-old shoots of mutant and wild-type plants. The levels of glucose and fructose in the *gcr1* mutant were significantly higher than those in the wild-type at the two developmental stages. The results of partial least squares discriminant analysis and variable importance in the projection showed that glucose and fructose contributed the most to the separation. These results suggest that GCR1 is linked to glucose sensing and affects glycolysis via cyclic AMP.

**Hea-Jong Chung, Hyeon-Jin Kim, Seong-Tshool Hong. Iron-dextran as a thermosensitizer in radiofrequency hyperthermia for cancer treatment.(2019) Appl. Biol. Chem. 62: 24**

Radiofrequency hyperthermia is a recently rediscovered oncotherapy rising in popularity. However, lack of a proper thermosensitizer limits current radiofrequency hyperthermia to be only slightly effective, mostly being used as a subsidiary to a standard oncotherapy. Here, we report that iron-dextran delivers iron ion to cancer cells for cancer-selective accumulation of the iron ion, which functions as a thermosensitizer for radiofrequency hyperthermia. Intravenous injection of iron-dextran to tumor-xenografted mice resulted in selective accumulation of iron ion in the targeted cancer cells. The accumulated iron ion in cancer cells dramatically reacted to radiofrequency wave to result in tumor-selective dielectric temperature increment without harming the surrounding normal tissue. The oncotherapeutic effect of was evaluated using tumor-xenografted mice. The overall anticancer efficacy of radiofrequency hyperthermia after injection of iron-dextran as a thermosensitizer in breast cancer-bearing mice was much better than the efficacy of paclitaxel, a standard chemotherapy drug for cancer. Moreover, hyperthermia using iron-dextran as a thermosensitizer completely eradicated cancer in the tumor xenografted mice. This work suggests that iron-dextran is an ideal thermosensitizer for radiofrequency hyperthermia. We believe that the application of iron-dextran as a thermosensitizer would be a major progress in hyperthermia cancer treatments.

**Yun-Seon Kwak, So-Hyun Joo, Enkhtaivan Gansukh, Bhunpendra M. Mistry, Young Soo Keum. Synthesis and anticancer activities of polymethylenedioxy analogues of combretastatin A-2.(2019) Appl. Biol. Chem. 62: 25**

Combretastatin A-4 is a highly potent natural stilbene that can inhibit cancer cell proliferation. Numerous analogues of combretastatin A-4 have been proposed for clinical applications. However, structural studies of combretastatin A-2, a methylenedioxy derivative of combretastain A-4, are not available. In this study, various analogues of combretastatin A-2 with polymethylenedioxy spacer were prepared and their antiproliferative activities to four human cancer cell lines (HeLa, SK-OV-3, A549, and HT-29) and two normal cells (HaCaT and MDCK) were evaluated. Binding characteristics were evaluated based on computational docking and previously reported experimental data. Results suggest that their binding conformations are highly dependent on steric volume and electrostatic properties of substituents.

**Youngjun Kim, Hyejung Mok. Citraconylated exosomes for improved internalization into macrophages.(2019) Appl. Biol. Chem. 62: 26**

Considering the close relation between macrophages and inflammatory diseases, the design of carriers for the delivery of drugs, genes, and small molecules into macrophages is crucial. In this study, the surface charge of exosome (EXO) was easily modified to highly negative charge by citraconylation. Prepared citraconylated EXO (cit-EXO) exhibited a significantly reduced surface charge down to − 50 from − 15 mV of EXO surface charge, despite similar hydrodynamic size. In the absence of serum proteins, both EXO and cit-EXO were similarly internalized into RAW264.7 cells and DC2.4 cells. However, cit-EXO exhibited superior intracellular uptake to that of EXO for RAW264.7 cells in the presence of serum proteins because of highly negative charges. However, there were no significant differences in intracellular uptake of EXO and cit-EXO for DC2.4 cells. Taken together, simple surface modification onto EXOs via citraconylation improved delivery of nanosized EXO (~ 50 nm) into macrophages, which could serve as a promising strategy for the development of carriers for efficient macrophage delivery.

**Jose Enrique Herbert-Pucheta, Cinthia Mejía-Lara, Benito Reyes-Trejo, Lino Reyes, Holber Zuleta-Prada. Nuclear magnetic resonance assignment strategy for pentacyclic triterpenes, using lup-20(29)-ene from *Pilotrichella flexilis* as model system, combining spectrally filtered proton-to-carbon schemes and DFT–GIAO approach.(2019) Appl. Biol. Chem. 62: 28**

The present work comprises a method to obtain full proton-to-carbon nuclear magnetic resonance chemical shift assignment of a C30H50 lup-20(29)-ene, for the first time obtained from the Mexican native mosses *Pilotrichella flexilis*, wherein said method consists in a combination of the following NMR schemes: 1D-13C (DEPT-135), 2D-{1H–13C} HMBC with a spectral filter for promoting only weak-c.a. 2 Hz-long-range scalar couplings, 2D-{1H–1H} EXSY with long mixing times to favour only weak H–H dipolar correlations and ultra-high resolution one- and two-dimensional 1H instant homodecoupling Psyche pure shift. Full set of assigned resonances were compared against the theoretical isotropic chemical shifts computed with a gauge invariant atomic orbital–density functional theory with self consistent reaction field calculation, retrieving accurate agreements, despite the intrinsic severe signal overlap that these C30 hydrocarbon triterpenes experimentally present. Therefore, a 3D-structure supported by experimental NMR data of this type of important metabolite precursor in plants can be proposed.

**Ji Sun Lim, Dongyup Hahn, Myeong Ju Gu, Jisun Oh, Jeong Soon Lee, Jong-Sang Kim. Anti-inflammatory and antioxidant effects of 2, 7-dihydroxy-4, 6-dimethoxy phenanthrene isolated from *Dioscorea batatas* Decne.(2019) Appl. Biol. Chem. 62: 29**

Our previous study showed that the ethanol extract of *Dioscorea batatas* Decne (Chinese yam) peel upregulated certain antioxidant enzymes and had the anti-inflammatory activity. In this study, 2, 7-dihydroxy-4, 6-dimethoxy phenanthrene (DDP) was isolated from yam peel extract as a potent antioxidative enzyme inducer through bioassay-guided fractionation using HepG2-ARE cells, and subjected to examination for its anti-inflammatory activity as well as antioxidant activity. DDP decreased the levels of inflammatory mediators in LPS-stimulated Raw 264.7 macrophage and reduced the level of reactive oxygen species in *tert*-butyl hydroperoxide-challenged Raw 264.7 cells. Moreover, DDP enhanced the expression of nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and its downstream heme oxygenase-1 proteins while it decreased the expression of iNOS, COX-2, proinflammatory cytokines via nuclear factor-κB pathway. However, the combinatorial treatment with DDP and the inhibitor of Nrf2 or HO-1 activity did not affect the levels of inflammatory biomarkers, suggesting that anti-inflammatory action by DDP is achieved by the mechanism independent of Nrf2 signaling pathway. In conclusion, DDP was found to be a strong antioxidant and anti-inflammatory agent and warrants further in vivo efficacy study for future use as a functional food ingredient.

**Jin-kyu Woo, Young Chul Park, Ju Won Lee, Su-Hyun Yun, Minju Kim, Sukman Park, Yi Lee, Kwan Jeong Song, Ho Bang Kim. Evaluation of polyembryony for genetic resources and efficacy of simple sequence repeat markers for the identification of nucellar and zygotic embryo-derived individuals in citrus.(2019) Appl. Biol. Chem. 62: 30**

Many citrus cultivars have the polyembryony trait that develops many nucellar embryos alongside a single zygotic embryo in an individual seed by sporophytic apomixis. This unique botanical trait hinders citrus breeding by genetic hybridization and affects breeding efficiency and cost. Techniques to efficiently identify nucellar and zygotic individuals in citrus are still very limited. For a systematic and targeted citrus breeding program, we collected 101 citrus genetic resources and determined their embryo types, which revealed 22 monoembryo, 54 polyembryo, and 25 mixed types. We also developed 17 simple sequence repeat (SSR) markers showing polymorphism among the genetic resources from the public resources and our own comparative genome analysis. Seventeen SSR markers detected a total of 181 alleles, ranging from 5 to 16 alleles per locus. The average polymorphism information content value was 0.67, ranging from 0.43 to 0.84. Genetic cluster analysis based on similarity matrices of alleles revealed that several genetic resources of the genus *Citrus* were fragmented and/or scattered throughout the entire dendrogram, not forming unique groups, due to frequent natural or intended genetic crossings. Application of these polymorphic SSR markers to F1 individuals derived from several genetic crosses using polyembryonic citrus cultivars as a female parent revealed that the polyembryony trait decreased the breeding efficiency due to the poor occurrence rate of zygotic individuals. Therefore, our results suggest that identification of nucellar and zygotic embryo-derived F1 individuals using SSR markers as a genotyping technology may be a powerful tool for establishing a systematic molecular breeding program in citrus.

**Kyong Mi Jun, Joung Sug Kim, Songhwa Chae, Yoon-Mok Pahk, Gang-Seob Lee, Joon-Hui Chung, Yeon-Ki Kim, Baek Hie Nahm. Development of *Tos17* insertion mutants from Korean cultivars ‘Ilmibyeo’ and ‘Baegjinju1ho’ (*Oryza sativa* L.).(2019) Appl. Biol. Chem. 62: 31**

Rice is one of the most important crops globally and a model plant for genomic studies of monocots. With the release of complete genome sequences, the next challenge is to develop various resources based on functional analyses of genes. In this study, we generated mutants via the insertion of *Tos17*, a mobile endogenous retrotransposon active during tissue culture. Two rice cultivars, *Oryza sativa* L. japonica **‘**Ilmibyeo**’** (IM) and **‘**Baegjinju1ho**’** (BJJ1), which represent white and brown rice in the Korean domestic market, respectively, were selected for this study. We analyzed 7608 flanking sequences of newly transposed *Tos17* insertions by the flanking adaptor-ligation polymerase chain reaction method and identified 1672 and 843 mutants (M2 generation) in IM and BJJ1, respectively. An analysis of these *Tos17* insertions showed the preferential insertion of *Tos17* into rice chromosome genic regions (approximately 70%). We found new insertional mutants in 830 genes among the 1533 genes representing 2515 IM and BJJ1 mutants that did not overlap with the 3280 genes affected in the ‘Nipponbare’ (NP) mutants from the National Institute of Agrobiological Sciences database. Of the 1000 lines of *Tos17* insertion mutants, we observed semi-dwarf and various leaf-type mutants, including those with narrow, pale-green, and striped leaves at the vegetative stage. At the reproductive stage, 10 lines showed a 17–56% increase in 100-grain weight compared with the wild type. This study demonstrates the potential utility of *Tos17* mutants via an efficient tissue culture method in various rice cultivars for improving agronomic traits, including seed weight.

**Sang Gon Kim, Jin-Seok Lee, Hwan Hee Bae, Jung-Tae Kim, Beom-Young Son, Sun-Lim Kim, Seong-Bum Baek, Seonghyu Shin, Weon-Tai Jeon. Physiological and proteomic analyses of Korean F1 maize (*Zea mays* L.) hybrids under water-deficit stress during flowering.(2019) Appl. Biol. Chem. 62: 32**

Despite the relevance of drought stress, the regulation of gene expression, protein accumulation, and plant physiology under water-deficit stress is not well understood in Korean F1 maize (*Zea mays* L.) hybrids. In this study, we investigated the effect of water deficit on the F1 maize hybrids, Ilmichal (Ilmi) and Gwangpyeongok (GPOK), by withholding water for 10 days during flowering. Water deficit severely reduced the relative water content, area, SPAD values, and stomatal conductance of leaves, stem length, and the dry matter content of aerial tissues in drought-stressed plants of both hybrids. However, the dry matter content of roots was reduced only in GPOK. Two-dimensional gel electrophoresis identified 24 spots representing proteins accumulated to differential levels in well-watered and drought-stressed plants of both hybrids. Further analysis of protein spots using matrix assisted laser desorption ionization–time of flight mass spectrometry and protein database searches revealed that nine proteins were involved in carbohydrate metabolism, seven in stress response, and two in photosynthesis. Among these proteins, delta 3,5-delta 2,4-dienoyl-CoA isomerase (spot 8) and bifunctional 3-phosphoadenosine 5-phosphosulfate synthetase 2 (spot 23) were present only in GPOK, whereas NAD-dependent epimerase/dehydratase (spot 13), NAD(P)H-quinone oxidoreductase subunit 2 A (spot 24), and an uncharacterized protein (spot 19) were present only in Ilmi, in response to water-deficit stress. Semi-quantitative reverse transcription PCR analysis showed that the transcript levels of most of the genes encoding these proteins correlated well with their protein levels, suggesting that water deficit affects gene transcription in F1 maize hybrids at the flowering stage.

**Dong Gun Lee, Eun Byeol Go, Mindong Lee, Pyo June Pak, Joong-Su Kim, Namhyun Chung. Gold nanoparticles conjugated with resveratrol induce cell cycle arrest in MCF-7 cell lines.(2019) Appl. Biol. Chem. 62: 33**

Resveratrol is a kind of phytoalexin produced in several plants with self-defense effect. It is known for its anti-inflammatory and ant-cancer effects. However, it has low efficacy due to its degradation before reaching the target. To heighten its delivery rate and efficacy, gold nanoparticles (GNPs) under 30 nm size were synthesized as drug carrier and conjugated with resveratrol via polyvinylpyrrolidone (PVP) as cross-linker. These gold nanoparticles conjugated with resveratrol (GRs) were used to estimate their anti-tumor effects through cell cycle arrest. It was found that resveratrol- and GRs-treated groups had decreased extent of G0/G1 phase but increased extent of S phase compared to control and GNP-treated groups, suggesting that the effect was due to resveratrol which was attached to gold nanoparticles. To estimate cytotoxicity after treatment with GNPs and GRs, the extent of lactate dehydrogenase (LDH) release was investigated. Results showed that GNPs and GRs-treated groups had almost no difference in LDH release compared to control group, suggesting that the extent of toxicity was not significant. Taken together, these results suggest that GRs could be potentially effective in treating cancer as anti-tumor drug with further development.

**Jeong-In Hwang, Da-Rong Seok, Jang-Eok Kim. Effects of cuticular waxes on permeation of fungicides azoxystrobin and chlorothalonil into apples.(2019) Appl. Biol. Chem. 62: 34**

Time-dependent permeation characteristics of two fungicides azoxystrobin and chlorothalonil into apples were investigated in the presence and absence of the cuticular waxes. Either apple samples which were subjected to wax-removing treatment or not (raw) were individually submerged in each dilution solution of the tested fungicides for a short time, and some of each submerged sample were then washed in running tap water. All apple samples were incubated under controlled conditions and collected sequentially after 1, 24, and 48 h. The collected apple samples were divided into four tissue parts before fungicide residue analysis: peel, pulp-1, pulp-2, and pulp-3. Most residues of azoxystrobin (70.7‒86.4%) in apples were present in the peel, and the residual extents in the pulps increased by removal of cuticular waxes. By washing treatment, 52.3–69.2% of azoxystrobin residues in raw apples were removed. Meanwhile, all chlorothalonil residues were determined in the peel of apples, and their concentrations slightly increased by wax removal. However, significant chlorothalonil residues (84.5–91.1%) were removed by washing the apple surface. Results in this study may be extensively utilized as basic data to understand characteristics of cuticular permeation and translocation of fungicides applied on fruit crops.

**Chang Hoon Lee, Seong Jin Park, Hyun Young Hwang, Myung Sook Kim, Ha il Jung, Deogratius Luyima, Suk Young Hong, Taek Keun Oh, Seong Heon Kim. Effects of food waste compost on the shift of microbial community in water saturated and unsaturated soil condition.(2019) Appl. Biol. Chem. 62: 36**

Despite the widespread use of food waste compost as a soil organic amendment, there is limited information on how it affects the composition of the microbial community as well as its relationship on soil environmental factors. This study investigated the effects of food waste compost on soil microbial-community composition by using FAME analysis. It was established that the application of food waste composts in water saturated paddy and unsaturated upland soils increased pH levels, electrical conductivity (EC) values, total carbon (TC), and ESP contents. It also increased the total phospholipid fatty acid (T-PLFA) and fungi resulting in a significant rise in the fungi to bacterial ratio. Furthermore, microbial community composition shifted depending on the quantity of food waste compost applied after crop harvest in both paddy and upland soils. Also, a positive correlation was found between changes in soil microbial community and changes in TC, EC, and water availability for crop growth. These results suggest that soil fertility and its microbial composition depends on the amount of food waste compost applied in both water saturated the paddy and unsaturated upland soils.

**Su-Lim Lee, Jong-Hwan Park, Seong-Heon Kim, Se-Won Kang, Ju-Sik Cho, Jong-Rok Jeon, Yong-Bok Lee, Dong-Cheol Seo. Sorption behavior of malachite green onto pristine lignin to evaluate the possibility as a dye adsorbent by lignin.(2019) Appl. Biol. Chem. 62: 37**

The objective of this study was to evaluate the adsorption characteristics of malachite green (MG) on pristine lignin as a dye adsorbent. The adsorption capacity of MG on lignin (31.2 mg/g) was described by Langmuir isotherm and pseudo second order models, and were higher than humic acid (6.4 mg/g). The adsorption of MG by lignin was rapid occurring within 15 min of the reaction, and then equilibrium was reached. The adsorption of MG by lignin based on an intraparticle diffusion model indicated that it was dominated by external boundary. Removal of MG by lignin can be applied at a wide range of pH’s (2–5), and optimal lignin dosage for MG removal was 3 g/L. In addition, the desorption efficiency of MG adsorbed on lignin was highest in methanol + acetic acid (95:5%, v/v) mixture of all solutions tested. The peaks attributed to the hydrogen-bonded stretching vibrations and sulphonyl groups in lignin before MG adsorption, were assigned at about 3400 and 620 cm−1, while the peaks in lignin after MG adsorption were attenuated or reduced. This result indicates that the adsorption of MG by lignin is closely related to the O–H and S–O bonds. Finally, this study suggests that pure lignin, which excludes active processes, can also be used as an adsorbent for dyes. However, in order to utilize the dye-adsorbed lignin repeatedly, further studies will be needed.

**Yongsung Joo, Hyun Ee Ok, Jihyun Kim, Sang Yoo Lee, Su Kyung Jang, Ki Hwan Park, Hyang Sook Chun. A statistical model for determining zearalenone contamination in rice (*Oryza sativa* L.) at harvest and its prediction under different climate change scenarios in South Korea.(2019) Appl. Biol. Chem. 62: 38**

Mycotoxin contamination of food grains is a food safety hazard, and zearalenone (ZEN) is one such mycotoxin affecting rice grains (*Oryza sativa* L.). A statistical model for estimating the impacts of climate change on ZEN contamination of rice grains in South Korea was constructed. Observational data on ZEN concentrations in rice grains at harvest and local weather information from 241 rice fields in South Korea were collected. To estimate the impact of weather variables on ZEN concentrations, multiple regression analyses were conducted along with variable selection procedure. The final model included the following variables: average temperature and humidity over the flowering period, daily (between days) change in temperature over the harvest period, degree of milling, and the climate region. On the basis of this regression model, maps showing ZEN contamination were produced for South Korea in the present day, the 2030s, and the 2050s, using the representative concentration pathway (RCP) emission scenarios RCP 2.6, 4.5, and 8.5. The predictive maps project that in the 2030s and 2050s, ZEN contamination in rice grains will increase nationwide, particularly more so on the western side of South Korea. Our research results might be helpful in developing effective control measures against ZEN contamination due to climate change.

**Jun Sung Seo, Pingzhi Zhao, Choonkyun Jung, Nam-Hai Chua. PLANT U-BOX PROTEIN 10 negatively regulates abscisic acid response in Arabidopsis.(2019) Appl. Biol. Chem. 62: 39**

MYC2 is well known as a positive regulator for abscisic acid (ABA) signaling but whether PLANT U-BOX PROTEIN 10 (PUB10) is involved in ABA responses has not been reported. Here, we show that the E3 ubiquitin ligase PUB10 modulates ABA signaling in Arabidopsis. *PUB10ox* (*35S:PUB10*-*myc*) and *myc2* loss-of-function mutants were hyposensitive to ABA during germination, whereas *pub10* loss-of-function and *MYC2ox* (*35S:MYC2*-*GFP*) mutants were hypersensitive. In addition, *pub10* mutants showed hypersensitivity to high salt and osmotic stress during germination; by contrast, *PUB10ox* line displayed the opposite phenotype. ABA-induced expression of *KIN2* (*Cold*- *and ABA*-*Inducible Protein*), *RD22* (*Responsive to Dehydration 22*), *ANAC019* (*NAC Domain*-*Containing Protein 19*), and *ANAC055* (*NAC Domain*-*Containing Protein 55*) was enhanced in both *pub10* and *MYC2ox* plants. Taken together, *pub10* plants phenocopied *MYC2ox* plants, whereas *PUB10ox* plants phenocopied *myc2* in ABA response. Our results provide evidence that PUB10 negatively regulates ABA signaling in Arabidopsis.

**Ye-Jin Kim, Eun-Ho Lee, Eun-Bi Cho, Dong-Hee Kim, Byung-Oh Kim, In-kyu Kang, Hee-Young Jung, Young-Je Cho. Protective effects of galangin against UVB irradiation-induced photo-aging in CCD-986sk human skin fibroblasts.(2019) Appl. Biol. Chem. 62: 40**

Photo-aging is caused by cumulative oxidative stress from ultraviolet B irradiation with up-regulating intracellular reactive oxygen species, 4-hydroxynonenal, and matrix metalloproteinases. MMPs are the enzyme that degrades collagens so that impair the function of the dermis. Galangin was identified by 1H-NMR and 13C-NMR spectroscopy and is a natural flavonol that recently known to have many pharmacological effects such as anti-viral, anti-inflammatory, anti-atopic dermatitis and anti-oxidative activities. In this study, the protective effect of galangin on UVB-induced photo-aging in human skin fibroblasts (CCD-986sk) was conducted by Western blot analysis and enzyme-linked immunosorbent assay. Activator protein 1 and nuclear factor-kappa B are the main transcription factors from activated mitogen-activated protein kinases that up-regulates MMPs. Galangin showed down-regulation of intracellular ROS, 4-HNE, and MMPs through inhibition of phosphorylation of the MAPK pathway and showed a protective effect against skin fibroblasts under oxidative stress caused by UVB irradiation. This lead to up-regulation of fibroblast growth factor 2 and type 1 pro-collagen. These findings suggest that galangin can be developed as a potential agent for functional food and cosmetics of UVB-induced skin photo-aging.

**Soo Ji Kang, Eun Ah Park, Dong Hun Lee, Kwang Won Hong. Comparison of the stability of eGFP displayed on the *Bacillus subtilis* spore surface using CotB and C-terminally truncated CotB proteins as an anchoring motif under extreme conditions.(2019) Appl. Biol. Chem. 62: 41**

We investigated the expression and stability of enhanced green fluorescent protein (eGFP) under extreme conditions using two types of high-copy-number vectors and two types of anchoring motifs (CotB and C-terminally truncated ∆CotB spore coat proteins) for the development of a spore surface display system in *Bacillus subtilis*. The fused *cotB*-*gfp* and *ΔcotB*-*gfp* DNA fragments were cloned into the pUB19 (pUB110-derived) and pHY300PLK vectors. Four types of expression vectors were transformed into *B.* subtilis 168. The expression level of eGFP on the surface of spores prepared from *B. subtilis* transformants was measured by flow cytometry. When pUB19 vector was used, the activities of ∆CotB-eGFP and CotB-eGFP were 17.9 and 5.6 times higher than those of the pHY300PLK vector, respectively. In addition, the activity of pUB19-∆CotB-eGFP was 1.76 times higher than that of pUB19-CotB-eGFP. Overall, the activity of eGFP was more stable under extreme conditions (heat, pH, and protease challenges) when ∆CotB was used as an anchoring motif instead of CotB. Compared to the control groups, the activities of ΔCotB-eGFP and CotB-eGFP were maintained at 56% and 41% at 80 °C and 88% and 55% at pH 10, respectively. The activities of ΔCotB-eGFP and CotB-eGFP were maintained at 62% and 41%, respectively, when treated with 0.03 U of proteinase K. In addition, the activities were maintained at 77% and 36%, respectively, when treated with 5.5 U of trypsin.

**Hee-Dong Eun, Sajid Ali, Hyeonjung Jung, Kihwan Kim, Won-Chan Kim. Profiling of ACC synthase gene (*ACS11*) expression in *Arabidopsis* induced by abiotic stresses.(2019) Appl. Biol. Chem. 62: 42**

Abiotic stress induce the production of 1-aminocyclopropane-1-carboxylate (ACC), the precursor of ethylene by activating the enzyme ACC synthase. There are twelve ACC synthase genes reported in the genome of *Arabidopsis,* and the *ACC synthase 11* (*ACS11*) gene encodes a polypeptide that is functional; however, its involvement in ethylene biosynthesis in response to abiotic stresses remains unclear. We evaluated the effects of higher ACC accumulation on *A. thaliana* seedlings in response to abiotic stressors such as flooding, salinity, cold, and drought. Transgenic plants were generated with *ACS11* (*ACS11*-OX), and they demonstrated that overexpression of *ACS11* reduces both root and shoot length observed in seedlings. RT-PCR analysis revealed that abiotic stressors induce the expression of the wild type *ACS11* gene. Histochemical staining revealed that GUS activity followed the same time course as induction of wild type *ACS11* gene expression, increased ACC levels, and production of stress hormone, ethylene. One finding showed that although induction of wild type *ACS11* gene occurs under drought stress, GUS activity was highest at 6 h of drought stress and decreased to levels similar to control seedlings at 12 and 24 h. Thus, Wild type *ACS11* expression is involved in ACC production, and abiotic stressors induce the expression of *ACS11* gene. Moreover, ACC increases in response to abiotic stress lead to the production of ethylene. All of the data presented here suggest that the overexpression of *ACS11* paves the way for the production of stress hormone, ethylene, which adversely affected the growth and development of the plant.

**Hahk-Soo Kang, Jong-Pyung Kim. Butenolide derivatives from the fungus *Aspergillus terreus* and their radical scavenging activity and protective activity against glutamate-induced excitotoxicity.(2019) Appl. Biol. Chem. 62: 43**

The organic extract of cultured *Aspergillus terreus* displayed scavenging activity against ABTS•+ and DPPH free radicals, and protective activity against glutamate-induced excitotoxicity in N18-RE-105 neuroblastoma-retina hybrid cells. Bioassay-guided fractionation of the active organic extract led to the isolation of total six butenolide derivatives, including one new metabolite, named butyroscavin (**1**), and five previously described metabolites, butyrolactones I (**2**), II (**3**), III (**4**), and VII (**5**), and aspernolide E (**6**). The planar structure of butyroscavin (**1**) was determined by the analysis of spectroscopic data including ESIMS (electrospray ionization mass spectrometry), and 1D and 2D NMR (nuclear magnetic resonance). The absolute configuration of butyroscavin (**1**) was assigned by comparison of the specific rotation with those of known compounds that share the same chiral carbon. All isolated compounds were active in the radical scavenging assay, whereas only butyrolactones I (**2**) and VII (**5**) exhibited protective activity against the glutamate-induced excitotoxicity with the EC50 of 130.1 and 91.9 *μ*M, respectively.

**Li Luo, Min-Joo Kim, Jihyun Park, Hee-Deuk Yang, Younglim Kho, Myung-Sub Chung, BoKyung Moon. Reduction of perfluorinated compound content in fish cake and swimming crab by different cooking methods.(2019) Appl. Biol. Chem. 62: 44**

Perfluorinated compounds (PFCs) are widely used in industries, and have become common environmental pollutants. Consumption of aquatic foods and its processed products can result in the accumulation and maintenance of PFCs in organs of human body, which can lead to toxic consequences and poisoning. The aim of this study was to evaluate the reducing effects of PFC contents in fish cake and swimming crab by different cooking conditions. Fish cake was processed with blanching, boiling, frying, stir-frying and swimming crab was pretreated with soaking and cooked by steaming and stewing. The change of PFCs were determined using LC–MS/MS. Boiling reduced the total PFCs in fish cake by up to 45.9%. As for swimming crab, soaking, steaming and stewing have reduced 65.7%, 17.6% and 13.3% of PFCs, respectively. These results suggest that cooking method involving water addition and high-temperature heating would be effective at reducing PFCs (PFOA especially) in food.

**Hyun Ho Lee, Do Young Heo, Hae Ri Han, Ye Lim Park, Chuanpit Ruangcharus, Sung Un Kim, Dong Cheol Seo, Taek-Keun Oh, Chang Oh Hong. Evaluation of the effects of mandarin (*Citrus reticulate*) by-products containing citric acid on immobilization of cadmium in arable soils.(2019) Appl. Biol. Chem. 62: 45**

In a pilot study, we observed cadmium (Cd) immobilization with citric acid (CA) and suggested that mandarin by-products (MB), which contain CA at ca. 1.65%, can be used as soil amendments that reduce Cd bioavailability. In the present study, we (1) elucidated mechanisms of Cd immobilization by CA and (2) evaluated the use of MB as a soil amendment for Cd immobilization. In Experiment 1, CA was mixed with Cd contaminated soil at 0 and 3.5 mmol kg−1. We then added MB to Cd-spiked soil at 10, 20, and 40 g kg−1. Addition of CA decreased F2 (surface adsorbed Cd fraction) contents by 2.64 mg kg−1 compared with the control but was associated with increases in Cd fractions F1 (bioavailable Cd fraction) and F5 (residual Cd fraction) of 1.04 and 1.49 mg kg−1, respectively. Addition of CA enhanced the concentration of fraction F5, likely reflecting Cd precipitation from soil solutions with increased HCO3− concentrations. However, although this treatment immobilized Cd, it also led to increasing residual and bioavailable Cd fractions. Unlike CA treatments, MB increased non-bioavailable Cd fractions without increasing the bioavailable Cd fraction. Moreover, at 40 g kg−1, MB decreased F1 contents by 8% compared with the control, but increased F2, F3, and F5 contents by 3.6%, 0.7%, and 4.5%, respectively. Cd may be immobilized by MB through H*x*CO3− mediated precipitation as CdCO3 following decomposition of CA and concomitant increases in the negative charge of soil due to the organic matter in MB. MB also improved the chemical properties of soils, with increased nutrient concentrations and cation exchange capacities.

**Da Hee Choi, Hyung Seo Hwang. Anti-inflammation activity of brazilin in TNF-α induced human psoriasis dermatitis skin model.(2019) Appl. Biol. Chem. 62: 46**

Psoriasis is a chronic inflammatory skin disease that causes erythema, scale, and invasion due to excessive proliferation of keratinocyte and vascular deformation of the upper part of the dermis. Recently, it has been reported that brazilin, an active compound of *Caesalpinia sappan* L., possesses anti-inflammatory activity in mouse macrophage. However, little is known about its effect or anti-inflammatory activity on psoriasis dermatitis. Thus, the objective of this study was to determine anti-inflammatory activity of brazilin in TNF-α-induced human keratinocyte (HaCaT) widely used as a model of psoriatic dermatitis. First, CCK-8 assay was performed to determine cytotoxicity of brazilin in HaCaT cells and cytotoxicity was not observed up to 7 μg/mL concentrations. Brazilin decreased mRNA expression levels of inflammatory cytokines such as IL-1α, IL-1β, IL-6, IL-8 and TNF-α in a concentration dependent manner. Brazilin also significantly reduced phosphorylation of I-κB, Akt, and MAPKs such as ERK, JNK, p38 and STAT3 in immortalized human keratinocytes (HaCaT) induced by TNF-α. In addition, inflammation causes the weakness of the skin barrier structure and increase cell permeability, stimulating serious problems in skin moisturizing. Thus, we observed changes of skin permeability in TNF-α induced inflammatory condition through transepithelial electrical resistance (TEER) assay. While TNF-α induced inflammation caused reduction of TEER value (ohm (Ω) × cm2), it was recovered by treatment with brazilin in a concentration-dependent manner. These results strongly imply that brazilin can reinforce the skin barrier due to its anti-inflammatory activity. Therefore, brazilin could be a promising candidate for treating psoriasis dermatitis.

**Dae Young Lee, Bo-Ram Choi, Jae Won Lee, Yurry Um, Dahye Yoon, Hyoung-Geun Kim, Young-Seob Lee, Geum-Soog Kim, Youn-Hyung Lee, Nam-In Baek. Simultaneous determination of various platycosides in Four *Platycodon grandiflorum* cultivars by UPLC-QTOF/MS.(2019) Appl. Biol. Chem. 62: 47**

In Platycodi Radix (root of *Platycodon grandiflorum*), there are a number of platycosides that consist of a pentacyclic triterpenoid aglycone and two sugar moieties. Due to the pharmacological activities of platycosides, it is critical to assess their contents in PR, and develop an effective method to profile various platycosides is required. In this study, an analytical method based on ultra performance liquid chromatography coupled with quadrupole time-of-flight/mass spectrometry (UPLC-QTOF/MS) with an in-house library was developed and applied to profile various platycosides from four different Platycodi Radix cultivars. As a result, platycosides, including six isomeric pairs, were successfully analyzed in the PRs. In the principal component analysis, several platycosides were represented as main variables to differentiate the four Platycodi Radix cultivars. Their different levels of platycosides were also represented by relative quantification. Finally, this study indicated the proposed method based on the UPLC-QTOF/MS can be an effective tool for identifying the detail characterization of various platycosides in the Platycodi Radix.

**Eun-Ha Kim, So-Young Lee, Da-Young Baek, Soo-Yun Park, Sang-Gu Lee, Tae-Hoon Ryu, Seong-Kon Lee, Hyeon-Jung Kang, Oh-Hun Kwon, Mira Kil, Seon-Woo Oh. A comparison of the nutrient composition and statistical profile in red pepper fruits (*Capsicums annuum* L.) based on genetic and environmental factors.(2019) Appl. Biol. Chem. 62: 48**

Red peppers are a remarkable source of nutrients in the human diet. However, comprehensive studies have not reported on the effects of genotype, cultivation region, and year on pepper fruit characteristics. To address this, 12 commercial pepper varieties were grown at two locations in South Korea, during 2016 and 2017, representing four environments, and concentrations of proximate, minerals, amino acids, fatty acids, capsaicinoids, and free sugars in pepper pericarps were determined. Variation in most nutrients was observed among the 12 varieties grown within each location in each year, indicating a significant genotype effect. Statistical analysis of combined data showed significant differences among varieties, locations, and years for the measured components. The % variability analysis demonstrated that environment (location and year) and genotype-environment interaction contributed more to the nutritional contents than genotype alone. Particularly, variation in many amino acids, capsaicinoids, free sugars, and myristic acid was attributed to location. Year effect was significant for palmitoleic acid, ash, tryptophan, copper, linolenic acid, crude fiber, and tyrosine. Insoluble dietary fiber, soluble dietary fiber, sodium, sulfate, linoleic acid, and alanine were primarily varied by genotype–environment interaction. Palmitic acid was the trait the most highly affected by genotype. Cultivation and the genotype–environment interaction have a major role in determining the composition of 12 pepper varieties across four environments. The data from this study could explain the natural variation in the compositional data of peppers by genotypes and environments.

**Yuqian Hu, Linlin Zheng, Jinhui Zhang, Lijuan Lin, Yue Shen, Xiaoyan Zhang, Buling Wu. Dual delivery of bone morphogenetic protein-2 and basic fibroblast growth factor from nanohydroxyapatite/collagen for bone tissue engineering.(2019) Appl. Biol. Chem. 62: 49**

Background

In bone tissue engineering, the fabrication and biocompatibility of scaffold are crucial. Among many scaffold materials, nanohydroxyapatite (nHAP) and collagen (COL) are chosen as building materials of scaffold. At the same time, growth factors were also used to modify the scaffolds.

Methods

In this study, blending and freeze drying methods were adopted together in order to build basic fibroblast growth factor (bFGF)-bone morphogenetic protein-2 (BMP-2)-nHAP/COL scaffolds. ELISA was applied to test the release of bFGF and BMP-2 on the scaffold. The flow cytometry was used to identify bone marrow mesenchymal stem cells (BMSCs). Scanning electron microscope was adopted to observe scaffolds and cells morphology. BMSCs were seeded on the scaffolds to test the biological compatibility in vitro. Cells were counted to detect early cell adhesion. Cell counting kit-8 assay was adopted to detect cell proliferation and alkalinephosphatase assay was applied to detect cell activity.

Results

The characterization of bFGF-BMP-2-nHAP/COL scaffolds meets the requirements of ideal bone tissue engineering scaffolds. BMSCs that were isolated, purified and passaged satisfied the needs of further experiments. The growth status of cells on bFGF-BMP-2-nHAP/COL scaffolds was satisfactory. Cell adhesion was the highest in the bFGF-BMP-2-nHAP/COL scaffolds group. The cell viability and ALP activity of bFGF-BMP-2-nHAP/COL scaffolds group were the highest.

Conclusion

Taken together, bFGF-BMP-2-nHAP/COL scaffolds have good biocompatibility in vitro and promote adhesion, proliferation, differentiation of BMSCs.

**Kyeongnam Kim, Yong Ho Lee, Gayoung Kim, Byung-Ho Lee, Jeong-Oh Yang, Sung-Eun Lee. Ethyl formate and phosphine fumigations on the two-spotted spider mite, *Tetranychus urticae* and their biochemical responses.(2019) Appl. Biol. Chem. 62: 50**

Two spotted spider mite, *Tetranychus urticae*, is a polyphagous pest to a variety of plants and they are hard to be controlled due to occurrence of resistance to acaricides. In this study, biochemical evaluation after ethyl formate (EF) and phosphine (PH3) fumigation towards *T. urticae* might help officials to control them in quarantine purposes. PH3 fumigation controlled eggs (LC50; 0.158 mg/L), nymphs (LC50; 0.030 mg/L), and adults (LC50; 0.059 mg/L) of *T. urticae*, and EF effectively affected nymphs (LC50; 2.826 mg/L) rather than eggs (LC50; 6.797 mg/L) and adults (LC50; 5.836 mg/L). In a longer exposure time of 20 h, PH3 fumigation was 94.2-fold more effective tool for control of *T. urticae* than EF fumigant. EF and PH3 inhibited cytochrome *c* oxidase (COX) activity differently in both nymphs and adults of *T. urticae*. It confirmed COX is one of target sites of these fumigants in *T. urticae* and COX is involved in the respiratory chain as complex IV. Molecular approaches showed that EF fumigation completely down-regulated the expression of *cox11* gene at the concentration of LC10 value, while PH3 up-regulated several genes greater than twofold in *T. urticae* nymphs treated with the concentration of LC50 value. These increased genes by PH3 fumigation are *ndufv1*, *atpB*, *para*, and *ace*, responsible for the expression of NADH dehydrogenase [ubiquinone] flavoprotein 1, ATP synthase, and acetylcholinesterase in insects, respectively. Lipidomic analyses exhibited a significant difference between two fumigants-exposed groups and the control, especially an ion with 815.46 m/z was analyzed less than twofold in the fumigants-treated group. It was identified as PI(15:1/18:3) and it may be used as a biomarker to EF and PH3 toxicity. These findings may contribute to set an effective control strategy on *T. urticae* by methyl bromide alternatives such as EF and PH3 because they have shared target sites on the respiratory chain in the pest.

**Joon-Goo Lee, Jung-Hyuck Suh, Hae-Jung Yoon. Occurrence and risk characterization of polycyclic aromatic hydrocarbons of edible oils by the Margin of Exposure (MOE) approach.(2019) Appl. Biol. Chem. 62: 51**

Polycyclic aromatic hydrocarbons (PAHs) are carcinogenic and genotoxic chemicals naturally derived from food during heat processing. Edible oil is one of the most frequently contaminated foods. Many researches were recently conducted to determine the contents of PAHs and to assess their risks, but there have been no studies characterising risks of PAHs by calculating Margin of Exposure (MOE) of total PAHs instead of toxic equivalency factors (TEFs) concept in Korea. To analyze the 4 PAHs including benz(a)anthracene (BaA), chrysene (CHR), benzo(b)fluoranthene (BbF), and benzo(a)pyrene (BaP) simultaneously, gas chromatography with mass spectrometry was optimized. Total 303 edible oils were investigated and contaminated by 4 PAHs at ND–12.91 ng g−1. The MOEs were estimated by PAHs contents, daily consumption, and were over 10,000. The risk of PAHs of edible oils in Korea was of low concern. Furthermore, the MOEs of the estimated equivalent BaP calculated by TEFs of other 3 PAHs were higher than those of mixed PAHs, which would be overestimated.

**Soon-Jae Eum, Il Ryong Kim, Hye Song Lim, Jung Ro Lee, Wonkyun Choi. Event-specific multiplex PCR method for four genetically modified cotton varieties, and its application.(2019) Appl. Biol. Chem. 62: 52**

Multiplex polymerase chain reaction (PCR) methods have been developed and validated for screening, tracing, and regulating genetically modified (GM) crops in quarantine and environmental monitoring. In this study, we aimed to develop a method to simultaneously detect four GM cotton varieties in order to establish a screening system for cotton volunteers. Based on the sequence of DNA in the junction between introduced gene and flanking genomic DNA of four GM cotton events, herbicide-tolerant MON88701 and DAS-81910-7 and insect-resistant COT102 and T304-40, event-specific primers were designed and a multiplex detection method was developed. The simplex PCR results supported the multiplex PCR results; the amplification efficiency of the novel multiplex PCR method was increased compared with that of the Joint Research Centre (JRC) method. Based on the accuracy and efficiency, the method can be applied to detect and identify randomly mixed reference materials and suspected cotton volunteers. To apply this multiplex PCR method to living modified (LM) environmental monitoring samples, we performed additional PCR analysis to identify whether the volunteers were the four LM cotton varieties. As a result, 66 cotton volunteers were identified with stack event, comprising one or two of the four LM cotton events, and all stacks have been approved in South Korea for food, feed, and processing. These results indicated that our novel multiplex method is suitable for LMO identification.

**Dong-Hyun Yoon, Won Seok Choi, Young Kyu Hong, Young Bok Lee, Sung Chul Kim. Effect of chemical amendments on reduction of bioavailable heavy metals and ecotoxicity in soil.(2019) Appl. Biol. Chem. 62: 53**

Heavy metal pollution in soil has been concerned because of toxicity in ecosystem and adverse effect on human health. Main objective of this study was to examine reduction of bioavailable heavy metals and consequently, decrease of ecotoxicity to biota when chemical amendments were applied in soil. Three chemical amendments, acid mine drainage sludge (AMDS), lime stone (LS), and steel slag (SS) were applied with varied application ratio (1, 3, 5%) in heavy metal polluted soil and bioavailable fraction of heavy metal was monitored. In addition, ecotoxicity test using earthworm (*Eisenia fetida*) was conducted for 28 days examining mortality, weight increase, and bioaccumulation of heavy metal in the earthworm. Result showed that AMDS was the most efficient amendment for reducing bioavailable heavy metals in soil while SS showed the least efficiency. Reduction ratio of bioavailable-As, Cd, and Pb was ranged 39.0–92.0% depending on application ratio and heavy metal species for AMDS application. However, only bioavailable-Pb was reduced at the range between 39.1% and 56.5% when SS was applied in soil. In contrast, the lowest concentration of As, Cd, and Pb and ecotoxicity effect in the earthworm was observed in SS treatment indicating that exposure route of heavy metals or particle size of amendments might effect on uptake of heavy metals to the earthworm. Overall, ecotoxicity test in combination with chemical concentration monitoring is a useful tool for evaluating remediation efficiency of heavy metal polluted soil.

**Hye Jin Kim, Do Young Kim, Ye Seul Moon, In Soon Pack, Kee Woong Park, Young Soo Chung, Young Joong Kim, Kyong-Hee Nam, Chang-Gi Kim. Gene flow from herbicide resistant transgenic soybean to conventional soybean and wild soybean.(2019) Appl. Biol. Chem. 62: 54**

Gene flow from transgenic crops to conventional cultivars or wild relatives is a major environmental and economic concern in many countries. South Korea is one of the major importer of transgenic crops for food and feed, although commercial cultivation of transgenic crops is not yet allowed in this country. This study evaluated gene flow from the herbicide glyphosate- and glufosinate-resistant transgenic soybean (*Glycine max*) to five non-transgenic soybean cultivars and three accessions of wild soybean (*Glycine soja*). Field trials were conducted over 2 years, and gene flow was monitored up to 10 m distance from the pollen source. The results indicated that the detectable rate of gene flow from transgenic to conventional soybeans varied between 0 and 0.049% in both 2014 and 2015 field trials, while no hybrids were detected among wild soybean progenies. The highest rate of gene flow was found in the progenies of the Bert cultivar, which exhibited the longest period of flowering synchronization between the pollen donor and the recipient. In addition, overall gene flow rates declined with increased distance from the transgenic soybean plot. Gene flow was observed up to 3 m and 8 m from the transgenic soybean plot in 2014 and 2015, respectively. Our results may be useful for developing measures to prevent gene flow from transgenic soybean.

**Seung Mi Choi, Do-Hee Kim, Kyung-Soo Chun, Joon-Seok Choi. Carnosol induces apoptotic cell death through ROS-dependent inactivation of STAT3 in human melanoma G361 cells.(2019) Appl. Biol. Chem. 62: 55**

Melanoma is the leading cause of skin cancer deaths, and the poor prognosis of metastatic melanoma has made needs for a novel pharmacological treatment or efficient intervention. Carnosol, a major polyphenolic compound from *Rosmarinus officinalis*, has a wide range of biological activities including anti-cancer effect. However, the underlying molecular mechanisms of its anti-cancer effect remain poorly understood in malignant human melanoma cells. In the present study, we investigate the apoptotic effect and the underlying anti-cancer mechanisms of carnosol. Our results revealed that carnosol strongly induced apoptosis against human melanoma G361 cells in a dose- and time-dependent manner, and caused dramatical elevation in cellular reactive oxygen species (ROS) level during apoptosis. In mechanistic studies, carnosol treatment decreased protein level of anti-apoptotic B‑cell lymphoma 2 (Bcl-2) and B cell lymphoma-extra large (Bcl-xL), however, increased level of pro-apoptotic Bcl-2-associated X protein (Bax) protein. Moreover, carnosol escalated cellular level of p53, which was accompanied by a decline of mouse double minute 2 homolog (MDM2) level. Also, carnosol inhibited activation of Src and signal transducer and activator of transcription 3 (STAT3), therefore down-regulated STAT3-dependent gene expression, such as D-series cyclin and survivin. These changes by carnosol were attenuated by pre-treatment of *N*-acetyl cysteine, and abolished progression of carnosol-induced apoptosis. In conclusion, carnosol induced apoptosis in human melanoma G361 cells through ROS generation and inhibition of STAT3-mediated pathway. Our results provide molecular bases of carnosol-induced apoptosis, and suggest a novel candidate for human melanoma treatment.

**Soon Ae Sim, Su Gyeong Woo, Dae Yeon Hwang, Jin-Hong Kim, Seung Sik Lee, Chae Oh Lim, Jong Chan Hong, Young Hun Song. FLOWERING HTH1 is involved in CONSTANS-mediated flowering regulation in *Arabidopsis*.(2019) Appl. Biol. Chem. 62: 56**

Flowering at the right time is essential for maximum reproductive fitness. In *Arabidopsis thaliana*, the CONSTANS (CO) protein facilitates the transition from the vegetative phase to the reproductive phase under long-day conditions. The formation of heterodimeric complexes between CO and DNA binding domain-containing transcription factors is important for the induction of day length-dependent flowering. Here, we report a myb-like helix turn helix (HTH) transcriptional regulator family protein as a new modulator of floral transition, which we have named FLOWERING HTH1 (FHTH1). We isolated FHTH1 as a CO-interacting protein by a yeast two-hybrid screen using an *Arabidopsis* transcription factor library. Our analysis showed that FHTH1 presented in the nucleus and the FHTH1-CO complex was formed in the same subcellular location. We also observed the expression of a *FHTH1:GUS* construct in the leaf vasculature, where CO exists. Transgenic plants overexpressing FHTH1 fused with the plant-specific repression domain SRDX showed a delayed flowering phenotype in long days, resembling the phenotype of the *co* mutant. Our results suggest that FHTH1 may contribute to CO-mediated photoperiodic flowering regulation.

**Hwa-Kyung Lee, Jonghwa Lee, Junghak Lee, Joon-Kwan Moon, Jeong-Han Kim. X-ray crystal structure of endosulfan sulfate. (2019) Appl. Biol. Chem. 62: 57**

X-ray crystallography is an important method used to confirm the three-dimensional structure of a chemical compound. In this study, the crystal structure of endosulfan sulfate was investigated. Endosulfan sulfate is the major metabolite of the insecticide endosulfan, which is composed of two stereoisomers (α and β). From GC–MS analysis, α- and β-endosulfan each gave a single peak in the endosulfan sample, but only one peak was observed for endosulfan sulfate. Interestingly, in X-ray crystallography, two conformers of endosulfan sulfate (A and B) were observed at a ratio of 2(A):1(B). A heterocyclic seven-membered ring of conformer B assumed a horizontal-chair form, differing from two twisted forms of α-endosulfan while a vertical-chair form was observed for conformer A, showing the very similar structure to β-endosulfan; this difference in conformation is caused by differing bond angles at O(1)–C(8)–C(3) and O(2)–C(9)–C(4). In space packing, two asymmetric units were obtained, and three molecules were aligned in the order of A–A–B conformers in each unit. The total potential energy of A was slightly lower (approximately 4 kcal/mol) than B, possibly resulting in the two molecules of A that exist in a rigid crystal state. However, A and B conformers should not exist at room temperature in a solution state for GC–MS analysis, likely due to the small energy difference.

**Hyeon-Seon Ji, Hua Li, Eun-Jin Mo, Un-Hee Kim, Young-Ho Kim, Ho-Yong Park, Tae-Sook Jeong. Low-density lipoprotein-antioxidant flavonoids and a phenolic ester from *Plectranthus hadiensis* var. *tomentosus*. (2019) Appl. Biol. Chem. 62: 58**

To investigate the effects of extraction solvents and drying methods on *Plectranthus hadiensis* var. *tomentosus* quality, eight compounds were isolated and the content of active compounds with their antioxidant activities were compared. Compounds **1** and **2** were known antioxidants, whereas the low-density lipoprotein (LDL)-antioxidant activities of compounds **3**, **5**, **6**, and **7** are reported for the first time, with IC50 values of 2.5, 3.8, 22.8, and 53.7 μM, respectively. Our analysis of 30‒95% ethanol extracts from freeze- and air-dried leaves and stems revealed a relationship between extract composition and antioxidant activity. The 95% ethanol extracts of freeze-dried stems (FDS) exhibited highest phenolic and flavonoid content, which were 1.40 and 2.67 times, respectively, greater than those of air-dried stems (ADS), and very high LDL-antioxidant and DPPH radical scavenging activities, which may have resulted from the phenolic ester rosmarinic acid (**2**), a major component of FDS extracts and potent antioxidant. In contrast, the 95% ethanol extracts of ADS exhibited relatively low antioxidant activity, possibly owing to the low antioxidant activity of the main components ayanin (**7**) and (+)-plectranthone (**8**). These results are important for the development of *P. hadiensis* var. *tomentosus* as an effective natural antioxidant material.

**Doaa A. Badr, Mohamed E. Amer, Wagih M. Abd-Elhay, Mohamed S. M. Nasr, Tamer M. M. Abuamara, Harbi Ali, Aly F. Mohamed, Maha A. Youssef, Nasser S. Awwad, Yi-Hsu Ju, Ahmed E. Fazary. Histopathological and genetic changes proved the anti-cancer potential of free and nano-capsulated sinapic acid. (2019) Appl. Biol. Chem. 62: 59**

Cancer is known to be a fierce disease that causes a large percentage of the deaths worldwide. The common cancer treatments; chemotherapy, radiotherapy and surgery are known for their severe side effects; therefore scientists are working on finding solutions to reduce these drawbacks. One of these treatment systems is the sustained released drugs formulations, these systems depend on the encapsulation of the chemotherapy within an emulsifying agent, in order to obtain a slow drug release of low doses over long time intervals. In this study, the anti-cancer effects of free and encapsulated sinapic acid was tested against lung (A549), and colon (CaCo2) cancer cell lines, along with normal fibroblast cells (HFB4) as a negative control. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was performed for IC50 evaluation, also cell cycle assay was performed to detect cell cycle arrest status and related anti-apoptotic and pro-apoptotic; Blc-2, BAX, and P53 gene profile fold changes post cellular treatment. Data recorded revealed that encapsulated SA showed a lower toxicity than the free form to both cell lines and also to the normal cells. The cell cycle analysis showed a cell cycle arrest at the G2/M phase post cell treatment with the free and encapsulated sinapic acid accompanied with up regulation of Bax and P53 and a down regulation of Blc-2 genes in both cell lines. The data suggest a promising anti-cancer and anti-proliferative potential of free and encapsulated sinapic acid. Also they show that the anti-cancer effect of free and encapsulated sinapic acid is quite close.

**Hyejun Jo, Jiwan Hong, Tatsuya Unno. Investigation of MiSeq reproducibility on biomarker identification. (2019) Appl. Biol. Chem. 62: 60**

MiSeq-derived artificial sequences appeared to be of good quality, thus bioinformatics tools failed to remove MiSeq artefacts. Even after removing singleton sequences or operational taxonomic units (OTUs), it is not clear how many sequence artefacts remained. Here, 16S rRNA genes were amplified from soil, human feces, pig feces, and groundwater. These were sequenced with five separate runs of MiSeq. Subsequently, each run of MiSeq was compared through alpha and beta-diversity analyses. We found more than half the OTUs were not in consensus through the multiple MiSeq runs, resulting in varying group-specific biomarker OTUs in each MiSeq run. Thus, differential abundance test should be interpreted with caution, and we suggest that results also should be verified further with other quantification methods such as qPCR.

**Hwang-Ju Jeon, Kyeongnam Kim, Yong-Deuk Kim, Sung-Eun Lee. Antimelanogenic activities of piperlongumine derived from *Piper longum* on murine B16F10 melanoma cells in vitro and zebrafish embryos in vivo: its molecular mode of depigmenting action. (2019) Appl. Biol. Chem. 62: 61**

In this study, the antimelanogenic activity of piperlongumine in murine B16F10 melanoma cells and zebrafish was investigated, and its mode of antimelanogenic action was elucidated using quantitative reverse transcription-polymerase chain reaction. A melanocyte-stimulating hormone (α-MSH, 200 nM) was used to induce melanin production in B16F10 melanoma cells, and kojic acid (200 μM) was used as a positive control. Piperlongumine had no inhibitory effects on cell growth at the treated concentrations (3 and 6 μM), and it significantly reduced total melanin production. Piperlongumine decreased the expression of *Mitf*, *Tyr*, *Trp*-*1*, and *Trp*-*2* and tyrosinase activity was also dramatically reduced by the piper amide addition under α-MSH treatment. With these findings, zebrafish embryos were used to confirm antimelanogenic activity of piperlongumine, and it showed the potent antimelanogenic activity at the concentration of 1 μM. Altogether, piperlongumine has potent antimelanogenic activity, and these results support it as a candidate for natural depigmentation agent in a cosmetic and pharmaceutical industries.

**Hyeon Jeong Seong, Seong Woo Kwon, Dong-Cheol Seo, Jin-Hyo Kim, Yu-Sin Jang. Enzymatic defluorination of fluorinated compounds. (2019) Appl. Biol. Chem. 62: 62**

Fluorine-containing compounds are widely used because they have properties required in textiles and coatings for electronic, automotive, and outdoor products. However, fluorinated compounds do not easily break down in nature, which has resulted in their accumulation in the environment as well as the human body. Recently, the enzymatic defluorination of fluorine-containing compounds has gained increasing attention. Here, we review the enzymatic defluorination reactions of fluorinated compounds. Furthermore, we review the enzyme engineering strategies for cleaving C–F bonds, which have the highest dissociation energy found in organic compounds.

**Hwang-Ju Jeon, Kyeongnam Kim, Yong-Deuk Kim, Sung-Eun Lee. Naturally occurring Piper plant amides potential in agricultural and pharmaceutical industries: perspectives of piperine and piperlongumine. (2019) Appl. Biol. Chem. 62: 63**

Piperaceae plants consist of about 3600 species, of which about 2000 are Piper plants. Their habitat is distributed across pantropical regions. The representative plant is *Piper nigrum*, known as black pepper. These plants have been widely used in folk medicine in Korean traditional medicine. This review collected papers identifying and separating the amides obtained from these Piper plants, with a focus on Piper amides potential to control the production and growth of fungal strains that cause plant disease and their insecticidal properties against agricultural pests. Piper amide benefits include antiaflatoxigenic activities, antiparasitic activities, anticancer properties, antiplatelet activities, and anti-inflammatory activities, among other therapeutic properties for the treatment of human diseases. In addition, this review paper provides a total synthesis study on the mass production of Piper amides and their derivatives, with a formulation study for industrial use. This review paper is designed to help inform future studies on Piper amide applications.

**Joon-Goo Lee, Jeong-Yun Hwang, Hye-Eun Lee, Tae-Hun Kim, Jang-Duck Choi, Gil-Jin Gang. Effects of food processing methods on migration of heavy metals to food. (2019) Appl. Biol. Chem. 62: 64**

Heavy metals including Lead (Pb), Cadmium (Cd), Arsenic (As) and Aluminium (Al) were analysed in oilseeds, noodles, tea leaves and their processed or cooked products to study the effects of food processing methods on migration of heavy metals. The heavy metals were determined with ICP-MS and ICP-OES following microwave-assisted acid digestion. Heavy metals in oilseeds, noodles and teas were reduced by extracting oils, boiling noodles, and infusing teas. And the transfer of heavy metals into boiling water and infusion tea was increased as the boiling and infusion time is increased. Heavy metals in foods are water soluble and heavy metals in foods would be decreased when foods are processed or cooked with water. Furthermore, it is needed to determine the migration rates in other cooked foods and assess the risk of heavy metals with concentrations calculated by the migration rates.

**Seong Eun Jeong, Soo Hyun Chung, Sung-Yong Hong. Natural occurrence of aflatoxins and ochratoxin A in *meju* and soybean paste produced in South Korea. (2019) Appl. Biol. Chem. 62: 65**

In this study, we investigated the occurrence of aflatoxins (AFs) and ochratoxin A (OTA) in *meju* and soybean paste produced in South Korea. Samples were collected from three regions divided on the basis of climate in South Korea. A total of 100 *meju* samples were analyzed over 3 years (2012–2015), and 45 soybean paste samples were analyzed in 2016. Mycotoxins were extracted with an immunoaffinity column method and quantified by high-performance liquid chromatography. AFs were detected in 10 of *meju* (10%) and 11 of soybean paste samples (24.4%) with concentrations of 0.2–48.3 μg/kg and 0.88–16.17 μg/kg, respectively. OTA was detected in 50 of *meju* (50%) and 22 of soybean paste samples (48.9%) with concentrations of 0.1–193.2 μg/kg and 0.88–26.29 μg/kg, respectively. Mycotoxin contamination in *meju* was more common in the central region than in the southern areas. Thus, more mycotoxins were produced in the central region owing to less fungal competition in *meju* during fermentation inside households. We also found that about 91% of AFs and 73% of OTA in *meju* were degraded after the production of soybean paste and soy sauce. Even after degradation of AFs and OTA, the levels of AFB1 and OTA were 0.5 µg/kg and 7.5 µg/kg in soy sauce and 11.9 µg/kg and 190.4 µg/kg in soybean paste, respectively. Thus, our results suggest the need for constant monitoring of *meju* and soybean paste for AFs and OTA.

**Yoon Chae Jeong, Ki Seog Lee. Overexpression, purification, crystallization and preliminary X-ray crystallographic characterization of the receiver domain of the response regulator PhoP from *Enterococcus faecalis* ATCC 29212. (2019) Appl. Biol. Chem. 62: 66**

Phosphate (Pho) regulon plays a critical role in bacterial phosphate homeostasis. It is regulated by two-component system (TCS) that comprises a sensor histidine kinase and transcriptional response regulator (RR). PhoP from *Enterococcus faecalis* (EfPhoP) belongs to the OmpR subfamily of RRs. It has not yet been structurally characterized because it is difficult to crystallize it to full-length form. In this study, a truncated form of EfPhoP containing the receiver domain (EfPhoP-RD) was constructed, purified to homogeneity and crystallized using the hanging-drop vapour-diffusion method. The crystal of EfPhoP-RD diffracted to 3.5 Å resolution and belonged to the orthorhombic space group *C*2221, with unit-cell parameters a = 118.74, b = 189.83, c = 189.88 Å. The asymmetric unit contains approximately 12 molecules, corresponding to a Matthews coefficient (*V*m) of 2.50  Å3 Da−1 with a solvent content of 50.9%.

**Jee-Yun Park, Khulan Amarsanaa, Yanji Cui, Ji-Hyung Lee, Jinji Wu, Yoon-Sil Yang, Su-Yong Eun, Sung-Cherl Jung. Methyl lucidone exhibits neuroprotective effects on glutamate-induced oxidative stress in HT-22 cells via Nrf-2/HO-1 signaling. (2019) Appl. Biol. Chem. 62: 67**

Oxidative stress causes neuronal cell death in various neurodegenerative diseases, such as Alzheimer’s disease, ischemia, and Parkinson’s disease. Therefore, reducing intracellular reactive oxygen species (ROS) has been evaluated as an effective treatment strategy for neurodegenerative disorders. Methyl lucidone (MLC) extracted from *Lindera erythrocarpa* Makino (Lauraceae) has been previously reported to exhibit microglial-mediated neuroprotective effects via inhibiting neuroinflammation. However, the antioxidant effects of MLC are still unclear. The aim of this study was to determine the neuroprotective mechanism of MLC in HT-22 neurons against oxidative stress induced by glutamate. In results, the pretreatment of MLC significantly enhanced the viability of HT-22 cells under glutamate-induced oxidative conditions, suggesting that MLC has a neuronal mechanism to protect neurons without microglial regulation. Also, the glutamate effect to increase ROS production was effectively blocked by MLC without any free radical scavenging activity. To induce this antioxidant effect, MLC upregulated the expression of heme oxygenase 1 (HO-1) and nuclear translocation of nuclear factor-E2-related factor 2 (Nrf-2), known as an intracellular antioxidant enzyme, and its transcription factor. Additionally, Akt phosphorylation regulating Nrf-2 was confirmed to be involved in the neuroprotective signaling activated by MLC. These results indicate that MLC may play a role as an antioxidant agent to inhibit neurodegenerative processes via activating antioxidant signaling pathways that include Nrf-2 and phosphatidylinositol 3-kinase (PI3K).

**Gyeong-Im Shin, Sun Young Moon, Song Yi Jeong, Myung Geun Ji, Joon-Yung Cha, Woe-Yeon Kim. Production, characterization, and cross-reactivity of a polyclonal antibody against Arabidopsis TARGET OF RAPAMYCIN. (2019) Appl. Biol. Chem. 62: 68**

TARGET OF RAPAMYCIN (TOR), a member of the phosphatidylinositol 3-kinase-related family of protein kinases, is encoded by a single, large gene and is evolutionarily conserved in all eukaryotes. TOR plays a role as a master regulator that integrates nutrient, energy, and stress signaling to orchestrate development. TOR was first identified in yeast mutant screens, as its mutants conferred resistance to rapamycin, an antibiotic with immunosuppressive and anticancer activities. In *Arabidopsis thaliana*, the loss-of-function *tor* mutant displays embryo lethality, but the precise mechanisms of TOR function are still unknown. Moreover, a lack of reliable molecular and biochemical assay tools limits our ability to explore TOR functions in plants. Here, we produced a polyclonal α-TOR antibody using two truncated variants of TOR (1–200 and 1113–1304 amino acids) as antigens because recombinant full-length TOR is challenging to express in *Escherichia coli*. Recombinant His-TOR1−200 and His-TOR1113−1304 proteins were individually expressed in *E. coli*, and a mixture of proteins (at a 1:1 ratio) was used for immunizing rabbits. Antiserum was purified by an antigen-specific purification method, and the purified polyclonal α-TOR antibody successfully detected endogenous TOR proteins in wild-type Arabidopsis and TOR orthologous in major crop plants, including tomato, maize, and alfalfa. Moreover, our α-TOR antibody is useful for coimmunoprecipitation assays. In summary, we generated a polyclonal α-TOR antibody that detects endogenous TOR in various plant species. Our antibody could be used in future studies to determine the precise molecular mechanisms of TOR, which has largely unknown multifunctional roles in plants.

**Mohammad Khajavian, David A. Wood, Ahmad Hallajsani, Nasrollah Majidian. Simultaneous biosorption of nickel and cadmium by the brown algae *Cystoseria indica* characterized by isotherm and kinetic models. (2019) Appl. Biol. Chem. 62: 69**

Biosorption is an effective way of extracting heavy metal ions from aqueous solutions of various compositions. The brown algae, *Cystoseria indica,* when treated with sodium chloride, demonstrates significant capacity to extract cadmium and nickel, simultaneously, from aqueous solutions. The batch system was running over wide ranges of initial metal ion concentrations (5–150 mg/L), pH (2–6), adsorbent mass (1–4 g/L), and contact times (20–300 min), at a temperature of 25 °C. The results obtained when applying the system in these conditions exhibit higher removal capacities for cadmium than nickel. The optimal conditions of the biosorption process were found as the adsorbent mass of 1 g/L, initial concentration of adsorbates of 100 mg/L and pH of 6. The equilibrium data obtained are better described by the extended-Freundlich isotherm for nickel and cadmium. The maximum biosorption of nickel and cadmium in binary-metal-component system were 18.17 and 55.34 mg/g, respectively. The kinetic data derived from these experiments were evaluated with pseudo-first-order, pseudo-second-order and intra-particle-diffusion kinetic models. Kinetic examination of the equilibrium data derived from these models suggest that the adsorption of nickel and cadmium both follow the intra-particle-diffusion kinetic model.

**Hyeon Ji Song, Jin Ho Lee, Hyun-Cheol Jeong, Eun-Jung Choi, Taek-Keun Oh, Chang-Oh Hong, Pil Joo Kim. Effect of straw incorporation on methane emission in rice paddy: conversion factor and smart straw management. (2019) Appl. Biol. Chem. 62: 70**

Straw incorporation is strongly recommended in rice paddy to improve soil quality and mitigate atmospheric carbon dioxide (CO2), via increasing soil organic carbon (SOC) stock. However, straw application significantly increased methane (CH4) emission during rice cultivation, and then its incorporation area was not expanded effectively. To find the reasonable straw management practice which can reduce CH4 emission without productivity damage, the effect of straw incorporation season and method on CH4 emission was investigated at six different textured paddy fields in South Korea for 2 years. A straw was applied right after rice harvesting in autumn, and the other right before rice transplanting in spring. In the autumn application, straw was applied with two different methods: spreading over soil surface or mixing with soil. Straw application significantly increased seasonal CH4 flux by average 28–122% over 197–590 kg CH4 ha−1 of the no-straw, but its flux showed big difference among straw applications. Fresh straw application before transplanting increased seasonal CH4 flux by approximately 120% over the no-straw, but the autumn application reduced its CH4 flux by 24–43% over 509–1407 kg CH4 ha−1 of the spring application. In particular, the seasonal CH4 flux was approximately 24% lower in straw mixing with soil after autumn harvesting than 423–855 kg CH4 ha−1 in straw spreading over surface. However, CH4 fluxes were not significantly discriminated by soil and meteorological properties in the selected condition. Straw application slightly increased rice grain yield by approximately 4% over the no-straw, but rice productivity was not statistically different among straw applications. Spring straw application increased CH4 intensity which means seasonal CH4 flux per grain yield by the maximum 220% over the no-straw. Autumn straw application significantly decreased CH4 intensity by average 24–65% over the spring straw application. In particular, CH4 intensity in straw mixing with soil treatment was not statistically different with the no-straw. Therefore, autumn straw application with mixing inner soil could be a reasonable straw management practice to decrease CH4 emission impact with improving soil productivity.

**Hyeon Ji Cho, Young Han Lee, Si-Lim Choi, Dong Cheol Seo, Sung Ran Min, Jae-Young Heo. Soil microbial communities of Japanese apricot (*Prunus mume*) orchard under organic and conventional management. (2019) Appl. Biol. Chem. 62: 71**

Organic farming has positive effects on soil microbial population, process, and activity. To examine effects of two different management methods (organic farming vs. conventional farming) on the cultivation of Japanese apricot, contents of fatty acid methyl ester (FAME), total glomalin, and soil chemical properties were analyzed and compared. The organic farming practice resulted in significantly higher contents of organic matter, total FAME, total bacteria, Gram-negative bacteria, arbuscular mycorrhizal fungi, and total glomalin than the conventional farming practice. Soil organic matter showed positive correlation with contents of soil microbial biomass, total bacteria, total glomalin, Gram-positive bacteria, Gram-negative bacteria, actinomycetes, and arbuscular mycorrhizal fungi. In 2018, the organic farming practice resulted in lower ratios of cy17:0 and 16:1ω7c than the conventional farming practice, indicating that microbial stress was reduced by the input of organic fertilizer into soil. Based on principal component analyses (PCA) of soil microbial communities, ratios of cy17:0 to 16:1ω7c in orchid soil can be used as microbial indicators to distinguish organically farmed orchard soil from conventionally farmed orchard soil.

**Peng Lu, Hong-ming Liu, Ai-min Liu.** **Biodegradation of dicofol by *Microbacterium* sp. D-2 isolated from pesticide-contaminated agricultural soil. (2019) Appl. Biol. Chem. 62: 72**

Dicofol is an organochlorine insecticide widely used to prevent pests worldwide. Consequently, serious environmental problems have arisen from the application of dicofol. Bioremediation is an effective solution for dicofol persistence in the environment. In this study, a bacterial strain D-2, identified to genus *Microbacterium,* capable of degrading dicofol was isolated from dicofol-contaminated agricultural soil. This represents the first dicofol degrading bacterium isolated from this genus. *Microbacterium* sp. D-2 degraded 50 mg/L dicofol within 24 h at a rate of 85.1%. Dicofol was dechlorinated by D-2 and the further degradation metabolite was indentified as p,p′-dichlorobenzophenone(DCBP). Soils inoculated with *Microbacterium* sp. D-2 degraded 81.9% of the dicofol, while soils without D-2 only degraded 20.5% of the dicofol present. This finding suggests that strain D-2 has great potential in bioremediation of dicofol-contaminated soils.

**Sung Jong Lee, Hong Joo Ha, Eun Hea Jho.** **Assessing ecotoxicological effects of 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, and 2,3,4,7,8-PeCDF in soil using *Allivibrio fischeri*. (2019) Appl. Biol. Chem. 62: 73**

The toxic effect of dioxins and dioxin-like compounds have largely been studied using in vivo techniques; however, in vivo studies can be limited when rapid screening is required. Microtox® can be used as a rapid ecotoxicity assessment tool for dioxins in the environment, but studies on the assessment of dioxins and dioxin-like compounds using bioluminescent bacteria *Allivibrio fischeri* are limited. This study investigated the potential of using *A. fischeri* for assessing different dioxins and dioxin-like compounds, and the toxic effects of soils contaminated with dioxins and dioxin-like compounds were tested using different fractions of dioxins in soil to determine the appropriate way of assessing the toxic effects of contaminated soils. The results show that *A. fischeri* can potentially be used as a test species for rapidly evaluating toxic effects of dioxins and dioxin-like compounds in the environment. With the soil used in this study, the toxic effects of the water extracts (i.e., mobile fraction of dioxins) and the soil slurries (i.e., bioavailable fraction of dioxins) were similar to that of the controls. This suggests that the toxicity assessment of the organic extracts (i.e., total amount of dioxins) can be inappropriate in a managerial perspective, as the mobile or bioavailable fraction of contaminants in soils is often more of concern than the total amount of contaminants present in soils. Overall, when *A. fischeri* are to be used for a rapid toxicity assessment of dioxins-contaminated soils, different fractions of dioxins need to be assessed for better management of the contaminated soils.

**Ki-Beom Moon, Hyunjun Ko, Ji-Sun Park, Jung-Hoon Sohn, Hye-Sun Cho, Youn-il Park, Hyun-Soon Kim, Jae-Heung Jeon.** **Expression of Jerusalem artichoke (*Helianthus tuberosus* L.) fructosyltransferases, and high fructan accumulation in potato tubers. (2019) Appl. Biol. Chem. 62: 74**

Fructans are polymers of fructose that are present as storage carbohydrates in various plants. Jerusalem artichoke (*Helianthus tuberosus* L.) contains a high amount of inulin. Two enzymes are involved in inulin biosynthesis. The sucrose:sucrose 1-fructosyltransferase (1-SST) enzyme mainly catalyzes the synthesis of 1-kestose from sucrose. In the next step, fructan:fructan 1-fructosyltransferase (1-FFT) catalyzes the synthesis of inulin from 1-kestose. In this study, the *Ht1*-*SST* and *Ht1*-*FFT* genes were isolated from Jerusalem artichoke and expressed in potato (*Solanum tuberosum* L.), either separately or together, via *Agrobacterium*-mediated transformation. Transgenic potato tubers overexpressing *Ht1*-*SST* accumulated 1-kestose to a high level (up to 3.36 mg/g), while tubers overexpressing both *Ht1*-*SST* and *Ht1*-*FFT* accumulated up to 3.14 mg/g short-chain inulin-type fructans, with the degree of polymerization (DP) ranging from 3 to 5, excluding high DP inulins. Transgenic potato plants accumulated fructo-oligosaccharides to a high level, following the fructan biosynthetic pathway of Jerusalem artichoke, and therefore present a high potential for the mass production of inulin through established potato breeding and cultivation methods.