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**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.

**Mi Sun Cheong, Young-Eun Yoon, Jin Wook Kim, Young Kyu Hong, Sung Chul Kim, Yong Bok Lee.** **Chlortetracycline inhibits seed germination and seedling growth in *Brassica campestris* by disrupting H2O2 signaling. (2020) Appl. Biol. Chem. 63: 1**

Antibiotics have been identified as a new type of environmental contaminant because of their increased use in farm animal production systems. Those drugs that animals are not absorbed mostly are excreted in the feces and urine and contaminates soils. However, the effects of antibiotics on crop plants are still largely unknown. In this study, we determined the effects of chlortetracycline (CTC), a veterinary drug released into the agricultural field by grazing animals or through manure application, on the growth and physiology of *Brassica campestris* seedlings. Differently from animals, *Brassica campestris* seedlings have accumulated 5–10-fold higher CTC during cultivation rather than excretion. Morphologically, CTC delays seed germination and inhibits seedling growth such as shortening primary root length and decreasing chlorophyll level. At the molecular level, CTC accumulation in plants downregulated the expression of *superoxide dismutase* (SOD) genes and decreased the production of hydrogen peroxide (H2O2). Since H2O2 is one of the signaling components involved in the regulation of root growth, exogenous application of H2O2 partially restored the growth and physiology of CTC-treated seedlings. These results suggest that application of CTC-containing manure or compost to soil delays seed germination and inhibits plant growth.

**Adriana Rivera-Piza, Sung-Joon Lee.** **Effects of dietary fibers and prebiotics in adiposity regulation via modulation of gut microbiota. (2020) Appl. Biol. Chem. 63: 2**

The microbiota is indispensable for human health and the regulation of various body functions, including energy metabolism. The harmonic crosstalk between the microbiota and the intestinal epithelial barrier determines gut homeostasis and health status in the healthy subject. Obesity and type 2 diabetes risk are, to some extent, explained by alterations in the microbiota. Since recent data indicate that the population of gut microorganisms can influence nutrient absorption and energy storage thus prevalence on obesity and metabolic disorders. Moreover, metabolic disease conditions, such as obesity, may be stimulated by genetic, environmental factors and by pathways that link metabolism with the immune system. On the basis of the above considerations, this review compiles the current results obtained in recent studies indicating the gut microbiota contribution to obesity development.

**Hyun Young Hwang, Seong Heon Kim, Myung Sook Kim, Seong Jin Park, Chang Hoon Lee.** **Co-composting of chicken manure with organic wastes: characterization of gases emissions and compost quality. (2020) Appl. Biol. Chem. 63: 3**

Co-composting of organic wastes is globally recognized to be effective method to dispose two or more wastes at once and minimize drawbacks of composting such as gases emissions and nutrient reduction. In this study, pilot-scale experiments were conducted to characterize the co-composting process of chicken manure with cow manure (CC), swine manure (CS), plant residues plus mushroom media (CRM), on emissions of greenhouse gas, and ammonia, compost quality, maturity and their correlations. The results showed that cumulative flux of carbon dioxide (CO2), methane (CH4), nitrous oxide (N2O) and ammonia (NH3) widely ranged like 38,211–50,830, 172–417, 98–142 and 118–927 g kg dm−1 day−1 respectively. It indicated the importance of selection for co-composting material. The NH3 emission was significantly increased by 4.3–7.9 times in CS and CRM, compared to OC and CC. Both of CS and CRM also showed longer thermophilic phase and later maturation were also observed in both treatments. Temperature was positively correlated with gases (*P*< 0.001) except CH4, and nitrogen content, C/N ratio and nitrate nitrogen significantly affected emission of carbon and nitrogen (*P*< 0.001). In conclusion, for chicken manure composting, sole chicken manure or combination with cow manure could be suitable composting method to improve compost quality and minimize gases losses.

**Karthika Muthuramalingam, Moonjae Cho, Youngmee Kim.** **Role of NAPDH oxidase and its therapeutic intervention in TGF-β-mediated EMT progression: an in vitro analysis on HeLa cervical cancer cells. (2020) Appl. Biol. Chem. 63: 4**

Epithelial to mesenchymal transition (EMT) is a complex biological event, wherein polarized epithelial cells lose their integrity resulting in a mesenchymal phenotype with enhanced motility, a phenomenon known as metastasis. However, the underlying mechanisms of EMT are still poorly understood in cervical carcinomas. In this study, we investigated the molecular signalling events responsible for the effect of TGF-β, a potent inducer of EMT, on HeLa cervical cancer cells. We observed that TGF-β treatment (5 ng/mL) upregulates the expression of EMT-associated transcription factors such as Snail and Slug and downregulates the expression of epithelial markers such as ZO-1 and E-cadherin. Furthermore, treatment with TGF-β activates both Smad-dependent and Smad-independent signaling pathways, which subsides upon addition of Diphenyleneiodonium (DPI), a potent ROS inhibitor that inhibits NAPDH oxidase (NOX). TGF-β treatment enhanced cellular migration and invasion ability was diminished in the presence of ROS inhibitors. In addition, we also observed that ROS-mediated, TGF-β-induced EMT progression was inhibited using therapeutic candidates that target the key signal transduction mediators, including PI3K/AKT, ERK, and P38/MAPK. Accordingly, we demonstrated the involvement of redox biology (NOX2 and NOX4 mediate migration and invasion) in TGF-β-mediated EMT advancement and explored suitable therapeutic interventions.

**Wisarut Payoungkiattikun, Anupong Joompang, Suyanee Thongchot, Boonpob Nowichai, Nisachon Jangpromma, Sompong Klaynongsruang.** **Evidence of multi-functional peptide activity: potential role of KT2 and RT2 for anti-inflammatory, anti-oxidative stress, and anti-apoptosis properties. (2020) Appl. Biol. Chem. 63: 5**

Although several explications of anti-inflammatory therapeutic substances for treating inflammatory-related diseases have been broadly discussed within the last few decades, peptide-based compounds display the potential to be novel inflammation treatment agents. Here, we evaluated the anti-inflammatory activity and other inflammation-associated activities, including anti-oxidative stress and anti-apoptosis properties, of the cationic peptides KT2 and RT2. Nitric oxide (NO) and other inflammatory markers were evaluated in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells co-incubated with peptides. The levels of interrelated gene and protein expressions were quantified. Peptides formed complexes with LPS and displayed anti-inflammatory properties by reducing NO and pro-inflammatory cytokine production in inflamed RAW 264.7 cells. These peptides also exhibit a strong suppressive effect on mRNA expression levels of inducible nitric oxide synthase, tumor necrosis factor-α, signal transducer and activator of transcription 1, c-Jun N-terminal protein kinase (JNK)-1, nuclear factor kappa B (NF-κB), and p38 mitogen-activated protein kinase (MAPK), which affects the decay of phosphorylated JNK-1, p38 MAPK, and NF-κB p65 protein expression. Both peptides induce up-regulation of anti-inflammatory mRNA and protein expression levels of extracellular signal-regulated kinase and mRNA expression levels of MAPK phosphatase-1. Also, the production of reactive oxygen species was observed to be markedly reduced. Furthermore, peptides exhibited an anti-apoptotic property. To our knowledge, this is the first report of the multi-functional peptides KT2 and RT2 exerting broad biological activity related to anti-inflammatory effects. These peptides have potential for delivering a medical method for the handling of inflammation-related diseases.

**Seyyed Mohammad Hossein Razavian, Ali Kashfi, Zohreh Khoshraftar.** **Purification of bovine liver transglutaminase by gel filtration. (2020) Appl. Biol. Chem. 63: 6**

Transglutaminases (TGases) are enzymes that catalyze transfer of acyl group and covalent crosslinks formation between peptide-bound glutaminyl residues and amino groups. TGases have many industrial applications and have been purified from various sources. TGase was purified from the bovine liver extract by gel filtration on Sephacryl S-200 HR column. TGase activity was measured using CBZ-L-glutaminylglycine & hydroxylamine and the enzyme was characterized with respect to its response to different temperatures, pHs and salt concentrations. TGase was purified by yield 36.7%, had a weight 74 kDa, a high pH (pH = 8) and temperature (45 °C) optimum. The enzyme was observed to be stable at temperatures below 55 °C and was stable within a narrow pH range of 6.5–8.0. Purified TGase showed Ca2+ dependent characteristics and tended to retain activity at a high NaCl concentration. These results revealed that purified TGase can be used as a potential alternative to other sources.

**Tanyarath Utaipan, Piyawan Boonyanuphong, Thipphawan Chuprajob, Apichart Suksamrarn, Warangkana Chunglok.** **A trienone analog of curcumin, 1,7-bis(3-hydroxyphenyl)-1,4,6-heptatrien-3-one, possesses ROS- and caspase-mediated apoptosis in human oral squamous cell carcinoma cells in vitro. (2020) Appl. Biol. Chem. 63: 7**

The leading causes of oral cancer treatment failure are cancer metastasis and chemotherapeutic resistance. Thus, developing novel anticancer agents that are effective against those aggressive cancer cells would be important for complementary or alternative treatments. The objective of this study was to investigate cytotoxicity and anticancer mechanisms of a synthetic trienone analog of curcumin, 1,7-bis(3-hydroxyphenyl)-1,4,6-heptatrien-3-one (trienone 11), against human oral squamous cell carcinoma (OSCC) cells exhibiting multidrug resistance (CLS-354/DX). The study of cytotoxicity showed that trienone 11 exerted threefold stronger cytotoxicity to CLS-354/DX cells than curcumin. Trienone 11 (15–30 μM) markedly induced intracellular reactive oxygen species (ROS) resulting in apoptotic cell death within 24 h, through activation of caspase-3/7 and caspase-9. A ROS inhibitor, N-acetylcysteine (NAC) prevented apoptotic cell death via decreasing caspase activation. Thus, the cytotoxicity of trienone 11 against CLS-354/DX cells was ROS-mediated intrinsic apoptosis. Overall, trienone 11 could be an interesting lead for developing anti-cancer agents against multidrug resistant OSCC cells.

**Yinfeng Xia, Ming Zhang, Daniel C. W. Tsang, Nan Geng, Debao Lu, Lifang Zhu, Avanthi Deshani Igalavithana, Pavani Dulanja Dissanayake, Jörg Rinklebe, Xiao Yang, Yong Sik Ok.** **Recent advances in control technologies for non-point source pollution with nitrogen and phosphorous from agricultural runoff: current practices and future prospects. (2020) Appl. Biol. Chem. 63: 8**

Eutrophication of natural water is a universal problem. Nitrogen (N) and phosphorus (P) from agricultural runoff are the main sources of nutrient input, provided that emissions from industrial point sources (IPS) are under control. Therefore, it is of great environmental importance to reduce pollution associated with agricultural runoff as a means of regulating eutrophication levels in natural water. Numerous methods proposed for treating agricultural runoff can be classified into three categories: source control, process control, and end treatment. In this review, major technologies for N and P control from agricultural runoff are summarized along with discussion of newly proposed technologies such as biochar biomimetics and microbial catalyst. Because agricultural runoff (from farmlands to receiving waters) is a complicated pollution process, it is difficult to regulate the nutrients discharged via such process. This review will thus offer a comprehensive understanding on the overall process of agricultural runoff and eutrophication to help establish control strategies against highly complicated agricultural non-point sources.

**Sokho Kim, Seo-Hyun Ahn, Jong-Heum Park, Chan Hum Park, Yu Su Sin, Gee-Wook Shin, Jungkee Kwon.** **Anti-adipogenic effects of viscothionin in 3T3-L1 adipocytes and high fat diet induced obesity mice. (2020) Appl. Biol. Chem. 63: 9**

*Viscum album* subsp. *Coloratum*, also known as Korean mistletoe, is a traditional herb that has more recently been used for the treatment of nervine, hypertensive and cardiovascular diseases. Therefore, this study was undertaken to access the anti-obesity effect of Korean mistletoe-derived polypeptide viscothionin using 3T3-L1 adipocytes in vitro and in vivo mouse experimental model. Viscothionin (up to 5 μM) was used to treat mouse 3T3-L1 pre-adipocytes during adipocyte differentiation. Adipocyte differentiation in 3T3-L1 cells was confirmed by Oil Red O staining. Obesity was induced by a high-fat diet (HFD) in C57BL/6J mice, followed by oral administration of viscothionin (up to 10 mg/kg) for 3 weeks. As a result, viscothionin (5 μM) inhibited differentiation of adipocyte cells and attenuated accumulation of intracellular lipids through activation of 5′-adenosine monophosphate-activated protein kinase (AMPK), by down-regulating phosphorylation in AKT and glycogen synthase kinase 3β (GSK3β). Treatment of viscothionin also decreased the levels of sterol regulatory element binding protein-1 (SREBP-1) and its target gene, fatty acid synthase (FAS). Moreover, viscothionin (10 mg/kg) significantly suppressed body weight and fat content, and improved serum lipid concentration, compared with the standard drug simvastatin (10 mg/kg), a well-known anti-obesity agent. The present study suggests, that viscothionin exerts anti-adipogenic effect through the activation of AMPK and has potential to prevent HFD-induced obesity.

**N. H. M. Rubel Mozumder, Yeong-Ran Lee, Kyeong Hwan Hwang, Min-Seuk Lee, Eun-Hee Kim, Young-Shick Hong.** **Characterization of tea leaf metabolites dependent on tea (*Camellia sinensis*) plant age through 1H NMR-based metabolomics. (2020) Appl. Biol. Chem. 63: 10**

The chemical or metabolic compositions of tea (*Camellia sinensis*) varies according to numerous factors, such as geographical origin, cultivar, climate, plucking position, and horticultural practices. However, how the age of tea plants affects the metabolite compositions of tea leaves has not been reported yet. Therefore, we extended the metabolomic approach to the investigation of the age-related differences of tea leaf metabolites in the fresh leaves collected from tea plants aged 8 and 25 years. Multivariate statistical analysis with comprehensive metabolite profiles analyzed by 1H NMR spectroscopy showed the clear metabolic differentiation between the fresh tea leaves from different ages of the tea plants. Of the various tea leaf metabolites varied according to the age of the tea plants, theanine, glutamine, catechin, and gallocatechin were uniquely dependent on the age of tea plants, demonstrating a difference of theanine metabolism between young and old tea plants. These results suggest that leaves from 25-year-old tea plants would still be worthy as a functional ingredient for the production in the food or cosmetic industry rather than quality-enhanced tea infusions for human consumption.

**Hwa-Kyung Lee, Junghak Lee, Jonghwa Lee, Hyeri Lee, Jeong-Han Kim.** **Metabolite profiles of live or dead carp (*Cyprinus carpio*) exposed to endosulfan sulfate using a targeted GC–MS analysis. (2020) Appl. Biol. Chem. 63: 11**

Endosulfan sulfate is a major oxidized metabolite of endosulfan, which is a broad-spectrum chlorinated cyclodiene insecticide. In this study, GC–MS-based metabolic profiles of dead or live carp (*Cyprinus carpio*) exposed to endosulfan sulfate were investigated to elucidate the molecular toxicological effects of endosulfan sulfate on carp. Three different extraction methods were compared, and a 50% methanol solution was chosen as an efficient extraction method. Carp was exposed to endosulfan sulfate at a concentration of 8 ppb for 2 days. After exposure, the whole body of the fish was homogenized with liquid N2, extracted with the 50% methanol solution and dried before TMS derivatization for GC–MS analyses of the dead and live carp. A SIM (selected ion monitoring)-library of 373 metabolites was applied after GC–MS analysis to detect 146 metabolites in carp. Based on the one-way ANOVA results (P < 0.001) and fold changes of metabolites in dead carp versus control (fold change > 1.5 or < 0.667), 30 metabolites were identified as biomarkers that were significantly different in the metabolic profiles among the control, dead and live carp. A metabolic pathway analysis using MetaboAnalyst 4.0 revealed that those biomarkers were important for the living or death response to endosulfan sulfate. The pathways indicated by the metabolic pathway analysis included starch and sucrose metabolism, galactose metabolism, glycerolipid metabolism, the citrate cycle and linoleic acid metabolism. These results suggest that these pathways underwent significant perturbations over the exposure period.

**Adetunji Ajibola Awe, Beatrice Olutoyin Opeolu, Olalekan Siyanbola Fatoki, Olushola Sunday Ayanda, Vanessa Angela Jackson, Reinette Snyman.** **Preparation and characterisation of activated carbon from *Vitisvinifera* leaf litter and its adsorption performance for aqueous phenanthrene. (2020) Appl. Biol. Chem. 63: 12**

The adsorption of phenanthrene onto activated carbons produced from *Vitisvinifera* leaf litter (a waste plant biomass) was investigated in this study. Zinc chloride (ZnCl2) and phosphoric acid (H3PO4) were utilised as activating agents in producing the activated carbons. The characterisation of the activated carbons was achieved with Fourier transform infrared spectroscopy (for surface functional groups), scanning electron microscopy (for surface morphology) and Brunauer–Emmett–Teller (BET) (for surface area determination). The adsorption of phenanthrene onto the activated carbons was optimised in terms of solution pH, adsorbent dosage, initial concentration of adsorbate solution and contact time. Experimental results showed that H3PO4 modified activated carbon gave better yield (up to 58.40%) relative to ZnCl2 modified activated carbon (only up to 47.08%). Meanwhile, surface characterisation showed that ZnCl2 modification resulted in higher BET surface area (up to 616.60 m2/g) and total pore volume (up to 0.289 cm3/g) relative to BET surface area of up to 295.49 m2/g and total pore volume of up to 0.185 cm3/g obtained from H3PO4 modified activated carbons. Adsorption equilibrium data fitted well into Freundlich isotherm model relative to other applied isotherm models, with maximum Kf value of 1.27 for ZnCl2 modified activated carbon and 1.16 Kf value for H3PO4 modified activated carbon. The maximum adsorption capacity for ZnCl2 and H3PO4 activated carbons for the removal of phenanthrene were 94.12 and 89.13 mg/g, respectively. Kinetic studies revealed that dynamic equilibrium was reached at 80 min contact time. Experimental data fitted best into the Elovich kinetic model relative to other kinetic models, based on the correlation coefficient (*R*2) values obtained from kinetic studies. Chemisorption was deduced as a major phenanthrene removal pathway from aqueous solution and the physicochemical characteristics of the adsorbents have major influence on phenanthrene removal efficiencies.

**Aro Lee, Cao Son Trinh, Won Je Lee, Minseo Kim, Hyeri Lee, Duleepa Pathiraja, In-Geol Choi, Namhyun Chung, Changhyun Choi, Byung Cheon Lee, Hojoung Lee.** **Characterization of two leaf rust-resistant *Aegilops tauschii* accessions for the synthetic wheat development. (2020) Appl. Biol. Chem. 63: 13**

*Aegilops tauschii* (*Ae. tauschii*) is a diploid (2n = 2x = 14) wild grass species, which has been reported as the progenitor of hexaploid wheat (*Triticum aestivum*) with D-genome. In this study, 68 *Ae. tauschii* accessions with diverse geographical backgrounds were investigated for their resistance to infection by the leaf rust fungi *Puccinia triticina*. Two *Ae. tauschii* accessions that exhibited hyper-resistance to leaf rust at both seedling and adult stages were identified. Utilizing two susceptible *Ae. tauschii* ecotypes and keumkang, a common Korean wheat cultivar known to be susceptible to leaf rust, as the negative control, further investigations were conducted for understanding the mechanism underlying immunity to leaf rust disease of these two resistant accessions. Resistant accessions displayed the increased β-1,3-glucanase activity to prevent fungal penetration and the better peroxidase activity to cope with leaf rust-induced oxidative stress. Moreover, transcriptional analyses reveal the important role of the LRR receptor-like serine/threonine-protein kinase FLS2 (*lrr*) to the disease resistance of the two ecotypes. *Ae. tauschii* is a remarkable genetic source, especially for abiotic and biotic stress resistance genes, as the plant is known for its wide-ranging geographical habitat and adaptability to different environments. This, combined with the fact that *Ae. tauschii* and wheat share a close evolutionary relationship, is indicative of the immense benefit of using *Ae. tauschii* as a material for improving the quality of synthetic wheat. Our aim was to identify and evaluate the strongest *Ae. tauschii* contenders for breeding leaf rust-resistant synthetic wheat.

**Jinhoon Jang, Sanjida Khanom, Youngkook Moon, Sooim Shin, Ok Ran Lee.** **PgCYP76B93 docks on phenylurea herbicides and its expression enhances chlorotoluron tolerance in *Arabidopsis*. (2020) Appl. Biol. Chem. 63: 14**

The phenylurea herbicides are used to control annual and perennial weeds on crop cultivating fields. The excessive usage of these agrochemicals increase many environmental problems. Thus, engineering transgenic plant for herbicide metabolism can provide efficient and eco-friendly means for enhanced phytoremediation capacity. Cytochrome P450 enzymes comprise one of the major plant enzyme families that mediate the oxidative degradation of xenobiotic chemicals, including herbicides. Considering these notions, phytoremediation properties of transgenic ginseng-derived *PgCYP76B93* in *Arabidopsis* to phenylurea herbicides were assessed. Phylogenetic tree of PgCYP76B93 clustered in between close to the herbicide metabolism-related enzyme families and terpenoid biosynthesis-related. The expression of *PgCYP76B93* was considerably upregulated upon treatment with phenylurea herbicide, chlorotoluron. Simulated docking using Autodoc program predicted possible interaction with chlorotoluron. Transgenic *Arabidopsis* plants overexpressing *PgCYP76B93* were resulted in slightly reduced plant height with relatively small leaves. The lower plant height in the *PgCYP76B93*-overexpressing line than in the control revealed that it was linked to the expression of gibberellin oxidases (*GAox*). The bioassay of transgenic plants growing on herbicide-containing media revealed enhanced resistance against chlorotoluron.

**Yingshu Peng, Guibin Wang, Fuliang Cao, Fang-Fang Fu.** **Collection and evaluation of thirty-seven pomegranate germplasm resources. (2020) Appl. Biol. Chem. 63: 15**

Pomegranates *(Punica granatum* L.) are gaining popularity among consumers because of their high antioxidant activity and multiple medical benefits. China is rich in pomegranate genetic resources, but how to use them effectively is a problem worthy of deep consideration. In this article, thirty-seven pomegranate varieties from seven provinces in China were collected and analyzed for twelve phenotypic traits and twelve biochemical indicators (seeds and juices). The fruit and aril fresh weight ranged between 210.5 and 576.5 g and 121.0 to 327.5 g, respectively, and the edible rate (42.58–64.80%), seed weight (1.80–3.41 g), seed number (249.1–838.9), fruit height (10.51–15.48 mm), fruit diameter (11.46–17.50 mm), skin thickness (2.14–6.98 mm), and shape index (0.82–0.96) varied among the different genotypes. The pomegranate juice total phenolic content ranged from 40.91 to 132.47 µg/mL, and the total flavonoid content (14.08–137.72 µg/mL), vitamin C content (12.80–66.63 µg/mL), pH (3.10–4.34), total soluble solids (13.13–17.50°Brix), and titratable acidity (0.26–2.71%) also varied; the pomegranate seed total phenolic content ranged from 0.62 to 1.78 mg/g, and the total flavonoid content (0.39–0.99 mg/g), vitamin C content (7.55–13.90 mg/g), DPPH radical scavenging capacity (85.98–98.24%), and ABTS scavenging ability (28.72–51%) were also measured. The coefficients of variation of the studied traits ranged from 5.62 to 54.02%, and the phenotypic traits’ Shannon–Weaver diversity indexes ranged from 0.67 to 1.53. Cluster analysis divided the 37 varieties into three categories, providing a reference for improved variety breeding. In addition, genotypic and environmental effects mainly affected the pomegranate flavor and antioxidant activity, respectively.

**Worku Dinku, Johan Isaksson, Fredrik Garnås Rylandsholm, Petr Bouř, Eva Brichtová, Sang Un Choi, Sang-Ho Lee, Young-Sik Jung, Zae Sung No, John Sigurd Mjøen Svendsen, Arne Jørgen Aasen, Aman Dekebo.** **Anti-proliferative activity of a novel tricyclic triterpenoid acid from *Commiphora africana* resin against four human cancer cell lines. (2020) Appl. Biol. Chem. 63: 16**

Myrrh, a resin derived from the damaged bark of *Commiphora* genus, has traditionally been used for treatment of various human diseases, such as amenorrhea, ache, tumors, fever, and stomach pains. In spite of this widespread use of the myrrh in Ethiopia, the pharmacological activity and chemical composition have not been studied in detail. A new tricyclic triterpene acid (3*S*,4*S*,14*S*,7*E,*17*E*,21*Z*)-3,30-dihydroxypodioda-7,17,21-trien-4-carboxylic acid (commafric A) has been isolated from a crude methanolic extract of *Commiphora africana* (A. Rich.) Engl. resin along with the known pentacyclic triterpene α-amyrin. The structure of commafric A was characterized using different spectroscopic techniques such as 1D and 2D NMR, IR, and VCD combined with computations. The anti-proliferative activity of both isolated compounds was evaluated using SRB based colorimetric cellular assay against four human cancer cell lines. Etoposide was used as a positive control. Commafric A showed significant anti-proliferative effects against non-small cell lung cancer (A549) with IC50 values of 4.52 μg/ml. The pentacyclic triterpene α-amyrin showed a weak anti-proliferative activity against A2780 (ovarian cancer), MIA-PaCa-2 (pancreatic cancer), and SNU638 (stomach cancer) cell lines tested with IC50 values ranging 9.28 to 28.22 μg/ml. Commafric A possessed anti-proliferative activity against non-small cell lung cancer (A549), which suggests that commafric A has potential to be further optimized being a lead compound in the search for new drugs against cancer diseases.

**Anamika Khanal, Ji-Hoon Lee.** **Functional diversity and abundance of nitrogen cycle-related genes in paddy soil. (2020) Appl. Biol. Chem. 63: 17**

The nitrogen cycle and the associated microbes play an important role in natural ecosystems, including terrestrial habitats; they also have a major effect on climate change. The aim of this study was to explore microbial communities in rice paddy soil by detecting and quantifying some key functional genes involved in the nitrogen cycle using molecular techniques such as conventional polymerase chain reaction (PCR), clone library construction, sequencing, phylogenetic analysis, and real-time PCR. The genes analyzed were as follows: nitrogenase reductase gene (*nif*H), hydrazine synthase gene (*hzs*A), nitrous oxide reductase gene (*nos*Z), copper-containing (*nir*K) and cytochrome cd1-containing (*nir*S) nitrite reductase genes, nitrite oxidoreductase gene (*nxr*B), and ammonium monooxygenase gene (*amo*A). The sequence assessment using the clone library targeting these genes revealed high diversity and dominance of bacterial communities. Furthermore, real-time PCR using SYBR green dye and some primers specific for each gene revealed the high abundance of *nxr*B (4.1 × 109 ± 0.4 × 109 copies g−1 soil) and low abundance of *hzs*A (4.0 × 105 ± 1.1 × 105 copies g−1 soil). The findings of our study will be useful to explore microbial communities in terrestrial habitats, such as agricultural paddy fields.

**Cuiping Li, Weidong Jiang, Yang Zhou, Xuanping Huang, Nuo Zhou.** **PF4V1 affects the progression of oral squamous cell carcinoma by regulating Wnt/β-catenin pathway and angiogenesis. (2020) Appl. Biol. Chem. 63: 18**

Platelet factor-4 variant 1 (PF4V1) was recently described as a natural non-allelic gene variant of platelet factor-4 (PF4), which has been closely associated with the growth and metastasis of various cancers. Our previous research showed that PF4V1 was related to oral squamous cells carcinoma (OSCC) metastasis. Howerver, it is still not clear about the functional role of PF4V1 in OSCC. In this study, stably transfected cell lines were constructed and the expression level of PF4V1 was verified by real‐time polymerase chain reaction (RT-PCR) and western blot. The effect of PF4V1 on proliferation, migration, invasion, and apoptosis of oral cancer (OC) cells were detected. Moreover, a xenograft tumor model was constructed to evaluate the effect of PF4V1 on OSCC in vivo. Indicators of Wnt/β-catenin, angiogenesis and epithelial-mesenchymal transition (EMT) pathways were also examined. Stable cell lines with overexpression and inhibited expression of PF4V1 were constructed successfully. After stable transfection, PF4V1 significantly promoted the proliferation, migration, and invasion of OC cells in vitro, and their tumor formation in vivo. Furthermore, PF4V1 remarkably promoted the expression of β-catenin, VEGF, and FGF but suppressed the expression of GSK-3β. There was no statistically significant correlation between PF4V1 and EMT pathway. This study provides evidence that PF4V1 promotes the proliferation, migration, invasion and tumor formation of OC cells by regulating the Wnt/β-catenin pathway and angiogenesis. Our findings suggest that PF4V1 could be a very promising target of OSCC therapy in the future.

**Do-Won Park, Ga-yeon Lim, Young-duck Lee, Jong-Hyun Par.** **Characteristics of lytic phage vB\_EcoM-ECP26 and reduction of shiga-toxin producing *Escherichia coli* on produce romaine. (2020) Appl. Biol. Chem. 63: 19**

Foodborne Shiga toxin-producing *Escherichia coli* (STEC) cause severe diarrhea and hemolytic uremic syndrome (HUS) in humans. However, traditional methods for STEC sterilization are difficult to apply to fresh food. To control the pathogen, phage infecting *E. coli* O157:H7 were isolated and characterized. The isolated phage vB\_EcoM-ECP26 had an icosahedral head and a contractile tail, and was classified as belonging to the *Myoviridae* family. The phage showed a broad host range against STEC and exhibited a large burst size of 1914 PFU/cell. The phage was highly stable at high temperatures (65 °C) and wide ranges of pH (4–10). The genome of vB\_EcoM-ECP26 consists of 136,993 nucleotides, 214 open reading frames, and does not contain lysogenicity-related genes. Phylogenetic analysis showed that vB\_EcoM-ECP26 is a V5-like species. STEC O157 growth was inhibited by vB\_EcoM-ECP26 for 8 h. Furthermore, this phage not only significantly decreased the STEC population (p < 0.05), but also persisted in fresh lettuce at 4 °C for 5 days. Therefore, these results reveal that the novel lytic phage vB\_EcoM-ECP26 could be a useful agent for the control of foodborne STEC.

**Jong-Hwan Park, Su-Lim Lee, Se-Wook Hwang, Ju-Hyun Eom, Seong-Heon Kim, Se-Won Kang, Ju-Sik Cho, Dong-Cheol Seo.** **Characteristics of ammonia gas emissions from soybean cultivation soils treated with mixed microorganisms. (2020) Appl. Biol. Chem. 63: 20**

This study was conducted to evaluate (i) the characteristics of ammonia gas emissions from soybean cultivation soils amended with varying levels of urea and soil water, and (ii) the rate of reduction in ammonia emissions that could be obtained by applying mixed microorganisms (MM) to the urea-treated soils. The ammonia gas emissions from all treatments except the control were highest on day 2 of a laboratory-scale experiment and decreased gradually thereafter. The ammonia gas emissions from the soils increased with increasing urea and soil water contents. However, there were less emissions from soils treated with MM than those from the urea only treatment, and emissions also decreased significantly as the concentration of MM increased. In a field-scale experiment, the total cumulative emissions of ammonia from soil treated with a combination of chemical fertilizers and MM was reduced to 85.8% of that from the soil treated with chemical fertilizers only. Although we infer that MM can be used as an agent to reduce ammonia gas emissions from actual soils used for soybean cultivation, our knowledge of the processes involved in reducing ammonia emissions using microbial treatment is still limited. Consequently, further studies are required to investigate the efficient control of ammonia gas emissions from agricultural soils through the application of microorganisms.

**Ying-Hao Han, Dong-Qin Chen, Mei-Hua Jin, Ying-Hua Jin, Jing Li, Gui-Nan Shen, Wei-Long Li, Yi-Xi Gong, Ying-Ying Mao, Dan-Ping Xie, Dong-Seok Lee, Li-Yun Yu, Sun-Uk Kim, Ji-Su Kim, Taeho Kwon, Yu-Dong Cui, Hu-Nan Sun.** **Anti-inflammatory effect of hispidin on LPS induced macrophage inflammation through MAPK and JAK1/STAT3 signaling pathways. (2020) Appl. Biol. Chem. 63: 21**

Severe inflammatory reactions caused by macrophage activation can trigger a systemic immune response. In the present study, we observed the anti-inflammatory properties of hispidin on LPS induced RAW264.7 macrophage cells. Our results showed that hispidin treatment significantly reduced the production of cellular NO, IL-6 and reactive oxygen species (ROS) while has not inhibitory effect on TNF-α productions. Excitingly, hispidin treatment retains the phagocytosis ability of macrophages which enabling them to perform the function of removing foreign invaders. Signaling studies showed, hispidin treatment dramatic suppressed the LPS induced mitogen activated protein kinases (MAPK) and JAK/STAT activations. In conclusion, our findings suggest that hispidin may be a new therapeutic target for clinical treatment of macrophages-mediated inflammatory responses.

**Seungyeop Baek, Sohui Park, Jisu Shin, Jun-Seok Lee, Hye Yun Kim, Gyoonhee Han, YoungSoo Kim.** **Investigation of memory-enhancing botanical mixture and their isolated compounds for inhibition of amyloid-β and tau aggregation. (2020) Appl. Biol. Chem. 63: 22**

Alzheimer’s disease (AD) is a neurodegenerative disease characterized by the abnormal assembly of amyloid-β (Aβ) and tau aggregates in the brain. When Aβ and tau proteins misfold, progressive brain cell death, synaptic loss, atrophy, and cognitive decline are observed. Here, we report that the memory-enhancing botanical natural product mixture, HX106N, efficiently inhibits formation of Aβ oligomers and fibrils and aggregation of tau. HX106N is a botanical mixture extract of *Dimocarpus longan*, *Liriope platyphylla*, *Salvia miltiorrhiza* and *Gastrodia elata*. In previous clinical studies, HX106N showed increased working memory performances of individuals of subjective memory complaints. However, the drug mechanism and responsible ingredients of HX106N has been unclear yet. In this study, we expanded the investigation of the drug mode of action to the single chemical level by identifying four active components of HX106N, among 14 isolated molecules, with significant inhibitory function against Aβ aggregation. We found that salvianolic acids A, B, E and rosmarinic acid, isolated from the botanical mixture, have potency to inhibit the protein misfolding.

**Jaewhan Kim, Namjoon Cho, Eun-Mi Kim, Ki-Sun Park, Yeon Woo Kang, Joong Hyeon Nam, Myoung Soo Nam, Kee K. Kim.*****Cudrania tricuspidata* leaf extracts and its components, chlorogenic acid, kaempferol, and quercetin, increase claudin 1 expression in human keratinocytes, enhancing intercellular tight junction capacity. (2020) Appl. Biol. Chem. 63: 23**

Dysfunction of tight junctions and their components can cause diverse skin diseases. Here, we investigated the expression of claudin 1, a major tight junction protein, and changes of tight junction capacity upon treatment of the extracts of *Cudrania tricuspidata* (*C. tricuspidata*) and its components, chlorogenic acid, kaempferol, and quercetin. The effects of ethanol extracts of *C. tricuspidata* (EECT) and water extracts of *C. tricuspidata* (WECT) on the viability of human keratinocyte HaCaT cells were assessed by cell proliferation assay. Quantitative reverse transcription polymerase chain reaction (qRT-PCR) was conducted to measure the expression of claudin 1 mRNA. The protein expression of claudin 1 was analyzed by western blot and its tight junctional distribution was observed with immunofluorescence microscopy analysis. The tight junction capacity was analyzed by dispase assay. Upon treatment of WECT to HaCaT cells, the mRNA and protein expressions of claudin 1 were increased. In addition, chlorogenic acid, kaempferol, and quercetin increased claudin 1 protein expression levels in a dose-dependent manner. WECT and these three compounds enhanced the tight junction capacity of HaCaT cells in dispase assay. WECT, and its components, such as chlorogenic acid, kaempferol, and quercetin, upregulates both mRNA and protein expressions of claudin 1, which leads to the enhancement of tight junction capacity. Thus, WECT could be a therapeutic approach for treating tight junction-disrupted conditions such as atopic dermatitis and psoriasis.

**Boran Hu, Jin Gao, Shaochen Xu, Jiangyu Zhu, Xuemei Fan, Xiaoyan Zhou.****Quality evaluation of different varieties of dry red wine based on nuclear magnetic resonance metabolomics. (2020) Appl. Biol. Chem. 63: 24**

The metabolites that provide the aroma and flavor to wine are the products of several influences, such as grape cultivar, geographic location and associated environmental features, viticultural practices, and vinification techniques, which are central to production protocols, quality evaluation and development of wine regions. Accordingly, we initiated the requisite studies to investigate the differences in the dry red wine metabolites of different grape varieties. The proton-nuclear magnetic resonance technique (1H-NMR) combined with multivariate statistical analysis was used to investigate the changes of metabolite levels in Cabernet Sauvignon, Merlot and Cabernet Gernischt dry red wines vinified in Changli, Hebei province, China, in 2017. The results showed that the types of metabolites in different varieties of dry red wines were similar, but the content was significantly different. The main contributors to the differences in Cabernet Sauvignon, Merlot and Cabernet Gernischt dry red wines were ethyl acetate, lactic acid, alanine, succinic acid, proline, malic acid, and gallic acid, indicating 1H-NMR method combined with multivariate statistical analysis can distinguish these three types of dry red wines from each other. It provides a benchmark for further comparative study on wine quality and the verification of wine authenticity.

**Woo Tae Park, Sun Kyung Yeo, Ramaraj Sathasivam, Jong Seok Park, Jae Kwang Kim, Sang Un Park.****Influence of light-emitting diodes on phenylpropanoid biosynthetic gene expression and phenylpropanoid accumulation in *Agastache rugosa*. (2020) Appl. Biol. Chem. 63: 25**

*Agsatache rugosa* (Korean mint), belongs to the mint family and it has various medicinal properties. In addition, it has several valuable compounds such as monoterpenes and phenylpropanoid compounds. Amongst these, two compounds viz., rosmarinic acid (RA), and tilianin are well-known natural compounds that have numerous pharmacological properties. The phenylpropanoid biosynthetic gene expression under stress conditions and the subsequent accumulation of phenylpropanoid content has not been extensively studied in Korean mint. Here, we investigated the effect of light-emitting diodes (LEDs) on the expression levels of phenylpropanoid biosynthetic pathway genes and the accumulation of phenylpropanoid compounds such as RA and tilianin in *A. rugosa*. Real-time PCR analysis showed that the phenylpropanoid pathway genes responded to the LED lights. The transcript levels of downstream genes (*C4H*, *CHS*, *CHI*, and *RAS*) were comparatively higher than those of upstream genes (*PAL*, *TAT*, and *HPPR*). In addition, HPLC analysis showed that the content of RA and tilianin were significantly higher in plants cultivated under white light than those grown under red, blue, green, and orange lights. The RA and tilianin content were the highest in the plantlets after three weeks of exposure to white light. These results suggested that white LED lights significantly enhanced the accumulation of phenylpropanoid compounds in *A. rugosa*.

**Kwang-Sik Lee, So-Yeun Woo, Mi-Ja Lee, Hyun Young Kim, Hyeonmi Ham, Dong-Jin Lee, Sik-Won Choi, Woo Duck Seo.****Isoflavones and soyasaponins in the germ of Korean soybean [*Glycine max* (L.) Merr.] cultivars and their compound-enhanced BMP-2-induced bone formation. (2020) Appl. Biol. Chem. 63: 26**

Soybeans are used worldwide as food and as a healthy ingredient. Specifically, soy germ (SG) has received considerable attention owing to its abundant nutritional and biological components. This study aimed to elucidate the contents of isoflavone and soyasaponin of SG in 24 Korean soybean cultivars and the osteogenic activity of individual compounds. The isoflavone content in the SG ranged from 1110.9 to 3131.1 mg/100 g, and the soyasaponin content in SG ranged from 1173.5 to 3582.3 mg/100 g. The isoflavone and soyasaponin content depended on soybean cultivars. All isoflavone and soyasaponin compounds enhanced bone morphogenetic protein-2-mediated osteoblast differentiation in a dose-dependent manner, especially soyasaponin Ab. In conclusion, our results suggest that Seonpung cultivar with high soyasaponin Ab is beneficial for developing functional materials.

**Sunil Ghatge, Youri Yang, Jae-Hyung Ahn, Hor-Gil Hur.** **Biodegradation of polyethylene: a brief review. (2020) Appl. Biol. Chem. 63: 27**

Plastic waste management and recycling became a serious global issue as it affects living beings from all the ecosystems. Researchers investigated biodegradation of polyethylene (PE) by measuring changes in various physico-chemical and structural characteristics using techniques like as fourier transform infrared spectroscopy (FTIR), scanning electron microscope (SEM), etc. However, these evidences are not enough to prove the exact biodegradation of PE. In this review, we summarized microbial biodegradation of polyethylene and discussed recent developments for the candidate microbial enzymes and their possible roles in PE degradation. In addition, we conversed the advanced technologies correctly used for measuring PE degradation using isotope-labeled PE to figure out its metabolism into the end products like as 13CO2.

**Ahsan Hameed, Syed Ammar Hussain, Shaista Nosheen, Zafarullah Muhammad, Yang Wu, Samee Ullah, Hafiz Ansar Rasul Suleria, Yuanda Song. Microencapsulation of microbial antioxidants from *Mucor circinelloides*, their physico-chemical characterization, in vitro digestion and releasing behaviors in food. (2020) Appl. Biol. Chem. 63: 28**

This study aimed at increasing the stability of heat-labile and pH-sensitive microbial antioxidants by the microencapsulation. Microbial antioxidants from *Mucor circinelloides* were microencapsulated. The physico-chemical and powder flowing properties of resulting microcapsules were evaluated. The initial safety studies were evaluated by in vivo acute oral toxicity tests. The bio-accessibility of powders vs. extracts was analyzed in in vitro digestion models with further application of microcapsules to model food system. Physico-chemical properties were significantly different (p < 0.0001) for all microcapsules regardless of their non-substantial variations (p > 0.05) in powder flowing properties. The microencapsulation of extract with 5% whey protein hydrogels (WPHG) + 5% pectin (TA) showed higher retain-ability of polyphenols accompanying low degradation in gastric and intestinal digestion and with no major toxicity signs. The addition of TA microcapsule did not produce any nutritional, physico-chemical, compositional, and nutritional distinctions in cheese. Microencapsulation proved to be appropriate approach for not only protecting the thermo-labile and pH-sensitive microbial antioxidants but also for enhanced bioavailability, and targeted release of bioactive extracts.

**Ji-Su Park, Han Sol Lee, Sung Min Cho, Su Jung Lee, Hye-Sun Shin, Jae-Han Shim, Sang Soon Yun, Yong-hyun Jung, Jae-ho Oh. Simultaneous determination of the metabolites of the herbicide metazachlor in agricultural crops by LC–MS/MS. (2020) Appl. Biol. Chem. 63: 29**

To manage the safety of the herbicide metazachlor, analytical methods are required for the determination of metazachlor metabolites in agricultural crops. Herein, a liquid chromatography–tandem mass spectrometry (LC–MS/MS) method was developed for the simultaneous determination of metazachlor metabolites (479M04, 479M08, and 479M16) in various agricultural commodities. After extraction using acetonitrile and adjusting the pH to 3, the samples were purified using a hydrophilic–lipophilic balance cartridge. The matrix-matched calibration curves (0.002–0.2 μg/mL) were linear (*r*2 > 0.99). For validation, recovery tests were carried out at three fortification levels (limit of quantification (LOQ), 10 LOQ, and 50 LOQ) in various agricultural samples. The recoveries of 479M04, 479M08, and 479M16 were 79.6–113.0, 76.9–97.7, and 79.1–102.1%, respectively, with relative standard deviation values of less than 17.0%. Furthermore, inter-laboratory testing was conducted to validate the method. All the values corresponded to the criteria of both the CODEX (CAC/GL 40-1993, 2003) and Ministry of Food and Drug Safety guidelines. Therefore, the proposed LC–MS/MS method can be used as an analytical method for the determination of metazachlor.

**Ying-Hao Han, Ying-Ying Mao, Nan-Nan Yu, Mei-Hua Jin, Ying-Hua Jin, Ai-Guo Wang, Yong-Qing Zhang, Gui-Nan Shen, Yu-Dong Cui, Li-Yun Yu, Dong-Seok Lee, Yu-Jin Jo, Hu-Nan Sun, Jeongwoo Kwon, Taeho Kwon. RNA sequencing reveals that *Prx II* gene knockout can down-regulate the allograft rejection of dermal mesenchymal stem cells. (2020) Appl. Biol. Chem. 63: 30**

In this study, we used RNA sequencing (RNA-seq) to analyze and compare bulk cell samples from wild-type (WT) dermal mesenchymal stem cells (DMSCs) (n = 3) and *Prx II* knockout DMSCs (n = 3). The purpose of the study was to elucidate the role of *Prx II* on allogeneic immune rejection of transplanted DMSCs. The results revealed differential expression of 472 genes (176 up-regulated and 296 down-regulated; *p* ≤ 0.05) between the PrxII+/+ (WT) and PrxII−/− sample groups. When highly regulated genes were categorized according to the Gene Ontology (GO) molecular function classification and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis, the PrxII−/− samples showed a robust downward trend in allograft rejection. The study identified 43 all immunologically rejected differentially expressed genes, of which 41 showed lower expression in the PrxII−/− vs. PrxII+/+ (WT) samples. These findings suggest that *Prx II* gene knockout may down-regulate the allograft rejection that occurs during DMSCs transplantation and improve the survival rate of DMSCs in the host. This study provides a new perspective on the clinical treatment of stem cell transplantation.

**Hyunuk Kim, Mina Lee, Jae-Hwang Lee, Kye-Hoon Kim, Gary Owens, Kwon-Rae Kim. Distribution and extent of heavy metal(loid) contamination in agricultural soils as affected by industrial activity. (2020) Appl. Biol. Chem. 63: 31**

In Korea, rapid industrialization has often caused severe soil and water pollution near industrial complexes. Particularly, heavy metal(loid) contamination of agricultural lands could induce serious long-term problems for crop safety and productivity, requiring continual safety assessment. This study investigated heavy metal(loid) contamination of agricultural lands near fifteen industrial complexes. At each of industrial sites in Gyeongsangbuk-do, topsoils and subsoils were collected at two different distances from each site (0–500 m and 500–1000 m). For comparison, at each site, non-polluted soils were also collected more than 1000 m away from each industrial complex. With the exception of one sample, heavy metal(loid) concentration of all soils were lower than the Korean guidelines for soil contamination. However, the difference between the heavy metal(loid) concentrations of Cu, Pb and Zn in topsoil and subsoil increased the closer the samples were the industrial complexes, which implied that these elements were being generated by industrial activities and were freshly loaded on to near surface soils. While the heavy metal(loid) concentration in the studied sites did not exceed the Korean guideline, the geoaccumulation index of each soil indicated that the degree of Cd, Cu, and Pb contamination was heavily or extremely serious in more than twenty of the examined soils. The elevation of specific metals associated with industrial activity in soils in close proximity to industrial sites is of some concern and should be taken into consideration for the future management of agricultural soils around such complexes as well as the industrial complex operation itself.

**Jing Zhang, Hong Wang, Sha Yi, Zemei Guo, Yue Huang, Weifeng Li, Xin Zhao, Huazhi Liu. Protective effect of Insect tea primary leaf (*Malus sieboldii* (Regal) Rehd.) extract on H2O2-induced oxidative damage in human embryonic kidney 293T cells. (2020) Appl. Biol. Chem. 63: 32**

In this study, Insect tea primary leaf (*Malus sieboldii* (Regal) Rehd.) was used as the research object to investigate the protective effect of Insect tea primary extract (ITPLE) on hydrogen peroxide (H2O2)-induced oxidative damage in human embryonic kidney 293T cells (HEK 293T cells) and the mechanism of action of the main active components. The 3-(4,5-dimethyl-2-thiazolyl)- 2,5-diphenyl-2-H-tetrazolium bromide (MTT) assay was used to determine the toxicity of ITPLE to HEK 293T cells in vitro as well as its protective effect against (H2O2)-induced oxidative damage in HEK 293T cells. In addition, various assay kits were used to measure oxidation-related indicators in HEK 293T cells, and quantitative polymerase chain reaction (qPCR) analysis was used to determine the mRNA expression levels of oxidation-related genes in HEK 293T cells. High performance liquid chromatography (HPLC) analysis was used to characterize active components in ITPLE. The experimental results revealed that the ITPLE had no toxic effect on cells in the range of 0–200 μg/mL, and, in this range, exhibited a concentration-dependent protective effect against H2O2-induced oxidative damage in HEK 293T cells. It was also found that the ITPLE can reduce the malondialdehyde (MDA) level and increase the levels of superoxide dismutase (SOD), glutathione (GSH), glutathione peroxidase (GSH-Px), and catalase (CAT)in oxidative damage HEK 293T cells. The qPCR analysis results also showed that the ITPLE upregulated the mRNA expression levels of *SOD*, *CAT*, GSH and GSH-Px in HEK 293T cells damaged by H2O2-induced oxidative stress. The HPLC analysis identified 7 bioactive components in the ITPLE, including neochlorogenic acid, cryptochlorogenic acid, rutin, kaempferin, isochlorogenic acid B, isochlorogenic acid A and hesperidin. This study reveals that ITPLE is rich in active compounds and has good antioxidant effect in vitro, thus it has the potential to be developed into a traditional Chinese medicine and functional drinks.

**Li Nan, Hyeon-Hwa Nam, Byung-Kil Choo. Costunolide inhibits inflammation in LPS-induced RAW264.7 cells and ameliorates gastric acid reflux-induced esophageal injury in rat model. (2020) Appl. Biol. Chem. 63: 33**

As one of the gastroesophageal reflux disease (GERD), reflux esophagitis (RE) severely affects patients’ daily lives. Costunolide (Cos), pertains to a sesquiterpene lactone, performs multiple pharmacological activities including inhibited acute liver injury, anti-inflammation and anti-oxidant. We carried out our study to investigate the anti-inflammatory effect and protective effects of Cos against esophageal tissue damage caused by gastric acid refluxing. The determination of anti-inflammatory effects of Cos were conducted using lipopolysaccharide (LPS)-induced RAW 264.7 cell inflammatory model. The ameliorative effects of Cos on RE were confirmed on RE controlled rats model. The results indicated that Cos reduced nitrite production and inhibited cellular inflammation via regulating the activation of NF-κB. In addition, gastric acid reflux increased expression levels of inflammatory proteins (COX-2, TNF-α and IL-1β) in esophageal tissues, while Cos treatment significantly downregulated the expression of these proteins by inhibiting activation of NF-κB. Furthermore, through observing histological stain, Cos significantly improved esophageal damage caused by gastric acid reflux. Therefore, we suggested that Cos has the potential to be a material of natural drug for the treatment of reflux esophagitis caused by acid reflux.

**Chan-joo Park, Hyun-sang Kim, Dong Woon Lee, Jinho Kim, Yong-hwa Choi. Identification of antifungal constituents of essential oils extracted from *Boesenbergia pulcherrima* against Fusarium wilt (*Fusarium oxysporum*). (2020) Appl. Biol. Chem. 63: 34**

With the aim of developing environment-friendly agricultural products with antifungal activity against *Fusarium oxysporum* f. sp. lycopersici, a causative agent of Fusarium wilt, active substances from *Boesenbergia pulcherrima* roots were isolated. The hexane fraction from *B. pulcherrima* root extract was analyzed by GC/MS. The main peaks were estimated and identified to be methyl eugenol, methyl isoeugenol, elemicin, α-asarone, and 1,2-dimethoxy-4-(2-methoxyethenyl)benzene based on the Wiley library and by comparing retention times and mass spectra with their corresponding standards using GC/MS. For the identification of the compound in peak D that was estimated to be 1,2,4-trimethoxy-5-vinylbenzene, for which no reference standard was available, the hexane fraction was processed by column chromatography before NMR analysis. The result confirmed the compound to be 1,2,4-trimethoxy-5-vinylbenzene. Almost all compounds showed antifungal activity against *F. oxysporum* based on bioassays, and α-asarone had the highest activity. Therefore, *B. pulcherrima* root extract can be a potential source of environment-friendly agricultural products with antifungal activity against *F. oxysporum*.

**Joon-Goo Lee, Young Chae, Youngjae Shin, Young-Jun Kim. Chemical composition and antioxidant capacity of black pepper pericarp. (2020) Appl. Biol. Chem. 63: 35**

*Piper nigrum* L. is a widely used spice because of its flavour and health effects. It is prepared as black and white pepper, according to the harvest time and inclusion of the outer skin. Pepper pericarp is usually considered waste when making white pepper. In this study, bioactive and flavour compounds and minerals in the pericarp of black pepper were determined to identify its applications. The pericarp contained total phenol, total flavonoid and piperine contents of 1421.95 ± 22.35 mg GAE/100 g, 983.82 ± 8.19 mg CE/100 g and 2352.19 ± 68.88 mg/100 g, respectively. There were higher levels of total phenols and total flavonoids in the pericarp compared with black pepper and white pepper. Piperine content was lower in the pericarp than in black pepper. The principal monoterpene compounds in the pericarp were α-pinene (9.2%), 2-β-pinene (14.3%), δ-3-carene (21.5%) and DL-limonene (18.8%), and the primary sesquiterpenes were α-copaene (5.1%) and caryophyllene (17.2%). The higher percentages of flavour compounds found in the pericarp would impart a more potent odour, and the pericarp exhibited higher minor and tiny differences based on electronic nose analysis. It had more minerals than black pepper and peeled black pepper.

**Chuanpit Ruangcharus, Sung Un Kim, Chang Oh Hong. Mechanism of cadmium immobilization in phosphate-amended arable soils. (2020) Appl. Biol. Chem. 63: 36**

Little is known about the exact mechanism of cadmium (Cd) immobilization by phosphate (P) in arable soil containing low Cd concentration. This study was designed to describe this mechanism in detail. We determined the phosphorus (P) addition rate allowing Cd to precipitate as Cd minerals in Cd-contaminated arable soils and identified the main species of Cd minerals formed by the reaction of Cd and P, using the chemical equilibrium model MINTEQ. To determine the amount of Cd adsorption to soil adsorbed P, 0 –10,000 mgPL−1 of K2HPO4 solution was reacted with soil, then the P adsorbed soil was reacted with a CdCl2 solution (500 mg Cd L−1). Cadmium might not precipitate as Cd minerals such as Cd3(PO4)2 and CdCO3 with a recommended application rate of P fertilizer in field scale. Cadmium might be immobilized by Cd2+ adsorption instead of precipitation under a low P application system. Phosphate adsorption increased the negative charge of soil and Cd adsorption. The contributions of the increase in pH- and P-induced negative charges to the total increase in the soil negative charge were 93.2 and 6.8%, respectively. The increase in Cd adsorption caused by P adsorption was mainly attributed to the increase in pH-induced negative charge.

**Joo Tae Hwang, Jin Ah Ryuk, Hye Jin Kim, Dong Ho Jung, Byoung Seob Ko. Validation study on the geometric isomers from bulbs of *Allium fistulosum* and their conversion. (2020) Appl. Biol. Chem. 63: 37**

To discover new standard for the standardization of bulbs of *Allium fistulosum* (Chongbaek, Korean herbal name), twelve compounds (**1**–**12**) were isolated. Among them, a new HPLC/UV analysis method by selecting the five cinnamic acid amides (**5**–**9**) and two decursidate isomers (**10** and **11**), was fully validated. The developed analysis method showed sufficient reproducibility (< 2.58%) and accuracy (96.00–106.72%). Moreover, among compounds **5**–**11**, only *trans*-isomers were verified from all four Chongbaek samples which produced in different regions, and this finding implied that the *cis*-forms were not originally nature compounds, thus, it led us to verify the conversion processes. The four *trans*-standard solutions and extracts of Chongbaek were converted to the *cis*- derivatives after 96 h of UV (254 nm) light exposure as 78.74% (**6**), 82.29% (**8**), and 63.99% (**11**) in solution and 82.38% (**6**), 62.91% (**8**), and 61.64% (**11**) in extracts. A verified analysis method using new indicators was developed for quality control of Chongbaek, as well as their stability control under UV light exposure. These results might be important for the industrial use of Chongbaek.

**Ji-Eun Ra, So-Yeun Woo, Hui Jin, Mi Ja Lee, Hyun Young Kim, Hyeonmi Ham, Ill-Min Chung, Woo Duck Seo. Evaluation of antihypertensive polyphenols of barley (*Hordeum vulgare* L.) seedlings via their effects on angiotensin-converting enzyme (ACE) inhibition. (2020) Appl. Biol. Chem. 63: 38**

Angiotensin-converting enzyme (ACE) is an important therapeutic target in the regulation of high blood pressure. This study was conducted to investigate the alterations in blood pressure associated with ACE inhibition activity of the polyphenols (**1–10**), including 3-*O*-feruloylquinic acid (**1**), lutonarin (**2**), saponarin (**3**), isoorientin (**4**), orientin (**5**), isovitexin (**6**), isoorientin-7-*O*-[6-sinapoyl]-glucoside (**7**), isoorientin-7-*O*-[6-feruloyl]-glucoside (**8**), isovitexin-7-*O*-[6-sinapoyl]-glucoside (**9**), and isovitexin-7-*O*-[6-feruloyl]-glucoside (**10)**, isolated from barley seedlings (BS). All the isolated polyphenols exhibited comparable IC50 values of ACE inhibition activity (7.3–43.8 µM) with quercetin (25.2 ± 0.2 µM) as a positive control, and their inhibition kinetic models were identified as noncompetitive inhibition. Especially, compound **4** was revealed to be an outstanding ACE inhibitor (IC50 = 7.3 ± 0.1 µM, Ki = 6.6 ± 0.1 µM). Based on the compound structure–activity relationships, the free hydroxyl groups of flavone-moieties and glucose connections at the A ring of the flavone moieties were important factors for inhibition of ACE. The alcohol extract of BS also demonstrated potent ACE inhibition activity (66.5% ± 2.2% at 5000 µg mL−1). The polyphenols from BS had strong inhibitory activity on ACE and this study results suggest that BS can be used as an effective blood pressure regulator through ACE inhibition.

**Sung-Kyu Choi, Yeong-Geun Lee, Rong Bo Wang, Hyoung-Geun Kim, Dahye Yoon, Dae Young Lee, Yeon-Ju Kim, Nam-In Baek. Dibenzocyclooctadiene lignans from the fruits of *Schisandra chinensis* and their cytotoxicity on human cancer cell lines. (2020) Appl. Biol. Chem. 63: 39**

Repeated chromatographic separations of the EtOAc fraction of *Schisandra chinensis* fruits on silica gel, octadecyl silica gel, and Sephadex LH-20 led to the isolation and identification of seven dibenzocyclooctadiene lignans (**1**–**7**). The NMR data reported in the literature for angeloyl gomisin H (**5**) were shown to be incorrect. We unambiguously identified the compounds based on detailed analysis of the 1D and 2D NMR data, especially from HMBC and NOESY experiments. In addition, MTT assays and cell viability experiments verified the cytotoxicity of the isolated dibenzocyclooctadiene lignans against the human cancer cell lines AGS, HeLa, and HT-29.

**Jeong-A Lim, Nari Lee, Hyang-Sook Chun, Hyun-Joo Chang. Characterization of a novel endolysin from bacteriophage infecting *Vibrio parahaemolyticus,* vB\_VpaP\_KF2. (2020) Appl. Biol. Chem. 63: 40**

The antimicrobial resistance of food-borne pathogenic bacteria, including *Vibrio parahaemolyticus,* has been reported globally, warranting the need to identify promising alternative antibiotics such as endolysins that originate from bacteriophages. In our previous study, we characterized a bacteriophage infecting *V. parahaemolyticus*, vB\_VpaP\_KF2, at the molecular level. In this study, an open reading frame encoding putative endolysin was cloned from the complete genome data and expressed in the *Escherichia coli* expression system. The recombinant endolysin, vB\_VpaP\_KF2\_Lys, exhibited a novel lytic property against Gram-negative bacteria regardless of pretreatment with an outer-membrane permeabilizer. It was also stable over a wide range of temperatures, pH, and NaCl concentrations, and its hydrolytic spectrum was broader than that of the parent bacteriophage. From the results, vB\_VpaP\_KF2\_Lys could be used as a biocontrol agent against food-borne pathogens in the field of food safety.

**Martha Guillermina Romero-Garay, Emmanuel Martínez-Montaño, Adrián Hernández-Mendoza, Belinda Vallejo-Cordoba, Aarón Fernando González-Córdova, Efigenia Montalvo-González, María de Lourdes García-Magaña. *Bromelia karatas* and *Bromelia pinguin*: sources of plant proteases used for obtaining antioxidant hydrolysates from chicken and fish by-products. (2020) Appl. Biol. Chem. 63: 41**

In the present study, we evaluated new sources of plant proteases from fruits of *Bromelia karatas* (BK) and *Bromelia pinguin* (BP) to obtain antioxidant hydrolyzates/bioactive peptides (BPs) derived from chicken by-products (CH) and fish by-products (FH). The profile of the peptides was identified by reverse-phase high-resolution liquid chromatography (RP-HPLC) and the size weight distribution by molecular exclusion chromatography (SEC). The hydrolysates obtained with BK in both sources of by-products showed greater antioxidant capacity compared to those obtained with BP, presenting similar or higher values when compared to a commercial plant enzyme. The use of new sources of plant proteases allowed to obtain hydrolysates of hydrophilic character with a high percentage (> 50%) of peptides with molecular weights < 17.5 kDa from chicken and fish by-products. Therefore, based on the results obtained in antioxidant capacity it is possible to consider the hydrolysates as potential ingredients, food additives, and pharmaceutical products.

**Young Kyu Hong, Jin Wook Kim, Sang Phil Lee, Jae E. Yang, Sung Chul Kim, Efigenia Montalvo-González, María de Lourdes García-Magaña. Heavy metal remediation in soil with chemical amendments and its impact on activity of antioxidant enzymes in Lettuce (*Lactuca sativa*) and soil enzymes. (2020) Appl. Biol. Chem. 63: 42**

Chemical amendments have been used to remediate soils contaminated with heavy metals. However, there is little understanding on the impacts of these amendments on the physiological and biochemical functions of plants and soil. This study used in situ microcosm experiment to understand the effect of chemical amendments on antioxidant and soil enzyme activity in plant and soil with respect to heavy metal reduction. Three chemical amendments—acid mine drainage sludge (AMDS), limestone (LS), and steel slag (SS)—were applied to soil at 3, 5, and 10% mixing ratios, and lettuce (*Lactuca sativa*) was cultivated in that soil for 30 days. The results showed that bioavailable Cd and Pb in soil was reduced by 9.8–40.5% and 4.2–92.5%, respectively. The most efficient amendment for heavy metal reduction was AMDS. The uptake of Cd and Pb also decreased by 0.5–66.1 and 21.6–79.5%, respectively, depending on the amendment type and application ratio. The activity of three antioxidants—catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR)—was generally higher than the control with no amendments. This result indicated that there was minimal inhibition of antioxidant activity due to the reduction of heavy metal uptake. Also, no significant difference was observed in chemical amendments applied soil compared to control in terms of soil enzyme activity. However, correlation analysis between heavy metal concentration in soil and two soil enzyme activities showed that significantly negative correlation (p < 0.01) was observed between bioavailable Pb in soil and acid-phosphatase activity. This result might indicate that impact of bioavailable Pb was much higher than Cd in terms of inhibition of soil enzyme activity. Overall, the application of chemical amendments to heavy metal polluted had a positive effect on plant physiological function and soil enzyme activity with a reduction in bioavailable heavy metals in soil and plants.

**Sijia Zhao, Xiaoduo Zhao, Qingbo Liu, Yujun Jiang, Yanhua Li, Wenxiao Feng, Honghua Xu, Meili Shao. Protective effect of *Lactobacillus plantarum* ATCC8014 on acrylamide-induced oxidative damage in rats. (2020) Appl. Biol. Chem. 63: 43**

Acrylamide (AA), which is mainly found in fried foods, causes neurotoxicity, genetic toxicity, carcinogenic effects, and DNA damage. This study confirms that a strain of lactic acid bacteria (*Lactobacillus plantarum* ATCC8014) could alleviate the toxicity of rats by inhibiting the AA-induced oxidative damage. Forty-eight adult male SD rats were randomly divided into eight groups: control group, AA group (40 mg/kg), three different doses (1 × 107 CFU/ml, 1 × 108 CFU/ml, 1 × 109 CFU/ml of *Lactobacillus plantarum* ATCC8014) of prevention groups and therapeutic groups, respectively. At the end of three-week experiment, AA treatment produced a significant reduction in the rate of weight gain along with the symptoms of hind limb splay and ataxia. Histological examinations revealed various degrees of injury in five tissues. Levels of superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) in group AA rats were significantly decreased, but the level of lipid peroxidation (LPO) was significantly increased (p < 0.05). Both prevention and therapeutic groups with 1 × 109 CFU/ml of *Lactobacillus plantarum* ATCC8014 could effectively reduce the injury of AA to the body. However, reductions in both groups were not statistically significant.

**Venkatesa Prabhu Sundramurthy, Baskar Rajoo, Natesan Rajendran Srinivasan, Rajan Kavitha. Bioleaching of Zn from sphalerite using *Leptospirillum ferriphilum* isolate: effect of temperature and kinetic aspects. (2020) Appl. Biol. Chem. 63: 44**

Biological methods for leaching of nonferrous and noble metals from its sulfide ores are widely applied at industrial enterprises of different countries. This process is based on the use of the oxidative activity of acidophilic microorganisms. Since all bio systems are quite sensitive to the temperature, bacterial leaching process also significantly effects. In the present study, the impact of temperature on bacterial leaching of Zn from its sulphide ore, sphalerite, was investigated using ore adapted iron oxidizing bacteria. The bacteria were isolated from mine drainage samples and subjected to gene sequencing. The acquired nucleotide sequence revealed that the isolate was *Leptospirillum ferriphilum*. The nucleotide sequence of *L. ferriphilum* isolate was submitted to National Center for Biotechnology Information (NCBI) and accession number KF743135 was assigned. Using the isolate, the Zn leaching data were collected in the 298–318 K temperature range. The results showed that leaching of Zn increases with temperature until optimum temperature of 313 K and achieves highest leaching efficiency of 96.96% within 20 days. Since bioleaching of minerals have become increasingly applied in different mining industries, there is immense important to analyze mechanistically-based kinetics for the design, optimization, operation, and control of biochemical processes. The kinetic study showed that the rate of Zn leaching was maximized at the optimum temperature. Further, the leaching data were analyzed using shrinking core model which revealed that the rate of leaching was inhibited by diffusion through product layer. Reaction kinetics is also to be contrasted with thermodynamics. Using Arrhenius law of thermodynamics, it was found that activation energy for Zn bioleaching reaction was 39.557 kJ mol−1. Such investigations will be necessitated for designing and implanting the ideal bioleaching system for metal bio-mining industries.

**Kyung-A Kim, Chung Hyun Lee, Tae Kyeom Kang, Sung Jae Yang, Chang Yong Lee, Wook-Bin Lee, Sang Hoon Jung. Effect of persimmon leaves (*Diospyros kaki*) on goblet cell density and inflammation in experimental dry eye model. (2020) Appl. Biol. Chem. 63: 45**

The efficacy of ethanol extract of *Diospyros kaki* (EEDK) on dry eye (DE) was determined using an experimental mouse model. Experimental groups included three treated with various amounts of EEDK and one treated with omega-3 for 2 weeks. Damage to the ocular surface was evaluated, and the presence of conjunctival goblet cells was determined. Moreover, the inflammatory response was analyzed via RT-PCR analysis and a reporter gene assay. Fluorescein staining intensity decreased in the EEDK treatment group, and goblet cell density increased significantly in a dose-dependent manner. Pro-inflammatory cytokines were upregulated in human corneal epithelial cells treated with Pam3Cys-Ser-(Lys)-4. However, pro-inflammatory cytokines were downregulated at the mRNA level upon treatment with EEDK. Furthermore, EEDK regulated Pam3CSK4-induced gene expression through interferon regulatory factors. EEDK effectively improves the conjunctival goblet cell density and reduces the inflammatory response by reducing interferon regulatory factor activation downstream of Toll-like receptors in DE. Therefore, EEDK could be beneficial agents for preventing and treating DE.

**Min Ji Gu, Pyeongjae Lee, Sang Keun Ha, Jinyoung Hur. Zerumbone attenuates lipopolysaccharide-induced activation of BV-2 microglial cells via NF-κB signaling. (2020) Appl. Biol. Chem. 63: 46**

The brain is considered an immune-privileged organ. However, it has been found that inflammation mediated by microglia, which were once believed to support the brain structure, plays important roles in neuronal cell survival and death. Whether activated microglia has beneficial or detrimental effects on neurons remain controversial. Activated microglia could contribute to maintaining homeostasis in the brain by removing damaged cells. Nonetheless, dysregulation of microglial activation leads to neuronal cell death. Therefore, much attention has been paid to compounds that regulate microglial activation. Zerumbone, a constituent of *Zingiber zerumbet*, has been reported to exert several biological activities such as anticancer, anti-bacterial, and anti-inflammatory effects. In this study, we aimed to determine the anti-inflammatory effect of zerumbone on lipopolysaccharide-induced activation of BV-2 microglial cells and elucidate the underlying mechanism of action. Zerumbone suppressed nitric oxide and prostaglandin E2 production induced by lipopolysaccharides through inhibiting the expression of inducible nitric oxide synthase and cyclooxygenase-2. Blocking of mitogen-activated protein kinase and NF-κB activation, if not completely, is considered to be due to the anti-inflammatory effect of zerumbone against microglial activation.

**Amira Ragab EL Barky, Tarek Mostafa Mohamed, Ehab Mostafa Mohamed Ali. Detoxifying and antioxidant effect of ellagic acid nano particles in rats intoxicated with sodium nitrites. (2020) Appl. Biol. Chem. 63: 47**

Sodium nitrite is used as a preservative in food products to stabilize color and reduce rancidity. Its absorption into the body causes many diseases. Ellagic acid is a natural polyphenol that contains powerful antioxidants, but it is taken as a poorly absorbed food. Therefore, chitosan-coated nanoparticles (EANP@CS) were loaded to enhance their bioactivity and bioavailability after oral administration. EANP@CS was administrated in rats given water containing sodium nitrite to reduce toxins. Ellagic acid was extracted from pomegranate and manufactured EANP@CS. EANP@CS was identified by FT-IR, UV, X-ray diffraction and TEM. Average EANP@CS size ranges from 20–62 nm. Rats were divided into five groups: normal, treated using EANP@CS; rats were receiving nitrite for 8 weeks, and the last two groups were treated with EANP@CS. Serum and liver NO, MDA, and DNA fragmentation were reduced. Liver thiol and GSH levels, and Gpx, catalase and GST activities increased in rats treated with EANP@CS rats compared to rats drinking nitrites. Liver NOS activity was reduced 7 and 4.9 times in rats treated with EANP@CS during or after discontinuation of nitrite administration, respectively. Liver arginase activity was raised in rats that drink nitrite or were treated using EANP@CS. Inflammatory infiltrations of the liver, kidney, and spleen were observed in the tissues of rats that received nitrites and improved when the rats were given EANP@CS. EANP@CS improved oral bioavailability and reduce the risk of sodium nitrite in rats. EANP@CS can be used as a therapeutic goal to detoxify any unwanted toxic substance in food.

**Sang Gyu Lee, Hyeri Lee, Byung Cheon Lee, Hojoung Lee, Jun Cheol Moon, Changhyun Choi, Namhyun Chung. Effect of sodium silicate on early growth stages of wheat under drought stress. (2020) Appl. Biol. Chem. 63: 48**

Wheat yield is decreasing due to climate change, and a method to prevent decreasing yield during drought stress is desirable. In this study, wheat cultivars (Koso and Jokyung) were treated with 15% polyethylene glycol-6000 (PEG) and PEG + Si solution (6.5, 8.7, 13.1 and 26.1 mM). The effect of Si treatment on the alleviation of drought stress was measured using the germination test, shoot relative water content (RWC), seedling stage observation, and quantitative real time polymerase chain reaction (qRT-PCR). The results of root/shoot length ratio and shoot length ratio showed that Si treatment induced the alleviation of drought stress in Jokyung cultivar. The result of qRT-PCR showed the alleviation of drought stress in Koso cultivar. In addition, the results with shoot RWC and seedling stage observation showed that the alleviation effects of Si treatment was observed with both Koso and Jokyung cultivar at the high concentration of Si (26.1 mM). All these results suggest that Si treatment at a high concentration could be employed to alleviate drought stress in wheat.

**Fang Li, Hailan Sun, Guangjun Ran, Xinhong Liu, Ruokun Yi, Fang Tan, Xin Zhao, Huazhi Liu. Preventive effect of *Lactobacillus plantarum* HFY09 on HCl/ethanol-induced gastric injury in mice. (2020) Appl. Biol. Chem. 63: 49**

The aim of this study was to investigate the effect of *Lactobacillus plantarum* HFY09 on gastric injury induced by HCl/ethanol in Kunming mice. The results showed that HFY09-H inhibited any increases in gastric juice volume, maintained the normal pH value of gastric acid, and reduced the damage caused to the gastric mucosa and gastric wall, the inhibition rate on the injury area reaches 63.70%. Compared with the negative control group, HFY09 increased the levels of serum somatostatin (SS) and vasoactive intestinal peptide (VIP), and also decreased the levels of substance P (SP), endothelin-1 (ET-1), interleukin-6 (IL-6), interleukin-12 (IL-12), tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ). In addition, real time fluorescent quantitative PCR (Q-PCR) also confirmed that high-dose HFY09 (109 CFU/kg/day) upregulated the mRNA expression of copper/zinc superoxide dismutase (Cu/Zn-SOD), manganese superoxide dismutase (Mn-SOD), catalase (CAT), endothelial nitric oxide synthase (eNOS), and neuronal nitric oxide synthase (nNOS), and downregulated the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). At the same time, the results of the HFY09 treatment group were similar to those of the ranitidine treatment group. These results indicate that HFY09 can prevent gastric injury induced by HCl/ethanol in vivo. Therefore, HFY09 may play a potential role in the treatment of gastric diseases.

**Hyunjeong Park, Young-Jun Kim, Youngjae Shin. Estimation of daily intake of lycopene, antioxidant contents and activities from tomatoes, watermelons, and their processed products in Korea. (2020) Appl. Biol. Chem. 63: 50**

Tomatoes, watermelons, and processed tomato products contain abundant antioxidant compounds, including lycopene. In spite of the interest in the role of lycopene, little is known about the contribution of tomatoes and tomato products commonly consumed in Korea to the intake of lycopene. In this study, the daily per capita intake values of lycopene and antioxidant compounds and activities of tomatoes, watermelons, and their processed products in Korea were estimated. The daily per capita intake values of lycopene from raw tomatoes, watermelons, tomato ketchup, and tomato juice were measured to be 1.07, 0.54, 0.26, and 0.08 mg/capita/day, respectively. The average lycopene intake of male and female were 1.98 and 1.92 mg/capita/day, respectively. The daily per capita intake value of total phenolics was highest in raw tomatoes (7.21 mg/capita/day), followed by watermelons (1.72 mg/capita/day), tomato ketchup (1.41 mg/capita/day), and tomato juice (0.80 mg/capita/day). The daily per capita intake value of total antioxidant activities also showed a similar pattern to total phenolic results. The overall daily per capita intake of antioxidant content and activities was affected by both the daily per capita intake of each product and the levels of antioxidant content and activities of each product. The daily per capita intake of these products in Korea is currently considerably low, compared to the U.S. and Europe. Thus, an adequate consumption of lycopene-rich products is recommended.

**Hu-Zhe Zheng, Chun-Lan Cui, Woo-Sik Jeong, Shin-Kyo Chung. Anti-inflammatory effect of unripe apple polyphenols-chitooligosaccharides microcapsule against LPS-induced RAW 264.7 cells. (2020) Appl. Biol. Chem. 63: 51**

In order to improve the synergistic effect of unripe apple polyphenols (APP) and chitooligosaccharides (COS), apple polyphenols-chitooligosaccharides microcapsule (APCM) were prepared by spray-drying method. The effects of APCM on the release of polyphenols in simulated gastrointestinal digestion model, as well as the anti-inflammatory effect against LPS-induced RAW264.7 cells were also evaluated. Scanning electron microscope (SEM) and HPLC analysis of APP and APCM showed that during the spray-drying process, most of the polyphenols are successfully encapsulated in COS. The simulated gastrointestinal digestion model results showed that about 98% of polyphenols released from APCM within 60 min. Anti-inflammatory effect of APCM on LPS induced RAW 264.7 cells showed that although APP showed a strong inhibitory effect on cell viability at 0.6 mg/mL, the effect of APCM on cell viability was less and could maintain a high level at the same concentration. In addition, APCM significantly inhibited nitric oxide (NO) and TNF-α production via the elevation of cytokine IL-10 as the concentration increases, respectively. The results suggest that APCM alleviate the intensity of inflammatory processes by inhibiting the production of pro-inflammatory cytokines TNF-α, as well as additionally by promoting the production of anti-inflammatory cytokines IL-10. These findings provide scientific and theoretical support for the claim that traditional medicine treats inflammation-related diseases.

**Deogratius Luyima, Jwakyung Sung, Jae-Han Lee, Seong-Ah Woo, Seong-Jin Park, Taek-Keun Oh. Sorption of urea hydrogen peroxide by co-pyrolysed bone meal and cow dung slowed-down phosphorus and nitrogen releases but boosted agronomic efficiency. (2020) Appl. Biol. Chem. 63: 52**

Co-pyrolysis of animal manure biomass with bone meal (BM) and soaking of the resultant biochar in urea containing solutions may offer a sustainable and cheap way of formulating slow-release nitrogen (N) and phosphorus (P) fertilisers. This method can lead to optimisation of the carbon sequestration capacity of the biochar, abatement of environmental pollution by P and N and alleviation of the severity of the projected future scarcity of P. A few studies have indicated that sorption can create efficient slow-release fertilisers although all of them utilised charged moieties such as ammonium ions to formulate them and as a result, there is a paucity of data concerning the efficiency of fertilisers formulated using uncharged compounds like urea. It’s against that background that we examined the possibility of leveraging co-pyrolysis and sorption with urea containing solutions to formulate slow-release N and P fertilisers along with assessing the agronomic efficiency of the formulated fertilisers through cultivating lettuce in pots for two seasons. Both urea-hydrogen peroxide (UHP) and urea were utilised as N sources. UHP (CDBM-UHP) and urea (CDBM-Urea) containing biochars averagely released 64.40% and 87.00% of the added N, respectively over the 28-day incubation period with the amount of N released decreasing with increasing concentrations of BM in the biochar. Lettuce yields and nutrient use efficiencies of N and P were higher in the CDBM-UHP than in the CDBM-Urea treatments. It’s therefore clear that sorption of UHP by BM containing biochar concomitantly slows-down releases of N and P and boosts the agronomic efficiency of the fertilisers.

**Nguyen Bao Hung, Woon-Ra Park, Bohyun Yun, Dong Cheol Seo, Won-Il Kim, Hyun-Ju Kim, Sanghyun Han, Se-Ri Kim. Effect of sequential presoaking and chlorine dioxide treatment on the inactivation of pathogenic *Escherichia coli* and *Salmonella* spp. on sprout seeds. (2020) Appl. Biol. Chem. 63: 53**

This study was conducted to evaluate the effect of sequential presoaking and chlorine dioxide (ClO2) on the reduction of pathogenic *Escherichia coli* and *Salmonella* spp. in alfalfa. When unsoaked and presoaked alfalfa were exposed to 200 ppm ClO2 for 15 min, the population of *E. coli* and *Salmonella* spp. on presoaked seeds reduced more than those on unsoaked seeds by 2.07 and 1.43 log CFU g−1 (*p*< 0.05), respectively. To determine the optimal concentration and treatment time to reduce pathogenic *E. coli* and *Salmonella* spp. in alfalfa seeds immersed in water for 5 h, presoaked seeds were exposed to four different concentrations of ClO2 (50, 100, 150, and 200 ppm) for 15, 30, 45, and 60 min. The most effective condition to eliminate *E. coli* and *Salmonella* spp. from alfalfa seeds was sequential immersion in water for 5 h and 200 ppm ClO2 treatment for 1 h. After the optimal condition was applied to eight kinds of sprout seeds, the pathogens were completely inactivated in all seeds, except radish seeds. Growth of pathogenic *E. coli* and *Salmonella* spp. during sprouting after ClO2 treatment of alfalfa seeds was also completely inactivated. However, the germination rate of seeds did not significantly decrease after ClO2 treatment. In addition, ClO2 residues were not present in any sprout during 3 days of cultivation. These results demonstrated that sequential presoaking and 200 ppm ClO2 treatment is the optimal seed disinfection treatment to prevent foodborne diseases associated with sprout consumption.

**Jung-Tae Kim, Gibum Yi, Mi-Jung Kim, Beom-Young Son, Hwan-Hee Bae, Young Sam Go, Sun-Lim Kim, Seong-Bum Baek, Seung-Hyun Kim, Ill-Min Chung. Glycolysis stimulation and storage protein accumulation are hallmarks of maize (*Zea mays* L.) grain filling. (2020) Appl. Biol. Chem. 63: 54**

Maize (*Zea mays* L.) is a major dietary source of human caloric intake. Grain filling, the developmental stage of the seed during which starch and proteins accumulate, is of great interest in plant biology and agronomy. However, proteomic datasets covering maize seed development, especially during grain filling, are much scarcer than transcriptomic datasets, largely due to the labor-intensive and costly nature of the large-scale analysis required for proteomics. Here, we searched for proteins that showed changes in abundance during four time-points covering the middle stages of grain filling by two-dimensional electrophoresis, MALDI-TOF, and database searches. We detected 1384 protein spots, of which 48 exhibited differential accumulation during grain filling. Of those, we identified the underlying protein for 32 spots: they included enzymes of carbohydrate metabolism, stress-related proteins, and storage proteins, the latter of which represented 34% of all changing proteins during grain filling. Proteins related to carbohydrate metabolism reached their maximum accumulation around 15–20 days after pollination (DAP) and subsequently dropped until 30 DAP. The rise of stress-related proteins such as heat shock proteins demonstrated their involvement in grain filling and seed maturation. This study catalogues the proteome changes during grain filling and provides basic but critical information regarding the biological changes during maize kernel development.

**Seung Hoon An, Gyu-Sik Choi, Joong-Hoon Ahn. Biosynthesis of fraxetin from three different substrates using engineered *Escherichia coli*. (2020) Appl. Biol. Chem. 63: 55**

Fraxetin, which is a simple coumarin, is a phytochemical present in medicinal plants, such as *Fraxinus rhynchophylla*, and *Cortex Fraxini*. In plants, it serves as a controller of iron homeostasis. The health-enhancing activities of fraxetin, such as anticancer, neuroprotective and antibacterial activities, are known. Scopoletin 8-hydroxylase (S8H) is a key enzyme involved in the synthesis of fraxetin from scopoletin. Scopoletin can be synthesized either from esculetin by *O*-methylation or from ferulic acid by feruloyl CoA 6′-hydroxylase (F6′H) and 4-coumaric acid CoA ligase (4CL). To enable fraxetin synthesis, the fraxetin biosynthesis pathway was introduced into *Escherichia coli*. Three distinct routes, from ferulic acid, esculetin, and scopoletin, were designed for the synthesis of fraxetin. In the first approach, *E. coli* strain harboring S8H was used and found to synthesize 84.8 μM fraxetin from 100 μM scopoletin. Two *E. coli* strains were used for the other two approaches because these approaches required at least two enzymatic reactions. Through this approach, 41.4 μM fraxetin was synthesized from 100 μM esculetin, while 33.3 μM fraxetin was synthesized from 100 μM ferulic acid.

**Chi-Eun Oh, Gap-Jin Kim, Seung-Jin Park, Seunghoon Choi, Min-Joo Park, O-Mi Lee, Jeong-Woo Seo, Hong-Joo Son. Purification of high purity docosahexaenoic acid from *Schizochytrium* sp. SH103 using preparative-scale HPLC. (2020) Appl. Biol. Chem. 63: 56**

High purity polyunsaturated fatty acids (> 95%) are essential for the synthesis of specialized pro-resolving lipid mediators (SPMs), such as protectins, resolvins, and maresins, which are used for clinical application. To date, high purity (> 95%) eicosapentaenoic acid (EPA; C20:5n3) and docosahexaenoic acid (DHA; C22:6n3) have been produced through various manufacturing steps using fish oil. In this study, we optimized preparative high performance liquid chromatography (HPLC) process to purify high-purity DHA ethyl ester (DHAee; > 98%) from oleaginous microalgae *Shizochytrium* sp. SH103 containing at least 34% DHA content. The purity and yield of DHA were determined by reverse phase chromatography with changing the mobile phase velocity, loading amount, and mobile phase composition. On a semi-preparative scale, optimal DHA separation in isocratic elution was obtained with a mobile phase velocity of 0.5 mL/min, a loading amount of 10 mg/mL, and mobile phase composition of methanol/water (96:4, v/v), wherein the purity of DHA was 98.5%. This separation was scaled up to a preparative column, resulting in 99.0% DHA fraction with a yield of 79.8%. This result suggests that a large amount of high purity DHA can be produced from microalgae when scaling up a preparative column to an industrial column.

**Jinyoung Hur, Yeonmi Lee, Chang Jun Lee, Ho-Young Park, Sang Yoon Choi. 6-shogaol suppresses oxidative damage in L6 muscle cells. (2020) Appl. Biol. Chem. 63: 57**

Ginger (*Zingiber Officinale Roscoe*) has been known reduce muscle pain after exercise, and 6-shogaol {(E)-1-(4-Hydroxy-3-methoxyphenyl)dec-4-en-3-one)} is the major essential oil contained in ginger. In this study, the protective effect of 6-shogaol on L6 muscle cells against oxidative damage was measured. 6-shagol inhibited the damage of L6 cell induced by H2O2, and allowed the increase in mRNA and protein expression levels of intracellular HO-1 and NRF2. 6-shogaol also reduced the production of intracellular ROS. These results suggested that 6-shagol effectively inhibits oxidative damage of skeletal muscle cell.

**Jia-qi Tan, Peng-cheng Li, Qian Li, Jin-tian Tang, Hong-kun Xue. Protective effect of procyanidin B2 on hydrogen peroxide (H2O2)-induced oxidative damage in MCF-7 cells. (2020) Appl. Biol. Chem. 63: 58**

The aim of this study is to assess the cytoprotection and potential molecular mechanisms of procyanidin B2 (PCB2) on hydrogen peroxide (H2O2)-induced oxidative damage in MCF-7 cells. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed to determine the viability of MCF-7 cell exposure to H2O2 or PCB2. We measured the antioxidant properties of PCB2 by determining the activities of SOD, GSH-Px, LDH and MDA levels, and evaluated apoptosis and intracellular reactive oxygen species (ROS) levels. The related proteins expression levels were monitored by Western blot. MCF-7 cells induced with H2O2 had a remarkable decrease in cell viability that was suppressed when it was interfered with PCB2 (0.1–10.0 μM). PCB2 interference memorably and dose-dependently inhibited H2O2-induced LDH leakage, ROS and MDA overproduction, while PCB2 markedly increased H2O2-induced the activities of SOD and GSH-Px. Eventually, H2O2 prominently down-regulated the ratio of Bcl-2/Bax and the relative proteins expression levels of Nrf2, GCLC, NQO1 and HO-1, and up-regulated the relative proteins expression levels of cytochrome c, caspase-3 and Keap1. However, the relative expression levels of these proteins were reversed in PCB2-interfered MCF-7 cells. This study implied that protective effect of PCB2 on H2O2-induced oxidative damage in MCF-7 cells might be related to inhibition of mitochondria-dependent apoptosis, activation of Keap1/Nrf2/HO-1 signaling pathway and improvement of the antioxidant enzymes activities.

**Boyoung Kim, Yoo Yeon Kim, Phuong Thi-Thanh Nguyen, Hajin Nam, Jun Gyo Suh. Sex differences in glucose metabolism of streptozotocin-induced diabetes inbred mice (C57BL/6J). (2020) Appl. Biol. Chem. 63: 59**

Considering that sex differences in glucose metabolism are observed in mice, researchers unconsciously use male mice to reduce variations by an estrogen cycle in female mice. In this study, we investigated the sex differences in glucose homeostasis in streptozotocin (STZ)-induced diabetes inbred mice (C57BL/6J). The C57BL/6J male and female mice were injected with or without STZ (40 mg/kg) for 5 consecutive days. Levels of fasting blood glucose (FBG), glycosylated hemoglobin (HbA1C), lipid profiles, oral glucose tolerance, and insulin resistance were measured at 3 and 6 weeks after STZ treatment. The FBG level in the STZ-induced male (M-STZ) group was significantly higher than that in the STZ-induced female (F-STZ) group during the entire experimental period. Furthermore, HbA1C and glucose tolerance levels in the M-STZ group were significantly higher than those in the F-STZ group at 3 and 6 weeks after STZ treatment. The glucagon/insulin ratio in the M-STZ group was significantly higher than that in the F-STZ group. Values of the homeostatic model assessment-insulin resistance, an indicator of β-cell function and insulin resistance, significantly increased in both the M-STZ and F-STZ groups at 3 weeks after STZ treatment. However, insulin resistance was observed in the M-STZ group, but not in the F-STZ group, at 6 weeks after STZ treatment. Taken together, our results indicate that glucose metabolism in the M-STZ group was worse than that in the F-STZ group, indicating that estrogen may have an important role in glucose metabolism by STZ treatment.

**Dan Gao, Le Ba Vinh, Chong Woon Cho, Kyoung Won Cho, Young Ho Kim, Jong Seong Kang. Discrimination and quality evaluation of fifteen components in *Stauntonia hexaphylla* leaves from different harvest time by HPLC–PDA–ESI–MS/MS and ELSD coupled with multivariate statistical analysis and anti-inflammatory activity evaluation. (2020) Appl. Biol. Chem. 63: 60**

The leaves of *Stauntonia hexaphylla* (SHL) are a very popular herbal medicine in Korea because it can be used to treat rheumatic osteoporosis and other diseases. However, owing to the inconsistency in harvesting time and growth years, their quality is uneven, which indirectly negatively affects the safety of this medication. Therefore, the difference of constituents in SHL harvested at different seasons and years were analyzed by high-performance liquid chromatography coupled to photodiode-array and electrospray ionization mass spectrometry detectors (HPLC–PDA–ESI/MS). A total of 15 components were tentatively characterized in samples of SHL, including 5 compounds reported for the first time in this plant. Moreover, the relative content of these constitutions was simultaneously determined by HPLC coupled with evaporative light scattering detection (ELSD). Hierarchical clustering analysis (HCA) and principal component analysis (PCA) revealed that the quality has a certain extent difference in different harvest times, the best harvest time was 3 years old growing in autumn. The same harvest time was also suggested based on the anti-inflammatory evaluation.

**Ya Wu, Fang Tan, Tianyu Zhang, Binglin Xie, Lixian Ran, Xin Zhao. The anti-obesity effect of lotus leaves on high-fat-diet-induced obesity by modulating lipid metabolism in C57BL/6J mice. (2020) Appl. Biol. Chem. 63: 61**

Lotus leaves (*Nelumbo nucifera*) are widely used in medicines and foods. The investigate systematically studied the anti-obesity effect of lotus leaf extracts. It could reduce body weight, alleviate liver damage, and inhibit fat accumulation in high-fat-diet-induced obese mice. Lotus leaf extracts reduced serum alanine aminotransferase (ALT), aspartate transaminase (AST), and alkaline phosphatase (AKP) levels; decreased total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C) levels in the serum; and increased high-density lipoprotein cholesterol (HDL-C) levels to improve dyslipidemia. Lotus leaves also inhibited inflammation accompanied by obesity via decreasing inflammatory cytokine interleukin (IL)-1β, tumor necrosis factor-α (TNF-α), interferon gamma (IFN-γ), and IL-6 levels and increasing anti-inflammatory cytokine IL-4 and IL-10 levels. qPCR analysis revealed that lotus leaves upregulated peroxisome proliferator-activated receptor alpha (PPAR-α), lipoprotein lipase (LPL), carnitine palmitoyltransferase 1 (CPT1), and cholesterol 7 alpha hydroxylase (CYP7A1) mRNA expressions and downregulated peroxisome proliferator-activated receptor gamma (PPAR-γ) and CCAAT/enhancer-binding protein alpha (C/EBP-α) mRNA expressions, to reduce adipocyte differentiation and fat accumulation, promote oxidation of fat and decomposition of triglyceride and cholesterol. So, lotus leaves effectively regulated lipid metabolism, alleviated inflammation and liver injury in obese mice; thus, lotus leaves could be further developed as a food to combat obesity.

**Ji Su Bae, Yeo Myung Yoon, Seon Kyoung Shin, Dong Jin Lee, Dong Cheol Seo. Biogas potential and methanogenic community shift in in-situ anaerobic sewage sludge digestion with food waste leachate additions. (2020) Appl. Biol. Chem. 63: 62**

The objective of this study was to determine methane yields (MY) of organic wastes in biogasification facilities according to the mixing ratio of food waste/food waste leachate and sewage sludge. One biogasification facility that treated sewage sludge only was compared with three biogasification facilities treating sewage sludge and food waste. The theoretical MY was derived based on analyses of carbohydrate, fat, and protein to examine the efficiency of the biogasification facility. The average actual MY was 0.424 Sm3CH4/kg volatile solids, which corresponded to 83.7% of theoretical MY. In the case of combined anaerobic digestion (CD) mixing with food waste/food waste leachate, inhibitory factors (volatile fatty acids [VFAs], total nitrogen [TN], and organic matter contents) showed the tendency to have relatively higher values in CD facilities than in the biogasification facility treating sewage sludge only. Mean concentrations of VFAs and TN in the anaerobic digester effluent, and the organic loading rate were 406 mg/L, 3,721 mg/L, and 1.62 kg volatile solids/m3 day, respectively. The influence of anaerobic digester effluent was in charge of 10% within the influent environmental loading rate from the sewage treatment plants associated with the biogasification facilities. Analyses of the microbial community showed that a remarkable change in the structure of methanogens was directly related to different MY in each plant. In particular, *Methanoculleus* and *Methanosaeta* increased with an increasing ratio of food waste/food waste leachate to sludge, while *Methanococcus* and *Methanosarcina* decreased. In conclusion, CD showed steady operational conditions and high efficiency of MY by injecting food waste/food waste leachate into the anaerobic digester. It met the current criteria for integrated treatment of organic waste in biogasification facilities in South Korea.

**Yeong Jun Ban, Aizhamal Baiseitova, Mohd Azlan Nafiah, Jeong Yoon Kim, Ki Hun Park. Human neutrophil elastase inhibitory dihydrobenzoxanthones and alkylated flavones from the *Artocarpus elasticus* root barks. (2020) Appl. Biol. Chem. 63: 63**

Neutrophil elastases are deposited in azurophilic granules interspace of neutrophils and tightly associated with inflammatory ailments. The root barks of *Artocarpus elasticus* had a strong inhibitory potential against human neutrophil elastase (HNE). The responsible components for HNE inhibition were confirmed as alkylated flavones (**2**–**4**, IC50 = 14.8 ~ 18.1 μM) and dihydrobenzoxanthones (**5**–**8**, IC50 = 9.8 ~ 28.7 μM). Alkyl groups on flavone were found to be crucial functionalities for HNE inhibition. For instance, alkylated flavone **2** (IC50 = 14.8 μM) was 20-fold potent than mother compound norartocarpetin (**1**, IC50 > 300 μM). The kinetic analysis showed that alkylated flavones (**2**–**4**) were noncompetitive inhibition, while dihydrobenzoxanthones (**5**–**8**) were a mixed type I (*K*I < *K*IS) inhibitors, which usually binds with free enzyme better than to complex of enzyme–substrate. Inhibitors and HNE enzyme binding affinities were examined by fluorescence quenching effect. In the result, the binding affinity constants (*K*SV) had a significant correlation with inhibitory potencies (IC50).

**Wonho Lee, Dahye Yoon, Seohee Ma, Dae Young Lee, Jae Won Lee, Ick-Hyun Jo, Taekwang Kim, Suhkmann Kim. Machine learning for a rapid discrimination of ginseng cultivation age using 1H-NMR spectra. (2020) Appl. Biol. Chem. 63: 64**

The scientific and systematic classification of cultivation age is important for preventing age falsification and ensuring the quality of ginseng. Therefore, we applied deep learning to classify the cultivation age of ginseng. Deep learning, which is based on an artificial neural network, is one of the new class of models for machine learning, and is state-of-the-art. It is a powerful tool and has been used to solve complex problems in many fields. In the present study, powdered samples of 4-, 5-, and 6-year-old ginseng were measured using high-resolution magic angle spinning nuclear magnetic resonance (HR-MAS NMR) spectroscopy. NMR data were analyzed with deep learning and partial least-squares discriminant analysis (PLS-DA) to improve accuracy. The accuracy of the PLS-DA was 87.1% and the accuracy of the deep learning model was 93.9%. NMR spectroscopy with deep learning can be a useful tool for discrimination of ginseng cultivation age.

**Do Young Kim, Min Sik Eom, Hye Jin Kim, Eun Mi Ko, In-Soon Pack, Jung-Ho Park, Kee Woong Park, Kyong-Hee Nam, Sung Duk Oh, Jae Kwang Kim, Ju Seok Seo, Chang-Gi Kim. Gene flow from transgenic soybean, developed to obtain recombinant proteins for use in the skin care industry, to non-transgenic soybean. (2020) Appl. Biol. Chem. 63: 65**

Soybean has been recognized as a useful platform for heterologous protein production. This study compared the pollen characteristics of transgenic and non-transgenic soybean and investigated the rate of gene flow from transgenic soybean events, developed to obtain recombinant proteins (such as human epidermal growth factor, insulin-like growth factor 1, or thioredoxin) for use in the skin care industry, to non-transgenic soybean under field conditions, and determined the distance at which gene flow could occur. The lack of significant differences in pollen grain size, viability and pollen germination rates between transgenic and non-transgenic cultivars indicates that the overexpression of transgenes did not alter pollen characteristics in soybean. The highest rates of gene flow from the three transgenic soybean events to non-transgenic soybean ranged from 0.22 to 0.46% at the closest distance (0.5 m). Gene flow was observed up to 13.1 m from the transgenic plots. Our data fell within the ranges reported in the literature and indicate that an isolation distance greater than at least 13 m from transgenic soybean is required to prevent within-crop gene flow in soybean. As the potential markets for transgenic crops as a recombinant protein factory increase, gene flow from transgenic to non-transgenic conventional crops will become a key decision factor for policy makers during the approval process of transgenic crops. Our study may provide useful baseline data for the prevention of transgenic soybean seed contamination caused by transgene flow.

**Hyung Churl Bae, Joong Hyeon Nam, Gereltuya Renchinkhand, Suk-Ho Choi, Myoung Soo Nam. Physicochemical changes during 4 weeks ripening of Camembert cheeses salted with four types of salts. (2020) Appl. Biol. Chem. 63: 66**

The objectives of this study were to compare physicochemical, rheological and sensory characteristics of Camembert cheeses salted with 4 types of salts (refined salt, baked refined salt, sun-dried salt, and Himalayan rock salt) during 4 weeks ripening period. The pH of Camembert cheese was 7.2 in the sun-dried salt, which was the highest than the other three types of salts. The viable lactic acid bacteria (LAB) counts after 4 weeks ripening of the cheeses with 4 types of salts were 1.4 × 108, 1.6 × 108, 1.3 × 108, and 1.3 × 108 CFU/ml, respectively. The water soluble nitrogen level of the cheese salted with Himalayan rock salt was the highest (109.23 µg/g) among those of all the cheeses at 4 weeks ripening. Organic acids in all the cheeses decreased as the ripening period advanced. Protein hydrolysis proceeded faster refined salted cheese than other salted cheeses after 4 weeks ripening. Octadecanoic acid and hexadecanoic acid (volatile fatty acid) which were detected in the cheeses with refined salt and Himalayan rock salt were lower than those with baked refined salt and sun-dried salt. In terms of textural characteristics there were substantially steep decreases in hardness, gumminess, and chewiness after 4 weeks ripening, while springiness and cohesiveness decreased less in all the Camembert cheeses. With respect to sensory properties, the taste score of the cheese with refined salt were significantly higher than those with baked refined salt, sun-dried salt and Himalayan rock salt. These results suggested that the refined salt should be recommended for Camembert cheese making.

**Jia Jia, Xigang Kang, Yanfang Liu, Jianwei Zhang. Inhibition of human liver cancer cell growth by evodiamine involves apoptosis and deactivation of PI3K/AKT pathway. (2020) Appl. Biol. Chem. 63: 67**

Evodiamine is an active alkaloid member found in Traditional Chinese Herb (TCH) *Evodia rutaecarpa.* It has been reported to exhibit remarkable biological and medicinal activities including anticancer and anti-inflammatory. This study was designed to investigate the anticancer effects of evodiamine against human liver cancer and evaluate its effects on cell migration, cell invasion, cellular apoptosis and PI3K/AKT pathway. The results showed that evodiamine exhibits potent antiproliferative effects against two human liver cancer cell lines (HepG2 and PLHC-1) with an IC50 of 20 µM. Nonetheless, the cytotoxic effects of evodiamine were comparatively low against the normal cells as evident from the IC50 of 100 μM. The growth inhibitory effects of evodiamine were found to be due to the induction of apoptosis as revealed by the DAPI, AO/EB and annexin V/PI staining assays. The induction of apoptosis was also associated with upregulation of Bax and downregulation of Bcl-2 expression in a concentration dependent manner. The wound healing and transwell assay revealed that evodiamine caused a significant decline in the migration and invasion of the HepG2 and PLHC-1 cells. Investigation of the effects of evodiamine on the PI3K/AKT signalling revealed that evodiamine inhibited the phosphorylation of PI3K and AKT proteins. Taken together, the results showed that evodiamine inhibits the growth of human liver cancer via induction of apoptosis and deactivation of PI3K/AKT pathway. The results point towards the therapeutic potential of evodiamine in the treatment of liver cancer.

**Jae-Han Lee, Deogratius Luyima, Chang-Hoon Lee, Seong-Jin Park, Taek-Keun Oh. Efficiencies of unconventional bulking agents in composting food waste in Korea. (2020) Appl. Biol. Chem. 63: 68**

Sawdust is the main bulking agent used to compost food waste in Korea but it is not an economically desirable choice because its availability entirely depends on imports. Since food waste composting provides agricultural, environmental and economic benefits, it is vital that we search for suitable replacements to sawdust from the locally available materials. In this study, we assessed the composting characteristics of food waste amended with various bulking agents including sawdust, ginkgo leaves, insect feces, and mushroom waste. Each of the bulking agents was mixed with the food waste in ratios of 3:7, respectively. Even though the initial temperatures were highest in the mixture of the food waste and insect feces whose temperature stood at 65 °C against 39, 58 and 51 °C in the sawdust, ginkgo leaves and mushroom waste mixtures, respectively on the third day of the experiment (DAT 3), it was terminated on the 21st day (DAT 21) because of excessively high water content (70.92%). The water content of the composted food waste supplemented with sawdust, mushroom waste, and ginkgo leaves stood at 51.28, 39.81, and 44.92%, respectively at the end of the experiment and therefore, the fully mature composts satisfied the water content requirement of less than 55% established by the Rural Development Agency of the ministry of Agriculture of Korea. The results of the CoMMe-101, Solvita and seed germination index indicated that the composted food waste amended with the mushroom waste and ginkgo leaves matured relatively quicker than that of the sawdust amendment. Based on the above observations, it is clear that the mushroom waste and ginkgo leaves are actually more effective bulking agents than the sawdust and as such, are recommended as suitable replacements for sawdust in food waste composting.

**Min-Kyu Park, Joo-Yeon Oh, Sung-Eun Lee, Sung-Deuk Choi. Determination of veterinary pharmaceutical runoffs from a swine manure pile using LC–MS/MS. (2020) Appl. Biol. Chem. 63: 69**

The mass usage of veterinary pharmaceuticals in farms has contributed to environmental pollution in vicinity waters, soils, and sediments from farms and composting facilities. In the present study, we investigated the usage of four antibiotics (viz., lincomycin, sulfamethazine, sulfamethoxazole, and trimethoprim) to understand their contamination routes from livestock manure piles. Residual levels of these antibiotics in a nearby reservoir were set as a positive control (Site 1), and a swine manure pile in a farm (Site 2) and a soil sample around the manure pile (Site 3) were selected for this study. Artificial rainwater was flowed into the manure sample (Site 2), the soil sample around the manure pile (Site 3), and a soil sample around the vicinity river (Site 4). A stream sample (Site 5) around the manure pile and river water near the manure pile (Site 6) were also collected. For qualitative and quantitative analyses, analytical validation was performed, and all the four antibiotics were detected at Site 1 in the concentration range of 0.03–1.6 µg/L. Lincomycin was the antibiotic with the highest detection level. At Site 2, the detection level of all antibiotics remained at 0.3–17.3 µg/L, and their residual amounts were continuously detected in subsequent samples with approximately 30-fold decrease. The migration of antibiotics was confirmed to be independent of pH value. Therefore, this study indicates that farm manure pile should be thoroughly managed for antibiotic contamination in vicinity areas with periodical monitoring, especially waterways.

**Guihun Jiang, Zhaogen Wu, Kashif Ameer, Shanji Li, Karna Ramachandraiah. Particle size of ginseng (*Panax ginseng* Meyer) insoluble dietary fiber and its effect on physicochemical properties and antioxidant activities. (2020) Appl. Biol. Chem. 63: 70**

Dietary fibers (DFs) and associated phytochemicals in ginseng species are known to provide various functional and health benefits. The incorporation of ginseng insoluble dietary fiber (IDF) in food products often result in undesirable physicochemical properties. Thus, to overcome such demerits, micronization of IDF has been considered. This study investigated the effect of particle size on the physicochemical properties, antioxidant activities, structure and thermal analysis of ginseng IDF. Micronized IDF powder with median particle diameter of 15.83 μm was produced through fine grinding. Reduction of ginseng IDF resulted in increased brightness, water holding capacity and solubility. Decreasing particle sizes also lowered bulk, tapped density, Carr index and Hausner ratio. Reduction of particle size caused greater extractability of mineral and phenolic content and thereby increasing the DPPH radical scavenging activity and ferric reducing antioxidant power. Increased polyphenol extraction with smaller particle size also lowered the mice erythrocytes hemolysis percentage while the hemolysis inhibition rate was increased. Particle size also influenced the thermal stability of ginseng IDF powders. FTIR spectra revealed lack of impact on the major phenolic structures due to superfine grinding. Hence,micronized ginseng IDF powders with improved physicochemical properties and antioxidant activities possess the potential to be used in food and pharmaceutical industries.

**Won-Pyo Park, Kong-Man Chang, Hae-Nam Hyun, Kyung-Hwan Boo, Bon-Jun Koo. Sorption and leaching characteristics of pesticides in volcanic ash soils of Jeju Island, Korea. (2020) Appl. Biol. Chem. 63: 71**

It is important to evaluate leaching behavior in agricultural soils to prevent the pollution of groundwater by pesticides. We identified the distribution coefficients (Kd) of ten pesticides with different physicochemical properties and compared their leaching characteristics using wick lysimeters from three distinct soil types on Jeju Island. The Kd values varied by pesticide and soil, but were within the range of 1.2 to 4231 L kg−1. Based on the European standard (Kd < 10 L kg−1), six pesticides (alachlor, ethoprophos, carbofuran, napropamide, tebuconazole, and etridiazole) were mobile in at least one tested soil, and their soil organic carbon affinity was ≤ 5.811. This value differed greatly from the other pesticides (16.533 and higher). The solubility of the six mobile pesticides was ≥ 32 mg L−1, which substantially differed from the other pesticides (≤ 0.71 mg L−1). Thus, we conclude that our mobility assessment, which is based on Kd values, can be used to predict the leaching of pesticides in the volcanic ash soils of Jeju Island. The use of pesticides should be strictly controlled to reduce the possibility of groundwater contamination.

**Zaihua He, Qiang Li, Xiaoyi Zeng, Kai Tian, Xiangshi Kong, Xingjun Tian. Impacts of peat on nitrogen conservation and fungal community composition dynamics during food waste composting. (2020) Appl. Biol. Chem. 63: 72**

Peat, as a heterogeneous mixture of decaying plant debris and microbial residues, has been widely used in many fields. However, little research focused on the impact of peat addition on food waste composting. To fill this gap, a composting experiment of food waste mixed with five varying percent peat 0, 5, 10, 15, and 20% (w/w, dry weight) was designed to investigate the effect of different dosages of peat on nitrogen conservation, physiochemical parameters, and fungal community dynamics during composting. The results showed that adding peat elevated the peak temperature of composting, lowered final pH, reduced ammonia emissions and increased the final total nitrogen content. Compared to control, adding 5, 10, 15, and 20% peat decreased ammonia emissions by 1.91, 10.79, 23.73, and 18.26%, respectively, during 42 days of composting. Moreover, peat addition increased fungal community diversity especially during maturation phase. The most two abundant phyla were Basidiomycota and Ascomycota in all treatments throughout the composting process. At the end of composting, in treatments with adding 10 and 15% peat, the richest fungi were *Scedosporium* spp. and *Coprinopsis* spp*.*, respectively. Simultaneously, canonical correlation analyses showed that pH, moisture content, and seed germination index had significant association with fungal community composition. The study also showed that fungal community and nitrogen conservation had no direct obvious relation during composting. Overall, the results suggest that the addition of peat could efficiently enhance nitrogen conservation through reduction of ammonia emissions and 15% peat addition is the optimal formula for food waste composting.

**Saoraya Chanmuang, Orawan Meemalai, Kitipong Promyo, Kyung-Hee Park, Suthipong Pongworn, Dal-Seong Gong, Min-Ho Oak, Jeong-Yong Cho, Seong-Gook Kang, Kyung-Sik Ham. Ameliorative effects of ark clams (*Scapharca subcrenata* and *Tegillarca granosa*) on endothelial dysfunction induced by a high-fat diet. (2020) Appl. Biol. Chem. 63: 73**

Endothelial dysfunction is directly involved in consequence of various metabolic syndromes such as diabetes and hypertension. In this study, we investigated the preventive effects of two ark clams [ark shell (AS, *Scapharca subcrenata*) and granular ark (GA, *Tegillarca granosa*)] on endothelial dysfunction induced by a high-fat diet. Wistar rats were divided into four groups as follows: control (normal diet), HF (high-fat diet), AS (high-fat diet + 5% AS powder), and GA (high-fat diet + 5% GA powder) for 12 weeks. AS and GA diets enhanced vascular reactivity of the rat thoracic aorta and significantly increased expression levels of vascular relaxation-related proteins (p-Akt-ser473 and p-eNOS-ser1177). Ark clam supplement reduced endothelin-1 expression level, as compared to the HF group. Additionally, AS and GA showed a trend of improving insulin sensitivity compared to HF. Our results suggest that AS and GA enhance vascular reactivity and ameliorated endothelial dysfunction induced by a high-fat diet.

**Hyun Ji Eo, Youngki Park, Seong Su Hong, Gwang Hun Park. Anti-inflammatory effects of 18-nor-*ent*-pimara-9(11),15-diene-4β-ol isolated from the roots of *Aralia continentalis* on LPS-induced in RAW264.7 cells. (2020) Appl. Biol. Chem. 63: 74**

*Aralia continentalis* (*A. continentalis)* is a medicinal plant belonging to Araliaceae, it has been reported to exert anti-cancer, anti-bacterial, anti-inflammatory, anti-platelet and anti-oxidative activities. But the potential mechanism for the anti-inflammatory effect of compounds isolated from the roots of *A. continentalis* is still insufficient. So, we evaluated whether compounds isolated from the roots of *A. continentalis* exert anti-inflammatory effects and elucidated its potential mechanism in RAW264.7 cells. The concentrated residue was subsequently suspended in H2O and partitioned with *n*-hexane, methylene chloride (CH2Cl2), ethyl acetate (EtOAc) and *n*-butanol (n-BuOH). The fractions were subjected to sequential column chromatography over silica-gel, RP-18, MPLC, recycling and preparative HPLC to isolated the novel compound. The novel compound was identified as 18-nor-*ent*-pimara-9(11),15-diene-4β-ol and confirmed anti-inflammatory activity. The 18-nor-*ent*-pimara-9(11),15-diene-4β-ol dose-dependently blocked NO production and inhibited iNOS, COX-2, TNF-α and IL-1β expression in LPS-stimulated RAW264.7 cells. The 18-nor-*ent*-pimara-9(11),15-diene-4β-ol inhibited LPS-stimulated degradation of IκB-α and nuclear accumulation of p65, which resulted in the suppression of NF-κB activation in RAW264.7 cells. Also, the 18-nor-*ent*-pimara-9(11),15-diene-4β-ol attenuated the phosphorylation of p38 and ERK1/2 in LPS-induced RAW264.7 cells. These results suggest that the nor-*ent*-pimara-9(11),15-diene-4β-ol isolated from the roots of *A. continentalis* may have grate potential for the development of anti-inflammatory drugs.

**Sooyeon Lim, Jin-Chul Ahn, Eun Jin Lee, Jongkee Kim. Antiproliferation effect of sulforaphene isolated from radish (*Raphanus sativus* L.) seeds on A549 cells. (2020) Appl. Biol. Chem. 63: 75**

Sulforaphene (SFE), a major isothiocyanate in radish seeds, is a close chemical relative of sulforaphane (SFA) isolated from broccoli seeds and florets. The anti-proliferative mechanisms of SFA against cancer cells have been well investigated, but little is known about the potential anti-proliferative effects of SFE. In this study, we showed that SFE purified from radish seeds inhibited the growth of six cancer cell lines (A549, CHO, HeLa, Hepa1c1c7, HT-29, and LnCaP), with relative half maximal inhibitory concentration values ranging from 1.37 to 3.31 μg/mL. Among the six cancer cell lines, SFE showed the greatest growth inhibition against A549 lung cancer cells, where it induced apoptosis by changing the levels of poly(ADP-ribose) polymerase and caspase-3, -8, and -9. Our results indicate that SFE from radish seeds may have significant anti-proliferative potency against a broad range of human cancer cells via induction of apoptosis.

**Wu Jing, Shahab Uddin, Rupak Chakraborty, Duong Thu Van Anh, Donah Mary Macoy, Si On Park, Gyeong Ryul Ryu, Young Hun Kim, Joon‑Yung Cha, Woe-Yeon Kim, Min Gab Kim. Molecular characterization of HEXOKINASE1 in plant innate immunity. (2020) Appl. Biol. Chem. 63: 76**

Hexokinase1 (HXK1) is an Arabidopsis glucose sensor that has a variety of roles during plant growth and devlopment, including during germination, flowering, and senescence. HXK1 also acts as a positive regulator of plant immune responses. Previous research suggested that HXK1 might influence plant immune responses via responses to glucose. Plant immune responses are governed by two main pathways: PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI). PTI involves the recognition of Pathogen-Associated Molecular Patterns (PAMPs) and leads to increased callose formation and accumulation of pathogenesis response (PR) proteins. ETI acts in response to effectors secreted by Gram-negative bacteria. During ETI, the membrane-localized protein RPM1-interacting protein 4 (RIN4) becomes phosphorylated in reponse to interactions with effectors and mediates the downstream response. In this study, the effects of glucose on plant immune responses against infection with *Pseudomonas syringae* pv. *tomato* DC3000 and other *P. syringae* strains were investigated in the presence and absence of HXK1. Infiltration of leaves with glucose prior to infection led to decreases in bacterial populations and reductions in disease symptoms in wild-type Arabidopsis plants, indicating that glucose plays a role in plant immunity. Both PTI and ETI responses were affected. However, these effects were not observed in a *hxk1* mutant, indicating that the effects of glucose on plant immune responses were mediated by HXK1-related pathways.

**Hyoung-Geun Kim, Ki Sun Kim, Minji Kim, Sang-Hwan Shin, Yeong-Geun Lee, Myun-Ho Bang, Dong-Geol Lee, Nam-In Baek. *β*-Glucogallin isolated from *Fusidium coccineum* and its enhancement of skin barrier effects. (2020) Appl. Biol. Chem. 63: 77**

Soil has been used for treatment of wound and skin diseases and for cosmetic purposes. *Fusidium coccineum* (FC) SA-1FC (Ascomycota) is a fungus found in nature, and its by-products are present in humid soils with plant humus. This study investigates the medium of fermented FC as a covering for all skin problems, including dryness, inflammation, and wounds. A preliminary study revealed that an alcohol extract of FC had a skin-enhancing effect, and thin-layer chromatography revealed a major component in a non-polar fraction. Here we identify a major compound isolated from a non-polar fraction as *β*-glucogallin. The mRNA levels of filaggrin and HAS3 are upregulated by FC and *β*-glucogallin treatment in keratinocytes and immortalized human keratinocytes cells. In addition, FC and *β*-glucogallin exert anti-inflammatory effects by suppressing expression of interleukin-4/poly(I:C)-induced chemokines and inflammatory cytokines. In fibroblasts, Hs68 cells, FC and *β*-glucogallin stimulate cell migration. These results suggest that FC and *β*-glucogallin can enhance skin barrier function.

**Hee-Weon Lee, Sang Keun Ha, Yoonsook Kim. Bisphenol A disrupts inflammatory responses via Nod-like receptor protein 3 pathway in macrophages. (2020) Appl. Biol. Chem. 63: 78**

Bisphenol A (BPA) is a harmful endocrine disruptor that is found in polycarbonate plastics such as plastic food containers and in epoxy resins such as dental resins. In the current study, we investigated the effect of BPA on function of inflammatory responses involving activation of Nod-like receptor protein 3 (NLRP3) inflammasome. Treatment with BPA decreased nitric oxide (NO) production and expression levels of inducible NO synthase (iNOS), prostaglandin E2 (PGE2), and cyclooxygenase (Cox)-2 in RAW 264.7 macrophages. BPA also suppressed activation of mitogen-activated protein kinases (MAPKs) and nuclear factor-kappa B activity (NF-κB). BPA significantly down-regulated the secretion of pro-inflammatory cytokines including tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-1β, and IL-18. The decreased production of IL-1β and IL-18 induced by BPA was associated with inactivation of the activity of the NLRP3 inflammasome. Collectively, these data suggested that BPA could act as a disruptor of the inflammation activity by regulating the NF-κB/MAPK pathways and NLRP3 inflammasome activation.

**Ghazala Muteeb, Adil Alshoaibi, Mohammad Aatif, Md. Tabish Rehman, M. Zuhaib Qayyum. Screening marine algae metabolites as high-affinity inhibitors of SARS-CoV-2 main protease (3CLpro): an in silico analysis to identify novel drug candidates to combat COVID-19 pandemic. (2020) Appl. Biol. Chem. 63: 79**

The recent dissemination of SARS-CoV-2 from Wuhan city to all over the world has created a pandemic. COVID-19 has cost many human lives and created an enormous economic burden. Although many drugs/vaccines are in different stages of clinical trials, still none is clinically available. We have screened a marine seaweed database (1110 compounds) against 3CLpro of SARS-CoV-2 using computational approaches. High throughput virtual screening was performed on compounds, and 86 of them with docking score <  − 5.000 kcal mol−1 were subjected to standard-precision docking. Based on binding energies (< − 6.000 kcal mol−1), 9 compounds were further shortlisted and subjected to extra-precision docking. Free energy calculation by Prime-MM/GBSA suggested RC002, GA004, and GA006 as the most potent inhibitors of 3CLpro. An analysis of ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties of RC002, GA004, and GA006 indicated that only RC002 (callophysin A, from red alga *Callophycus oppositifolius*) passed Lipinski’s, Veber’s, PAINS and Brenk’s filters and displayed drug-like and lead-like properties. Analysis of 3CLpro-callophysin A complex revealed the involvement of salt bridge, hydrogen bonds, and hydrophobic interactions. callophysin A interacted with the catalytic residues (His41 and Cys145) of 3CLpro; hence it may act as a mechanism-based competitive inhibitor. Docking energy and docking affinity of callophysin A towards 3CLpro was − 8.776 kcal mol−1 and 2.73 × 106 M−1, respectively. Molecular dynamics simulation confirmed the stability of the 3CLpro-callophysin A complex. The findings of this study may serve as the basis for further validation by in vitro and in vivo studies.

**Bala Murali Krishna Vasamsetti, Nam-Seok Kim, Kyongmi Chon, Hong-Hyun Park. Teratogenic and developmental toxic effects of etridiazole on zebrafish (*Danio rerio*) embryos. (2020) Appl. Biol. Chem. 63: 80**

Etridiazole (EDZ), a thiadiazole-containing toxic chemical, is widely used as a fungicide. Regular usage of EDZ may reach and contaminate water bodies, but its adverse effects on aquatic vertebrates have not been well studied. Therefore, the present study aimed to evaluate the harmful effects of EDZ using zebrafish (ZF) (*Danio rerio*) embryos. ZF embryos were treated with 3.75, 7.5, 15, 30, and 60 mg/L of EDZ. Subsequently, mortality and developmental toxicities were quantified at 24, 48, 72, and 96 h post fertilization (hpf). The results showed that embryo mortality was concentration- and time-dependent. The median lethal concentration (LC50) of EDZ at 96-h was 25.58 ± 1.49 mg/L. Besides, EDZ induced a series of morphological deformities, including abnormal somite formation, abnormal eye pigmentation, abnormal tail morphology, tail kinks, skeletal malformations (lordosis, kyphosis, and scoliosis), and yolk sac edema in a concentration-dependent manner. Among the deformities, the most significant were reduced heartbeat and increased incidence of pericardial edema. The median effective concentration (EC50) of EDZ at 96-h was 17.93 ± 2.22 mg/L and the 96-h teratogenic index (TI) value was 1.52. Taken together, these results indicate that EDZ is a teratogen, and primarily affects the cardiovascular system of ZF.

**Muhammad Haroon, Sun Chul Kang. Celastrol-mediated autophagy regulation in cancer. (2020) Appl. Biol. Chem. 63: 81**

In the last few decades, studies on autophagy regulation and its potential role in cancer therapeutics have expanded to include detailed mechanisms. Since apoptosis exhibits drug resistance in some cancers, efforts have focused on searching for compounds with autophagy modulating properties. Numerous natural compounds have been used in cancer treatment and are considered a significant research area due to their remarkable anti-cancer properties. Celastrol, a quinone methide triterpene, derived from *Tripterygium wilfordii*, has recently drawn much attention because of its anticancer potential. It enhances tumor suppression and induces autophagy in cancer cells by regulating signaling pathways such as Beclin-1, Akt/mTOR, ROS, NF-κB, MAPK, HSP90, and the proteasome. In the current study, we address the anticancer potential of celastrol, its effect on various cellular pathways, and describe how it functions as an autophagy modulator in cancer therapeutics and helps diminish multidrug resistance in cancer cells.

**Soon Young Shin, Jihyun Park, Yearam Jung, Young Han Lee, Dongsoo Koh, Youngdae Yoon, Yoongho Lim. Anticancer activities of cyclohexenone derivatives. (2020) Appl. Biol. Chem. 63: 82**

We designed 21 ethyl 3,5-diphenyl-2-cyclohexenone-6-carboxylate derivatives to identify compounds exhibiting anticancer activity. To measure the inhibitory effects of the compounds on cancer cell growth, a long-term survival clonogenic assay was performed. Since compounds containing a cyclohexenone moiety inhibit the enzyme acetylcholinesterase, an in vitro acetylcholinesterase assay was performed for all 21 cyclohexenone derivatives. To examine the effect of the derivative that exhibited the best cancer cell growth inhibition on the induction of apoptosis by demonstrating the activation of caspases and apoptosis regulatory proteins, immunoblotting and immunofluorescence microscopic analyses were performed. The binding mode between the cyclohexenone derivatives and acetylcholinesterase was elucidated at the molecular level using in silico docking. Druggability was evaluated based on ligand efficiency.

**Byeongwook Choi, Sungjong Lee, Eun Hea Jho. Removal of TPH, UCM, PAHs, and *Alk*-PAHs in oil-contaminated soil by thermal desorption. (2020) Appl. Biol. Chem. 63: 83**

Oil-contaminated soils from a former landfill and gas station site in Korea were treated by thermal desorption. The removal efficiencies of the different oil components such as total petroleum hydrocarbons (TPH), polyaromatic hydrocarbons (PAHs), unresolved complex mixtures (UCM), and alkylated PAHs (*Alk*-PAHs) by thermal desorption were determined. The effects of temperatures (200, 400, and 600 °C) and treatment times (15, 30, 45, and 60 min) on the thermal desorption efficiency were studied. The treatment efficiency increased with increasing temperature from 200 to 400 °C and with increasing treatment time. Almost complete removals of TPH, UCM, PAHs, and *Alk*-PAHs were observed after 15 min at 400 °C. The treatment temperatures of 400 and 600 °C did not show a significant difference (*p*-value > 0.05). Overall, this study shows that the different components of oil in the oil-contaminated soils can be treated effectively in a relatively short time by thermal desorption, and such high removal efficiency in a relatively short time for the oil-contaminated soils can be of advantage for the treatment of highly contaminated or weathered soils where biological treatment efficiency is low.

**Ju Sung Lee, Leo Adrianne Paje, Won-Hee Choi, Eun Ju Cho, Hyun Young Kim, Sonia D. Jacinto, Sanghyun Lee. Validation of an optimized HPLC/UV method for the quantification of flavonoids in lotus. (2020) Appl. Biol. Chem. 63: 84**

Flavonoids present in the leaves of lotus (*Nelumbo nucifera*) grown in different regions of South Korea (Yeongcheon, Haenam, and Seocheon) and at different harvest times (July to September) were determined. Flavonoid contents in lotus extracts were identified and analyzed using high-performance liquid chromatography (HPLC). The HPLC results revealed that the flavonoid contents of the lotus extracts varied at different harvesting times, with the highest content in July. Analysis of the flavonoid content in the leaves from the different regions showed the highest contents of isorhamnetin-3-*O*-glucoside, quercetin 3-*O*-glucuronide, and quercetin 3-*O*-glucoside in Yeongcheon, Korea, and highest contnts of rutin, myricetin, kaempferol 3-*O*-glucoside, and quercetin in Haenam, Korea. The HPLC method was validated and optimized to quantify quercetin 3-*O*-glucuronide; it showed good linearity (1000–62.5 µg/mL, *r*2 = 0.9999), accuracy (106%–108%), and precision (RSD ≤ 1.70%). Determination of flavonoid content in lotus is valuable for producing medicinal crops and identifying the optimal sources to increase the quantity of clinically available medicines.

**Guili Bao, Yinglong Zhang, Xiaoguang Yang. Effect of lemon peel flavonoids on anti-fatigue and anti-oxidation capacities of exhaustive exercise mice. (2020) Appl. Biol. Chem. 63: 85**

In this study, lemon peel flavonoids (LPF) were administered to investigate its effect on the anti-fatigue and antioxidant capacity of mice that undergo exercise until exhaustion. LPF (88.36 min in LPFH group mice) significantly increased the exhaustion swimming time compare to the untreated mice (40.36 min), increased the liver glycogen and free fatty acid content in mice and reduce lactic acid and BUN content in a dose-dependent manner. As the concentration of lemon peel flavonoids increased, the serum creatine kinase, aspartate aminotransferase, and alanine aminotransferase levels of mice gradually decreased. LPF increases superoxide dismutase (SOD) and catalase (CAT) levels in mice and reduces malondialdehyde levels in a dose-dependent manner. And LPF raises hepatic tissue SOD, CAT activities and reduces skeletal muscle tissue iNOS, TNF-α levels of mice compared to the control group. LPF also enhanced the expression of copper/zinc-superoxide dismutase (Cu/Zn-SOD), manganese-superoxide dismutase (Mn-SOD), and CAT mRNA in mouse liver tissue. LPF also enhanced the expression of alanine/serine/cysteine/threonine transporter 1 (ASCT1) mRNA and attenuate the expression of syncytin-1, inducible nitric oxide synthase (iNOS), and tumor necrosis factor (TNF)-α in mouse skeletal muscle. According to high-performance liquid chromatography (HPLC) analysis, it was found that LPF contains flavonoids such as rutin, astragalin, isomangiferin, naringin, and quercetin. Our experimental data show that LPF has good anti-fatigue effects and anti-oxidation ability. In summary, LPF has high prospects to be developed and added to nutritional supplements.

**Yunjeong Gwon, Jisun Oh, Jong-Sang Kim. Sulforaphane induces colorectal cancer cell proliferation through Nrf2 activation in a p53-dependent manner. (2020) Appl. Biol. Chem. 63: 86**

Sulforaphane is a well-known phytochemical that stimulates nuclear factor erythroid 2-related factor 2 (Nrf2)-mediated antioxidant cellular response. In this study, we found that sulforaphane promoted cell proliferation in HCT116 human colon cancer cells expressing a normal p53 gene in a dose-dependent but biphasic manner. Since p53 has been reported to contribute to cell survival by regulating various metabolic pathways to adapt to mild stress, we further examined cellular responses in both p53-wild-type (WT) and p53-knockout (KO) HCT116 cells exposed to sulforaphane in vitro and in vivo. Results demonstrated that sulforaphane treatment activated Nrf2-mediated antioxidant enzymes in both p53-WT and p53-KO cells, decreased apoptotic protein expression in WT cells but increased in KO cells in a dose-dependent manner, and increased the expression of a mitochondrial biogenesis marker PGC1α in WT cells but decreased in KO cells. Moreover, a low dose of sulforaphane promoted tumor growth, upregulated the Nrf2 signaling pathway, and decreased apoptotic cell death in p53-WT HCT116 xenografts compared to that in p53-KO HCT116 xenografts in BALB/c nude mice. These findings suggest that sulforaphane can influence colon cancer cell proliferation and mitochondrial function through a crosstalk between the Nrf2 signaling pathway and p53 axis.