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IS1-1

Novel roles for the ubiquitin system in the modulation of light-responsive chromatin remodelling and gene expression in Arabidopsis

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The relevance of protein ubiquitination as an integral mechanism of many signaling pathways in plants has been demonstrated extensively. Ubiquitin (Ub) conjugation to proteins (i.e. ubiquitination) may trigger degradation of protein targets at the 26S proteasome or changes in their properties (e.g., protein activity, localization, assembly and interaction ability), depending on the extent or specific Ub chain configurations. Ub attachment to proteins (ubiquitination) is mediated by an enzymatic cascade in which different types of E3 Ub ligases provide the substrate specificity. Cullin4 RING E3 ubiquitin ligases (CRL4) have been involved in biological processes spanning the plant's whole life, including embryogenesis, seedling photomorphogenesis, circadian clock function and flowering by promoting degradation of specific targets controlling those processes. Different substrate adaptors allow recognition of targets, including the so-called COP10, DDB1, DET1, DDA1 (CDDD) complex, which cooperates with CRL4 in response to developmental but also stress signals. Known targets whose stability depends on CRL4-CDDD include photomorphogenesis regulator HY5 and DNA damage sensor DDB2. More recently, we have shown that DDA1, as part of CRL4-CDDD complexes, acts as a novel type of substrate adaptor to recognize abscisic acid (ABA) receptors of the PYR/PYL/RCAR (pyrabactin resistance/pyrabactin resistance-like/regulatory components of ABA) family, triggering their ubiquitination and proteasomal degradation (Irigoven et al., The Plant Cell 2014). Therefore, DDA1-containing CRL4 complexes also function as repressors of ABA-mediated stress responses. We aim to characterize the potential mechanism enabling crosstalk between light signaling, DNA damage and ABA which should help us to evaluate the role of CRL4-CDDD complexes as hubs integrating signaling from different stresses and stimuli. This CRL4-CDDD function is likely performed in close proximity to chromatin, which should enable rapid translation of environmental and stress signals into changes in gene expression. Evidences about CRL4-CDDD association to chromatin have been previously shown as DET1 interacts with H2B and has a role as a co-repressor of TOC1 expression. However, the full consequences of DET1 function while bound to chromatin are unknown. Recent results from our laboratory offer a novel connection for CRL4-CDDD in the control of chromatin-associated processes and epigenetic regulation of gene expression in response to environmental cues. As part of the talk, our latest results in these regards will be presented.



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Synthetic gene circuits for genetic biocontainment

IS1-2

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Synthetic gene circuits and switches are increasingly used to control gene expression in various industrial and clinical applications. The remaining challenges in building synthetic gene circuits and switches for more practical, real-world applications are to minimize cellular burden to maintain the genetic circuits stably in the host but to provide simple biocontainment solutions. First, we use empirical design and iterative construction and testing to build single-copy, bistable toggle switches with improved performance and reduced metabolic load that can be stably integrated into the host genome. The theory to create the single-copy circuit has been implemented to develop biocontainment system that couples environmental sensing with circuit-based control of cell viability could be used to prevent escape of genetically modified microbes into the environment. The kill switch uses unbalanced reciprocal transcriptional repression to couple a specific input signal with cell survival. The synthetic gene circuits efficiently kill *Escherichia coli* and can be readily reprogrammed to change their environmental inputs, regulatory architecture and killing mechanism. Overall, the given synthetic biology platform that resolves important practical limitations facing many different applications demonstrate how synthetic biology can be used to develop future molecular and cellular devices.



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IS1-3

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Plant defense mechanism is a sophisticated and complex process that is controlled by both the transcriptional and posttranscriptional regulation of gene expression. Here, we identify an Arabidopsis noncoding RNA, designated elf18-induced noncoding RNA 1 (ELENA1), as a factor enhancing resistance against *Pseudomonas syringe* DC3000. We show that ELENA1 acts through *pathogenesis-related 1* (*PR1*), a key marker gene for SAR response. ELENA1 knockdown mutants lead to a significant decrease in the expression of *PR1* and pathogen susceptible phenotype. On the other hand, over-expressing ELENA1 transgenic plants show enhanced *PR1* gene expression and pathogen resistant phenotype. We find that ELENA1 directly interacts with mediator 19a (MED19a), a positive regulator of *PR1* expression, and double mutants between ELENA1 and MED19a show that *PR1* regulation of ELENA1 is controlled by MED19a. Recently we found that ELENA1 could dissociate the interaction between MED19a and MED36a. We thus concluded that ELENA1 is a previously uncharacterized noncoding RNA models.



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IS1-4

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Meristems contain groups of indeterminate stem cells, which are maintained by a feedback loop between CLAVATA (CLV) and WUSCHEL (WUS) signaling. CLV signaling involves the secretion of the CLV3 peptide and its perception by a number of Leucine-Rich-Repeat (LRR) receptors, including the receptor-like kinase CLV1 and the receptor-like protein CLV2 coupled with the CORYNE (CRN) pseudokinase. CLV2, and its maize ortholog FASCIATED EAR2 (FEA2) appear to function in signaling by CLV3 and several related CLV3/EMBRYO-SURROUNDING REGION (CLE) peptide ligands. Nevertheless, how signaling specificity is achieved remains unknown. Recently, we identified a distinct CLV receptor, FASCIATED EAR3 (FEA3) in maize and Arabidopsis, and found that FEA3 controls responses to the maize FCP1 (ZmFCP1) CLE peptide. Here, we show that the maize CLV2 ortholog FEA2 also participates in ZmFCP1 signaling, in addition to controlling responses to the maize CLV3 ortholog, ZmCLE7. To ask how specificity from these different CLE peptide inputs is achieved, we first isolated mutant alleles of the maize CRN gene. Consistent with results in Arabidopsis, we found that *fea2* was epistatic to Zmcrn in control of meristem size, but Zmcrn;ct2 double mutants showed an additive enhanced phenotype, suggesting they act in parallel pathways, despite the fact that FEA2 binds both ZmCRN and CT2 in co-immunoprecipitation (co-IP) experiments. Strikingly, ct2 and Zmcrn mutants were resistant to different CLE peptides, ZmCLE7 and ZmFCP1, respectively, but fea2 was resistant to both, suggesting that FEA2 controls responses to different CLE peptides by acting through different downstream effectors. Our data provide a novel framework to understand how diverse signaling peptides can activate different downstream pathways through common receptor proteins.





Transcriptional regulation of Brown Adipose Tissue Thermogenesis

IS2-1

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Brown adipose tissue (BAT) is appreciated as a potential therapeutic strategy against obesity and diabetes because of its ability to take up circulating fatty acids and glucose and transform the resulting energy into heat. Transcriptional coactivator PGC-1 α is a key regulator of adaptive BAT thermogenesis. PGC-1 α mediates its action by activating various transcription factors that regulate expression of UCP1 and mitochondrial genes involved in mitochondrial biogenesis and oxidative metabolism. We previously identified a splice variant of the PGC-1 α gene that encodes a functional <u>N</u>-terminal isoform of PGC-1 α (NT-PGC-1 α). Mice lacking both PGC- 1α and NT-PGC- 1α are unable to produce heat in response to cold due to impaired induction of UCP1 and mitochondrial gene expression in BAT. In contrast, mice selectively lacking PGC-1a are cold-tolerant. Mechanistically, NT-PGC-1 α activates cold-induced BAT thermogenesis in the absence of PGC-1 α by upregulating UCP1 and many mitochondrial genes involved in fatty acid oxidation (FAO), TCA cycle, and electron transport. Our recent studies using NT-PGC-1 α / mice further reveals that NT-PGC-1 α deficiency specifically downregulates FAO gene expression in BAT, decreasing the capacity to oxidize fatty acids. Reduced fatty acid oxidation in turn enhances glucose utilization in NT-PGC-1 α / BAT. In agreement with this finding, NT-PGC-1 α^{-1} mice exhibit a greater reliance on carbohydrates to support BAT thermogenesis. Collectively, our data demonstrate a particularly significant role for NT-PGC-1 α in regulating fatty acid oxidation in BAT and provide deeper insights into the relative contribution of PGC-1 α and NT-PGC-1 α to adaptive BAT thermogenesis.



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Investigation Bioactive Natural Product from Medicinal Plant of Kazakhstan

IS2-2

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The traditionally medicine have played an important role in developing of natural product chemistry. The World Health Organization (WHO) estimates that 80% of the 4 billion people in the world depend on plants or medicinal plant related products for primary health care. In Kazakhstan grow over six thousand kinds of plants in which more than 6000 species of highest vascular plants, about 5000 species of mushrooms, 4851 species of lichen, more than 2000 species of seaweed are registered. Meanwhile the plant resources have been efficiently used in the treatments of different kinds of emerging and re-emerging diseases such as diabetics, bronchial asthma, cancer, hepatitis, tuberculosis, chronic rheumatoid arthritis, nephritis, hypertension, gastropathy, hemostasis, and metrorrhagia in Kazakh medicine. Therefor our research has been focused on the biologically active chemical components of medicinal plants from Kazakhstan. Using these rich natural resources to study new biological active constituents that can be used as anti-diabetics, anti-tumor and anti-hypertension drug leads, as well as to solve the problems of creation and implementation of pharmaceutical production in Kazakhstan.

The *Limonium* genus have been widely used as an important medicinal herb due to its various health promoti ng effects including improvement of blood circulation, which relates with angiotensin-1 converting enzyme (ACE). We found ACE inhibitory potential from *L. michelsonii* Lincz. which is an endemic medicinal plant in Kazakhstan, but has not been explored its secondary metabolites. The principle phenolic compounds (1-20) were isolated to recognize the responsible components of ACE inhibition. The natural abundance of active constituents within target plant were characterized by UPLC-Q-TOF-MS analysis. All isolated compounds except gallate (10-12) showed significant inhibition against ACE with IC₅₀ values between 7.1 and 138.4 μ M. In kinetic study, flavonols (3-5, 16-20) and dihydroflavonols (8, 9) behaved as competitive inhibitors, whereas other flavones (1, 2, 13-15) and flavanones (6, 7) showed noncompetitive inhibition.



Pseudane-VII isolated from *Pseudoalteromonas sp*.M2 ameliorates LPS-induced inflammatory response in vitro and vivo

IS2-3

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The ocean is a rich resource of flora, fauna, food, and biological products. We found a wild-type bacterial strain such as *Pseudoalteromonas* sp.M2 from marine water and isolated various secondary metabolites. Pseudane-VII is a compound isolated from the Pseudoalteromonas sp.M2 metabolite that possesses antimelanogenic activity. Inflammation is a response of the innate immune system to microbial infections. Macrophages have a critical role in fighting microbial infections and inflammation. Recent studies reported that various compounds derived from natural products can regulate immune responses including inflammation. However, the anti-inflammatory effects and mechanism of pseudane-VII in macrophages are still unknown. In this study, we investigated the anti-inflammatory effects of pseudane-VII. In present study, lipopolysaccharide (LPS)-induced nitric oxide (NO) production was significantly decreased by pseudane-VII treatment at 6 µM. Moreover, pseudane-VII treatment dose-dependently reduced mRNA levels of pro-inflammatory cytokines including *inos*, cox-2, $il-1\beta$, $tnf-\alpha$, and il-6 in LPS-stimulated macrophages. Pseudane-VII also diminished iNOS protein levels and IL-1ß secretion. In addition, Pseudane-VII elicited anti-inflammatory effects by inhibiting ERK, JNK, p38, and nuclear factor (NF)-KB-p65 phosphorylation. Consistently, pseudane-VII was also shown to inhibit the LPS-stimulated release of IL-1 β and expression of iNOS in mice. These results suggest that pseudane-VII exerted anti-inflammatory effects on LPS-stimulated macrophage activation via inhibition of ERK, JNK, p38MAPK phosphorylation, and pro-inflammatory gene expression. These findings may provide a new approaches in the effort to develop anti-inflammatory therapeutics.





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Immunological network for skin inflammation in the conditional knockout animal model

IS2-4

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Skin is a barrier for maintaining homeostasis of physiological role to protect from outside invaders and has series of cytokines network. In the last couple of years, many researches are focused on the role of Th17 and its signature cytokine network, especially IL-17, in disease model such as rheumatoid arthritis, psoriasis, and multiple sclerosis. Psoriasis is an immune-mediated disease, affecting to the skin – increase proliferative keratinocytes, recruit immune related cells in the dermis, and produce inflammatory cytokines. In animal model of psoriasis as well as human experimental data shows that Th17 and IL-17 have a critical role in the pathogenesis of psoriatic disease. As a clinical treatment, blocking IL-17 cytokine and its network has an important role in T cell populations. We found that inflamed skin showed significantly increase the number of CD45 positive cells, specially CD3 positive, MHC class II positive, and NK cells than normal skin. However, IL-17A blockage did not increase Th1 and IFN-gamma producing T cells. Herein, we demonstrate that the majority of IL-17A producers are specific subpopulation of T-cells and provide a mechanistic link between the genetic defect in keratinocytes and the characteristic dermal inflammation.



Epigenetics in Cancer: p300-MITF Axis is a Potential Therapeutic Target in Human Melanoma

IS3-1

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Epigenetic regulation of chromatin modification, including post-translational modifications of histones and promoter DNA methylation, has been demonstrated to have important roles in carcinogenesis and malignant progression. The transcriptional coactivator p300/CBP possesses histone acetyltransferase (HAT) activity and scaffolding properties that directly control transcriptional activation of targeted genes. Despite significant effort, the mechanism by which the p300 transcriptional coactivator mediates tumorigenesis remains unclear. Here, we use a genetic and chemical approach to identify the Microphthalmia-associated transcriptional control of MITF) as a critical downstream target of p300 driving human melanoma growth. Direct transcriptional control of MITF by p300-dependent histone acetylation within proximal gene regulatory regions was coupled to cellular proliferation, suggesting a significant growth regulatory axis. Further analysis revealed Forkhead Box M1 (FOXM1) as a key effector of the p300-MITF axis driving cell growth, which is selectively activated in a subset of human melanoma patients. Targeted chemical inhibition of p300/CBP HAT activity confirmed the critical role of the p300-MITF-FOXM1 axis in melanoma. Analysis of clinical patient tissue samples of TCGA (The Cancer Genome Atlas) dataset also validated the existence of significant correlation between the transcriptional regulation of MITF and FOXM1 by p300 and overall patient survival. These data support p300 as a promising novel epigenetic therapeutic target in human melanoma



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Strategy for promoting utilization of kenaf through fractionation technique: Hydrochloric acid-ethanol organosolv fractionation

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Ethanol organosolv fractionation technique is promising procedure for production of organosolv lignin from kenaf as energy crop. However, the main problem of organosolv fractionation technique could not use the C5 sugars due to removal into black liqour during organosolv fractionation. The HCl fractionation has been proposed to resolve this problem prior to organosolv fractionation. In this study, we were fractionated with 0.1-0.6 wt% HCl (solid : liquid ratio 1:10) at 130-190 °C for 60 min. Then, the fractionated solids were secondary fractionated with 60 vol% ethanol under various conditions (solid: liquid ratio 1:10, 120-180 °C, 60 min), and the composition changes of fractionated solids, liquid (hydrolysate and black liquor), and lignin after each step were analyzed for comparison. Subsequent HCl-ethanol fractionation under optimal condition was scale-up in a 7-L reactor. Fractionation technique based on hydrochloric acid and ethanol indicated excellent effectiveness to fractionate the cellulose, hemicellulose, and lignin.



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IS3-3

Establishment of the methods for propylene glycol, vegetable glycerin and nicotine in the aerosol and the solution of E-cigarettes by gas chromatographic analysis

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Electronic cigarettes(known as E-cigarettes) are devices designed to feel like conventional cigarettes by converting solution into aerosol. Main components in E-cigarette solution are propylene glycol(PG), vegetable glycerin(VG) and nicotine. In this study, simple and reliable methods for analysis of PG, VG and nicotine in E-cigarette solution and aerosol were established using gas chromatograph with flame ionization detector(GC-FID). The aerosolized PG, VG and nicotine generated from E-cigarette was automatically collected on 44 mm cambridge filter pads from e-smoking machine with the following puff conditions: puff velocity(1 L/min), puff duration(2 s), puff frequency(10 s), and puff number(10 times). The analytes in the refill solution and the aerosol was extracted with isopropanol with quinoline(internal standard) and analyzed. The inter-day and the intra day precision of the method were under 4% and 6%, respectively. The accuracy ranged from 98 ~ 106%, the linearity was over 0.999, and the recovery ranged from 94 ~ 104%. This analytical methods could be suitable for the determination of contents of PG, VG and nicotine in the aerosol and the solution.





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Effect of dietary organic selenium on juvenile steelhead trout Oncorhynchus mykiss

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Effect of dietary selenomethionine (SeMet) (control, 10, 20, 40, 80 mg SeMet/kg diet) were evaluated on a 4week study using juvenile steelhead trout *Oncorhynchus mykiss* in a recirculating tank system. Growth performance, proximate composition of whole body, tissue Se accumulation (gill, liver, kidney, white muscle), and hematological parameters (hematocrit, RBC count, hemoglobin concentration, etc) were evaluated. There were significant (p<0.05) increases in mortality and depression in growth of the juvenile fed SeMet diets compared to the control.

Results are currently being analyzed and will be reported in this abstract soon.

IS3-4



Systems biological evaluation of lactic acid bacteria and their host interactions for probiotic applications

IS4-1

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Obligate heterofermentative lactic acid bacteria (LAB) are well-known for their beneficial health effect s in humans. However, this LAB group remains largely underexploited due to limited understanding of the interplay between cellular energetics and redox state that govern their metabolic capabilities unlike other homolactic or facultatively heterolactic fermenting groups of LAB. To delineate such intrinsic relationships at systems level, we newly reconstructed a genome-scale metabolic model of a representative obligate heterofermenting LAB, Leuconostoc mesenteroides (iLME620), followed by constr aint-based flux analysis to evaluate the model validity both qualitatively and quantitatively [1]. More importantly, we employed iLME620 to elucidate unique metabolic characteristics of L. mesenteroides, such as the limited ability to utilize amino acids as energy source, and to substantiate the role of malolactic fermentation (MLF) in the reduction of pH-homeostatic burden on F0F1-ATPase. Model simulations further revealed possible proton-symporter dependent activity of the energy efficient glucosephosphotransferase system in obligate heterofermentative LAB. Aided by integrative transcriptomic analysis, we indicate transcriptional regulatory bias towards processes influencing intracellular redox state in L. mesenteroides. Subsequently, comparative genomic, phenomic and transcriptomic analyses of six LAB strains falling under the genera of Lactobacillus, Lactococcus and Leuconostoc were carried out to elucidate their probiotic and postbiotic traits by resorting to novel computational framework for host-LAB interaction modeling. We believe that the insights gained from the current integrative systems biology approaches of various LAB strains could potentially aid strain design for probiotic and cell factory applications in future. [This work was supported by the Next-Generation BioGreen 21 Program (SSAC, No. PJ01334605), Rural Development Administration, Republic of Korea]

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Diversity of bacterial ubiquinone biosynthesis -Identification of new group ubiquinone biosynthetic flavin monooxygenases in *Rhodobacter capsulatus*

IS4-2

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Ubiquinone biosynthetic pathway contains three hydroxylation steps in eukaryote and prokaryote. This is well studied in *Escherichia coli* which has three enzymes, UbiF, UbiH, UbiI. All of them are flavin monooxygenase (FMO) and each of them serves in specific reaction. Eukaryote also possesses a homologue of these enzymes, called Coq6. On the other hand, another type of monooxygenase Coq7 which contains di-iron center participates in UQ biosynthesis. Homologue of Coq7 is not found in *Escherichia coli*, but found in some other bacteria. Even though UQ biosynthesis requires three steps of hydroxylation reactions, the number of UQ biosynthesis related monooxygenases are limited to one or two in some bacteria.

Photosynthetic bacteria, *Rhodospirillum rubrum* and *Rhodobacter capsulatus* have only two monooxygenases. *R. rubrum* has one Coq7 and one FMO, and *R. capsulatus* has two FMOs. In order to identify the correspondence of these enzymes to three reactions, we have prepared KO strains of Coq7 and FMO in *R. rubrum* by replacing genes with antibiotic resistant gene. Since these KO strains can grow in anaerobic photosynthesis, they could be maintained although they showed growth defect in aerobic respiration. Two FMO from *R. capsulatus* were tested whether to complement the UQ biosynthesis or not. This can judged easily by anaerobic growth.

In 2016, Pelosi et al have reported phylogenetic analyses of UQ biosynthetic FMOs. Based on that analysis, some FMOs were named UbiL or UbiM. Interestingly, some UbiL or UbiM performed two or three hydroxylation reactions. FMO from *R. rubrum* was classified to UbiL and shown to serve in the two reactions same as UbiH and UbiI of *E. coli*. Both two FMOs from *R. capsulatus* were classified to UbiL.

From the complementation experiments, *Rc-ubiL1* complemented aerobic growth of $\Delta coq7$ of *R. rubrum*. On the other hand, *Rc-ubiL2* nor two genes introduction didn't complement that of $\Delta ubiL$ of *R. rubrum*. These result suggested the existence of third UQ biosynthetic monooxygenase in *R. capsulatus*. As a candidate of that, a FMO gene (*Rc-fmo1*) which showed lower amino acid sequence similarity compared to other UQ biosynthetic FMOs was picked up by blast search. Finally, aerobic growth of $\Delta ubiL$ of *R. rubrum* was complemented by the combination of *Rc-ubiL2* and *Rc-fmo1*.

As a result, we have identified three UQ biosynthetic flavin monooxygenases in *R. capsulatus*. The third FMO showed lower amino acid sequence identities compared to other FMOs.



Oligomeric Proanthocyanidins with Potent Dentin Biomodification Activity

IS4-3

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Proanthocyanidins (PACs) are one of the major classes of polyphenols which represent the condensed tannins. While various bioactivities such as anti-oxidant, anti-inflammatory, anti-microbial, and anti-allergic effects have been frequently reported, little is known about their activities as dentin biomodifiers. The ability of certain oligomeric proanthocyanidins (OPACs) to enhance the biomechanical properties of dentin involves collagen cross-linking of the 1.3–4.5 nm wide space via protein–polyphenol interactions. A systematic interdisciplinary search for the bioactive principles from the OPAC-enriched plant sources has yielded active entities capable of enhancing dentin stiffness. The structures of isolated compounds were assigned by a combination of HRESIMS, ECD, 1D/ 2D NMR, and Quantum-mechanics-driven ¹H iterative full spin analysis (QM-HiFSA). QM-HiFSA of NMR spectra distinguished previously unrecognized details such as higher order *J* coupling and provided valuable information about 3D structure. This study also applied the DESIGNER (Depletion and Enrichment of Select Ingredients Generating Normalized Extract Resources) approach for the selective enrichment of trimeric and tetrameric PACs using centrifugal partition chromatography (CPC). This method plays a key role in the development of dental biomaterials considering its reliability and reproducibility, as well as its scale-up capabilities for possible larger-scale manufacturing.



Characterization of a novel yeast species *Metschnikowia persimmonesis* KCTC 12991BP (KIOM G15050 strain) isolated from the Korean persimmon calyx (*Diospyros kaki* Thumb)

IS4-4

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The yeast strain *Metschnikowia persimmonesis* Kang and Choi *et al.*, sp. nov. [type strain KIOM_G15050 = Korean Collection for Type Cultures (KCTC) 12991BP] was isolated from the stalk of native persimmon cultivars (*Diospyros kaki* Thumb) obtained from different regions of South Korea and was characterized phenotypically, genetically, and physiologically. The isolate grew between 4 and 40 °C (optimum temperature: 24-28 °C), pH 3–8 (pH optimum = 6.0), and in 0–4% NaCl solution (with optimal growth in absence of NaCl). It also exhibited strong antibiotic and antimicrobial activities. Morphologically, cells were characterized by the presence of long, needle-shaped ascospores. Based on 18S ribosomal DNA gene sequence analysis, the new species was found to belong to the genus *Metschnikowia as* a sister clade of *Metschnikowia fructicola*. We therefore conclude that this yeast isolate from *D. kaki* is a new member of the genus *Metschnikowia* and propose the name *M. persimmonesis* sp. nov. This strain has been deposited in the KCTC for future reference. This discovery provides a basis for future research on *M. persimmonesis* sp. nov., including its possible contribution to the medicinal properties of the host persimmon plant. [Acknowledgement: This work was supported under 2018 KIOM project (K18720) 'Development of Cosmetic Preservation using Korean Herbal Medicine Associated Patent Yeast (KIOM G15050 strain) based on demand of industry'.]

