SCHEDULE

October 16 (Tue), 2018. (Okayama International Center)

9:00-9:30	**Registration (2F International Conference Hall)**
9:30-9:40	**Opening of the Conference (2F International Conference Hall)**
9:40-10:40	**Session I (2F International Conference Hall)** "Food and Nutrition Research in East Asia and the Surrounds (1)"
10:40-10:55	**Coffee Break (7F Multipurpose Hall)**
10:55-11:55	**Session II (2F International Conference Hall)** "Food and Nutrition Research in East Asia and the Surrounds (2)"
11:55-13:15	**Luncheon Seminar** (Only for the members of "12th Joint Conference on Nutrition" and BAO2018 committee)
13:15-14:15	**Session III (2F International Conference Hall)** "Food and Technology (1)"
14:20-15:20	**Oral Presentation of Young Investigators (2F International Conference Hall)**
15:20-16:30	**Poster Presentation of Young Investigators & Coffee Break (7F Multipurpose Hall)**
16:30-17:30	**Session IV (2F International Conference Hall)** "Food and Technology (2)"
17:30-17:40	**Announcement of Student Awards & Closing of the Conference**
18:00-20:00	**Banquet (B1F Reception Hall)**

PROGRAM

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9:30-9:40 **Opening of the Conference (2F International Conference Hall)**

9:40-10:40 **Session I (2F International Conference Hall)**
"Food and Nutrition Research in East Asia and the Surrounds (1)"
Chairs: Ding Zhi Fang (Sichuan Univ.)
Yoshitaka Takahashi (Okayama Pref. Univ.)

9:40-10:00 O-1 Hongbing Chen (Nanchang Univ.)
Effects of C18 unsaturated fatty acids on the structure and allergenicity of bovine α-lactalbumin

10:00-10:20 O-2 Jetty Chung-Yung Lee (Univ. Hong Kong)
Neuroprostanes derived from non-enzymatic DHA peroxidation displays neuroprotective properties in the rodent brain

10:20-10:40 O-3 Jia Lin (Sichuan Univ.)
Effects of high-carbohydrate and low-fat diets on serum lipid profiles in healthy young subjects with different genetic variations

10:40-10:55 **Coffee Break (7F Multipurpose Hall)**

10:55-11:55 **Session II (2F International Conference Hall)**
"Food and Nutrition Research in East Asia and the Surrounds (2)"
Chairs: Hongbing Chen (Nanchang Univ.)
Yasuyuki Irie (Okayama Pref. Univ.)

10:55-11:15 O-4 Gyu-Hee Lee (Woosong Univ.)
Physicochemical and sensory characteristics of pan bread made with various squeezed perilla leaf juice amount during storage

11:15-11:35 O-5 Chi Bun Chan (Univ. Hong Kong)
Regulation of muscle physiology by 7,8-dihydroxyflavone

11:35-11:55 O-6 Yuki Kawakami (Okayama Pref. Univ.)
Mutations of anti-leukotriene C_4 single-chain antibody change the antigen binding specificity

11:55-13:15 **Luncheon Seminar**
(Only for the members of "12th Joint Conference on Nutrition" and BAO2018 committee)

13:15-14:15 **Session III (2F International Conference Hall)**
"Food and Technology (1)"
Chairs: Ryoji Mitsui (Okayama Univ. Sci.)
Koichi Tanaka (Okayama Pref. Univ.)

13:15-13:35 O-7 Takanori Yano (Okayama Univ. Sci.)
Isolation of wild yeasts from local region and their application to winemaking

13:35-13:55 O-8 Kensuke Arakawa (Okayama Univ.)
Sweetened egg white can be fermented with *Lactobacillus casei*-group lactic acid bacteria

13:55-14:15 O-9 Izumi Tsukayama (Okayama Pref. Univ.)
Anti-cancer effect of *Dioscorea japonica* and the constituent diosgenin through down-regulation of prostaglandin E_2 synthetic pathway

14:20-15:20 **Oral Presentation of Young Investigators**
(2F International Conference Hall)
Chairs: Yoshimasa Nakamura (Okayama Univ.)
Yuki Kawakami (Okayama Pref. Univ.)

14:20-14:22 P-1 Jun Chen (Nanchang Univ.)
Production of whole-bean soymilk by pilot-scale microfludization

14:22-14:24 P-2 Rikako Inoue (Okayama Pref. Univ.)
Effect of malted-rice amazake on intestinal environment

14:24-14:26 P-3 Yuji Iwaoka (Okayama Pref. Univ.)
Affinity resins as tools for identifying target proteins of ascorbic acid

14:26-14:28 P-4 Keita Kanzaki (Kawasaki Univ. Med. Welf.)
Ingestion of soy protein isolate attenuates eccentric contraction-induced force depression and muscle proteolysis via inhibition of calpain-1 activation in rat fast-twitch skeletal muscle

14:28-14:30 P-5 Ken-Ichi Kobayashi (Notre Dame Seishin Univ.)
Dynamic visualization of anti-colorectal cancer effect of resveratrol using fluorescence labeling strategies

14:30-14:32 P-6 Jing Li (Nanchang Univ.)
The effect and mechanism of elaidic acid and trans vaccenic acid on HUVECS function based on phospholipids profile

14:32-14:34 P-7 Hitomi Maruta (Okayama Pref. Univ.)
Effect of *Bifidobacterium* fermented milk on anti-obesity

14:34-14:36 P-8 Junko Masuda (Okayama Univ.)
Cytokine expression and macrophage localization in xenograft and allograft tumor models stimulated with lipopolysaccharide

14:36-14:38 P-9 Toshiyuki Nakamura (Okayama Univ.)
Comparison of bioavailability of quercetin monoglucoside and its aglycone

14:38-14:40 P-10 Michiko Nemoto (Okayama Univ.)
Identification of proteins involved in tooth biomineralization in the giant Pacific chiton by integrated transcriptomic and proteomic analysis

14:40-14:42 P-11 Guang Yue Ren (Henan Univ. Sci. Technol.)
The effect of glass transition temperature on the procedure of microwave–freeze drying of Mushrooms (*Agaricus bisporus*)

14:42-14:44 P-12 Shota Tokai (RIBS)
Comparison of *Streptomyces* transglutaminases on substrate specificities toward casein peptides

14:44-14:46 P-13 Yuki Yamamoto (Okayama Univ.)
Ion channels involved in the generation mechanism of phasic spontaneous contraction of bovine oviducts

14:46-14:48 P-14 Qiang Yu (Nanchang Univ.)
Immunomodulatory activity of *Ganoderma atrum* polysaccharide on purified T lymphocytes through Ca^{2+}/CaN and MAPK pathway based on RNA-seq

14:48-14:50 PY-1 Februadi Bastian (Okayama Pref. Univ.)
New polyphenols from *Punica granatum* and their anti-glycation activities

14:50-14:52 PY-2 Yoshiko Fujitani (Okayama Univ.)
Functional analysis of lanthanide inducible proteins of *Methylobacterium aquaticum* 22A

14:52-14:54 PY-3 Yuuki Haruna (Okayama Univ.)
Molecular mechanism of methylotaxis in *Methylobacterium aquaticum* strain 22A

14:54-14:56 PY-4 Masahiro Ide (Okayama Univ.)
Effects of orizabin from *Tithonia diversifolia* on vascular protection and suppression of foam cell formation in human umbilical vein endothelial cells and human monocytic THP-1 cells

14:56-14:58 PY-5 Mana Koide (Okayama Univ.)
Isolation of *p*-nitrophenyl *N*-acetyl-β-D-glucosaminide assimilating bacteria and their β-*N*-acetylglucosaminidase sensitivity to *p*-nitrophenyl *N*, *N*, *N*-trimethyl β-D-glucosaminium iodide

14:58-15:00 PY-6 Natsuko Komoto (Notre Dame Seishin Univ.)
Quinolinic acid phosphoribosyltransferase (QPRT) knockout mouse as a novel noninvasive chronic kidney disease (CKD) animal model and a possible animal model for the search of bioactive compounds with preventive effect in CKD

15:00-15:02 PY-7 Yun Ma (Okayama Pref. Univ.)
Study on mechanism of taurine function on skeletal muscle of aged rats

15:02-15:04 PY-8 Kana Matsukawa (Okayama Univ.)
Identification of enzymes derived from *Saccharomyces cerevisiae* catalyzing the reaction of olive leaf secondary metabolite, 3,4-dihydroxyphenylethanol-elenolic acid to reduced 3,4-dihydroxyphenylethanol-elenolic acid

15:04-15:06 PY-9 Asaduzzaman Md (Okayama Univ.)
Purification of several subunits from a peanut allergen, Ara h1

15:06-15:08 PY-10 Kiyoshi Miura (Kawasaki Univ. Med. Welf.)
Absorption and metabolism of short-chain fatty acids in rats during the night

15:08-15:10 PY-11 Yuki Nagasaki (Okayama Pref. Univ.)
Lipocalin-type prostaglandin D synthase as a potential biomarker of bovine mastitis

15:10-15:12 PY-12 Xian Wen Tan (Okayama Univ.)
Rice bran extracts (RBE) as natural ameliorative bioactive materials for oxidative stress and inflammation

15:12-15:14 PY-13 Keisuke Toda (Okayama Pref. Univ.)
Preventive effect of red rice proanthocyanidin on psoriasis via inhibition of arachidonate 5-lipoxygenase

15:14-15:16 PY-14 Chengduo Wang (Okayama Pref. Univ.)
Beneficial effect of functional foods on fatty liver

15:16-15:18 PY-15 Ai Yamada (Okayama Univ.)
Temperature sensing TRP channels are involved in increase of prostaglandin production of bovine endometrial stromal cells under heat stress

15:18-15:20 PY-16 Patcha Yanpirat (Okayama Univ.)
Formaldehyde oxidation in lanthanide-dependent methylotrophy in *Methylobacterium aquaticum* strain 22A

15:20-16:30 Poster Presentation of Young Investigators & Coffee Break (7F Multipurpose Hall)

16:30-17:30 **Session IV (2F International Conference Hall)**
"Food and Technology (2)"
Chairs: Hideyuki Ito (Okayama Pref. Univ.)
Hiromi Yamashita (Okayama Pref. Univ.)

16:30-16:50 O-10 Xu Duan (Henan Univ. Sci. Technol.)
New freeze drying technologies for food processing

16:50-17:10 O-11 Yoshiyuki Tanaka (Okayama Univ.)
Biosynthesis of low-pungent capsaicinoid analogs in chili pepper fruits is controlled by mutations in putative aminotransferase

17:10-17:30 O-12 Seiji Awane (Yamada Bee Company Inc.)
The effect of drink containing honey vinegar on blood flow

17:30-17:40 **Announcement of Student Awards & Closing of the Conference**

18:00-20:00 **Banquet (B1F Reception Hall)**

Abstract for Oral Session

(Session I-IV)

O-1 ~ O-12

O-1 Effects of C18 unsaturated fatty acids on the structure and allergenicity of bovine α-lactalbumin

Xuanyi Meng, Zheling Zeng, Jinyan Gao, Ping Tong, Yong Wu, Xin Li and Hongbing Chen*

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Bovine α-lactalbumin (BLA) is one of the major food allergens in milk, and it is known to bind C18 unsaturated fatty acids (C18 UFAs) to form protein-ligand complexes. Previous studies have reported that C18 UFAs-BLA complexes can induce apoptosis in tumor and immature cells but not in healthy differentiated cells. However, whether the C18 UFAs have impact on the structure of BLA and its allergenicity is poorly understood. Therefore, the objective of this study was to determine if the treated BLA with C18 UFAs would affect the structure changes of protein and the related allergenicity.

In this study, the BLA was treated with or without 5 different kinds of C18 UFAs (oleic acid (OA), linoleic acid (LA), conjugated linoleic acid (CLA), α-linolenic acid (ALA), and γ-linolenic acid (GLA); 50 molar equivalent ratio) at 60°C for 30 min. Afterwards, the structure of untreated and treated BLA was analyzed by native-PAGE, circular dichroism spectroscopy, ultraviolet absorption spectroscopy and fluorescence spectroscopy. The allergenicity of the treated BLA was evaluated through IgG/IgE inhibition ELISA, the mouse basophil activation test, the human basophil KU812 cell degranulation test, *in vitro* digestibility and *in vivo* BALB/c mouse model.

When compared with untreated BLA, C18 UFAs treatment efficiently caused the structure of BLA emerged a significant variation. With the increase in the degree of unsaturation, the tertiary structure gradually unfolded and promoted their hydrophobicity, while the secondary structure of C18 UFAs-treated BLA did not show obviously changes. Moreover, C18 UFAs-treated BLA induced a remarkable increase of the IgG binding ability and the IgG-dependent activation marker CD200R3 expression on basophils. The IgE binding ability and the degranulation capacity of human basophil KU812 cells (intracellular Ca^{2+}, histamine, β-Hex, and IL-6) were also enhanced. Additionally, C18 UFAs-treated BLA resistance to gastrointestinal digestion, and its digestion products have a higher IgE binding ability. Finally, the BALB/c mouse model confirmed that the C18 UFAs-BLA complexes promote both the sensitization and elicitation of the allergic reaction by a disruption of the Th1/Th2 balance towards a Th2- immune response with decreasing the number of regulatory T cells (Tregs) and enhanced Th2-related cytokines and serum specific antibodies.

Collectively, these results suggested that C18 UFAs changed the structure of BLA, which contributed to its increased allergenicity.

O-2 Neuroprostanes derived from non-enzymatic DHA peroxidation displays neuroprotective properties in the rodent brain

Yiu Yiu Lee[1], Jean-Marie Galano[2], Camille Oger[2], Thierry Durand[2] and Jetty Chung-Yung Lee[1,*]

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F_4-neuroprostanes (F_4-NeuroP), a non-enzymatically derived metabolite from the oxidation of docosahexaenoic acid (DHA), have shown to be associated to neurodegenerative diseases. Since its discovery, F_4-NeuroP has been widely considered as a sensitive biomarker for neuronal oxidative damages. Although the onset of neurodegenerative diseases in general is related to the elevation of F_4-NeuroP, it is hypothesized to play a novel role, but the exact molecular mechanism still remains largely unclear. In this study, F_4-NeuroP (4-F_4-NeuroP) standard that we have in-house synthesized, was tested for potential bioactivities. Determined by MTT assay, 4-F_4-NeuroP (up to 100 μM) in SH-SY5Y human neuroblastoma cultured cells showed no signs of cellular toxicity. However, cell viability was significantly reduced when SH-SY5Y were co-treated with 4-F_4-NeuroP and H_2O_2 as compared to control (H_2O_2), suggesting oxidized 4-F_4-NeuroP, but not native 4-F_4-NeuroP, is cytotoxic. On the contrary, qPCR results revealed that native 4-F_4-NeuroP, not oxidized 4-F_4-NeuroP treatment on SH-SY5Y cells activates the expression of antioxidant genes, such as hemeoxygenase-1 and catalase. Interestingly, infusion of 4-F_4-NeuroP in rats significantly induced the production of DHA and its hydroxylated product (HDHA) in liver and brain tissues. Also, the fact that exogenous infused 4-F_4-NeuroP were deposited in the rat brains suggest that the elevation of 4-F_4-NeuroP in different neurodegenerative diseases could also be attributed from other organs, and the mode of delivery is likely via the formation of a ligand complex with serum albumin, a transportation mechanism similar to those peripheral free fatty acids, as suggested by isothermal calorimetry experiment. These results propose that the endogenous neural 4-F_4-NeuroP has protective mechanisms by acting as an activator/mediator for the enzymatic antioxidant pathway and fatty acid synthesis pathways.

O-3 Effects of high-carbohydrate and low-fat diets on serum lipid profiles in healthy young subjects with different genetic variations

Jia Lin, Hui Tang, Xin Huang, Zhen Zhang and Ding Zhi Fang*

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More favorable serum lipid profiles have been documented and believed to contribute to lower prevalence of cardiovascular diseases (CVD) in Chinese population. Although diets and genetic backgrounds were among key players regulating serum lipid profiles, the effect of diets on and association of genetic variations with serum lipid profiles were not constantly reported. Our hypothesis is that interplays may occur between diets and genetic variations to influence serum lipid profiles. Therefore, 209 university students were recruited and 60 of those who met our criteria entered the study. They received low-fat diets for 6 days after wash-out diets for 7 days and 56 subjects completed whole study with good compliance. Fasting serum before and after low-fat diets were collected and lipid profiles were measured. Then, 21 genetic variations in 17 lipids metabolism-related genes of genomic DNA from white blood cells were analyzed and verified by DNA sequencing.

Unlike previous studies, the current study population displayed an obvious elevation level of high density lipoprotein cholesterol (HDL-C) and apolipoprotein (Apo) A1 after low-fat diets when comparing that before the diet. When considering gender, a significantly increased HDL-C level was only observed in males, not in females. Further analysis indicated that when comparing that before intervention, low-fat diets could induce to increased HDL-C levels in males carrying lipoprotein lipase (*LPL*) rs326 G allele, males carrying sterol regulatory element binding protein 1c (*SREBP-1c*) rs2297508 C allele, males carrying *LPL Hind*III H- allele, male *ApoC3*-482CC homozygotes, females carrying *ApoC3*-482T allele, males carrying hepatic lipase (*LIPC*)-250A allele, male cholesteryl ester transfer protein (*CETP*) *Taq*IB B1B1 homozygotes and male cholesteryl ester transfer protein (*LIPE*) -60CC homozygotes. Additionally, low-fat diets could result in elevation of ApoA1 levels in males carrying *LPL* rs326 G allele, males carrying *LPL*447X allele, males carrying *LPL Hind*III H- allele, females carrying *ApoC3*-482T allele, males carrying *LIPC*-250A allele, male *CETP* TaqIB B1B1 homozygotes and male *LIPE*-60CC homozygotes after the intervention. Meanwhile, low-fat diets also contributed to changes of serum lipids and lipid ratios induced by low-fat diets.

The current study indicates effects of low-fat diets on serum lipid profiles in healthy young subjects with different lipids metabolism-related genetic variations. It provides new insight into mechanisms involved in more favorable serum lipid profiles in Chinese population and paves the way to prevent CVD early in the life. It also has the potential to contribute to formulate personalized interventions of CVD.

O-4 Physicochemical and sensory characteristics of pan bread made with various squeezed perilla leaf juice amount during storage

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For wide application of perilla leaf *(Perilla frutescens* Britton var. *japonica* Hara), which have various healthy function and could be easily cultured on Korean whole area, the physicochemical and sensory properties of pan bread made with various squeezed perilla leaf juice amount were analyzed.

When dough characteristics were analyzed by using farinograph, consistency and dough development time (DDT) were not shown difference between control and the bread dough made with various squeezed perilla leaf juice amount (SPLJ), but dough stability time (DST) was increased to increase SPLJ amount. Expansion rate of dough was increased to increase SPLJ amount. The volume, specific volume, and baking loss rate of the pan bread made with various SPLJ amount were decreased to increase SPLJ amount. The pan bread crumb colors were getting thickened the greenish with increasing SPLJ amount. In physical properties of pan bread made with various SPLJ amount, springiness and cohesiveness were decreased to increase SPLJ amount, but brittleness, chewiness, and hardness were increased to increase SPLJ amount. In sensory strength analysis, pore uniformity and soft texture was decreased to increase SPLJ amount, but dark green crumb color, perilla leaf odor, perilla leaf taste, and chewing texture were increased to increase SPLJ amount. In overall acceptance analysis, 1.5% SPLJ was the most like as 7.1, however, the statistical differences between 1.5% and 1.0% SPLJ were not shown at $p<0.05$. In partial least squares (PLS) analysis, the consumers were liked the bread possessed the green crumb color, perilla leaf odor, perilla leaf taste, and soft and chewing texture.

During storage, moisture contents of whole breads were decreased, and those of the bread made with the more SPLJ were shown the less value. In physical properties, springiness and cohesiveness were decreased during storage, those of the bread made with the more SPLJ was shown the dramatically lower values. In sensory evaluation, soft texture was decreased during storage, that of the bread made with the more SPLJ was shown the less value. In overall acceptance analysis, consumers more like the bread made SPLJ until one day storage, however, they less like the bread made with SPLJ after 3 day storage than control. The correlations among physicochemical properties, sensory characteristics and consumer acceptability were analyzed by using principle component analysis (PCA). In PCA, just after baking bread made with imported wheat (IWC-0), with domestic wheat (DWC-0), with 0.5% SPLJ-0 (0.5% SPLJ-0), and with 1.0%-SPLJ (1.0%-SPLJ-0) and the bread made with 0.5%-SPLJ after one day storage (0.5%-SPLJ-1), which were located in both positive principal component (PC) 1 and PC 2 dimension, were shown the moistened and soft texture and high consumer acceptability. After 3 day storage, the bread made with SPLJ had the higher hardness, chewiness and brittleness than that of control (IWC, DWC).

As a conclusion, physicochemical properties of pan bread made with SPLJ were shown less desirable than control, however, consumer acceptance made with 1.5% SPLJ were shown the most. After storage, 0.5% SPLJ could be good bread making addition amount of SPLJ. If the SPLJ are used for making pan bread, the bread making method will be developed for decreasing the hardness, chewiness and brittleness.

O-5 Regulation of muscle physiology by 7,8-dihydroxyflavone

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Obesity is a result of imbalanced energy intake and expenditure. Since skeletal muscle is the largest contributor to overall metabolic rate, enhancing energy expenditure in this tissue is a potential method to reduce the body weight gain during energy surplus. In our attempts to identify new bioavailable compounds with anti-obesity activities, we found that 7,8-dihydroxyflavone (7,8-DHF), a naturally occurring flavone in *Godmania aesculifolia*, increased mitochondrial biogenesis and lipid oxidation in cultured muscle cells via the brain-derived neurotrophic factor (BDNF) receptor/ AMP-activated protein kinase (AMPK)/ peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) pathway. In obese mice, 7,8-DHF consumption increased the muscular mitochondrial content and uncoupling protein 1 (UCP1) expression. Systemic energy metabolism was thus elevated, which reduced their body weight gain. Consequently, hyperlipidemia, hyperglycemia hyperinsulinemia, ectopic lipid accumulation, and insulin resistance in the skeletal muscle of these obese animals were alleviated after 7,8-DHF treatment. 7,8-DHF also possesses a preventive activity against obesity development as high-fat diet (HFD) feeding induced less body weight gain in C57BL/6 mice receiving 7,8-DHF simultaneously. Together, our results demonstrate that 7,8-DHF is an effective anti-obesity agent in both normal and obese animals.

O-6 Mutations of anti-leukotriene C_4 single-chain antibody change the antigen binding specificity

Yuki Kawakami[1], Mai Kinoshita[1], Yoshiko Mori[1], Shuji Okochi[1], Shiori Hirano[1], Keita Kanzaki[1], Toshiko Suzuki-Yamamoto[1], Masumi Kimoto[1], Mitsuaki Sugahara[2], Tetsuya Hori[2], Hiromichi Saino[3], Masashi Miyano[2,3] and Yoshitaka Takahashi[1,*]

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Cysteinyl leukotrienes (LTs) including LTC_4, LTD_4 and LTE_4 are key lipid mediators in inflammation. Biosynthesis of these cysteinyl LTs is initiated by conversion of arachidonic acid to LTA_4 catalyzed by 5-lipoxygenase. Then, the unstable epoxide LTA_4 is conjugated with reduced glutathione to produce LTC_4 which is converted to LTD_4 and LTE_4 by the sequential cleavage for the tripeptide adduct of glutamate and glycine. Previously a mouse monoclonal antibody against LTC_4 (mAbLTC) was prepared for immunoaffinity purification and radioimmunoassay of this bioactive eicosanoid.

To explore the structure of the antigen-binding site of the antibody recognizing LTs, we isolated full-length cDNAs for heavy and light chains of the monoclonal antibody and constructed an expression plasmid encoding a single-chain variable fragment against LTC_4 (scFvLTC) comprising variable regions of heavy and light chains of the original monoclonal antibody. The recombinant antibody expressed in *Pichia pastoris* showed the antigen-binding affinity and specificity similar to those of the parental mAbLTC. Thus, scFvLTC would be a useful tool by using site-directed mutagenesis to identify amino acids contributing to recognition of the antigen. The X-ray crystal structure of an mAbLTC in complex with LTC_4 was determined, however, crystallographic studies alone are not enough to fully understand the structure-function relationship of the antibody.

To elucidate the individual contribution of amino acids of mAbLTC which interacted with the glutathione moiety of LTC_4, we examined whether substitution of the residues affects the antigen binding affinity and specificity using an scFvLTC. Among the mutants, N35(L)Q, Y37(L)F and Y54(L)W showed a dramatic increase in the affinity to LTE_4 which was comparable to that to LTD_4. We combined the three substitutions and the resultant triple mutant further increased the affinity to LTE_4 to the level exceeding that to LTC_4. Our results identified these amino acids contributing specific LT recognition by the antibody.

O-7 Isolation of wild yeasts from local region and their application to winemaking

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To produce an original wine, a wild yeast isolated from local region can be used instead of a commercial yeast. A flavor-producing wild yeast for practical use leads to the differentiation of wine quality.

Kofu, the capital of Yamanashi Prefecture in Japan, will be celebrating its 500th anniversary in 2019. In order to produce original sparkling wine to mark the occasion, we isolated 403 wild yeast strains from several sightseeing spots in Kofu. At least 77 isolates showed high fermentation ability in high-sucrose medium. Ten strains were selected from the 77 isolates on the basis of the results of fermentation tests using 'Koshu' juice. Finally, the wild yeast strain AU14-22 was selected from the enological aspects (sufficient fermentation in grape must, SO_2 tolerance, sensory examination). The strain was isolated from a reservoir in Takeda Shrine in October 2016.

AU14-22 belongs to the species *Saccharomyces cerevisiae* and possesses high ability to ferment 'Koshu' juice at 18°C equaling that of commercial yeast EC1118. AU14-22 has a potential to produce significant amount of β-phenylethyl alcohol in winemaking. The flavor compound has a floral type odor. The carbonated semi-sweet wine derived from AU14-22 received high marks as a result of wine tasting.

O-8 Sweetened egg white can be fermented with *Lactobacillus casei*-group lactic acid bacteria

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[Introduction] Egg is a typical nutritious food of animal origin as well as milk and meat, and one of the most important raw materials for making processed foods due to its coagulability, formability and emulsifiability. However, dissimilar to milk and meat, egg is hardly used as a material for fermentation particularly with lactic acid bacteria (LAB); because its sugar content is too low for fermentation, and various proteins to inhibit bacterial growth and proteolysis are rich in egg white (EW). LAB, the most major bacterial group frequently used as a fermentation starter, are strongly attracting attention to their bioregulatory function in the last two decades. In such a background, fermentation of sweetened liquid egg with LAB have been tried by a few food companies in order to change the flavor and texture and to add any biofunctionality. In most of the trials, some growth-promoting supplements such as yeast extract are necessarily used; but it's not preferable to use any supplements with unfavorable taste and flavor. In this study, we aimed to find LAB starter strains used for EW fermentation without any supplements except sugars, and to clarify the fermentation mechanism with the selected LAB strains.

[Experiments] First, we selected 7 strains that fermented EW supplemented with 5% glucose or sucrose from 45 LAB strains. The 7 strains all belonged to *Lactobacillus casei*-group LAB (*Lb. casei*, *Lb. paracasei* subsp. *paracasei*, *Lb. paracasei* subsp. *tolerans*, *Lb. rhamnosus*, and *Lb. zeae*). Second, we assayed susceptibility of some strains to 3 kinds of antibacterial EW proteins. As a result, the EW-fermenting strains was tolerant to all tested antibacterials including lysozyme, whereas the other non-fermentative strains were sensitive only to lysozyme. Then, *Lb. casei*-group members had higher tolerance capacity to lysozyme than many of the other LAB strains. But *Lb. paraplantarum*, *Lb. pentosus* and *Streptococcus thermophilus* strains were also lysozyme-tolerant, although they did not ferment sweetened EW. Next, to find another reason for the EW fermentation with *Lb. casei*-group strains, the lysozyme-tolerant strains were cultivated in EW supplemented with various nutritional ingredients. Supplementation with vitamins, minerals, nucleic acids, Tween 80 (oleate) and ovalbumin (protein) resulted in no or limited effects on their growth, whereas pepsinolytic hydrolysates of ovalbumin (ovalbumin peptides) promoted the fermentation. These shows that proteolysis by cell-envelope proteinases (CEP) of LAB necessary for the growth would be inhibited by any EW component, presumably one of the EW proteins. Actually, degradation of and peptide-release from EW proteins were very slow even when *Lb. casei*-group strains were used. Now we are trying to identify the CEP-inhibitory component in EW, and to clarify the reason why only *Lb. casei*-group strains could slowly but surely overcome the inhibitory effect and ferment EW.

[Conclusion] We selected 7 strains of *Lb. casei*-group LAB as a starter to ferment EW without any nutritional supplements except sugars. It's found that the specific EW fermentation should be actualized by virtue of tolerance capacity of *Lb. casei*-group members to lysozyme and any CEP-inhibitory comportment in EW. We hope that EW fermentation will be established as a processing method and contribute to creating novel egg products in the near future.

O-9 Anti-cancer effect of *Dioscorea japonica* and the constituent diosgenin through down-regulation of prostaglandin E_2 synthetic pathway

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Prostaglandin (PG) E_2 is one of the pro-inflammatory lipid mediators derived from arachidonic acid, and is hyperproduced by inducible cyclooxygenase (COX)-2 and microsomal PGE synthase (mPGES)-1 in several pathophysiological conditions. COX-2 and mPGES-1 are involved in the cancer developmental process including carcinogenesis, cancer cell differentiation, and metastasis. In this paper, we report that a wild yam *Dioscorea japonica*, and diosgenin, a plant sterol as its constituent had anti-cancer effect through the down-regulation of COX-2 and mPGES-1.

Dioscorea japonica, a wild yam, is a relative of the *Dioscoreaceae* family native to Japan. In human non-small cell lung carcinoma A549 cells, *Dioscorea japonica* extract (DJE) dose-dependently suppressed COX-2 and mPGES-1, and inhibited COX-2 activity and PGE_2 production. DJE induced the translocation of NF-κB as COX-2 transcriptional factor from nuclei to cytosol and the decrease of COX-2 promotor activity. Moreover, DJE induced apoptosis in cancer cells. To isolate the functional substances from DJE, we carried out some chromatographies, and found the candidate compound, a steroidal saponin, diosgenin. Diosgenin also suppressed COX-2 and mPGES-1. Diosgenin may down-regulate COX-2 expression via glucocorticoid receptor, because COX-2 suppression by diosgenin was recovered by an antagonist of glucocorticoid receptor, RU486. In addition, diosgenin induced the translocation of NF-κB from the nucleus to the cytosol, and the effect was recovered by the addition of RU486 in A549 cells.
In order to confirm the effects of *Dioscorea japonica in vivo*, we demonstrated its effect on squamous cell carcinoma of mouse skin exposed to 17,12-dimethylbenz [*a*] anthracene (DMBA) and 12-*O*-tetradecanoylphorbol 13-acetate (TPA). *Dioscorea japonica*-containing feed and DJE application inhibited tumor formation, and suppressed COX-2, mPGES-1 and the inflammatory cytokines. Lipid metabolome analysis by LC-MS/MS showed that *Dioscorea japonica* decreased PGE_2 and PGD_2 compared with carcinogenic control. Histopathological analyses showed that *Dioscorea japonica* inhibited hyperplasia and inflammatory cell infiltration. Immunohistochemical analyses showed the immunoreactivities of COX-2 and mPGES-1 in tumor keratinocytes and stronger immunoreactivities of COX-2 in epidermal dendritic cells, Langerhans cells. Treatment of *Dioscorea japonica* decreased the immunoreactivity of COX-2 and mPGES-1.

These results indicate that *Dioscorea japonica* down-regulate COX-2 and mPGES-1, and diosgenin as the candidate compound suppressed COX-2 via glucocorticoid receptor. Additionally, topical application and oral intake of *Dioscorea japonica in vivo* exhibited anti-cancer effect via down-regulation of PGE_2 synthetic pathway.

O-10 New freeze drying technologies for food processing

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Drying is a widespread concept in the food industry, typically used to convert a surplus crop into a shelf-stable commodity. With advancement of technology, however, there is interest in moving forward from the traditional convective air drying that is most widely used today for foods, to maintain at a very high level the nutritional and organoleptical properties of the initial fresh product. Freeze-drying (FD) produces the highest quality food product obtainable by any drying method, but it is considered the most expensive operation for manufacturing a dehydrated product owing to high energy consumption and high costs of both operation and maintenance. Microwave freeze-drying (MFD) and atmospheric freeze-drying (AFD) have been developed to reduce the FD energy consumption. The product quality of these two drying methods is similar to FD, due to removal of water content in materials by sublimation in both MFD and AFD. Although a significant amount of scientific research has been carried out in the field of sublimation drying, there are only a few comprehensive summarizations about the various sublimation drying methods. As a result, this review aims to highlight some of the latest and most notable advancements in sublimation-drying of foods, with main emphasis given to recent developments of reducing energy consumption of FD process and suggests future research areas on sublimation related drying.

O-11 Biosynthesis of low-pungent capsaicinoid analogs in chili pepper fruits is controlled by mutations in putative aminotransferase

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The pungent components in chili pepper fruits (*Capsicum*) are capsaicinoids. The chemical structure of capsaicinoids comprises an acid amide of vanillylamine with a fatty acid. Capsaicinoids are reported to have many bioactivities such as the enhancement of thermogenesis and suppression of fat accumulation. However, their use as ingredients in foods and supplements has been limited by their pungency. Capsinoids are low-pungent capsaicinoid analogs, which were first isolated from a low pungent mutant 'CH-19 Sweet' (*C. annuum*). They are similar in structure to capsaicinoids, but have an ester group instead of the amide moiety. Compared to capsaicinoids, capsinoids have similar bioactivity but considerably lower pungency. Because of their low pungency, capsinoids are more palatable than capsaicinoids. From this perspective, capsinoids are attractive ingredients for dietary supplements. The understanding of the controlling capsinoid biosynthesis is important to allow the breeding and improvement of chili peppers.

Putative aminotransferase (pAMT) catalyzes the formation of vanillylamine from vanillin in capsaicinoid biosynthetic pathway. This biosynthetic pathway should branch to vanillyl alcohol and then capsinoid after the formation of vanillin. To test the hypothesis that the capsinoid biosynthesis is caused by functional loss of pAMT, we investigated *pAMT* gene in low-pungent cultivars with capsinoids. The screening of germplasm for capsinoid content revealed that four low-pungent cultivars, 'Himo' (*C.annuum*) , 'Zavory Hot' , 'Aji Dulce Strain 2' , 'Belize Sweet' (*C.chinense*) contained the highest amounts of capsinoid among 54 cultivars. Analysis of cDNA sequence of *pAMT* revealed that there were mutations resulting in functional loss of *pAMT* in all low pungent cultivars with capsinoid. The cDNA sequence of 'CH-19 Sweet' contained a T nucleotide insertion at 1291 bp in the *pAMT* gene. This insertion formed a new stop codon of TGA. The cDNA contained a single nucleotide substitution (C to T) at 775 bp in 'Himo'. This nucleotide substitution results in a single amino acid substitution of cysteine to arginine in the pyridoxal 5-phosphate binding domain. The cDNA in 'Belize Sweet' contained 5-bp insertion. The cDNAs in 'Zavory Hot'and 'Aji Dulce Strain 2' had an insertion containing a *hAT* family transposon like sequence. It was shown that the *pAMT* genotype cosegregated with the capsinoid biosynthesis in the F_2 population. These results suggest that functional loss of *pAMT* is responsible for the low pungent *Capsicum* cultivars having capsinoid. In addition, novel leaky *pAMT* alleles (*pamt-leaky1* and *pamt-leaky2*) with intermediate activities between functional-type and loss-of -functional types were identified. Sequence analysis revealed that both alleles had *Tcc* transposon insertion in 3rd intron, but the locations of insertion were different within the intron. The *pAMT* mutations will be useful for genetic improvements to pungency and capsinoid content in *Capsicum* breeding programs.

O-12 The effect of drink containing honey vinegar on blood flow

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Honeybees products like propolis, royal jelly, and honey had been used commonly all over the world as traditional and ethno pharmacological nutrients since ancient times. Recently, various beneficial effect of honeybee products on health has been reported, so they are applied in a branch of complementary and alternative medicine—apitherapy.

The present study focuses on the potential health benefits of honey vinegar. Although various functionalities of vinegar have been reported, the effect of honey vinegar on blood flow has not been reported in clinical research.

The purpose of this study was to investigate the effect of drink containing honey vinegar (DHV) on blood flow. In this study, 10 healthy volunteers (men and women) aged 24-38 years have been recruited. An open-label cross-over trial design was used. DHV or water was administrated on day 1 or 7 with a week washout period. Before DHV intake, the hand blood flow of the volunteers was measured by blood flow meter. After DHV intake at 30, 60 and 90 min, the blood flow and sensible temperature were measured by visual analog scale. As a result, DHV intake increased peripheral blood flow and sensible temperature, especially in male. Our results suggested that DHV intake could increase the blood flow and sensible temperature in male.

Abstract for Poster Session

P-1 ~ P-14

PY-1 ~ PY-16

P-1 Production of whole-bean soymilk by pilot-scale microfludization

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Soymilk is a traditional consumed beverage in Asian countries and it has been accepted by Western countries recent years owing to its high nutritions and various health functions. Soymilk contains various high-quality proteins, essential fatty acids containing no cholesterol. Lecithin, isoflavones, vitamins and other nutrients in soymilk also contribute to the health function of soymilk.

In order to obtain superfine and smooth taste, the okara of traditional soymilk needs to be filtrated and wasted without appropriate utilization. However, okara contains plenty of high-quality protein, fat, dietary fiber and isoflavone, improper disposal of okara is likely to cause environmental pollution. Therefore, whole-bean soymilk is of great interest to the food industry duo to its cost saving, high nutrition values, and prevention of pollution.

A pilot-scale microfludizer was innovatively created and used to produce whole-bean soymilk, then the physical and chemical characteristics of whole-bean soymilk was investigated. Results showed that the particle sizes of whole-bean soymilk were sharply decreased and the viscosity were increased with the increasing pressure of microfludization, leading to a favorable stability that no precipitation was found during 30 days storage. The whole-bean soymilk presented higher soluble protein concentration compared with non-treated sample, while polyphenol and flavonoid content were slightly decreased. In addition, microfludization also have an excellent effect in decreasing lipoxygenase activity, the highest inactivation rate is able to reach 92%. This research indicated the newly created pilot-scale microfludizer could be an effective machine in producing high-nutrition and high-stability whole-bean soymilk.

Keywords Pilot-scale microfludizer; Whole-bean soymilk; Physicochemical properties; Stability; Nutrition

P-2 Effect of malted-rice amazake on intestinal environment

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Background: Constipation is regarded as a functional disorder with a decrease in the frequency of defecation, a sense of difficulty in defecation and a feeling of aftereffect. There are two types of amazake: malted-rice amazake and Sake lees amazake. It has been reported that Sake lees amazake improves human intestinal flora and relieves constipation. But there is no report on malted-rice amazake for improving constipation. The purpose of this study is to conduct a long-term ingestion test on humans and consider whether intake of malted-rice amazake will improve constipation. We also analyze the gut microbiota and investigate changes in the intestinal environment.

Methods: The subject is 30 adult females with a menstrual cycle of approximately 28 days. Feces were collected in the follicular phase to minimize the influence of premenstrual syndrome. The DNA extracts from feces were analyzed using quantitative PCR using specific primers. The defecation status was investigated by self-report questionnaire.

Results: In the defecation state, soft stool of the stool was found significantly by intake of malted-rice amazake in hard stool group. Symptoms of constipation improved in 83% of constipation group. Analysis of intestinal bacterial flora showed a significant decrease in *Clostridium cocoides* group, *C. ramosum* subgroup and the ratio of Firmiscutes / Bacteroidetes in constipation group due to intake of malted-rice amazake.

Conclusion: It was suggested that intake of malted-rice amazake changed the construction of gut microbiota as well as the intestinal environment, resulting in improvement in constipation.

P-3 Affinity resins as tools for identifying target proteins of ascorbic acid

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L-Ascorbic acid (AA), known as vitamin C, has diverse physiological functions such as collagen synthesis effect, antioxidant effect and catecholamine synthesis effect. AA is an essential nutrient for us, and we must take this vitamin from food on a daily basis. Unexpectedly, most functional mechanisms of AA have still not been clarified.

Affinity chromatography is a biochemical approach to directly identify target proteins of chemical compounds immobilized on an affinity resin. The target proteins can give us important clues for elucidating the functional mechanisms of the chemical compounds. However, an affinity resin on which AA is immobilized (AA-affinity resin) has not been prepared, because AA is very unstable and rapidly degraded in an aqueous solution.

In this study, we prepared two kinds of affinity resin on which AA is immobilized in a stable form. Using the prepared AA-affinity resins, cytochrome c (cyt c) was identified as an AA-targeted protein candidate from mouse brain protein extract. Cyt c has one c-type heme and it exists in oxidized cyt c (heme Fe^{3+}, oxd-cyt c) or reduced cyt c (heme Fe^{2+}, red-cyt c). Interestingly, oxd-cyt c exhibited higher affinity for the AA-affinity resins than did red-cyt c. In addition, AA reduced oxd-cyt c more efficiently and specifically than did other common reducing agents. These results indicated that oxd-cyt c shows specific affinity for AA. These AA-affinity resins can be powerful tools to identify new target proteins of AA.

P-4 Ingestion of soy protein isolate attenuates eccentric contraction-induced force depression and muscle proteolysis via inhibition of calpain-1 activation in rat fast-twitch skeletal muscle

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Eccentric contraction (ECC) is a contraction in which skeletal muscles are stretched while contracting and is prone to result in a larger and longer-lasting force deficit compared to concentric and isometric contractions. It has been shown that ECC-induced force deficit is, at least in part, due to calpain-mediated proteolysis of muscle proteins (e.g., Ca^{2+}-regulatory proteins) critical for muscle contraction and that soy protein ingestion enhances the activity of calpastatin, an endogenous calpain inhibitor, in skeletal muscles.

In this study, we investigated whether soy protein isolate (SPI) ingestion prior to ECC can ameliorate ECC-induced proteolysis of Ca^{2+}-regulatory proteins via inhibition of calpain activation and facilitate recovery of force production after ECC.

Male Wistar rats were randomly assigned to a control and a SPI group, which were fed a 20% casein and a 20% SPI diet, respectively, for 28 days before the ECC protocol. Anterior crural muscles underwent 200 repeated ECCs in situ and extensor digitorum longus muscles were excised 3 days later.

SPI ingestion attenuated ECC-induced force deficit and proteolysis of Ca^{2+}-regulatory proteins (ryanodine receptor, dihydropyridine receptor and junctophilin-1). SPI ingestion also inhibited ECC-elicited increase in calpain-1 autolysis (an indicator of its activation), but this was not accompanied by increased calpastatin content.

These results suggest that SPI ingestion inhibits ECC-elicited force deficit and proteolysis of Ca^{2+} regulatory proteins, which is caused by inhibited activation of calpain-1.

P-5 Dynamic visualization of anti-colorectal cancer effect of resveratrol using fluorescence labeling strategies

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[Background and Aims]
In recent years, much attention and interest have been directed toward pharmacokinetic approaches of bioactive compound in food. We reported the applicability of correlative light and transmission electron microscopy (CLEM) approach on colorectal cancer cell (Caco-2 cell) exposed to fluorescence labeled anti-cancer drug (Cisplatin)[1]. We thought that this fluorescence labeling strategies can be applied to a tool for kinetics of functional food constituents ("Nutrakinetics"). We are pushing forward research about dynamic intracellular imaging of fluorescence labeled polyphenol as hydroxytyrosol (olive polyphenol), caffeic acid (coffee polyphenol), and genistein (soy polyphenol). Here, we focused on resveratrol as a target of fluorescent labelling. Resveratrol, a polyphenol found in grape and wine, are known to possess a high antioxidant, anticancer capacity, and activation of NAD^+ dependent protein deacetylase Sirtuin. However, the uptake and localization of resveratrol in cancer cells is unknown. The aim of this study was to establish a novel method to assess the anti-colorectal cancer effect of food components using fluorescently labeling strategies.

[Methods]
Two types of resveratrol conjugated with fluorescein isothiocyanate (FITC) via the 3- or 4'- phenolic hydroxyl group (FITC-resveratrol 1 and 2) were synthesized. Caco-2 cells were cultured on glass bottom dishes, and then incubated with 500 μM of FITC-resveratrol for 24 hours subsequently incubated with LysoTracker (Lysosome marker) or MitoTracker Red (Mitochondria marker) with Hoechst 33342 (Nuclei marker). Thereafter, using OLYMPUS FV10i All-in-one confocal laser-scanning microscope, the imaging data was acquired.

[Results and Disscussion]
FITC-resveratrol 1 and 2 maintained fluorescent intensity for 8 hour imaging. FITC-resveratrol 1 was detected partly in lysosome, however, not in the mitochondria and nuclei. FITC-resveratrol 1 positive cells have no change of cell morphology. On the other hand, FITC-resveratrol 2 was detected in cytosol, not only in dots. Our results suggested that FITC-resveratrol 2 could mimic intracellular behavior of "intact" resveratrol. Interestingly, we observed that FITC-resveratrol 2 absorbed cell dynamically aggregates mitochondria and changes cell morphology. Thus, this imaging may show anti-colorectal cancer effect of resveratrol.

[Conclusion]
We concluded that cellular imaging using fluorescent labeled food compounds is a powerful tool to analyze the effect of functional food constituents.

[References]
1) Kobayashi K, Cheng D, Huynh M, Ratinac KR, Thordarson P, Braet F. Imaging fluorescently labeled complexes by means of multidimensional correlative light and transmission electron microscopy: a practical approach. *Methods Cell Biol*, **111**, 1-20 (2012)

P-6 The effect and mechanism of elaidic acid and trans vaccenic acid on HUVECS function based on phospholipids profile

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Epidemiological and clinical study showed that diet with rich industrial *trans* fatty acids could increase plasma lipids and cause AS and cardiovascular disease. Thus, many countries carried out policy to limit the contents of *trans* fatty acids in food products. However, *trans* fatty acids also found in the lipids of ruminant animals meat and milk products with little contents. And there is currently no policy to restrict its use. Under the global environment of limiting *trans* fatty acids, it is necessary to understand the effects of trans fatty acids in ruminants on human health, especially on cardiovascular disease, which plays an important role to clarify the safety and guidance of ruminant animal foods.

In this study we investigated the effect and mechanism of elaidic acid (9t18:1) and trans vaccenic acid (11t18:1) on human umbilical vein endothelial cells (HUVECs) function based on phospholipids profile. Here we reported that both 9t18:1 and 11t18:1 could induce cell injury and membrane damage. 9t18:1 could activated phospholipase A1 and phospholipase A2 to hydrolyzed SFA and PUFA of PI36:4 and then produce PI36:2, while 11t18:1 stimulated phospholipase A2 to hydrolyzed PC34:1 to produce PC34:2.MAPK pathways and three phospholipase A2(cPLA2, sPLA2, and iPLA2) were involved in 9t18:1 and 11t18:1 induced HUVECs inflammation which contributes to CHD

P-7 Effect of *Bifidobacterium* fermented milk on anti-obesity

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Obesity is one of the most serious health problem of the world. In recent years, it has been suggested that gut microflora is a contributing factor for the leading of obesity. Therefore, prevention of obesity using probiotics such as *lactic acid bacterium* and *Bifidobacterium* draw attention all over the world.

Bifidobacterium exist in gut of animals and it has been known to be effective in improving lipid metabolism. *Bifidobacterium* produces acetic acid as a final metabolite, which has been reported to improve glucose tolerance and insulin resistance and has function of anti-obesity and antidiabetic. In this study, we investigated novel function of *Bifidobacterium* that improve energy metabolism and prevent obesity.

Three *Bifidobacterium* strains (bif - 15, bif - 30, and bif - 39) were isolated from human feces, which have high ability to produce acetic acid and show good viability in milk. GAM broth culture medium of these each strains was inoculated into 12% nonfat milk, cultured until that acetic acid was produced at about 0.5 % in each fermented milk, and they were used for animal experiments. Each fermented milk was administered to Otsuka Long-Evans Tokushima Fatty (OLETF) rats, which are type 2 diabetes model animals that developed obesity and diabetes due to overeating, at 5 mL / kg of body weight for 5 days per week from 8 weeks to 24 weeks of age. Food consumptions and body weights were recorded every day. At 24 weeks of age, the rats were dissected, and muscles, white adipose tissues and cecums were immediately isolated for subsequent experiments. Blood samples were collected to analyze for blood biochemical test.

As a result, weight gain of rats was significantly suppressed and HDL-cholesterol level was significantly higher in bif-15 group than those in water group. Furthermore, genes expressions related to slow twitch muscle generation were significantly increased in soleus muscle of bif-15 group as compared with those of water group.

P-8 Cytokine expression and macrophage localization in xenograft and allograft tumor models stimulated with lipopolysaccharide

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T cell deficient mice such as nude mice are often applied to generate tumor xenograft for the development of anticancer agents. However, the functionality of the other immune cells including macrophages, dendritic cells (DCs), and myeloid derived suppressor cells (MDSCs) in the xenograft are largely unknown. Macrophages and dendritic cells (DCs) acquire functionally distinct properties in response to various environmental stimuli; the interaction of these cells with myeloid-derived suppressor cells (MDSCs) in tumor microenvironments regulates cancer progression. Nude mice are less likely to reject human cancer cells because of major histocompatibility complex (MHC) mismatches. The tumor microenvironment in xenograft, comprising human and mouse cells, exhibits more complex bidirectional signaling and function than that of allograft. Here, we evaluated the differences of myeloid cells between them. Plasma interferon-γ (IFN-γ) and interleukin-18 (IL-18) concentrations in the xenograft tumor model after lipopolysaccharide (LPS) administration were significantly higher than those in the allograft tumor model. MHC class I, II, and CD80 expression levels were increased in $CD11b^+$ and MDSC populations after LPS administration in the spleen of xenograft tumor model but not in that of allograft tumor model. Additionally, the number of CD80- and MRC1-expressing cells was decreased upon LPS administration in the tumor of the xenograft tumor. These results suggest that functions of macrophages and DCs are sustained in the xenograft, whereas their functions in response to LPS were suppressed in the allograft. The findings will encourage the consideration of the effects of myeloid cells in the xenograft for drug development.

P-9 Comparison of bioavailability of quercetin monoglucoside and its aglycone

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Quercetin, which is found as its glycosides in plants, is potentially beneficial for human health, such as anti-inflammatory and anti-obesity effects. The administered quercetin aglycone or its glycoside is rapidly absorbed as metabolites into plasma. Although numerous studies have shown the bioavailability of quercetin, there are limited reports describing the metabolite patterns of quercetin glycoside compared with that of its aglycone. In this study, we compared the metabolites in plasma derived from quercetin monoglucoside and its aglycone using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Quercetin (10 mg/kg BW) or quercetin-3-glucoside (equivalent to 10 mg quercetin aglycone/kg BW) was administered to Wistar/ST rats (male) after an overnight fasting. Peripheral venous blood was collected before and 0.5, 1, 2, 3, and 6 h after the administration of the test compound. Quercetin metabolites were extracted from blood plasma with methanol and acetonitrile, and their profiles were determined using LC-MS/MS.

Quercetin aglycone and its monoglucoside were absorbed in the small intestine and translocated in the plasma. Quercetin-glucuronide (Q-GA) and quercetin sulfate (Q-S) with or without methylation were detected in plasma after the administration of quercetin monoglucoside as well as quercetin aglycone. The amount of Q-GA was almost the same regardless of the administration forms. On the other hand, the amount of Q-S was relatively low after the administration of quercetin glucoside compared with its aglycone. The amount of methylated Q-S after administration of quercetin-3-glucoside was further lower than that after administration of its aglycone. These results indicate that the bioavailability of quercetin-3-glucoside is almost the same as that of quercetin aglycone, and the variety of quercetin metabolites after administration of quercetin-3-glucoside is similar to that after administration of its aglycone. However, the metabolic profile in the plasma was partly different upon administration of either quercetin-3-glucoside or quercetin aglycone. Thus, the chemical forms of quercetin in the diet may have an effect on the metabolic process by phase 2 detoxification enzymes, such as glucuronate and sulfate transferases, in intestinal absorption.

P-10 Identification of proteins involved in tooth biomineralization in the giant Pacific chiton by integrated transcriptomic and proteomic analysis

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The chitons have been known to deposit magnetite (Fe_3O_4) on their mineralized radular teeth. Recently, much attention has been paid to the superior mechanical property of radular teeth in chitons which is a promising model for the development of a novel abrasion resistant materials. We have studied radular teeth biomineralization using the world largest species of chitons, *Cryptochiton stelleri*. In this study, to reveal the proteins involved in the mineralization of radular teeth, transcriptome and proteome analysis of *C. stelleri* was conducted.

First, total RNA was extracted from radular tissue including radular teeth and epithelial cells. RNA-seq was conducted on the extracted RNA using illumina Hiseq. The obtained reads were then de novo assembled to build a transcriptome data. To identify highly expressed transcripts in mineralized and non-mineralized teeth regions, each set of reads from two regions were aligned to the assembled transcriptome. Furthermore, the radular teeth proteins were identified from MS/MS spectra by searching them against the transcriptome data.

Transcriptome analysis revealed that the most expressed transcripts in non-mineralized teeth region include ferritin genes, while those in mineralized teeth region include a number of mitochondrial respiratory chain enzymes. Proteome analysis identified 22 mineralized teeth specific proteins including a novel protein which are good candidates for future research on biomineralization of radular teeth.

P-11 The effect of glass transition temperature on the procedure of microwave–freeze drying of Mushrooms (*Agaricus bisporus*)

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Freeze drying (FD) yields the best quality of dried mushroom but at the cost of a long drying time and high overall cost. Air drying (AD) gives an unacceptably poor quality product. To achieve faster drying along with a high-quality product, a microwave–freeze drying (MFD) technique was developed to dry mushrooms. MFD combines the advantages of both microwave heating and traditional FD. During the MFD process, most water is removed by sublimation under a high-vacuum condition, resulting in almost the same product quality as traditional FD. In addition, microwave heating is generated rapidly, resulting in the potential to improve energy efficiency.

T_g can be taken as a reference parameter to characterize the properties, quality, stability, and safety of food systems. The glass transitions of carbohydrates and proteins are affected by water, with lower water content resulting in increased glass transition temperatures. As a result, a drying program with different temperatures based on the glass transition in different drying stages could be adopted. In the experiment, the product temperature was higher than the T_g up to a moisture content of 20% wb, showing that the material is in the rubbery state during most of the drying process. At the beginning of drying, the moisture content is high and the difference between product temperature and T_g (D_1, D_2, and D_3) is great, resulting in quality deterioration. As MFD progresses the difference decreases, which will lead to more stable product matrix. At the end stage of MFD, T_g increased to a high level. Therefore, when the microwave power was controlled precisely, allowing the material to pass from a rubbery to a glassy state, the dried material became more rigid, significantly decreasing the extent of shrinkage.

In this study, we investigated the influence of different microwave loading programs on the process of MFD of button mushrooms. Based on the measured glass transition temperatures under different moisture contents, a suitable microwave loading scheme for improving the product quality was suggested during MFD of mushrooms. Based on experimental tests reported in this article, the microwave power has a significant effect on product quality and drying efficiency during MFD. As the moisture content of mushrooms decreases, the glass transition temperature increases during MFD. As a result, a step-down microwave loading scheme could be adopted to reduce the difference between the product temperature and T_g. It was found that the step-down microwave loading scheme based on the glass transition temperature can significantly improve the product quality and does not increase the drying time.

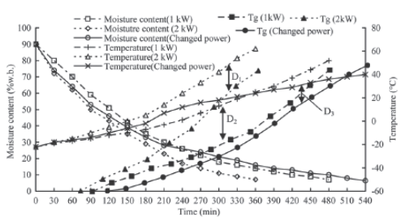

FIG. 4. Temperature and moisture content profiles of the samples under different microwave loading schemes during MFD.

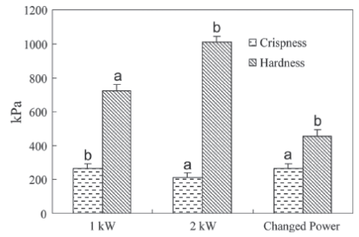

FIG. 5. Effect of different microwave loading schemes on the texture of the product.

P-12 Comparison of *Streptomyces* transglutaminases on substrate specificities toward casein peptides

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The protein cross-linking enzyme, transglutaminase (TGase), catalyzes an acyl transfer reaction between the γ-carboxyamide group of a glutamine residue on peptide as acyl donor and a several primary amines including lysine as acyl acceptor. Various organisms produce TGases, which are divided into two types by calcium dependent or independent for activity.

Because bacterial TGase has the calcium independent activity, the microbial TGase from *Streptomyces mobaraense* (SMTG) has used in various food industries such as cheese and ice cream product, Kefir, meat processing and bakery products. However, the detailed substrate specificities of TGases from the *Streptomyces* species toward the natural peptides contained Gln residue remains to be unclear.

In this study, we conducted the comparison of two different TGases from *Streptomyces mobaranensis* and *Streptomyces cinnamoneus* (SCTG). To clarify the region associated with the characteristics of enzymes, we constructed the fusion enzyme CM, of which sequence is SCTG at N-terminal and SMTG at C-terminal. We investigated the effects of pH and temperature on the activities of SCTG, SMTG and CM. The optimum pH of SCTG activity was at pH8.0 whereas that SMTG and CM were shifted to the side of alkali at pH9.0. The pH stability of CM at pH6.0 was less than those of SCTG and SMTG. In contrast, optimal temperatures of SCTG and CM activities were at 40°C and 45.4°C, respectively whereas that SMTG was higher than them at 50.9°C. In addition, the thermal stabilities of SCTG and CM showed similar Tm values 45.8°C and 46.0°C, respectively whereas that that of SMTG showed higher Tm value 51.7°C.

The substrate affinities and the reaction rates toward Z-Gln-Gly as acyl donor were increased in order of SCTG, CM, SMTG. The V_{max} of SCTG, SMTG and CM toward Z-Gln-Gly were 24.4, 17.5 and 20.0 μmol·min^{-1}·mg^{-1}, respectively. It was indicated that the TGases have little differences in reaction rates. In contrast, the K_m of SCTG for Z-Gln-Gly was 68.5 mM, approximately six-fold that of SMTG. Because of the low substrate affinity of SCTG for Z-Gln-Gly, the SCTG activity was likely less than SMTG. In contrast, the $V_{max}\cdot K_m^{-1}$ of them for L-lysine exhibited similar values each other.

To reveal the differences in the substrate specificity between SCTG and SMTG toward natural peptides, we investigated the time dependence of TGase activity on the productivity of cross-linking peptide with tryptic casein and lysine by LC-MS. We identified two peptides "VLPVPQK" and "AVPYPQR" as common substrate of both TGases SCTG and SMTG. Former peptide is known as an allergen and bitter peptide. We found that the products of these peptides bound lysine are less in SCTG than in SMTG. This result indicated that SCTG has high cross-linking activity among peptides than that of SMTG.

P-13 Ion channels involved in the generation mechanism of phasic spontaneous contraction of bovine oviducts

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[Introduction] Phasic spontaneous contraction of mammalian oviducts is essential for the transport of gametes and embryos. Previous studies have demonstrated that spontaneous membrane depolarization of "pacemaker" cells, the endogenous generator for contraction, induces Ca^{2+} increase in myocytes resulting in muscular contraction in the hearts and intestines. It is also known that several kinds of ion currents are responsible for generating contraction. The objective of this study is to clarify the basic mechanism of spontaneous contraction in bovine oviduct.

[Materials and Methods] Isthmic tissues of bovine oviduct were used for the following experiments. The effects of ion channel and gap junction blockers on the spontaneous contraction were investigated using Magnus system. Target factors were as follows; 1) voltage-dependent Ca^{2+} channels (VDCC) and the receptors responsible for Ca^{2+} release from endoplasmic reticulum (IP3R and RyR) as Ca^{2+} source, 2) Na^{+} and Cl^{-} channel as a depolarization initiator, 3) voltage-dependent K^{+} channel (VDKC) and Ca^{2+}-activated K^{+} channels (BK and SK channels) as a re-/hyper-polarization regulator, and 4) gap junction-mediated propagation of depolarization to neighboring cells.

[Results and Discussion] 1) The VDCC blocker decreased amplitude of contraction resulting in the loss of contraction. Inhibition of both IP3R and RyR decreased the number and amplitude. These results indicate that Ca^{2+} influx via VDCC or release from endoplasmic reticulum is necessary for spontaneous contraction. 2) Na^{+} channel blocker did not affect contraction, whereas Cl^{-} blockers decreased the frequency of contraction, suggesting that the Cl^{-} channel is involved in the initiation of depolarization. 3) The VDKC blocker decreased the number and amplitude. BK channel blocking decreased the number of contraction, although BK and SK channel blockers increased the amplitude. These results suggest VDKC and Ca^{2+}-activated K^{+} channels are involved in the regulation of re-/hyper-polarization. 4) A blocker of gap junction suppressed contraction, suggesting that depolarization propagates via gap junctions.

Our results revealed the ion channels which are responsible for phasic spontaneous contraction of bovine oviducts.

P-14 Immunomodulatory activity of *Ganoderma atrum* polysaccharide on purified T lymphocytes through Ca^{2+}/CaN and MAPK pathway based on RNA-seq

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Ganoderma atrum has been safely used as ingredient of traditional medicine and functional food for thousands of years in oriental countries. Recently, a polysaccharide, named PSG-1 with a purity of > 99.8%, was extract from *Ganoderma atrum* in our laboratory. Our recent studies have demonstrated that PSG-1 possesses a variety of biological functions, for example, anti-tumor, immunomodulatory, cardiovascular protection, chemoprotective and hypoglycemic activities. Among them, of note is that PSG-1 has immunomodulatory effect on spleen lymphocytes. However, the experimental conclusion was derived from the mix lymphocytes, which could not illustrate the immunomodulatory effect of PSG-1 on purified lymphocytes.

We first tried to purify T lymphocytes, then screen the key signal transduction pathways in purified T lymphocytes stimulated by PSG-1 via RNA-seq, and further investigate the underlying mechanism involved in the immunomodulatory activity of PSG-1 on purified T lymphocytes.

Our results showed that PSG-1 promoted T lymphocytes proliferation and increased the production of IL-2, IFN-γ and IL-12. Meanwhile, RNA-seq analysis found 394 differentially expressed genes. KEGG pathway analysis identified 20 significant canonical pathways and 7 biological functions. Furthermore, PSG-1 elevated intracellular Ca^{2+} concentration and calcineurin (CaN) activity, and raised the p-ERK, p-JNK and p-p38 expression levels. T lymphocytes proliferation and the production of IL-2, IFN-γ and IL-12 were decreased by the inhibitors of calcium channel and MAPKs. These results indicated that PSG-1 possesses immunomodulatory activity on purified T lymphocytes, in which Ca^{2+}/CaN and MAPK pathway play essential role.

PY-1 New polyphenols from *Punica granatum* and their anti-glycation activities

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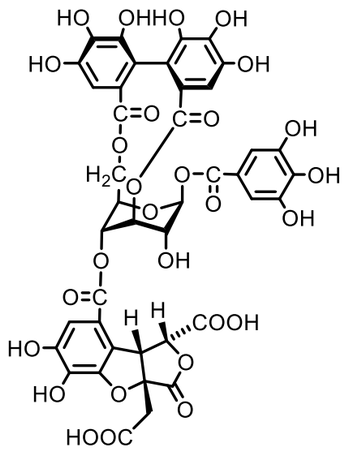
More than 1,000 hydrolyzable tannins, approximately half of them are ellagitannin have been reported. Among 200 or more ellagitannin oligomers have been isolated from different plant species and 80% of them are dimers. Fischer et al. has detected 48 compounds from pomegranate extract (peel, mesocarp, and aril). Ellagitannin mainly located in the fruit peel, arils, and mesocarp of pomegranate. We previously reported the isolation and characterization of two new ellagitannin oligomers (pomegraniins A and B) and a new neolignan (pomegralignan) from arils of *Punica granatum*. These compounds have been assessed the inhibitory activities toward the formation of advanced glycation end products (AGEs). In the present study, we isolated a new monomeric ellagitannin and three new phenolic compounds from the arils and leaves, and determined their structures.

The extract of arils juice and leaves of pomegranate was subjected to Diaion HP-20, and Toyopearl HW-40 column chromatographies with H_2O, MeOH, aqueous MeOH, and aqueous acetone to give a new monomeric ellagitannin, pomegraniic acid, and three new phenolic compounds. The structures of four new compounds were elucidated based on NMR, MS, and CD spectrometric analyses and chemical evidence.

Pomegraniic acid was obtained as a pale brown amorphous powder and exhibited a molecular ion peak at *m/z* 969.0853 $[M–H]^-$ by HR-ESI-MS, indicating that its molecular formula was $C_{41}H_{30}O_{28}$. The ^{1}H-NMR spectrum showed that pomegraniic acid consists of a galloyl, an hexahydroxydiphenoyl (HHDP) groups, a glucose, and a new acyl unit related dehydrohexahydroxydiphenoyl group. The 2D-NMR spectra indicated that the galloyl, HHDP and the new acyl group attached to O-1, O-3~O-6 and O-4 position at glucose, respectively. Pomegraniic acid was assigned as shown in the formula. Other new compounds were characterized as galloyl glucose derivatives based on NMR and MS spectroscopic analyses.



Pomegraniic acid

The ellagitannins and the related polyphenols isolated from pomegranate were assessed for inhibitory activities towards the formation of AGEs by the glycation reaction of HSA with glucose. Punicalagin and punicalin, which are known to be the main compounds of pomegranate were also evaluated. Among these inhibitors, pomegraniin A showed the highest level of inhibition with IC_{50} value of 0.03 μM.

PY-2 Functional analysis of lanthanide inducible proteins of *Methylobacterium aquaticum* 22A

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Methylobacterium species have PQQ-dependent methanol dehydrogenases (MDHs) for oxidizing methanol, and MDH is the most highly expressed periplasmic enzyme in the bacteria living in the phyllosphere. MDHs are encoded by two genes, *mxaF* and *xoxF*, in many of these bacterial genomes, and MxaF is known to be an enzyme containing calcium in its active site. The function of XoxF has not been known for a long time, however, just recently it was revealed to use lanthanide as a cofactor. The enzyme is the first enzyme found to be dependent on lanthanide. *M. aquaticum* strain 22A isolated from a moss, *Racomitrium japonicum,* promotes plant growth, and we use the strain as a model to study lanthanide-dependent methylotrophy. RNA-Seq analysis showed that *xoxF* was preferentially expressed in the presence of La^{3+} and *mxaF* was expressed only in the absence of La^{3+} when the cells were grown on methanol. Not only the genes for methylotrophy, some other genes were found to be up-regulated in the presence of La^{3+}. In this study, we analyzed the function of calcium-binding protein (CaBP) that was found to be highly expressed in the presence of La^{3+}.

The CaBP amino acid sequence has a Ca^{2+} binding domain called EF-hand. The gene is conserved in many of the genomes of *Methylobacterium* and *Bradyrhizobium* species. It contained a putative signal peptide and was considered to encode proteins of 12 kDa. Quantitative PCR results confirmed its upregulation in the presence of La^{3+}. We purified His-tagged recombinant CaBP expressed in *E. coli*. The UV absorption spectrum of the protein changed, and mobility in native PAGE changed in the presence of La^{3+}, suggesting that CaBP binds La^{3+}. The gene deletion mutant did not lose the ability to grow on methanol regardless of La^{3+}, suggesting that it is not necessary for methylotrophy. The mutant showed smaller amount of La^{3+} in solution when the cells were incubated with La^{3+}, suggesting that CaBP may be involved in lanthanide sequestration. Although precise function of the protein remains unclear, CaBP might be involved in lanthanide sensing and regulation of MDHs in *Methylobacterium* species.

PY-3 Molecular mechanism of methylotaxis in *Methylobacterium aquaticum* strain 22A

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Plants emit about 100 million tons of methanol annually into the atmosphere, and *Methylobacterium* species that can use methanol become dominant occupying 10-20% of the bacteria on the plant surface. They can promote growth of plants and are believed to be symbiotic bacteria beneficial to the plant. However, it is largely unknown what kind of chemical substances mediate the plant-bacteria recognition. We found that *M. aquaticum* strain 22A isolated from a moss, *Racomitrium japonicum,* shows chemotaxis to methanol (methylotaxis). The methylotaxis must be important for the bacteria to find plants to colonize, but its sensor and molecular mechanism have never been characterized.

The chemotaxis in bacteria is achieved by methyl-accepting chemotaxis protein (MCP) sensing the chemoattractant and multiple chemotaxis proteins controlling the flagellar rotation direction. There are 52 MCP genes in strain 22A genome. Based on RNA-Seq data, we chose several MCP candidate genes showing higher expression levels when the cells were grown on methanol. We checked methylotaxis of the MCP gene deletion mutants, and finally we found that three genes (*mcp1*, *mcp2*, and *mcp3*) are involved in methylotaxis. The methylotaxis was completely lost in the triple gene mutant, but the mutant retained chemotaxis toward organic acids. These three MCPs function independently, because methylotaxis was not lost in the single gene or double genes deletion mutants. Based on the prediction of MCP structures, Mcp1 was considered a cytoplasmic protein, which consists only of the MCP signal domain. Mcp2 contains a PAS domain that was responsible for energy taxis. Mcp3 has a typical MCP structure with a HAMP domain. The localization analysis of GFP-tagged MCPs in the cells showed that Mcp1 localizes in the cytoplasm, Mcp2 locates at a cell membrane or pole, and Mcp3 locates at the pole. Functional gene complementation could be done only for Mcp1. Methylotaxis in the presence of lanthanide showed that MCP2 is related to methanol metabolism, since *Δmcp2* decreased methylotaxis in the presence of La. The triple gene deletion mutant gathered slower than wild type to the roots of rice and *Arabidopsis thaliana*. We concluded that methanol and methylotaxis mediate the establishment of symbiosis between *Methylobacterium* species and plants.

PY-4 Effects of orizabin from *Tithonia diversifolia* on vascular protection and suppression of foam cell formation in human umbilical vein endothelial cells and human monocytic THP-1 cells

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In the diabetes prevention and health maintenance, *Tithonia diversifolia* (TD) used as Japanese folk medicine, but no study evaluating these effects had been conducted. Therefore, as a result of testing the anti-inflammatory effect of the TD methanol extract, there was a strong effect. In addition, water - ethyl acetate partitioning residue of the TD methanol extract resulted in a strong anti - inflammatory activity in the ethyl acetate layer.

In order to identify the active substance, the compound contained in the TD extract was isolated using preparative HPLC, and a compound having high content and high activity was identified as orizabin. The aim of this study was to evaluate anti-inflammatory effect and anti-atherogenic activity of orizabin isolated from TD in *vitro* experimental atherosclerosis model using Human Umbilical Vein Endothelial Cells (HUVEC) and human monocytic THP-1 cells.

Orizabin significantly inhibited the adhesion of THP-1 cells to HUVEC, and suppressed expression of adhesion molecules in THP-1 cells incubated with orizabine. In addition, phorbol (PMA) - stimulated THP-1 cells are induced to differentiate to macrophage-like cells, but differentiation induction is suppressed by culturing with orizabin and expression of CD36 which is one of oxidized LDL receptors is decreased. Also, orizabin was added to THP-1 macrophage-like cells, lipid uptake was suppressed significantly with compared to control without orizabin. These results revealed that Td- derived orizabin have a possibility of atherogenic activity.

KEYWORDS: *Tithonia diversifolia*; Atherosclerosis; orizabin; HUVEC; THP-1

PY-5 Isolation of *p*-nitrophenyl *N*-acetyl-β-D-glucosaminide assimilating bacteria and their β-*N*-acetylglucosaminidase sensitivity to *p*-nitrophenyl *N, N, N*-trimethyl β-D-glucosaminium iodide

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β-*N*-Acetylglucosaminidase (GlcNAcase, EC 3.2.1.52), a widely distributed glycoside hydrolase, catalyzes the release of *N*-acetylglucosamine from the non-reducing ends of substrates such as chitinoligomers and glycoconjugates. We found that family 20 GlcNAcases which hydrolyze *p*-nitrophenyl *N*-acetyl-β-D-glucosaminide (*p*NP-GlcNAc) was subdivided according to sensitivity against GlcNAcase inhibitor, TMG-chitotriomycin[1,2]. An *N, N, N*-trimethylglucosaminium (TMG) residue positioned at the non-reducing end of TMG-chitotriomycin will be contribute to inhibitory activity because TMG analogues, a mono- and di-saccharide TMG, showed specific inhibitory activity against certain GlcNAcases[3]. In this study, we synthesized *p*-nitrophenyl *N, N, N*-trimethyl β-D-glucosaminium iodide (*p*NP-TMG) as a TMG-chitotriomycin analogue. *p*NP-TMG was found to inhibit an insect GlcNAcase from *Spodoptera litura* and but not a prokaryotic GlcNAcase from *Streptomyces coelicolor*. This indicated that *p*NP-TMG showed similar inhibitory selectivity to TMG-chitotriomycin and other TMG analogues. In order to further confirm the inhibitory selectivity of *p*NP-TMG against GlcNAcases, *p*NP-GlcNAc assimilating bacteria were isolated by enrichment culture using *p*NP-GlcNAc as a carbon/nitrogen source and examined for sensitivity of their GlcNAcases to *p*NP-TMG. As a result, twenty-seven bacterial strains which showed an intracellular *p*NP-GlcNAc hydrolase activity were isolated. One strain showing high hydrolytic activity in resting cells among the obtained bacteria was cultured with a nutrient broth, and is found to constitutively express GlcNAcase. GlcNAcase in a cell-free extract prepared from the cultured cells was inhibited by *p*NP-TMG as well as the above insect GlcNAcase, but not the above prokaryotic GlcNAcase. Furthermore, GlcNAcase produced in *p*NP-GlcNAc containing medium showed higher specific activity than that produced in a nutrient broth medium. These results indicated that it is interesting to investigate the difference between *p*NP-TMG sensitivity of constitutively expressed GlcNAcases and that of induced ones, which are closely related to substrate recognition mechanism of GlcNAcases.

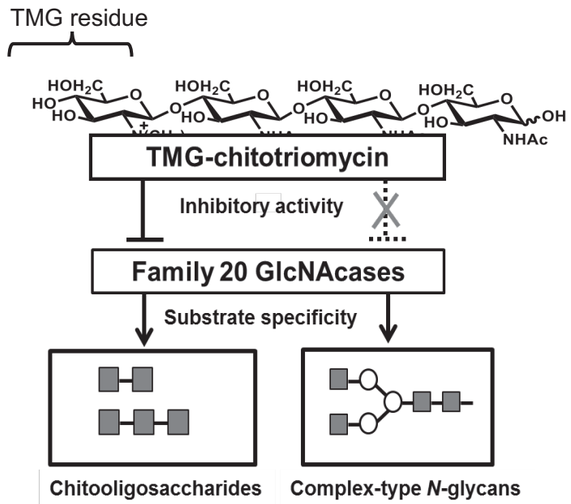

Fig. Inhibitory selectivity of TMG-chitotriomycin against GlcNAcases (GH20)
■: *N*-acetylglucosamine, ○:Mannose

1) H. Usuki *et al., J. Amer. Chem. Soc.,* **130**, 4146-4152 (2008)
2) H. Shiota *et al.*, *Carbohydr. Res.*, **375**, 29-34 (2013)
3) H. Usuki *et al.*, *Org. Biomol. Chem.*, **9**, 2943-2951 (2011)

PY-6 Quinolinic acid phosphoribosyltransferase (QPRT) knockout mouse as a novel noninvasive chronic kidney disease (CKD) animal model and a possible animal model for the search of bioactive compounds with preventive effect in CKD

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[Background and Aims]
Chronic kidney disease (CKD) describes the progressing loss of renal function, and the numbers of CKD patients continue to increase recently. Nutraceutical approach for preventing CKD have directed much attention and interest**, but is difficult because the** pathogenic mechanism **still remains obscure.** Quinolinic acid (QA) **which is** an **intermediary metabolite in Kynurenine pathway is known to be neurotoxic and uremic. There are a few reports about the relationship between QA and renal failure but its details are still an enigma.** The aim of this study is to investigate the effect of QA on renal function using quinolinic acid phosphoribosyltransferase (QPRT) knockout mice which is able to accumulate QA.

[Methods]
In this study, 60-week-old (middle-aged) QPRT knockout mice were used. Serum and urinary QA levels were measured by GC/MS. Serum metabolome analysis were performed using GC/MS. DNA microarray was used to evaluate the gene expression in the kidney of QPRT knockout mice. Renal functional markers of serum and urine were analyzed by respective methods. The mRNA expression of EPO, collagen-type-1-alpha-1 (col1a1) and alpha smooth muscle actin (a-SMA) were measured by real-time PCR. The collage deposition was determined by Sirius Red staining. And the levels of hemoglobin and hematocrit were measured.

[Results]
Serum QA was significantly increased and urinary QA tended to increase in QPRT knockout mice. Metabolome and biochemical analysis showed that creatinine, urea nitrogen and phosphate in QPRT knockout mice were significantly increased in serum and decreased in urine, suggesting that QPRT knockout mice have effect on renal function. The microarray data indicated that gene families involved in fibrosis were upregulated in QPRT knockout mice. Furthermore, col1a1 and a-SMA mRNA levels and collagen deposition were increased, suggesting that QPRT deletion has effect on renal fibrosis. In addition, QPRT knockout mice significantly decreased erythropoietin (EPO) mRNA expression ($p<0.05$), hemoglobin ($p<0.01$), and hematocrit ($p<0.05$).

[Conclusion]
Our results suggested that QA accumulation induces renal fibrosis, and causes EPO synthesis and glomerular filtration to be damage. We propose that QPRT knockout mouse is a novel noninvasive CKD model and can be used for the assessment of bioactive components in food with protective and/or improvement effects of CKD.

PY-7 Study on mechanism of taurine function on skeletal muscle of aged rats

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Taurine (2-aminoethane-sulfonic acid), is one of abundant free amino acids in mammalian tissues, which has many physiological roles such as membrane stabilization, osmoregulation and anti-oxidation. Numerous evidences were reported that taurine had a protective effect on skeletal muscle aging and atrophy. Muscle taurine content was significantly decreased in muscle of aged rats. Taurine depletion by knockout of taurine transporter (*Tau T*) gene led to shorten lifespan, accelerated functional defects of skeletal muscle, and decrease of total exercise capacity. Aged rats decreased serum and tissue levels of taurine, however, those conditions can be corrected by increased dietary taurine intake. Taurine administration maintained the taurine concentration in skeletal muscle on exercise and up-regulated physical endurance. Nevertheless, the mechanism of taurine anti-aging function on skeletal muscle has not been clarified.

In order to clarify the mechanism, male Sprague-Dawley rats (n=5/group) at 33 weeks of age were administrated water (control), 1% acetic acid, 0.5% taurine or 1% taurine (5 days/week, 5 ml/kg BW). The tissues were collected at 55 weeks of age and immediately frozen in liquid nitrogen and kept at -80°C.

Spontaneous locomotor activity in water group was decreased significantly as rats were getting older (from 33 weeks to 55 weeks). However, administration of 1% taurine significantly prevented the decline of spontaneous locomotor activity. Taurine contents in extensor digitorum longus (EDL) muscles were significantly higher in 1% acetic acid groups and 1% taurine group as compared with control group after 22-week administration. Taurine content in gastrocnemius muscle was higher in 1% taurine group as compared with 1% acetic acid group. Besides, taurine content in the intestine has a tendency to increase in taurine groups. *Tau T* expression in intestine was significantly increased in 1% acetic acid and 1% taurine groups than that in control group. Furthermore, taurine administration had effects on PGC-1 α, GLUT4 and MEF2A genes expression in gastrocnemius muscle.

From the results, it was suggested that chronic intake of taurine might stimulate to increase taurine absorption from intestine, increase taurine content in skeletal muscles, and has effect on anti-aging. Further investigation about other genes and proteins expressions is progressing to clarify the mechanism of taurine function.

PY-8 Identification of enzymes derived from *Saccharomyces cerevisiae* catalyzing the reaction of olive leaf secondary metabolite, 3,4-dihydroxyphenylethanol-elenolic acid to reduced 3,4-dihydroxyphenylethanol-elenolic acid

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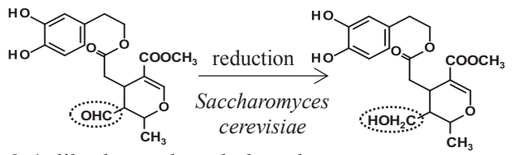
Structural conversion by microorganism is well-known as a high functionalization method of secondary metabolites. Previously, we revealed that 3,4-dihydroxyphenylethanol-elenolic acid (3,4-DHPEA-EA) in olive leaf extracts was bioconverted to reduced 3,4-DHPEA-EA by *Saccharomyces cerevisiae* [1)]. Reduced 3,4-DHPEA-EA has high antioxidant activity, which is expected to be applied for cosmetic and food materials. On the other hand, it remains to be clarified which redox enzymes from *S. cerevisiae* catalyzed this reaction. In this paper, we searched for the enzymes catalyzing the reduction.



3,4-dihydroxyphenylethanol-elenolic acid (3,4-DHPEA-EA)

Reduced 3,4-DHPEA-EA

Fig. Structural conversion of 3,4-DHPEA-EA

When cell-free extracts of *S. cerevisiae* were incubated with olive leaf extracts, the progress of the reaction could not be traced because of the protein aggregation. Therefore, we tried to search for candidate enzymes using the *S. cerevisiae* cells overexpressed with aldehyde reducing enzyme. 17 genes of alcohol dehydrogenases and 6 genes of aldehyde reductases were selected as candidate genes by the *Saccharomyces* Genome Database. The target enzyme was overexpressed by genetic Tug-Of-War (gTOW) method developed by Moriya *et al.*[2)] The reaction mixtures obtained by the incubation of olive leaf extracts containing 3,4-DHPEA-EA with resting cells expressing each candidate enzyme were analyzed by HPLC. As a result, alcohol dehydrogenase 6 (Adh6), aldehyde reductase intermediate 1 (Ari1) and aryl-alcohol dehydrogenase 4 (Aad4) overexpressing strains showed particularly high reduction activity, three times or higher than that of the control *S. cerevisiae*. It was speculated that two or more enzymes catalyzed this reduction reaction.

Among three enzymes selected above, Adh6 was purified to verify whether the reaction was observed at the protein level. Adh6 was expressed as a GST fusion protein and purified by affinity chromatography on Glutathione Sepharose 4B. Decrease in absorbance of NADPH at 340 nm was observed in the reaction mixture containing GST-Adh6, purified 3,4-DHPEA-EA and NADPH, indicating that GST-Adh6 is proved to catalyze the reduction reaction of 3,4-DHPEA-EA in the presence of NADPH. The reduction activity was about 4% when the cinnamaldehyde was used as a substrate. In addition, the formation of reduced 3,4-DHPEA-EA and following decrease in 3,4-DHPEA-EA were observed by HPLC analysis of the reaction mixture. From the above, we found for the first time that GST-Adh6 catalyzed reduction of 3,4-DHPEA-EA in a NADPH-dependent manner to produce reduced 3,4-DHPEA-EA. GST-Adh6 are proved to reduce aldehydes having with a relatively bulky group, since reduction of 3,4-DHPEA-EA in addition to those of cinnamaldehyde and benzaldehyde was catalyzed by this enzyme.

[1)] H.Kanzaki, T.Nitoda, *Fragrance J.*, **2**, 63-66 (2008). [2)] H.Moriya, K.Makanae, K.Watanabe, A.Chino, Y.Shimizu-Yoshida, *Mol.BioSyst.*, **8**, 2513-2522 (2012)

PY-9 Purification of several subunits from a peanut allergen, Ara h1

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[Introduction] Peanut allergy is one of the most severe food allergies, and many allergenic proteins have been identified from peanut seeds; Ara h1~ Ara h17. Most predominant species among these peanut allergens is Ara h1 consisting of three subunits, which accounts for nearly 15% of total proteins. The molecular characterization of Ara h1 has almost been completed, and the presence of two isoforms (clone 41B and clone P17) have been identified [1]. However, several variant molecules of Ara h1 have been found and the molecular and immunoreactive differences between each variant remain to be clarified. As a first step to clarify these differences, in this study, we purified several variant molecules of Ara h1 and analyzed their partial amino acid sequences and *N*-glycan structures.

[Methods] Ara h1 was extracted from the defatted peanut powder in 50 mM Tris-HCl buffer (pH8.3), containing 0.2 M NaCl. From the crude proteins, several variants of Ara h 1 were purified by a combination of $(NH_4)_2SO_4$ precipitation, anion exchange, gel-filtration, and hydrophobic interaction chromatography. The glycosylation of each subunit was analyzed by PNGase F digestion and SDS-PAGE. The structural features of *N*-glycan linked to each variant were analyzed after hydrazinolysis and pyridylamination (PA). The PA-labeled sugar chains were separated using RP- and SF-HPLC, and identified through MS and MS/MS analysis, and exo-glycosidase digestion.

[Results and discussion] The molecular mass of each subunit of Ara h 1 was estimated 68 kDa and 64 kDa and N-terminal amino acid sequence of each subunit was RS/HPPGER and EGREGEQ, respectively. These sequences found in the deduced amino acid sequences of Ara h1 (clone P41B and/or clone P17), suggesting that a different processing mechanism by endogenous protease(s) produced several variant subunits. Furthermore, each subunit was glycosylated, and four major structures ($Man_3Xyl_1GlcNAc_2$-PA, $Man_4Xyl_1GlcNAc_2$-PA, $Man_5GlcNAc_2$-PA, and $Man_6GlcNAc_2$-PA) were identified, as previously reported [1,2].

[1] Burks A.W. *et al.*, *J. Clin. Invest.*, 96, 1715-1721(1995)
[2] Ronald van Ree *et al*., *J.B.C.*, 275, 11451-11458 (2000)
[3] Kolarich D. *et al*., *Analyt. Bioc*., 285, 64-75 (2000)

PY-10 Absorption and metabolism of short-chain fatty acids in rats during the night

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Fructo-oligosaccharides (FOS) is fermented by intestinal bacterial flora, then short chain fatty acids (SCFAs) is produced. SCFAs are promptly absorbed in large intestine and largely used as calories source in host. In FOS intake, absorption of SCFAs in large intestine has been fully studied. However, absorption of SCFAs in large intestine during the night is still unknown.

In this study, we evaluated concentration of organic acids and pH in cecal contents to investigated intestinal condition in the rats fed FOS (FOS group). Rats were established ileum fistula for administration of experimental solutions into the cecum. Briefly, the ileum was cut at the site of the head side 5 cm from the ileocecal junction. The oral cut end of the ileum was sutured to the ileal segment by an end-to-side anastomosis. The aboral cut end of the ileum was sutured to a small opening made in the abdominal wall and then a fistula was created to the abdominal wall. Absorption and metabolism were measured with ^{13}C-SCFAs breath test in rats during the night (21:00~2:00). Rats were intravenously administered 15 μmol of 1-^{13}C propionic acid solution. And rats were intracecally administered 15 μmol of the solution from the ileal fistula. Rats fed diet not containing FOS (C group) were used as control.

In the C group, concentration of acetic acid, propionic acid and *n*-butyric acid in the cecal content were 27.8, 4.2 and 5.2 mM, respectively. In contrast, in the FOS group, concentration of acetic acid, propionic acid and *n*-butyric acid in the cecal content were 41.7, 10.7 and 17.4 mM, respectively. The cecal pH was lower in the FOS group than in the C group. Concentration of organic acids in the FOS group were higher than in the C group. In ^{13}C-SCFAs breath test, absorption of propionic acid in the FOS group was increasing more than in the C group. However, no significant differences were found between FOS group and C group in metabolism of propionic acid.

These results suggest that changes in the intestinal environment affect absorption of SCFAs during the night.

PY-11 Lipocalin-type prostaglandin D synthase as a potential biomarker of bovine mastitis

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Prostaglandin (PG) D_2 is one of the lipid mediators and is involved in sleep induction, pain, allergic inflammation, and so on. PGD_2 is isomerized from PGH_2 by PGD synthase (PGDS), which has two distinct types, namely lipocalin-type PGDS (L-PGDS) and hematopoietic-type PGDS (H-PGDS). One of them, lipocalin-type PGDS (L-PGDS), is expressed in central nervous system, heart, male reproductive organs, and so on, and is secreted in such as cerebrospinal fluid, seminal fluid, blood and urine.

Recently we found the existence and the high activity of PGDS in the cow mastitic milk. The purified enzyme was identified bovine L-PGDS, and was secreted into milk without N-terminal signal peptide of 28 amino acid residues. The purified enzyme had similar enzymological properties to human or rat L-PGDS, that is, the *K*m value, *V*max, and *K*cat for PGH_2 were 38 μM, 3.33 μmol/min/mg of protein and 1.17/s, respectively. The enzyme had the optimum pH 9, and exhibited thermostability and protease resistance.

In this study, we demonstrated L-PGDS levels were correlated with somatic cell count, a general mastitis parameter, and haptoglobin, an acute inflammatory marker. Profiles of lipid mediators analyzed by LC-MS-MS included several pro-inflammatory and pro-resolving lipid mediators, and indicated the mastitic milk had significant changes in some lipid mediators compared with normal one. The differences between normal and mastitic milk might have biochemical and nutritional influences on calves.

Somatic cell count is a simple and general parameter, but it often shows false-positive by aging, stress and so on. The present results suggest that L-PGDS and some lipid mediators have a potential to be biomarkers of bovine mastitis as alternatives to somatic cell count.

PY-12 Rice bran extracts (RBE) as natural ameliorative bioactive materials for oxidative stress and inflammation

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Natural antioxidants derived from plant-based biomaterials have the capacity to ameliorate oxidative stress associated complications. Such health benefits are generally attributed to the synergistic antioxidant protective effects of different bioactive constituents present in these biomaterials. Rice bran has been known to house different constituents of polyphenols and vitamins; yet it remained underexploit. Through recent emerging knowledge of rice bran in health and wellness, the present study was aimed to assess the bioactivities of rice bran extracts (RBE) derived from selected Japanese rice varieties via *in vitro* chemical and *in vitro* mammalian cell culture assay systems.

Phytochemical profiling of RBE revealed a significant variation in profiles of different bioactive constituents. The compositions of these bioactive constituents were found to affect the bioactivities of different RBE within the *in vitro* DPPH, ABTS, and H_2O_2 chemical radical scavenging assay systems. Dose-dependent free-radical scavenging and antioxidant properties of RBE were generally observed and the correlation studies further revealed that both phenolic acids and proanthocyanidins are potential bioactive constituents in RBE contributing to their respective free-radical scavenging properties against chemical radicals. Under *in vitro* mammalian cell culture system, the lipopolysaccharide (LPS)-induced inflammation in J774A.1 macrophage was used to evaluate the anti-inflammation properties of RBE. Pre-treatment of J774A.1 cells with RBE revealed a dose-dependent cytoprotective effect of RBE against excessive inflammatory response triggered by LPS-induced endotoxic shock. RBE were able to reduce overproduction of nitric oxide (NO), and downregulated both gene and protein expressions of targeted inflammatory cytokines: TNF-α, IL-1α, IL-1β, IL-2, IL-6, and iNOS.

Based on the present preliminary study, it has revealed the potential utilization of rice bran as a source of natural antioxidants to attenuate risks of inadvertent cellular oxidative damage and inflammatory responses in chronic diseases. Intrinsically, present study findings may provide global health prospects and future research directions for innovative utilization of rice bran in management of oxidative stress- and inflammation-associated complications, particularly as a potential adjunct in preventive or regenerative medicine to enhance treatment efficacies through amelioration of oxidative stress and inflammation.

Keywords: Bioactive materials, rice bran extracts (RBE), antioxidants, oxidative stress, anti-inflammation.

PY-13 Preventive effect of red rice proanthocyanidin on psoriasis via inhibition of arachidonate 5-lipoxygenase

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The hulls of red-kerneled rice (*Oryza sativa*) is abundant in polyphenols, and recently the details of the chemical structure were identified as proanthocyanidin composed of catechin octamer (average molecular weight 2338, N. Ganeko *et al.* ICPH2015).

In this report, we demonstrated that the red-kerneled rice proanthocyanidin (RRP) inhibited arachidonate 5-lipoxygenase (5-LOX) with IC_{50} of 7.0 μM in a mixed non-competitive manner *in vitro*. 5-LOX is a key enzyme for synthesis of leukotriene (LT) B_4 (regulation of immune response and chemotaxis) and cysteinyl LTs (induction of allergic inflammation) from arachidonate, and the produced LTs are involved in several inflammatory diseases such as psoriasis, asthma, and atherosclerosis. Therefore, we investigated novel functions of RRP on inflammation.

Psoriasis is a chronic inflammatory skin disease, and is thought to be promoted by LTB_4. In the psoriasis model mouse induced by an application of imiquimod (IMQ), the topical application of RRP decreased LTB_4 in the mouse skin. Consequently, RRP treatment suppressed the hyperplasia, and decreased inflammatory cell infiltration in the skin of psoriasis. In addition, RRP down-regulated the psoriasis-associated genes, *Il17a* (IL-17a), *Il22* (IL-22), *S100a9* (S100a9) and *Krt1* (Krt 1). Thus RRP may inhibit 5-LOX activity and prevent psoriasis.

PY-14 Beneficial effect of functional foods on fatty liver

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Bifidobacterium is a genus of gram-positive, anaerobic bacteria, and they have been used to ferment yogurt from long time ago. they are ubiquitous inhabitants of the gastrointestinal tract of mammals. Bifidobacterium can ferment carbohydrates to produce acetic acid and have a potential to improve intestinal environment and lipid metabolism. While, Taurine is an essential amino acid, which present a high concentration in liver, skeletal muscle, brain, heart, neutrophils, retina of mammals. Taurine is contained naturally in seafood and meat, but also used as a supplement for foodstuffs. Taurine supplementation may be beneficial in preventing various metabolic disorders, including obesity, insulin resistance, and atherosclerosis.

Lipid metabolism disorder such as hepatic steatosis is a serious problem in Japan and other countries. In order to investigate the function of Bifidobacterium fermented yogurt and taurine for preventing fatty liver, we examined those effect with using aged rats or type 2 diabetes model animal, Otsuka Long Evans Tokushima Fatty (OLETF) rats.

Fermented milks were administered to OLETF rats at 5 mL / kg of body weight for 5 days a week from 8 weeks to 24 weeks of age. Sprague-Dawley (SD) rats at 33 weeks of age were administrated water, 0.5% taurine or 1% taurine at 5 mL / kg of body weight for 5 days a week. Livers were collected from OLEFT rats at 25 weeks of age and SD rat at 55 weeks of age, then made into sections, and stain with HE and Oil-O-Red.

In OLETF rat, Bifidobacterium yogurt (bif-15) presented suppression of weight gain and hepatic lipid accumulation. Aged rats supplemented with 0.5% taurine showed less body weight gain and less lipid accumulation in liver according to histological analysis and steatosis score. It is indicated that 0.5% taurine reduces steatosis level and obesity of aged rats. These data suggest that Bifidobacterium fermented yogurt and taurine may have an effect on prevention of fatty liver.

PY-15 Temperature sensing TRP channels are involved in increase of prostaglandin production of bovine endometrial stromal cells under heat stress

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Recently, the global warming makes a big impact on the livestock industry. Especially, pregnancy rate in cattle dramatically decreases during the summer season. It is known that attenuation of endometrial secretion of PGF2α is necessary for establishment of pregnancy in ruminant species, resulting in maintaining of corpus luteum (CL). However, our previous study showed that heat stress (HS) enhances the secretion of both PGF2α and PGE2 (PGs) in cultured bovine endometrial cells (Sakai *et al.*, 2018), although the underlying mechanisms of this effect have not been manifested.

Cells are able to sense and react to various environmental stimuli such as osmolality, temperature, and mechanical stimuli by signaling pathways and cascades. Transient receptor potential (TRP) channels are ion channels located on the plasma membrane and some of which are involved in temperature sensing of the cell. The present study investigated the role of temperature sensing via TRP channel on the secretion of PGs from bovine endometrial cells.

The first experiment investigated the location of TRP channels (TRPV3, V4, and M2, which are sensitive to bovine body temperature) in bovine endometrium by immunohistochemistry. Slaughterhouse derived uteri classified at the late luteal stage (day 14–17) were used for the experiment. Uterine horns ipsilateral to CL were fixed and sliced. Each section was immunostained with antibodies against TRPV3, TRPV4, or TRPM2.

While TRPV3 was not clearly observed in the bovine endometrium, TRPV4 was slightly detected in luminal epithelial cells. TRPM2 was observed in each region of the uterine tissues, especially in luminal and glandular epithelial cells, which presented stronger signals than stromal cells, myometrium, and endothelial cells.

The second experiment investigated the effect of inhibition of TRP channels on the production of PGs from endometrial cells under HS. Only stromal cells were used in the present experiment since our previous study showed that HS does not influence the secretion of PGs from epithelial cells (Sakai *et al.*, 2018). Bovine endometrial stromal cells were collected and cultured in the presence of each of the antagonists for TRPV3, V4, and M2 (TRPV3: Icilin, TRPV4: HC-067047, TRPM2: 2-APB, respectively) for 34 hours, at either 38.5℃ (control) or 40.5℃ (HS). After incubation, the concentrations of PGs in the culture media were measured by EIA. When stromal cells were cultured in the presence of TRPV3 or TRPV4 antagonists, HS significantly increased the secretion of PGs ($p < 0.05$) compared to the controls, suggesting that inhibition of TRPV3 and TRPV4 does not reduce the effect of HS on the secretion of PGs from bovine stromal cells. On the contrary, TRPM2 antagonist significantly inhibited the secretion of PGs ($P < 0.05$) under HS.

Taken together, the present study suggests that temperature-sensing TRP channels were located on the bovine uterine endometrium and involved in the increase of PG production of endometrial stromal cells under HS.

PY-16 Formaldehyde oxidation in lanthanide-dependent methylotrophy in *Methylobacterium aquaticum* strain 22A

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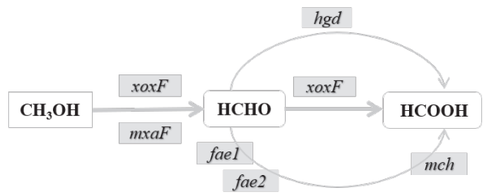
Methylobacterium species are one of the most predominant methylotrophic bacteria in the phyllosphere (plant aerial surfaces). It can utilize methanol emitted from plants as a sole carbon source. Methanol is oxidized by methanol dehydrogenases (MDHs), encoded by *mxaF* and *xoxF,* which are calcium- and lanthanide-dependent enzymes. XoxF is the first enzyme to be revealed as lanthanide-dependent. Since the oxidation of methanol yields formaldehyde that is toxic to the cell, it is necessary to detoxify the compound before it causes damage. Formaldehyde is then transported into the cytoplasm, where it is oxidized through formaldehyde oxidation pathways into formate. This oxidation is done through two crucial pathways, tetrahydromethanopterin (H_4MPT) pathway and glutathione (GSH) pathway. Finally, formate is then oxidized into CO_2, completing the oxidation process.

Our transcriptomic analysis revealed that in the presence of lanthanide, *xoxF* is highly induced and *mxaF* is repressed. In addition, H_4MPT and GSH pathways were downregulated whereas there was high formaldehyde dehydrogenase activity in the cells. These results suggested that formaldehyde oxidation other than H_4MPT and GSH pathways takes place. It is said that XoxF is capable of oxidizing formaldehyde as well as methanol, while MxaF cannot oxidize formaldehyde. The objective of this research is to reveal the exact roles of XoxF in methylotrophy in the presence of lanthanide, especially focusing on its formaldehyde oxidation.

We have generated single/multiple deletion mutants for formaldehyde-oxidizing enzyme genes, namely *fae1* and *fae2* (formaldehyde activating enzyme), *mch* (methenyl-H_4MPT cyclohydrolase), and *hgd* (S-hydroxymethyl glutathione dehydrogenase), as well as *mxaF* and *xoxF1*. Although Δ*xoxF1* mutant cannot grow on methanol irrespective of lanthanide because xoxF1 is necessary for mxaF expression, but we could isolate a suppression mutant named Δ*xoxF1*sup that regained growth on methanol expressing *mxaF* independent of *xoxF1*. The mutants were grown on methanol in the absence and presence of lanthanide. As results, we found followings: 1. Δ*hgd* didn't show any phenotype in any genetic backgrounds and growth conditions, suggesting that GSH pathway has less importance compared to H_4MPT. 2. In wild type (WT) and Δ*xoxF1*sup backgrounds, *fae1* was crucial for the growth but *fae2* was not, but in Δ*maxF* background Δ*fae1* showed better growth and Δ*fae2* did not show clear phenotype, suggesting that the roles of *fae1* and *fae2* differ depending on the presence of lanthanide. 3. In WT and Δ*xoxF1*sup backgrounds, Δ*mch* exhibited no growth suggesting formaldehyde accumulation, but Δ*mch* in Δ*mxaF* exhibited growth, suggesting that *mxaF* may play a role in repressing *xoxF1* and that XoxF1 can oxidize methanol to formate. 4. In *mxaF* background, Δ*mch*Δ*hgd* mutant exhibited, even if weak, growth in the presence of lanthanide, suggesting capability of XoxF to oxidize formaldehyde. These results genetically supported that XoxF is capable of oxidizing methanol directly to formate. In addition, our results suggested that H_4MPT and GSH pathways are necessary only when *mxaF* is active, since it produces formaldehyde.



Methylotrophy pathway in *M. aquaticum* strain 22A

最先端分子イメージング研究設備の共同利用を提供しています。

- 利用相談から機器操作、さらに実験後のデータ解析まで、**専任のスタッフが研究をサポート**します。
- **産学官共同研究**、学術研究機関所属の研究者による単独利用に加え、企業等に所属する研究者による**成果非公開**、**成果専有**でもご利用いただけます。
- 分子イメージング研究を実施する企業等に向けて**インキュベーション施設**を提供しています。

分子イメージング技術を用いた創薬・医療機器開発に関連する研究を支援します。

利用可能な分子イメージング研究設備群

サイクロトロン
HM-12S
（住友重機械工業）

小動物用PET装置
Clairvivo PET
（島津製作所）

中動物用PET/CTシステム
Eminence STARGATE
（島津製作所）

【その他設備】
ホットセル・PET用核種合成装置
発光・蛍光 in vivo イメージングシステム
飛行時間型質量分析装置

＊マウス、サル一時飼育ケージ、
動物乾燥室なども備えています。
＊利用料は、HPをご参照ください。

詳細はホームページをご覧ください。
http://www.crc.okayama-u.ac.jp/index.html

※利用相談および課題提案は、随時受け付けています。お気軽にお問い合わせください。

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おかやまバイオアクティブ研究会

Okayama Bioactive Research Society

生理活性物質に関する知識・技術の
向上のための研修・研究、
会員相互の情報交換を行っています。

おかやまバイオアクティブ研究会とは？

岡山県下の大学・公的研究機関における研究者および企業が会員として参加し、生理活性およびそれに関連する物質に関する研鑽や情報交換および人的交流などを行い、岡山県下の食品・医療品関連技術および産業の発展に寄与することを目的として平成19年5月に設立されました。

♣ 活動内容 ♣

- ○ 生理活性に関するセミナーおよびシンポジウム等の開催
- ○ 生理活性に関する共同研究の推進
- ○ 会員に対する生理活性に関する技術・開発に係る相談の実施
- ○ 会員相互の交流・情報交換
- ○ その他

♣ 実施事業 ♣

- ○ シンポジウム　年2回
- ○ 研究室訪問　年1回
- ○ 見学会　年1回

生理活性に関するさまざまな活動をおこなっています。

本会は岡山県生理活性物質研究会を前身とし、バイオアクティブおかやまの財源を吸収し、設立されました。
前研究会が行ってきた大学教員・学生を中心とした学術的かつ教育的な活動と、バイオアクティブおかやまが行ってきた岡山県下のバイオ産業育成と機能性食品の開発を融合させることを目標としています。

「産学共同研究の極意とはMutual Interestではなく Mutual Respectを大切にすることである」
【産】は研究者と経営者、【官】は研究職と行政職で、立場や考えが違うことが多くあります。大学教員は少なからず研究者ですから、経営や行政の立場についての理解が不足して産学官連携がうまくいっていない例もあります。産学官が共同研究を推し進めていく為にはお互いの立場を理解して行う事が大切と考えており、本研究会がその潤滑剤の役割を果たしていきたいと考えています。

会長　神崎　浩

ご入会のお申込み、お問合せはホームページより
http://www.optic.or.jp/bioactive/index.php

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Executive Endorsement

Okayama Prefecture Industrial Promotion Foundation

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