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Total phenolics, total anthocyanins, antioxidant capacity and anthocyanin profiles of new cultivars of red supersweet corns (*Zea mays L. saccharata*)

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Total phenolics, total anthocyanins and antioxidant capacity of new 16 conventionally bred cultivars of red supersweet corn were investigated. The results showed that total phenolic contents of red supersweet corn varied in a range of 322.3 - 439.9 mg gallic acid equivalents/ 100g dry weight (DW), while total anthocyanin contents (pH-differential method) ranged from 58.0 – 156.5 mg of cyanidin -3-glucoside/ 100g DW. Antioxidant capacity (DPPH assay) of red supersweet corn kernel were in the range of 321.92 – 464.29 mg Trolox equivalents/ 100g DW. Among 16 cultivars, SR 3052 had the highest total phenolic content and antioxidant capacity, while SR 3007 had the highest total anthocyanin content. Anthocyanin profiles of red supersweet corn cultivars were also identified using HPLC-DAD and HPLC-ESI/MS. The results indicated that pelargonidin-3-(6-malonylglucoside) was a predominant anthocyanin in red supersweet corn kernals. Other anthocyanins detected were cyanidin-3-o-glucoside, pelargonidin-3-glucoside, cyanidin-3-(malonylglucoside), cyanidin-3-o-glucoside-2-malonylglucoside pelargonidin-3-(dimalonylglucoside) and peonidin-3-(malonylglucoside).





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Molecular biology and biotechnology of secondary metabolite biosynthesis in buckwheat

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Development of an efficient protocol for successful hairy root induction by *Agrobacterium rhizogenes* is a key step in *in vitro* cultures for the mass production of secondary metabolites. Selection of an effective *Agrobacterium* strain for the production of hairy roots is highly dependent on the plant species and must be determined empirically. Therefore, we aimed to investigate the transformation efficiency of different *A. rhizogenes* strains for the induction of efficient transgenic hairy roots in *Fagopyrum tataricum* 'Hokkai T10' cultivar and determine the expression levels of polypropanoid biosynthetic pathway genes such as *FtPAL*, *FtC4H*, *Ft4CL*, *FrCHS*, *FrCH1*, *FrF3H*, *FtFLS1*, *FtFLS2*, *FtF3*:H1,*FtF3*:H2,*FtANS*, and *FtDFR* and quantify the *in vitro* synthesis of phenolic compounds and anthocyanins. Among the different strains, R1000 was the most promising candidate for hairy root stimulation as it induced the highest growth rate, root number, root length, transformation efficiency, and total anthocyanin and rutin content. However, strains R1000, 15834, and A4 provided higher transcript levels for most of the metabolic pathway genes for the synthesis of rutin (22.31, 15.48, and 13.04 µg/mg DW, respectively), cyanidin 3-*O*-glucoside (0.8, 0.75, and 0.65 mg/g DW, respectively), and cyanidin 3-*O*-rutinoside (2.41, 1.53, and 1.17 mg/g DW, respectively). In conclusion, a suitable *A. rhizogenes* strain could play a vital role in the fast growth of the bulk amount of hairy roots and secondary metabolites. Overall, R1000 performed the best as a promising strain for hairy root induction in buckwheat.







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Development of S-Allyl cysteine-Enriched Black Garlic Juice and Its Antidiabetic Effects in Streptozotocin-Induced Insulin-Deficient Mice

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S-Allyl cysteine (SAC), produced in large amounts during the aging process of garlic via enzymatic hydrolysis, is known as a key compound responsible for the multiple pharmacological activities of aged black garlic. In this study, we investigated the effects of enzyme- and high hydrostatic pressure (HHP)-assisted extraction on the content of the bioactive compounds, including SAC, in black garlic juice (BGJ) and evaluated the antidiabetic effects of SAC-enriched BGJ in streptozotocin (STZ)-treated mice. The aging process increased the contents of SAC, total polyphenols, and flavonoids in garlic juice. More importantly, pretreatment of pectinase cocktail with HHP resulted in a greater increase in those compounds during aging. Enzyme-treated BGJ reduced hyperglycemia and improved islet architecture and β -cell function in STZ-treated mice. Moreover, these effects were more potent than those of BGJ prepared by the conventional aging process. These findings provide useful information for the production of black garlic with improved bioactivities.





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Gut microbiota, host defense and immunity

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Animal gut is colonized with a huge number of commensal bacteria; for example, the human colon is colonized with more than 40 trillions of commensal microbes classified into several hundred species, collectively called gut microbiota. These microbes closely interact with the host to establish the unique and complicated gut ecosystem, which deeply impacts host physiology and pathology including host defense and immunity. However, the underlying mechanisms of how gut ecosystem influences host defense and immune system have poorly understood.

We have proposed an integrated multi-omics approach, where different levels of exhaustive analyses such as (meta)genomics, (meta)transcriptomics and metabolomics are combined.

By applying the integrated multi-omics approach, we have shown that *Bifidobacterium*-derived acetate can modify gene expression of the colonic epithelium to confer resistance against enterohemorrhagic *Escherichia coli* O157, which ultimately protects mice from O157-infectious death.

We have also found that butyrate produced by gut micirobiota can enhance differentiation of colonic regulatory T (Treg) cells from naïve T cells, via epigenetic modification through its histone deacetylase inhibitory ability.

We have also found that butyrate produced by gut micirobiota can enhance differentiation of colonic regulatory T (Treg) cells from naïve T cells, via epigenetic modification through its histone deacetylase inhibitory ability.

Finally, I would like to discuss the role of gut microbiota in the pathogenesis of multiple sclerosis (MS). MS is a demyelinating disease in the central nervous system. While the precise mechanism of pathogenesis is not clear, it is thought to be an autoimmune disorder caused by the combination of host genetic factors and environmental factors; among the latter is the gut microbiota. We are studying experimental autoimmune encephalomyelitis (EAE), an animal model for MS with the integrated multi-omics approach, which will be introduce in my talk.





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Preventive effects of decaffeinated green tea extract on metabolic syndrome in high fat fed mice

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Obesity and metabolic syndrome are growing public health problems. We investigated the effects of decaffeinated green tea extract (GTE) against obesity and metabolic syndrome in high fat (HF) fed C57BL/6J mice. After 16 wk, GTE reduced final body weight (9.4%). Although GTE did not show effect of fasting blood glucose, it significantly reduced fasting plasma insulin. In the liver, GTE increased the expression of proliferator-activated receptor- α (*Ppara*), carnitine palmitoyl transferase-1 α (*Cpt1a*). There was no significant change in stearoyl-CoA desaturase 1 (Scd1) and sterol regularoty element binding transcription factor 1 (Srebf1). Only the expression of acyl-CoA oxidase (Acox) was reduced by GTE treatment but not the expressions of proliferator-activated receptor- γ coactivator-1 α (pargc1a), mitochondrial NADH dehydrogenase 5 (mt-Nd5), mitochondrial cytochrome c oxidase subunit III (*mt*-Co3), mitochondrial cytochrome b (*mt*-Cytb). There was no change of the expression of peroxisome proliferator-activated receptor gamma (*Pparg*), sterol regularoty element binding transcription factor 1 (Srebf1), lipoprotein lipase (Lipe), patatin-like phospholipase domain-containing protein 2 (Pnpla2) in adipose tissue. However, the ratio of these expression show the increase of lipolysis rather than lipogenesis. Therefore the effects of GTE are due in part to modulation of genes related to energy metabolism and de novo lipogenesis.





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Current Topics of Relationships between Gut Microbiota and Host

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Two topics about the relationships between gut microbiota composition (GMC) and host will be shown in my talk. First topic is the gut microbota-gut-brain axis; second topic is the relationships between human GMC and personal and lifestyle characteristics.

Recent studies suggest that GMC influences gut-brain communication. In our study, we aimed to clarify the influence of GMC on cerebral metabolism. We analyzed the cerebral metabolome of germ-free (GF) mice and conventionalized (CV) mice, which were inoculated with suspension of feces obtained from specific pathogen-free mice, using capillary electro- phoresis with time-of-flight mass spectrometry (CE-TOFMS)*. CE-TOFMS identified 196 metabolites from the cerebral metabolome in both GF and CV mice. The concentrations of 38 metabolites differed significantly (p<0.05) between GF and CV mice. Approximately 10 of these metabolites are known to be involved in brain function, whilst the functions of the remainder are unclear. Furthermore, we observed a novel shows that normal GMC through association between cerebral glycolytic metabolism influences cerebral metabolites and GMC. Our work indicates that normal GMC was closely connected with brain health and disease.

Recent technological advancements have yielded information on GMC and function; however, the use of GMC analysis in predictive medicine is still preliminary. As a first step towards the practical application of GMC analysis, we used terminal restriction fragment length polymorphism analysis of GMC in 2,806 Japanese adults (16–92 years old) to examine the associations between GMC and 26 personal and lifestyle characteristics, including age, gender, BMI, dietary habits, and other habits.

The 2,806 gut microbial communities were divided into 8 clusters (1–8) based on their operational taxonomic units (OTUs). For all of the clusters, significant correlations were observed between the clusters and personal and lifestyle characteristics, as revealed by the chi-square test and odds ratio. Cluster 1, which was driven by *Clostridium*, correlated with "eat seaweed \geq 4 times/week". Cluster 2, which was driven by *Blautia* and *Clostridium* subcluster XIVa, correlated with BMI <25 kg/m². Cluster 3, which was driven by Coriobacteriaceae, correlated with age <60 years. Clusters 4, 5, and 6 were predominantly found in women (p < 0.05). Furthermore, clusters correlated with age \geq 60 years old and cluster correlated with age <60 years old and SMI 25 kg/m². Clusters 7 and 8 were correlated with opposite ends of the age spectrum, i.e., age \geq 60 and <60 years old, respectively. Interestingly, each cluster also had correlations with other personal and lifestyle characteristics besides gender, age, and BMI, such as defecation, frequency of eating vegetables, and smoking habits.





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In conclusion, we demonstrated multiple associations between GMC and personal and lifestyle characteristics. Our results suggest that these characteristics multidimensionally shape the characteristics of GMC and indicate a potential use for GMC analysis in preventive medicine.

* Matsumoto M., Kibe R., Ohga T., Aiba Y., Sawaki E., Koga Y., and Benno Y.: Cerebral lowmolecular metabolites influenced by intestinal microbiota : a pilot study. Frontiers in Syst. Neurosci. 7: 9. doi: 10.3389/fnsys (2013)





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Is continuous cropping a problem? OR, is the problem of continuous cropping a problem?

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Agrarian societies (Agricultural societies) have existed in various parts of the world as far back as 10,000 years ago and continue to exist today since 'sapient' humans realized that crop cultivation is much more efficient than simple fruit picking. Our ancestral farmers tried to grow a single crop species on a field year after year. And then realized that the 'continuous cropping' result in serious decline of yield. After that, humans would probably have chosen 'fire–fallow cultivation'. As time goes by, and as the cropland decreases, humans start to use crop rotation and mixed cropping methods to prevent problem of 'continuous cropping'.

In the 21st century Korea, ginseng is cultivated as the most profitable crop per unit area. However, ginseng is also suffered from continuous cropping. Once it is cultivated, ginseng can not return to the same land for 10 years. So what is the key factor in causing damage to the ginseng when it is tried to cultivate same land? A large number of previous studies have been indicated that continuous cropping changes soil physiochemical parameters, enzymes and microorganism communities, causing "replant problem". In the case of ginseng, there were no significant changes in the physical or chemical properties of soil. Therefore, we were interested to prove that bad microbes causing ginseng roots rot is dominant in the soil after the ginseng cultivation.

The relative count of ginseng root rot fungus was investigated by sampling the "unhealthy" soil with serious ginseng root rot disease and the "healthy" soil without any disease from the near field. Surprisingly, the results of the experiment showed that there was no evidence that there were more "pathogens" in "diseased" soils. After repeating the same experiment, we had a fundamental question: what is the "ecological health" of the soil? To solve this question, the diversity and dominance of microorganisms, which are generally used as indicators of soil ecology, have been studied. To make a long story short, it has been shown that microbial diversity and abundance in "diseased by continuous cropping" soils are higher and even more stable (ie, healthy?) than "so called healthy" soils that ginseng has never been cultivated. As a result, there has been a renewed concern about whether the continuous cropping is a problem or the problem of continuous cropping is a problem. Through this presentation, I would like to share and discuss the concern with you from agricultural, environmental and economical points of view.





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Metabolomics applied for the quality control of herbal products

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Recently, metabolomics approaches have been used to assess the metabolite contents of individual herbal products. The metabolic composition of herbal products differs depending on various factors such as cultivar, growing conditions, climate, soil type, and plant maturity. Metabolic profiling is defined as the identification and quantification of selected metabolites, and it can reflect the phenotype and quality of herbal products. Thus, many researchers have high interests in the profiling of not only primary metabolites but also secondary metabolites from herbal products and functional foods. For the detailed profiling of various metabolites, it is required to construct an effective analytical platform. Liquid chromatography coupled to mass spectrometry (LC-MS) has been widely used to analyze various metabolites from a complex mixture. In this study, we applied a metabolomics approach based on ultra-performance LC (UPLC) coupled to quadrupole time of flight (QTOF)/MS to the metabolic profiling of herbal products such as Panax ginseng, Platycodon grandiflorum, Cynanchum wilfordii, and Cynanchum auriculatum. P. ginseng is one of the most important herbal products which are widely used as a constituent of traditional medicine in Korea and other countries. In particular, ginsenosides from P. ginseng show various pharmacological properties such as anti-tumor, anti-diabetes, antioxidative, etc. Due to its utility, interest in the therapeutic potential of ginseng root has been growing. Moreover, it is economically critical to evaluate and control the quality of ginseng roots in the food industry and herbal markets. Here, we constructed the ginsenoside profiling method for the quality control for P. ginseng roots. Next, we constructed the platycosides profiling method for the quality control for Platycodi Radix (PR). Platycosides are the primary constituents of PR, having pharmacological activities including anti-oxidant, antiinflammatory, anti-obesity, and anti-cancer effects. Thus, the determination of platycosides is critical for the quality control and clinical use of PR. Finally, we applied the metabolomics approach to discriminate C. wilfordii (CW) and C. auriculatum (CA) appear morphologically similar. In Korea, it has been a critical issue to misuse these two roots in the herbal market and food industry. Thus, it is required to establish a robust tool for the discrimination and quality control of them. Here, we identified and validated the 14 biomarker candidates to discriminate CW and CA. These results indicated the utility of UPLC-QTOF/MS based metabolomics approaches for the quality control of herbal products.





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Usefulness of nano-sized bee pollen as a new natural resource for healthcare application

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Bee pollen is produced by honeybees and is considered one of the most balanced and nourishing nutritional supplements available. Historically, bee pollen has been prescribed for its healing properties and consumed for its high energy supply. Recent research has provided evidence that bee pollen has diverse biological activities, such as antioxidant, anti-inflammatory, antibacterial and even anticancer effects. However, the outer membrane of the pollen grain, exine, is highly resistant to most acidic solutions, high pressure, and even digestive enzymes, and the resulting low bioavailability limits its nutritional and clinical applications. This study applied a wet grinding method to effectively destroy the exine, then examined the pollen's potential as a new resource for healthcare products. First, microscopic observations provided strong evidence that wet grinding destroyed the exine time-dependently. In addition, the content of polyphenols, well-known ingredients of bee pollen and used as internal standards for the quality control of commercial pollen preparations, increased up to 11-fold with wet grinding. Further, the antioxidant activity demonstrated on the ABTS antioxidant assay as well as the DPPH radical scavenging assay was also dramatically increased. The enhanced biological activity of nano-sized bee pollen led us to investigate its potential as a new resource for cosmetics and health supplements. To this end, we prepared cosmetics containing nano-sized bee pollen and in clinical study we identified its beneficial effects on skin roughness, hydration, tragnsparency, wrinkles around the eyes and the melanin index. Together, the results presented here support nano-sized bee pollen as a new resource for medical, nutritional and cosmetic applications.

