#### International Institute of Rare Sugar Research and Education

#### https://www.kagawa-u.ac.jp/IIRSRE/en/index.html

The world leading "Rare Sugar Research" of Kagawa University strengthens a rare sugar production technology, and promotes a study on developments of rare sugar uses for various aspects including foods for specified health, pharmaceutical products, agriculture materials, etc. We will implement these research results into practical use aiming at what society could provide widely for the improvement of the society. For this purpose, Kagawa University reorganized an existing "Rare Sugar Research Center" and maintained it followed by installation of "International Institute of Rare Sugar Research and Education" as a new organization to promote this plan in April, 2016.

#### **Research Outline**

#### Innovation of New Field in Life Science.

The 21st century is called the century of Biotechnology. Rapid development of researches with DNA and protein analyses has been showing a huge contribution to the global community. While the "macromolecules" of DNA and proteins are leading the science of the 21st century, Kagawa University focuses on a "micromolecule" of rare sugars, which has never been paid an attention for a long time. Small molecules such as monosaccharides had been considered as "nonfunctional" other than energy sources and less meaning in signal transduction pathways in living beings until our series of findings of unexpected functions and roles of rare sugars.

Under a background of these circumstances, Kagawa University strengthens an innovation of the new face in life sciences using rare sugars as a research tool. The figure below shows the abundance of monosaccharides of the natural world schematically. The large green parts are

existing monosaccharides in the natural world, and the small red circles indicate "rare sugars" with the definition of monosaccharides and their derivatives being rarely found in nature by the International Society of Rare Sugars. Headquarter of the International Society of Rare Sugar is located in Kagawa University, Kagawa, Japan.



Kagawa University pushes forward various types of rare sugar studies in cooperation with research institutions and companies in the world with our aim of an innovation of the new face in life sciences using rare sugars as a research tool.

#### **Rare Sugar Production**

Since rare sugars are defined as monosaccharides and their derivatives being rarely found in nature, production of rare sugars is the essential first step to start rare sugar studies. The things necessary for production of various kinds of rare sugar are affordable raw materials, production tools, and a blueprint design of the production.

The most affordable raw material is D-glucose, which is the most abundant monosaccharide exists on the earth and can easily be obtained by hydrolysis of starch. However, a new source of raw material for rare sugar production can be developed with a progress of research.

An enzyme is a basic tool for rare sugar production and various updated biotechnology has been adapted for this processes. Additional tool is sugar organic chemistry, and the combined technology of enzymology and organic chemistry gives a powerful tool on rare sugar production with many aspects.

A blueprint design of rare sugar production starts from D-glucose following multiple series of enzymatic reactions and is summarized as "Izumoring". The figure below shows carbon six Izumoring but the Izumoring is also able to expand for even carbon five or four as well.

#### Izumoring

Izumoring, a blueprint design of rare sugar production, describes enzymatic reactions to produce aldose (•), ketose (•), and sugar Izumoring



alcohol (•). All hexoses lined up as a point symmetry from the central star, and left side is D-form as well as L-form in the right side.

Izumoring navigates the production method of a target rare sugar. For example, a plan to produce D-allulose starts from D-glucose at the right top following two enzymatic reactions to D-fructose and the final product.

Important point of [zumoring] is that [zumoring] is not only a simple design of producing rare sugars but also a useful tool based on their structures in bundling of multiple information such as expansion of sugars to modified sugars, physical characters of respective rare sugars, and even functions of the sugars. Production of deoxy sugars at carbon one or six position can be introduced by deoxy Izumoring. Physical characters and physiological functions of respective sugars can also be added to physical Izumoring or physiological Izumoring, and there is always a possibility to analyze the information with the correlation of respective rare sugar structures.

#### **Application Development of Rare Sugars**

Because the study of rare sugars has just begun, application development is expected to grow more and rapidly. Kagawa University started a challenge for an innovation of new face in life sciences using "micromolecule" of rare sugars as a research tool, while "macromolecules" of DNA and proteins are dominant research subjects.

Discoveries of physiological functions of rare sugars to microbes, plants, animals, insects, human, etc., are directly connected to application developments for economic and societal benefits in a global community. We had provided a functional sweetener composition comprising non-caloric natural sweetener such as D-allulose, and a commercialization of this had already been initiated. Without being satisfied with this one example of success, we look forward to providing more fruitful products from our findings. Direction of basic researches aiming application development is expanding more and more including physical sciences or molecular tool development, etc. Research experiences for such an innovation also guide human development as educational aspects, and a field of teaching material development was placed in the division of Application Development in our Institute.

Rare sugar production and its application development are tightly connected, and both run as two wheels for progress of rare sugar studies.

#### Approaches in Rare Sugar Studies

Kagawa University aims to discover the research area using rare sugars as a new tool in current life sciences, in which DNA and protein analyses have been showing a major contribution. Direction of researches is now expanding wider academic fields, and Kagawa University would like to contribute with solid scientific approaches for rare sugar studies, which was born in Kagawa, in the International Institute of Rare Sugar Research and Education.

#### **Rare Sugar Production Station**

Rare Sugar Production Station is the central facility for researches on "production of rare sugars" which is a basis of various types of rare sugar studies in the International Institute of Rare Sugar Research and Education, Kagawa University.

Rare Sugar Production Station was built in July, 2006, and located in the Faculty of Agriculture Campus.



Dr. Yoshihara operates Jar fermenter



Sugar analyses with HPLC



Sugar separation with various types of chromatography



Full set of equipments



Sugar analysis facility



Rare Sugar Production Station



#### (1)Genome and Gene Resource Research Center

# Advanced research genome and gene resources of organisms in the field of medicine, agriculture and engineering

The Genome and Gene Resource Research Center promotes the analysis of huge information of genomes obtained by next-generation sequencing from higher plants and animals to microorganisms in the fields of medicine, agriculture and engineering. The purpose of this research center is to establish a research and educational foundation for genome analysis technology at the University, and furthermore, to form a regional bioinformatics center and contribute to the regional problems through genome analysis technology.



International Symposium

Members	Specialized fields
TAKAMURA, Takejiro	Horticultural breeding
BEPPU, Kenji	Pomology
KOSUGI, Yusuke	Postharvest Horticulture
NARUMi, Takako	Floriculture
AKIMITSU, Kazuya	Plant Pathology
ICHIMURA, Kazuya	Plant Functional Genomics and Plant immunity

#### About each research center

KYO, Masaharu	Plant Cellular and Developmental Biology
GOMI, Kenji	Plant Pathology
NOMURA, Mika	Molecular Plant Nutrition
KONISHI-SUGITA, Saeko	Molecular Breeding in Plant
MOCHIZUKI, Susumu	Rare sugar molecular biology
FUKUDOME, Mitsutaka	Molecular Plant Nutrition
MATSUZAWA, Tomohiko	Enzymology

# Research Area: Horticultural Science Research Specialization: Horticultural breeding Name: TAKAMURA, Takejiro

Keywords: Flower color, Flower pigment, inter-specific hybridization, polyploidy breeding

# **Recent Research**

# 1. Genetic control and improvement of flower-color expression in cyclamen

Although petal color of cyclamen was red, purple, white, and their neutral tint until the 1980s, the yellow-flowered cultivars and individuals with bluish-violet petals in cyclamen are available now. We have clarified the main factor causing the bluish-violet petals and inheritance of the yellow-flowered characteristics. We presently study the mechanism of cyclamen petals becoming bluish color, expression of anthocyanin and flavonoid synthesis-related gene in cyclamen, and so on.

# 2. Interspecific hybridization and polyploidy breeding in the genus *Cyclamen*

The genus *Cyclamen* contains more than 20 species. Almost all the species, except *C. persicum*, have not contributed to the breeding of horticultural cyclamen cultivars, whereas some *Cyclamen* species have useful characteristics. In our laboratory, interspecific hybridization by using such *Cyclamen* species is studied. Polyploidy breeding by using such species and interspecific hybrids is also researched.

# 3. Analyses of mechanisms of pigmentation and flower-color expression in some ornamental plants

Mechanisms of flower-color expression and pigmentation in some ornamental plants (e.g., Persian buttercup and glory lily) are studied. Effects of environmental factors on the flower-color expression and pigmentation in some ornamental plants are also researched.

# Publications

Takamura, T. : Cyclamen, Flower Breeding & Genetics: Issues, challenges, and opportunities for the 21st century (N. O. Anderson ed.), 459-478, Springer-Verlag, Dordrecht (2006).

Takamura, T. *et al.*: Ploidy levels of degenerated embryos in the crosses between diploid and tetraploid cyclamen, Acta Horticulturae 855 ,261-266 (2010).

Takamura, T. *et al.*: Effects of  $CO_2$  enrichment on in vitro plant regeneration through somatic embryogenesis in cyclamen (*Cyclamen persicum* Mill.) Technical Bulletin of Faculty of Agriculture, Kagawa University 62, 1-4 (2010). Takamura, T. *et al.*: Effect of carbon source on *in vitro* plant regeneration in anther culture of cyclamen (*Cyclamen persicum* Mill.). Acta Horticulturae 923: 129-134 (2011).









Research Area: Horticulture Research Specialization: Pomology Name: BEPPU, Kenji

#### Keywords: peach, plum, cherry, reproductive physiology, breeding

#### **Recent Research**

#### 1. Breeding and utilization of lower-chilling peach

Forcing culture of peach under plastics is intended to ensure early shipping of production aimed at obtaining a higher market value. Usually, peach growers start forcing after attaining 900 chilling hours required by the high-chill cultivars. For achieving earlier forcing, lower-chilling peach with a high tasting quality would be necessary. Therefore, we bred a new cultivar named 'KU-PP1' and 'KU-PP2' that contained these traits by crossing Japanese high-chill and introduced low-chill cultivars. Currently we investigate effective methods for forcing culture with these cultivars.

#### 2. Breeding of self-compatible Japanese plum

Most commercial cultivars of Japanese plum are self-incompatible and cross-compatible cultivars need to be interplanted. Thus, information about *S*-haplotypes of each cultivar is important. We demonstrated that the *S*-RNase genes of Japanese plum could be amplified effectively by *S*-RNase gene-specific primers, and identified 14 different *S*-RNase genes. In addition, we revealed the involvement of the *S*<sup>e</sup>-haplotype in self-compatibility in Japanese plum and developed the *S*<sup>e</sup>-RNase gene-specific primer. Currently, we are breeding self-compatible Japanese plum cultivars with good fruit quality.

#### 3. Regulation of fruit set of sweet cherry in warm region

Poor fruit set due to physiological fruit drop have become serious problems preventing stable production of sweet cherry in warm regions. Under controlled conditions, we demonstrated that the fruit set rate decreased markedly due to the rapid degeneration of the embryo sacs when the trees were exposed to high temperatures above  $20 \,^{\circ}$ C, and that gibberellin may be involved in this phenomenon. We also found that extremely high temperature in summer reduced photosynthesis and cause early defoliation, which result in low fruit set rate in the following spring. We have developed several methods to improve fruit set in warm regions.







- Beppu, K. and Kataoka, I. (2016) Registration of new plant varieties, Peach, 'KU-PP2' (*Prunus persica* (L.) Batsch), Registration number: 24983.
- Beppu, K. et al. (2014) Examination of time of heating in forcing culture of lower-chilling peach selection HKH×FLP3, Acta Hort. 1059, 201-204.
- Beppu, K. et al. (2012) Molecular and genetic analyses of the S-haplotype of the self-compatible Japanese plum (*Prunus salicina* Lindl.) 'Methley', J. Hort. Sci. Biotech. 87, 493-498.
- Beppu, K. and Kataoka, I. (2011) Studies on pistil doubling and fruit set of sweet cherry in warm climate, J. Jap. Soc. Hort. Sci. 80, 1-13.

Research Area: Horticultural Science Research Specialization: Postharvest Horticulture Name: KOSUGI, Yusuke

Keywords: postharvest quality, flower longevity, senescence, ethylene (as a plant hormone), gene expression

#### **Recent Research**

Most of horticultural produce (fruits, vegetables and do ornamentals) easily deteriorate after harvest and lose their commercial value. We conduct basic studies that focus on the mechanisms inducing the postharvest deterioration. We mainly work on the flower senescence in ornamental plants including cut flowers.

# 1. Substances that improve vase-life of cut flowers

The plant hormone ethylene play a crucial role in inducing flower senescence in many ornamental plants. Carnation (*Dianthus caryophyllus* L.) is one of the major cut flower items, and the flowers show ethylene-dependent senescence. We examine the effect of new substances as inhibitors of ethylene biosynthesis, and evaluate them as potential preservatives for the cut flowers.

#### 2. Identification of genes responsible for flower longevity

Petal wilting is one of key events determining flower longevity in ornamental plants. To understand the molecular basis governing petal wilting, we isolate and analyze the genes associated with the process in carnation and balloon flower (*Platycodon grandiflorus* (Jacq.) A. DC.), both of which show flower senescence with their petals (corollas) wilting severely.

#### Publications

• 2-Aminooxyisobutyric acid inhibits the *in vitro* activities of both 1-aminocyclopropane-1-carboxylate (ACC) synthase and ACC oxidase in ethylene biosynthetic pathway and prolongs vase life of cut carnation flowers, Journal of Plant Biology, **57**, 218-224, 2014, **Kosugi, Y**., Matsuoka, A., Higashi, A., Toyohara, N., Satoh, S.

• 2,4-Pyridinedicarboxylic acid prolongs the vase life of cut flowers of spray carnations, Japanese Society for Horticultural Science, **83**,72–80, Satoh, S., **Kosugi, Y.**, Sugiyama, S., Ohira, I.

• *Escherichia coli*-based expression and *in vitro* activity assay of 1-aminocyclopropane-1-carboxylate (ACC) synthase and ACC oxidase. *In* Ethylene Signaling: Methods and Protocols. Edited by Binder, B.M. and Schaller, G.E. pp.47-58, 2017, Humana Press, Satoh, S. and **Kosugi,Y.** 







# Research Area: Horticultural Science Research Specialization: Floriculture Name: NARUMi, Takako

Keywords:ornamental plant, petal epidermal cell, floral morphogenesis, flowering

### **Recent Research**

#### 1. Identification of novel petal epidermal cell-related gene

Flower color is determined not only pigments but also the shape of petal epidermal cells, which influence the tone and texture of petals owing to the different reflection and refraction of light. We revealed that the known petal epidermal cell-related genes are not main factor though those have been identified in snapdragon and torenia. Therefore, we have performed identification of novel petal epidermal cell-related gene by RNA-seq analysis of torenia.

# 2. The effects of temperature on flowering

The recent high temperature during the summer and fall months has known to cause flowering delay in marguerite. We study about flowering characteristics in marguerite of a specialty of Kagawa Prefecture through flowering response by light or temperature control and flowering-related gene analysis.

#### 3. The others

- Elucidation of regulation mechanisms of floral zygomorphy in torenia
- Molecular mechanisms of flower color mutation derived from bud mutation of carnation

#### Publications

- Narumi, T. et al. (2007) Chimeric *AGAMOUS* repressor induces serrated petal phenotype in *Torenia Fournieri* similar to that induced by cytokinin application. Plant Biotechnology, 25: 45-54.
- Narumi, T. et al. (2011) *Arabidopsis* chimeric TCP3 repressor produces novel floral traits in *Torenia fournieri* and *Chrysanthemum morifolium*, Plant Biotechnology, 28: 131-140.
- Sasaki, K. et al. (2011) Utilization of a floral organ-expressing AP1 promoter for generation of new floral traits in *Torenia fournieri* Lind, Plant Biotechnology, 28: 181-188.
- Oda, A. et al. (2012) *CsFTL3*, a chrysanthemum *FLOWERING LOCUS T-like* gene, is a key regulator of photoperiodic flowering in chrysanthemums. J. Exp. Bot. 63: 1461-1477.
- Higuchi,Y. et al. (2013) The gated induction system of a systemic floral inhibitor, antiflorigen, determines obligate short-day flowering in chrysanthemums PNAS 110, 17137–17142,





TCP3-SRDX

WT

# Research Area: Plant Science Research Specialization: Plant Pathology Name: AKIMITSU, Kazuya

Keywords: Host-selective toxin, Rare sugar, Genome, Mitochondria, Citrus

#### **Recent Research**

#### 1. Biosynthesis gene cluster of Host-selective Toxin from Alternaria species

Host-selective toxins are second metabolic compounds with a low molecular weight, which are produced by several different pathotypes of *Alternaria* species. The host-range of toxin producer is equal to the host-selectivity of the toxin, and a loss of the toxin production leads a loss of pathogenicity. In our laboratory, we identified one dispensable chromosome carrying biosynthetic gene cluster for esters of 9,10-epoxy-8-hydroxy-9-methyl-decatrienoic acid (ACT-toxin) and another chromosome carrying that for polyketide (ACR-toxin). Target gene disruption and RNA silencing of the genes in the respective cluster caused a loss of the toxin production and pathogenicity, indicating the necessity of these genes in the toxin production as well as the pathogenicity.

# 2. Mechanism for appearance of plant mitochondrial disease cause by disfunction in modification of internal region of tRNA-Ala

ACRS gene encoding ACR-toxin receptor protein is located in the internal region of tRNA-Ala in mitochondrial genome, and the transcript of the region is processed in the toxin-insensitive cultivars of citrus. We examine the processing protein complex of the receptor gene transcript, and qualify the mechanism of host selectivity from both pathogen and host sides.

#### 3. Mechanism of rare sugar effects in plants

Rare sugars give effects on plants; such as a transient growth inhibition and induction of defense-related gene expression. The mechanism controlling these effects was identified and we are trying to use them for agricultural practices. *Itea* plant producing rare sugar is also examined on the processes of the sugar production as well as the evolutional role of rare sugar production.

HP http://www.ag.kagawa-u.ac.jp/plantpathology/index.html

#### References

- Endopolygalacturonase is essential for citrus black rot caused by *Alternaria citri* but not brown spot caused by *Alternaria alternata*(2001) A Isshiki, K Akimitsu, M Yamamoto, H Yamamoto, Molecular Plant-Microbe Interactions 14: 749-757.
- Host-selective toxins produced by the plant pathogenic fungus *Alternaria alternata* (2012) T Tsuge, Y Harimoto, K Akimitsu, K Ohtani, M Kodama, Y Akagi, M Egusa, ..., FEMS Microbiology Reviews 37: 44-66.
- Molecular, ecological and evolutionary approaches to understanding Alternaria diseases of citrus (2003) K Akimitsu, TL Peever, LW Timmer, Molecular Plant Pathology 4: 435-446.
- Sensitivity to *Alternaria alternata* toxin in citrus because of altered mitochondrial RNA processing (2002) K Ohtani, H Yamamoto, K Akimitsu, Proceedings of the National Academy of Sciences 99: 2439-2444.
- The rare sugar D-allose acts as a triggering molecule of rice defence via ROS generation (2013) A Kano, T Fukumoto, K Ohtani, A Yoshihara, T Ohara, S Tajima, K Izumori, . K Akimitsu, Journal of Experimental Botany 64: 4939-4951.







# Research Area: Plant Science Research Specialization: Plant Functional Genomics and Plant immunity Name: ICHIMURA, Kazuya

Keywords: Plant immunity, Environmental stress, signal transduction, *Ralstonia solanacearum*, effector, *Arabidopsis thaliana*, *Nicotiana benthamiana* 

# 1. Plant immunity signaling mediated by MAP kinase pathways 1.1 Autoimmunity phenotype of MEKK1 $\rightarrow$ MKK1 / MKK2 $\rightarrow$ MPK4 pathway mutants.

*Arabidopsis* MEKK1, a MAPKKK, comprises specific MAPK pathway with downstream MKK1/MKK2 (MAPKKs) and MPK4 (MAPK). This pathway is activated in the downstream of PRRs and proposed to be involved in a part of PTI signaling. In contrast, loss-of-function of the pathway results in severe dwarfism and constitutive ETI responses such as cell death and ROS accumulation. To elucidate complex mechanism underlying the phenotype, we use genetic and molecular biological approaches using *Arabidopsis thaliana* genome resources.

# 1.2 Ubiquitin ligase involved in regulation of MEKK1 $\rightarrow$ MKK1/MKK2 $\rightarrow$ MPK4 pathway

We identified a ubiquitin E3 ligase as a MEKK1-binding protein by yeast two-hybrid screening. Molecular biological analyses are in progress to clarify function of E3 ligase in immunity signaling through the MEKK1 $\rightarrow$  MKK1/MKK2  $\rightarrow$  MPK4 pathway

**1.3 Identification of novel pathway related to an innate immunity singling in** *Arabidopsis* According to the annotation, *Arabidopsis* genome contains over 80 MAPKKKs, 10 MAPKKs, and 20 MAPKs. This suggests that the MAP kinase pathways have to be composed of numerous combinations of protein kinases and that transduce various extracellular stimuli to activate physiological responses. We performed binding assay for the *Arabidopsis* MAP kinase pathway components towards identification of a novel signaling pathway, and we analyze possible pathway to find out its biological role.

**2.** *Ralstonia* functional effectomics towards identifying novel immunity signaling in plants *Ralstonia solanacearum* is the causative agent of bacterial wilt disease against more than 200 of plant species mainly *Solanaceae*. The broad host range of bacterial wilt is thought to be conferred by relatively large effector repertoire (> 70) to other pathogenic bacteria. Using the advantage of *Ralstonia* effector repertoire, we performed functional screening to find effectors suppress defense responses. With these effectors, we aim to identify a novel immunity signaling components in plants.

- Ichimura, K. *et al.*, SGT1 contributes to maintaining protein levels of MEK2<sup>DD</sup> to facilitate hypersensitive response-like cell death in *Nicotiana benthamiana*, *Physiological and Molecular Plant Pathology*, 94, 47-52, 2016.
- Yamada, K. et al., The Arabidopsis CERK1-associated kinase PBL27 connects chitin perception to MAPK activation, EMBO Journal, 35, 2468-2483, 2016.
- Nakamura, S. et al., Mitogen-Activated Protein Kinase Kinase 3 Regulates Seed Dormancy in Barley, Current Biology, 26, 775-781, 2016.
- Stegmann, M. et al. (2012) The ubiquitin ligase PUB22 targets a subunit of the exocyst complex required for PAMP-triggered responses in *Arabidopsis*. *Plant Cell*, 24, 4703-4716.
- Takahashi, F. et al. (2011) Calmodulin-dependent activation of MAP kinase for ROS homeostasis in *Arabidopsis*. *Molecular Cell*, 41, 649-660.







Keywords: immature pollen, cell culture, embryogenesis, totipotency

#### **Recent Research**

#### 1. Pollen embryogenesis

Since the first observation in 1964, embryogenesis of immature pollen through anther culture is well known as an important means to producing haploid useful in a high-efficient breeding method. However, with the exception of a limited number of species, the frequency of the phenomenon is generally low and the breeding method is not popularized. To examine the process from immature pollen to totipotent embryogenic cells we developed a pollen culture system [1,5], where freshly isolated tobacco pollen grains at midbicellular stage (Panel1) dedifferentiate showing a specific aspect (Panel2) in 2 days under a starveling condition. Their totipotency could be verified through the subsequent culture (Panel3,A,B,C). Previously, we found various events highly associated with the dedifferentiation. for example, appearance of specific phosphorylated proteins [4], specific gene transcripts [3] and activation of specific promoters [2]. Recently, we revealed that the isolated pollen vegetative cells proceed to S phase within 24 h as the first step towards embryogenesis, deviating from G1 arrest bound to maturation [1]. We hope these observations will be integrated to understand the induction mechanism of androgenesis.

#### 2. Regeneration-promoting genes

It is a key subject in plant biotechnology to find "regenerationpromoting genes" because such genes are useful for producing transgenic plants more efficiently, especially in crops recalcitrant for traditional regeneration protocols in vitro using cytokinin. Recently, we found co-expression of specific two genes promotes the regeneration competency in tobacco leaf segment culture without cytokinin. We are working on transformation of some recalcitrant crops using an expression vector harboring the two genes.

#### References

- <sup>1)</sup> Kyo, et al. (2014) Timing of the G1/S transition in tobacco pollen vegetative cells as a primary step towards androgenesis in vitro. Plant Cell Reports 33:1595-1606
- <sup>2)</sup> Yamaji and Kyo (2006) Two promoters conferring active gene expression in vegetative nuclei of tobacco immature pollen undergoing embryogenic dedifferentiation. Plant Cell Reports 25:749-757
- <sup>3)</sup> Kyo, et al.(2003) Cloning and characterization of cDNAs associated with embryogenic dedifferentiation of tobacco immature pollen grains. Plant Science 164:1057-1066
- <sup>4)</sup> Kyo and Harada (1990) Specific phosphoproteins in the initial period of tobacco pollen embryogenesis. Planta :182:53-63
- <sup>5)</sup> Kyo and Harada (1986) Control of the developmental pathway of tobacco pollen in vitro. Planta 168: 427-432





Research Area: Plant Science Research Specialization: Plant Pathology Name: GOMI, Kenji

#### Keywords: Jasmonate, Plant volatiles, Plant hormone, rice

#### **Recent Research**

#### 1. Role of plant volatiles in plant defense response

Terpenoids such as monoterpenes and sesquiterpenes are one of the most common volatiles emitted from plants in response to herbivory. Some terpenoids are emitted in response to treatment with abiotic or biotic elicitors and to fungal. However, there is a dearth of research about the role of individual volatiles in plant defense. We investigate roles of each volatile in plant defense response.

# 2. Mechanism of jasmonate siganlings in defense response

The plant hormone jasmonic acid (JA) or its derivative, an amino acid conjugate of JA, is a plant signaling compound involved in the regulation of plant defense and development. There are many studies about jasmonate signalings in dicot plants. However, there is a dearth of research about the role of jasmonte signalings in monocot plants including rice, which is one of the most important crops worldwide. We investigate mechanisms of jasmonate signalings in rice defense responses.

#### 3. Crosstalk between plant hormones

Jasmonic acid (JA) and salicylic acid (SA) play important roles in plant defense systems. JA and SA signaling pathways interact antagonistically in dicotyledonous plants, but, the status of crosstalk between JA and SA signaling is unknown in monocots. We investigate relationship between JA and SA in rice defense responses.

# Publications

- •Overexpression of OsMYC2 results in the upregulation of early JA-responsive genes and bacterial blight resistance in rice. Uji et al., *Plant and Cell Physiology* 57, 1814-1827, 2016.
- Jasmonate-induction of the monoterpene linalool confers resistance to rice bacterial blight and its biosynthesis is regulated by JAZ protein in rice. Taniguchi et al., *Plant Cell Environment* 37: 451-461. 2014.
- Isolation of jasmonate-induced sesquiterpene synthase of rice: product of which has an antifungal activity against *Magnaporthe oryzae*. Taniguchi et al., *Journal of Plant Physiology* 171, 625-632. 2014.
- Jasmonic acid and salicylic acid activate a common defense system in rice. Daisuke Tamaoki, et al., *Plant Signaling Behavior* 8, e24260, 2013.
- Involvement of OsJAZ8 in jasmonate-induced resistance to bacterial blight in rice. Shoko Yamada, , et al., *Plant and Cell Physiology* 53, 2060-2072, 2012.



Defense responses







Research Area: Plant Science Research Specialization: Molecular Plant Nutrition Name: NOMURA, Mika

Keywords:symbiosis, legume, nodule, nitrogen fixation

#### **Recent Research**

#### 1. Membrane fusion by SNARE in Lotus japonicus nodule

Legume produces nodules when rhizobia infect with root and fixes atmospheric nitrogen to produce ammonia. In the nodule these rhizobia are enclosed by plant derived differentiate into the symbiosome. organelles. and bacteroid.

We are studying the SNARE protein which has function for the nodule formation and could find one of the SNAREs which has important role to produce symbiosome in Lotus nodules. We are now analyzing for the SNARE complex proteins for the symbiosome membrane.

#### 2. Transcription factors for the nitrogen fixation

When rhizobia fix nitrogen in the nodule, legume has to provide carbon source. We have previously found that PEPC

which expresses in the nodule for the production of Phizobia is enclosed by symbiosome membrane derived from plant respiratory substrate, malate (Nomura et al, PCP 2006). However, there is no report of the transcription factors for the regulation of carbon metabolism in the nodule. We are now analyzing for the transcription factors for the regulation of nitrogen fixation.

#### 3. Analysis of ferritin for the nodule senescence

We have performed the transcriptome analysis for the nodule senescence and found that an iron accumulating protein, ferritin, accumulates in the aging nodule (Cungopast et al., Plant Biotech, 2014, J Plant Physiol, 2017). We speculates these iron would be transported to shoot. We are analyzing iron transporter in the nodule.

#### Publications

• Iron-induced nitric oxide leads to an increase in the expression of ferritin during the senescence of *Lotus japonicus* nodules, Chungopast, S., Duangkhet, M., Tajima, S., Ma, J.F., Nomura, M. J Plant Physiol. 208, 40-46 (2017)

M. J Plant Physiol., 171, 104-108 (2014)

Lotus japonicus (Left) and nodule (Right)

Rhizobia

Infectio





Transgenic plants by suppression of PEPC genes. Left: Plants were grown by the infection of M, loti (Left) and by addition of KNO3 (Right). C: control, aLjpc; transgenic plants by suppression of nodule Pepc gene

#### Click here for more information.



<sup>•</sup> A MYB-related transcription factor affects nodule formation in Lotus japonicus, Supriadi, Duangkhet, M., Thepsukhon, A., Widyastuti, R., Santosa, D.A., Tajima, S., Nomura, M. Plant Biotech. 33, 187-194 (2016)

<sup>•</sup> Transcriptomic profiles of nodule senescence in Lotus japonicus and Mesorhizobium loti symbiosis, Chungopast, S.,

Hirakawa, H., Sato, S., Handa, Y., Saito, K., Kawaguchi, M., Tajima, S., Nomura, M. Plant Biotech, 31, 345-349 (2014) · Glutamine synthetase I-deficiency in Mesorhizobium loti differentially affects nodule development and activity in Lotus japonicus, Chungopast, S., Thapanapongworakul, P., Matsuura, H., Dao, T.V., Asahi, T., Tada, K., Tajima, S., and Nomura,

# Research Area: Plant Science Research Specialization: Molecular Breeding in Plant Name: KONISHI-SUGITA Saeko

Keywords: rice, seed shattering, imaging, breeding

# **Recent Research**

# 1. Analysis of molecular mechanism about abscission layer formation and degradation in seed shattering in rice

Seed shattering is a trait of seed shedding from panicles (Fig.A). It is an important trait as a breeding strategy by the spread of seeds in nature. On the other hand, when considered as crops, strong seed shattering leads to a reduction in crop yield. Therefore, it is considered to be one of important agricultural traits even today. Our laboratory has isolated seed shattering gene *qSH1* in rice and it is clear that it is essential for abscission layer formation (Fig.B,C) In addition, a candidate gene downstream of *qSH1* was found by performing a site-specific microarray analysis using the laser microdissection method. Furthermore, we are searching for new genes by analyzing mutant strains using gamma ray irradiation.

#### 2. Characteristics extraction of leaves in rice using image analysis

In the early growth of plants, chemical energy obtained by photosynthesis is consumed to maintain life activities of plants by respiration and the like. The surplus is used for the formation and growth of new plants. Newly formed leaves will also photosynthesize and contribute to further individual growth. In this way, initial growth exhibits a complex positive feedback aspect. Therefore, slight differences in the natural environment during initial growth can lead to large differences in individual size. In our laboratory, we used a measurement system (Fig. D, E) for nondestructively monitoring plant growth (growth rate) of plants under natural environment. We are aiming at the production of high CO2-absorbing rice lines with rice as a crop material as an indicator of improvement of plant growth rate.

#### 3. Selection of rice variety for rice flour and challenge to application

In recent years the price of wheat has soared, the use of rice as rice flour, improvement of food selfsufficiency rate, effect of eliminating cultivation abandonment area is beginning to be expected. However, since wheat flour and rice flour have various components such as amylose content and protein content, it is essential to develop and use varieties corresponding to each application. Therefore, selection and characterization of rice varieties suitable for foods using rice flour are being conducted.

# **Publications**

Nao Tada, Katsuyuki Nii, Saeko Konishi-Sugita (2015) Mutant breeding of a Japanese traditional black rice cultivar 'Yayoi Murasaki' to improve seed shattering trait,The Nucleus, 58(3):217-223.

Konishi, S. *et al.* (2008) Inference of japonica rice domestication process from the distribution of six functional nucleotide polymorphisms of domestication-related genes in various landraces and modern cultivers. Plant & Cell Physiology, 49(9) 1283-1293.

Konishi, S. et al. (2006) An SNP caused loss of seed shattering during rice domestication. Science, 312, 1392-1396.











#### Research Area: Plant Science Research Field: Rare sugar molecular biology Name: MOCHIZUKI, Susumu

#### Keywords: Rare sugars, Genetic Functions, Plant biology, Microbiology, Plant-Microbe interactions

### **Recent Research**

1. Rare sugar production Several sugar converting enzymes were isolated at Kagawa University using the Izumoring strategy. These enzymes made it possible to produce many types of rare sugars, including Dallulose. Prompted by the growing industrial potential of rare sugars, the present study aims to select the best enzymes for production of rare sugars, and to improve their production efficiency by using genetic engineering methods.

2. Functional mechanisms of rare sugars These rare sugars can potentially be used for many applications, including in health food products, pharmaceutical products, and agricultural materials. However, the functional mechanisms of rare sugars have not yet been clarified. To gain a better understanding of how rare sugars act upon living cells, we are analyzing gene functions in sugar-treated plants and microbes.

**3. Plant-microbe interaction** Microorganisms have the ability to produce these sugars, and plants are able to store them. Many primary and secondary metabolism-related genes function in both plants and microbes during their interaction. To understand sugar and sugar-related metabolisms in more detail, we are analyzing the functions of these genes during interactions between plants and plant pathogenic fungi (*Magnaporthe* and *Alternaria*).

- Nishimura, T., Mochizuki, S. *et al.* (2016) *Magnaporthe oryzae* Glycine-Rich Secretion Protein, Rbf1 Critically Participates in Pathogenicity through the Focal Formation of the Biotrophic Interfacial Complex, *PLOS Pathogens*, 12(10), e1005921.
- Mochizuki, S. et al. (2015) Live-cell imaging of rice cytological changes reveals the importance of host vacuole maintenance for biotrophic invasion by blast fungus, *Magnaporthe oryzae*, *MicrobiologyOpen*, 4(6), 952-966.
- Mochizuki, S. *et al.* (2014) Ubiquitin ligase EL5 maintains the viability of root meristems by influencing cytokinin-mediated nitrogen effects in rice, *Journal of Experimental Botany*, 65(9), 2307-2318.
- ◆ Kouzai, Y. *et al.* (2014) Targeted Gene Disruption of *OsCERK1* Reveals Its Indispensable Role in Chitin Perception and Involvement in the Peptidoglycan Response and Immunity in Rice, *Molecular Plant-Microbe Interactions*, 27(9), 975-982.
- ◆ Sakai, T., Mochizuki, S. *et al.* (2012) The WAVY GROWTH 3 E3 ligase family controls the gravitropic response in Arabidopsis roots, *The Plant Journal*, 70, 303-314.
- Mochizuki, S. *et al.* (2011) Localization of probe-accessible chitin and characterization of genes encoding chitin-binding domains during rice–*Magnaporthe oryzae* interactions, *Journal of General Plant Pathology*, 77, 163-173.









Research Area: Plant Science Research Specialization: Molecular Plant Nutrition Name: FUKUDOME, Mitsutaka

Keyward: root nodule symbiosis, plant hemoglobin, reactive molecules

#### **Recent Research**

#### 1. Functions and roles of plant hemoglobin

Legumes can use atmospheric nitrogen molecules as nitrogen nutrition through symbiotic nitrogen fixation with rhizobia. This symbiosis is maintained successfully by leghemoglobin (Lb), which regulates the oxygen concentration in the nodules. In this laboratory, we are focusing on various plant hemoglobins (Phytogb) including Lb, and are working to elucidate the functions of various Phytogb. We also aim to clarify the role of nitric oxide scavenging activity of many Phytogb in plant-microbe interactions and environmental responses.

#### 2. Role and production mechanism of reactive sulfur species in symbiosis

In various processes of root nodule symbiosis, reactive molecular species such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) function as signaling molecules. Recently, it has been shown that reactive sulfur species (RSS), which are known to interact with ROS and RNS, can also be detected during the symbiosis process. In this laboratory, we are investigating the effects of RSS molecules on root nodule symbiosis. We are also analyzing which pathways and how RSS molecules are produced by host plants and symbiotic rhizobia.

#### 3. Agricultural application and search for novel symbiosis-related genes

Our laboratory aims to develop agricultural applications such as symbiosis enhancement and stress tolerance by controlling the expression and activity of factors that are deeply involved in root nodule symbiosis and environmental stress. We are also attempting to find new factors involved in symbiosis.

# Publications

• Nitric oxide detoxification by Mesorhizobium loti affects root nodule symbiosis with Lotus japonicus. Fukudome, M et al., Microbes Environ., 36(3): ME21038, (2021)

• Reactive sulfur species interact with other signal molecules in root nodule symbiosis in *Lotus japonicus*. Fukudome, M *et al.*, *Antioxidants*, 9(2): 145, (2020)

• Ectopic or over-expression of class 1 phytoglobin genes confers flooding tolerance to the root nodules of *Lotus japonicus* by scavenging nitric oxide. Fukudome, M *et al.*, *Antioxidants*, 8(7): 206, (2019)

• Stably transformed *Lotus japonicus* plants overexpressing phytoglobin LjGlb1-1 show decreased nitric oxide levels in roots and nodules as well as delayed nodule senescence. Fukudome, M *et al.*, *Plant Cell Physiol.*, 60(4): 816-825, (2019)

• Hemoglobin LjGlb1-1 is involved in nodulation and regulates the level of nitric oxide in the *Lotus japonicus–Mesorhizobium loti* symbiosis. Fukudome, M *et al., J. Exp. Bot.*, 67(17): 5275-5283, (2016)



RSS-specific fluorescence can be detected in infection

cells in the root nodule (yellow arrow).

Recombinant Phytogb



Section of root nodule

**RSS-specific fluorescence** 

Nodule Color

Bright field



#### Key ward: Enzyme, Sugar, Fungi, Yeast, Metagenome

# **Recent Research**

# 1. Plant Polysaccharides Degradation-Related Enzymes

Plants produce many kinds of polysaccharides and microorganisms produce plant polysaccharides degradation-related enzymes to assimilate plant polysaccharides. Each enzyme has distinct functions in the degradation, and cooperative actions of these enzymes are crucial to degrade plant polysaccharides.

Our study focus on the identification and characterization of novel plant polysaccharides degradation-related enzymes from microorganisms, including fungi and metagenome.



# 2. Evolution of Enzymes

Random and site-direct mutagenesis can change enzymatic properties, such as thermal stability and substrate specificity. We use the mutagenesis techniques to improve the enzymatic activities and to elucidate the molecular mechanism of enzymatic reactions.

# **Publications**

Matsuzawa, T., *et al.* (2021) Enzymatic degradation of xyloglucans by *Aspergillus* species: a comparative view of this genus. *Appl. Microbiol. Biotechnol.* 105:2701–2711

Matsuzawa, T., et al. (2020) Identification and characterization of two xyloglucan-specific endo-1,4-glucanases in Aspergillus oryzae. Appl. Microbiol. Biotechnol. 104:8761–8773

Matsuzawa, T., *et al.* (2020) Identification and characterization of  $\alpha$ -xylosidase involved in xyloglucan degradation in *Aspergillus oryzae*. *Appl. Microbiol. Biotechnol.* 104:201–210

Matsuzawa, T., *et al.* (2019) Cooperation between  $\beta$ -galactosidase and an isoprimeverose-producing oligoxyloglucan hydrolase is key for xyloglucan degradation in *Aspergillus oryzae*. *FEBS J.* 286:3182–3193

Matsuzawa, T., *et al.* (2019) Crystal structure and substrate recognition mechanism of *Aspergillus oryzae* isoprimeverose-producing enzyme. *J. Struct. Biol.* 205:84–90

Matsuzawa, T., *et al.* (2016) Identification of the gene encoding isoprimeverose-producing oligoxyloglucan hydrolase in *Aspergillus oryzae. J. Biol. Chem.* 291:5080–5087

Matsuzawa, T., *et al.* (2016) Crystal structure and identification of a key amino acid for glucose tolerance, substrate specificity, and transglycosylation activity of metagenomic  $\beta$ -glucosidase Td2F2. *FEBS J.* 283:2340–2353

#### (2) Advanced Biochemistry and Chemical Biology Center Advanced research with a marge of biochemistry, chemistry and chemical biology

In Kagawa University Faculty of Agriculture, biochemistry research of life science, chemistry and chemical biology of natural products are in progress. In our center, we are providing research infrastructures such as NMR or X-ray crystal structure analysis apparatus for the promotion of these studies, as well as mutual supports among center members for research skills and technology. Our goal is to contribute to providing drug lead compounds, health functional materials, and agrichemicals by various joint researches for such as search of functional natural compounds, synthesis and mode action analyses, elucidation of drug target protein or gene expression analyses.



Members	Specialized fields
KATO, Hisashi	Plant Biochemistry
SATO, Masashi	Bioactive Natural Products Chemistry
SUZUKI, Toshisada	Biomass Chemistry
FUKADA, Kazuhiro	Biophysical Chemistry & Colloid Science
FURUMOTO, Toshio	Functional Phytochemistry
KONG , Lingbing	Rare sugar organic chemistry

#### About each research center

YANAGITA, Ryo C.	Chemical Biology
HANAKI, Yusuke	Organic and Bioregulation Chemistry
HARA, Yasumasa	Natural Products Chemistry
KIMURA, Yoshi	MicrobialPhysiology
SAKURABA, Haruhiko	Enzyme Engineering
SUEYOSHI, Noriyuki	Molecular and Cellular Biology
TANAKA, Naotaka	Applied Microbiology
TABUCHI , Mitsuaki	Applied Molecular Cell Biology
WATANABE, Akira	Microbial Biochemistry
KATO, Shiro	Rare Sugar Enzymology
SUGIYAMA, Yasunori	Animal cell biology
MATSUZAWA, Tomohiko	Enzymology

Research Area: Bioresource & Functional Chemistry **Research Specialization: Plant Biochemistry** Name: KATO Hisashi

Keywords : Allelopathy, Allelochemical, Tropical and subtropical plants



The negative impacts of commercial herbicide use on the environment make it desirable to diversify weed management options. Many investigations have been attempted to exploit allelopathy of plants for weed control purposes in a variety of agricultural settings, since allelopathy is regarded as the direct influence of an organic chemical released from one living plant on the growth and development of other plants.

We have been extensively studied with respect to plant allelopathy as part of a strategy for sustainable weed management. We have already found and isolated several allelopathic substances from various plant sources and determined their chemical structures. We also try to understand the mode of action of the allelopathic substances for their biological activities by biochemical techniques such as protein, enzyme and gene levels.







barnyard grass



One of our students got the best oral presentation award on International Conference.

- $\geq$ Kato-Noguchi, H., Nakamura, K., Ohno, O., Suenaga, K., and Okuda, N.: Asparagus decline: autotoxicity and autotoxic compounds in asparagus rhizomes. Journal of Plant Physiology 213: 23–29 (2017).
- Kato-Noguchi, H.: Allelopathic chemical interaction of bryophytes with vascular plants. Mini-Reviews in Organic  $\geq$ Chemistry 13: 422 - 429 (2016).
- Kato-Noguchi, H., Saito, Y., Ohno, O. and Suenaga, K.: A phytotoxic active substance in the decomposing litter of the  $\geq$ fern Gleichenia japonica. Journal of Plant Physiology 176: 55-60 (2015).
- Kato-Noguchi, H., Salam, M.A., Ohno, O. and Suenaga, K.: Nimbolide B and nimbic acid B, phytotoxic substances in  $\geq$ neem leaves with allelopathic activity. Molecules 19: 6929-6940 (2014).
- $\geq$ Kato-Noguchi, H., Ai Kobayashi, A., Ohno, O., Kimura, F., Fujii, Y. and Suenaga, K.: Phytotoxic substances with allelopathic activity may be central to the strong invasive potential of Brachiaria brizantha. Journal of Plant Physiology 171: 525-530 (2014).
- $\geq$ Kato-Noguchi, H. and Peters, R.J.: The role of momilactones in rice allelopathy. Journal of Chemical Ecology 39:175-185 (2013).



Research Area: **Biological Molecular Chemistry** Research Specialization: **Bioactive Natural Products Chemistry** Name: **SATO, Masashi** 

Keywords: Biologically active substance, C. elegans, Rare sugar, Anti-aging

#### **Recent Research**

#### 1. Exploration of anti-aging rare sugars

Restriction of calorie intake is known to prolong the life span of various experimental animals. Even in humans, calorie restriction retards the onset of age-related diseases such as diabetes and cancer, and as a result it is thought to prolong life span. We thought that rare sugars having a metabolic suppression effect would make the body calorie restricted and prolong the life of the animal. We conducted the research using the nematode *Caenorhabditis elegans*, a model animal of aging research, and have reported that the rare sugar D-allulose (a stereoisomer of D-fructose) extended the lifespan of *C. elegans*. Currently, we are working on searching for novel anti-aging substances from over 50 kinds of rare sugars and their derivatives, and are developing screening methods to find active substances conveniently and quickly.

#### 2. Mechanism of the anti-aging effect of rare sugars

We are working on elucidating the mechanism of action of rare sugar anti-aging effect by biochemical, molecular biological techniques, and bio-organic chemical approaches such as derivatives synthesis.

• Sakoguchi, H. et al.: Screening of biologically active monosaccharides: growth inhibitory effects of D-allose, D-talose, and L-idose against the nematode *Caenorhabditis elegans*, Biosci. Biotechnol. Biochem., 80 1058-1061 (2016).



C. elegans







<sup>•</sup> Sato, M. et al.: Potential anthelmintic: D-psicose inhibits motility, growth and reproductive maturity of L1 larvae of *Caenorhabditis elegans*. J. Nat. Med., 62, 244-246, (2008).

<sup>•</sup> Sato, M. et al.: D-Ribose competitively reverses inhibition by D-psicose of Larval growth *in Caenorhabditis elegans*. Biological and Pharmaceutical Bulletin 32, 950-952 (2009).

<sup>•</sup> Sato, M., et al.: Structural characteristics for superoxide anion radical scavenging and productive activities of green tea polyphenols including proanthocyanidin dimers. Chemical and Pharmaceutical Bulletin 58, 98-102 (2010).

<sup>•</sup> Sakoguchi, H. et al.: Growth inhibitory effect of D-arabinose against the nematode *Caenorhabditis elegans*: Discovery of a novel bioactive monosaccharide, Bioorg. Med. Chem. Lett., 26 726-729 (2016).

Research Area: Biological Molecular Chemistry Research Specialization: Biomass Chemistry Name: SUZUKI, Toshisada

Keywords: Wood, Extractives, Bioactive substances, Biomass, Chemistry

#### **Resent research**

#### 1. Stereochemistry and Biosynthesis of lignans

Lignan exists to conifer and broadleaf tree heartwoods. It has the C6-C3-C3-C6 frame which consist of two phenyl propane unit (C6-C3) bounds. They are often exist as the glucoside in plants and shows optical activities. In the medical field, some of them are paid attention as bioactive substances. We investigate stereochemistry and biosynthesis of sesamin in sesame seeds, and lignans contained in *Eucommia ulmoides* and *Saururus chinensis*.

#### 2. Chemistry of bark compounds

Suberin is one of the main components in outer bark of woody plants, wound surface of higher plants and periderm of underground organs. The plant biopolymer provides resistance against water loss, and is proposed to be a defensive barrier against invasion by bacteria, fungi and insect. The structure of suberin is expected to be composed of an aliphatic domain and an aromatic domain. While the structure and composition of the aliphatic domain are well investigated, those of the aromatic domain are unclear. We investigate the chemical structure and biosynthesis of the aromatic domain of suberin in outer bark of woody plants.

#### 3. Bioactive substances of tropical trees

In tropical and subtropical regions, wide variety of plants are valuable genetic resources to provide bioactive constituents. Our current studies on isolation and identification of extractives of some Indonesian trees and their biological activity have been conducted in collaboration with some Indonesian researchers.

#### 4. Fast-growing trees in Indonesia

In Indonesia, fast-growing trees such as falcata, acacia and eucalyptus have been planted for production of wood-based materials, pulp and paper. We investigate on effective utilization of waste wood and bark of the fast-growing trees.

#### Publications

Suzuki, *et al.*: Antioxidative catechol lignans/neolignans isolated from defatted kernel of *Jatropha curcas*. *Journal of Wood Science* 62, 339-348 (2016)

Suzuki, et al.: Extractives from Spanish Cherry and their Antioxidant Activity. Journal of the Forest Biomass Utilization Society 9, 57-60 (2014)

Suzuki, *et al.*: Furanoditerpenes from *Arcangelisia flava* (L.) Merr. and their antifungal activity. *Phytochemistry Letters* 4, 333-336 (2011)











# Research Area: Biological Molecular Chemistry Research Speciality: Biophysical Chemistry & Colloid Science Name: FUKADA, Kazuhiro

Key words: surfactant, emulsion, bio-colloids, phase transition, rare sugar

#### **Recent Research**

#### 1. Protein-based O/W emulsions

The oil-in-water (O/W) emulsions are aqueous liquids containing dispersed small oil droplets (usually < 10  $\mu$ m diameter). A large variety of industrial products such as processed-foods, medicines, pesticides, and cosmetics are produced as O/W emulsions. To prepare fairly stable emulsions, it is crucial to add some amphiphilic material as an emulsifier. We are studying on emulsifying properties of proteins (whey or egg white proteins, casein, and so on) to understand formation and stabilization mechanisms of small oil droplets in protein solutions by the emulsification processes.

#### 2. Molecular assemblies of aqueous amphiphiles

When amphiphilic materials such as surfactants or phospholipids are mixed with water, they spontaneously form molecular assemblies, i.e., micelles or multi-lamellar vesicles, both of which are classified as associate colloidal system. In our laboratory, physico-chemical aspects on these colloids are studied focusing on the geometry of the molecular assemblies and effects of temperature and additives (salts, alcohols, and sugars) for the formation of assemblies.

#### 3. Physico-chemical properties of rare sugars

Rare sugars are monosaccharides rarely existing in nature. Some of rare sugars, for example D-allulose, are recently found to have biological activities with benefit, and broad range of studies including molecular structure and rheological, spectroscopic, and thermodynamic properties in solution have been in progress. We are studying on basic phsico-chemical properties of rare hexoses to understand why living organisms did not select rare hexoses, but Dglucose and D-fructose, in their metabolisms.









# **Click here for more information**

- A. Yoshihara, M. Sato, K. Fukada. Evaluation of the Equilibrium Content of Tautomers of Deoxy-ketohexoses and their Molar Absorption Coefficient of the Carbonyl Group in Aqueous Solution. *Chem. Lett.*, **45**, 113-115 (2016).
- T. Kozakai, K. Fukada, R. Kuwatori, T. Ishii, T. Senoo, K. Izumori. Aqueous Phase Behavior of the Rare Monosaccharide D-Allose and X-ray Crystallographic Analysis of D-Allose Dihydrate. *Bull. Chem. Soc. Jpn.*, **88**, 465-470 (2015).
- K. Fukada. Effects of Addition of Amphiphilic Molecules on the Dispersion Stability of Colloidal Systems. *Oleoscience*, **16**, 51-56 (2016). (in Japanese)
- N. Cheetangdee, K. Fukada, Emulsifying activity of bovine  $\beta$ -lactoglobulin conjugated with hexoses through the Maillard reaction. *Colloids Surfaces, A*, **450**, 148-155 (2014).



Key words: natural product chemistry, plant, chemical structure, biosynthesis, bioactivity

- Recent research
- 1. Secondary metabolites produced by plants
- Plants possess the ability to produce a variety of secondary metabolites (natural products). Our laboratory has carried out the isolation, structure determination, quantitative analysis, research for biosynthetic pathway and production mechanism, etc., about several plat secondary metabolites.
- For example, sesame seeds have been considered a valuable health food, and it is assumed that black seeds are more effective as health food than white seeds in Asia. Assay-guided fractionation of the extract from black seeds led to the isolation of a characteristic antioxidant that had a potent radical scavenging activity. Quinones are characterized by two carbonyl groups on a fully unsaturated ring and have various biological activities and wide utility. We isolated and elucidated the structures of many naphthoquinone and anthraquinone derivatives in *Sesamum indicum* roots and hairy roots. Moreover, the biosynthetic relationship among these quinone derivatives is under investigation.

#### • 2. Plant growth regulators in rhizoshere

 Plants produce and accumulate many organic compound in the roots, and the substances secreted or released from the roots affect other living organisms. We recently isolated a novel germination stimulant for the seeds of root parasitic weeds from sunflower root exudates.





#### Publications

- Furumoto, T. et al. (2003). Anthrasesamones from roots of Sesamum indicum. Phytochemistry 64: 863-866.
- Furumoto, T. et al. (2011). Biosynthetic origin of 2-geranyl-1,4-naphthoquinone and its related anthraquinone in a *Sesamum indicum* hairy root culture. Phytochemistry 72: 871-874.
- Furumoto, T. et al. (2012). Effect of chloride ions on anthrasesamone C production in a *Sesamum indicum* hairy root culture and identification of the precursor for its abiotic formation. Biosci. Biotechnol. Biochem. 76: 305-308.
- Furumoto, T. et al. (2016). Identification of a characteristic antioxidant, anthrasesamone F, in black sesame seeds and its accumulation at different seed developmental stages. Biosci. Biotechnol. Biochem. 80: 350-355.

• Ueno, K. et al. (2014). Heliolactone, a non-sesquiterpene lactone germination stimulant for root parasitic weeds from sunflower. Phytochemistry 108: 122-128.

Research Area: Biological Molecular Chemistry Research Specialization: Rare sugar organic chemistry Name: KONG, Lingbing

Keywords: sugars, rare sugars, organic chemistry, sugar transporters

# **Recent Research**

# 1. Rare sugar production

We use organic chemistry to strengthen rare sugar production with a combinational technology of sugar enzymology and sugar chemistry

### 2. Functional analysis of sugar transporters

We analyse sugar transporters, especially those related to rare sugars,<sup>K3</sup> with single-molecule electrical channel recording to allow functional analysis and development of novel therapeutics.

#### sugars, K30 capsules hannel nalysis eutics. Wza <u>E. coli</u> Wza <u>Wza</u> <u>Wza</u> <u>Wza</u> <u>Wza</u> <u>Wza</u> <u>Wza</u> <u>Wza</u> <u>Wza</u> <u>Human complement</u>

# Publications

•A monodisperse transmembrane α-helical peptide barrel, *Nature Chemistry*, 9, 411-419, 2017, Kozhinjampara R. Mahendran, Ai Niitsu, **Lingbing Kong**, Andrew R. Thomson, Richard B. Sessions, Derek N. Woolfson and Hagan Bayley

•Chemical polyglycosylation and nanolitre detection system enables single-molecule recapitulation of bacterial sugar export, *Nature Chemistry*, 8, 461-469, 2016, **Lingbing Kong**, Andrew Almond, Hagan Bayley and Benjamin G. Davis

• An antibacterial vaccination strategy based on a glycoconjugate containing the core lipopolysacchride tetrasaccharide Hep<sub>2</sub>Kdo<sub>2</sub>, *Nature Chemistry*, 8, 242-249, 2016, **Lingbing Kong**, Balakumar Vijayakrishnan, Michael Kowarik, Jin Park, Alexandra N. Zakharova, Larissa Neiwert, Amirreza Faridmoayer and Benjamin G. Davis

·Single-molecule interrogation of a bacterial sugar transporter allows the discovery of an

extracellular inhibitor, *Nature Chemistry*, 5, 651-659, 2013, **Lingbing Kong**, Leon Harrington, Qiuhong Li, Stephen Cheley, Benjamin G. Davis and Hagan Bayley.





Research Area: Biological Molecular Chemistry Research Specialization: Chemical Biology Name: YANAGITA, Ryo C.

Keywords: Natural product, tumor promoter, analog synthesis

# **Recent Research**

1. Development of simplified analogs of naturally-occurring tumor promoters

Tumor promoters are compounds which enhance tumorigenesis in cells initiated by a carcinogen. Recently, design and synthesis of simplified analogs of naturallyoccurring tumor promoters with desirable therapeutic effects including anticancer effect have been reported.

We are currently studying on the development of simplified analogs of aplysiatoxin, a polyacetate isolated from sea hare, and thapsigargin, a sesquiterpene lactone isolated from Apiaceae plant, being synthetically accessible and having less side effect.

#### 2. Screening of new protein kinase C ligands with anti-proliferative activity

Protein kinase C (PKC) is a family of serine/threonine kinase that involves in many cellular function, and are attracted as potential therapeutic target for the treatment of cancer, Alzheimer's disease, and AIDS.

We are searching for new PKC ligands from plant sources by an assay using synthetic PKC C1 peptides.

# 3. Development of biologically active derivatives of naturally-rare monosaccharide

Several of naturally-occurring monosaccharides are found to exhibit biological activities including anti-cancer activity. We currently focus our attention on the development of rare monosaccharide derivatives with "unnatural" moiety to improve their cell permeability and biological activities.

# **Publications**

Ueno, S., *et al.* Identification and Biological Activities of Bryostatins from Japanese Bryozoan. *Biosci. Biotechnol. Biochem.* **2012**, *76*, 1041–1043.

Yanagita, R. C., *et al.* Effects of the methoxy group in the side chain of debromoaplysiatoxin on its tumor-promoting and anti-proliferative activities. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 4319–4323.

Yanagita, R. C., *et al.* Anti-proliferative activity of 6-*O*-acyl-D-allose against the human leukemia MOLT-4F cell line. *Biosci. Biotechnol. Biochem.* **2014**, 78, 190–194.

Ashida, Y., *et al.* Binding mode prediction of aplysiatoxin, a potent agonist of protein kinase C, through molecular simulation and structure–activity study on simplified analogs of the receptor-recognition domain. *Bioorg. Med. Chem.* **2016**, *24*, 4218–4227.





Research Area: Biological Molecular Chemistry Research Specialization: Organic and Bioregulation Chemistry Name: HANAKI, Yusuke

Keywords: Bioactive molecule, Migration inhibitor, Organic synthesis

#### **Recent Research**

#### 1. Establishment of a novel chemical library

Chemical libraries contribute to drug discoveries. We are collecting bioactive molecules from natural sources and synthesizing unnatural compounds to create a novel small molecule library.

# 2. Screening for migration/proliferation inhibitors

Migration of endometrial cells is a cause behind common diseases such as adenomyosis and endometriosis. We are working on developing a rapid screening method for migration/proliferation inhibitors as novel medicinal seeds.

# 3. Mechanisms of action of bioactive compounds

We are trying to elucidate the mode of action of isolated bioactive molecules by biochemical and cell biological approaches.

- Hanaki, Y. *et al.*, Structure–activity studies at position 27 of aplog-1, a simplified analog of debromoaplysiatoxin with anti-proliferative activity. *Tetrahedron*, **69** (36), 7636-7645 (2013).
- Hanaki, Y. *et al.*, Loss of the Phenolic Hydroxyl Group and Aromaticity from the Side Chain of Anti-Proliferative 10-Methyl-aplog-1, a Simplified Analog of Aplysiatoxin, Enhances Its Tumor-Promoting and Proinflammatory Activities. *Molecules*, **22** (4), 631 (2017).
- Hanaki, Y. *et al.*, Identification of protein kinase C isozymes involved in the anti-proliferative and pro-apoptotic activities of 10-Methyl-aplog-1, a simplified analog of debromoaplysiatoxin, in several cancer cell lines. *Biochem. Biophys. Res. Commun.*, **495** (1), 438-445 (2018).







Research Area: **Biological Molecular Chemistry** Research Specialization: **Natural Products Chemistry** Name: **HARA, Yasumasa** 

Key words: natural products, plant, microorganism, biological activity

# **Recent Research**

# 1. Establishment of natural resource collection

We collect natural resources such as plants and microorganisms, and establish natural resource collections and the extract collections. We search for new natural products from these collection.

# 2. Activation of microbial biosynthetic genes

Many of the biosynthetic genes involved in the production of natural products in microorganisms are cryptic or silent. Therefore, we conduct and develop various microbial culture methods that lead to the activation of these genes in order to obtain new natural products.

Recently, we suggested a new method to search for natural products by culturing microorganisms in the presence of animal cells.



# 3. Search for new natural products and bioactive natural products

The collected natural resources are screened by various biological activity tests and analysis by TLC and HPLC. We isolate new natural products and biologically active natural products from selected natural resources. Also, we try to elucidate the functions and estimate the biosynthetic genes of the isolated compounds.

- Hara, Y. *et al.* Dehydropropylpantothenamide isolated by a co-culture of *Nocardia tenerifensis* IFM 10554<sup>T</sup> in the presence of animal cells. *J. Nat. Med.* **72**, 280-289 (2018).
- Hara, Y. *et al.* Coculture of a pathogenic actinomycete and animal cells to produce nocarjamide, a cyclic nonapeptide with Wnt signal-activating effect. *Org. Lett.* **20**, 5831-5834 (2018).
- Hara, Y. *et al.* Acacienone, a terpenoid-like natural product having an unprecedented C20 framework isolated from *Acacia mangium* leaves. *J. Nat. Med.* **75**, 99-104 (2021).
- Hara, Y. et al. Isolation of ikahonone, 4-methyl-2,4-dihydroxy-3-pentanone from *Bacillus cereus* IFM12235. *Heterocycles* **105**, 523-531 (2022).
- Hara, Y. *et al.* Two bioactive compounds, uniformides A and B, isolated from a culture of *Nocardia uniformis* IFM0856<sup>T</sup> in the presence of animal cells. *Org. Lett.* 24, 5867 and 4998-5002 (2022).

### Research Area: Applied Life Science Research Specialization: Microbial Physiology Name: KIMURA, Yoshio

Keywords: Myxobacteria, signal transduction, ATP synthesis, Novel microbial resources

# **Recent Research**

#### 1. Signal molecules of Myxobacteria.

When environmental conditions become unfavorable, organisms produce signal molecules and can respond to changes in their environment.

Diadenosine polyphosphate (Ap<sub>n</sub>A; n=3-6) and polyphosphate are produced during amino acid-starvation. We try to identify and characterize key enzymes involved in these signal molecules

of synthesis and degradation. Further, we generate gene-broken

strains in which the enzyme genes are broken, and we will clarify the function of these enzymes and signal molecules.

On the other hand, we are revealing that these signal molecules are substrates for ATP synthesis enzymes. We now studying that ATP synthesis system using these signal molecules under amino acid-starvation.

# 2. Isolation of *Sorangium* from the natural world and Search for useful substances.

Gram-negative myxobacteria are an important source of novel classes of secondary metabolites. Of these, the genus *Sorangium* is particularly valuable. Therefore, we are isolating the genus *Sorangium* form soil.

Natural products are extracted from *Sorangium* cultures, and are provided for microbial growth inhibition experiments. In addition, the extracts are analyzed by LC-MS.

we are exploring useful substances from these cultures of Sorangium.

# **Publications**

- Kimura, Y. et al. (2017). High concentrations of intracellular Ap4A and/or Ap5A in developing *Myxococcus xanthus* cells inhibit sporulation. Microbiology 163:86-93.
- Oka, M. et al. (2016). Lysyl-tRNA synthetase from *Myxococcus xanthus* catalyzes the formation of diadenosine penta- and hexaphosphates from adenosine tetraphosphate. Archieves of Biochemistry and Biophysics. 604:152-158.
- Sasaki, M. et al. (2014). Enzymatic characteristics of an ApaH-like phosphatase, PrpA, and a diadenosine tetraphosphate hydrolase, ApaH, from *Myxococcus xanthus* enzymes. FEBS Letters 588:3395-3402.
- Kimura, Y. et al. (2012). Function analysis of a bacterial tyrosine kinase, BtkB, in *Myxococcus xanthus*. *FEMS Microbiological Letters* 336(1):45-51 (2012).
- Mori, Y. et al. (2012). PhpA, a tyrosine phosphatase of *Myxococcus xanthus*, is involved in the production of exopolysaccharide. *Microbiology*158(10):2546-2555.
- Kimura, Y. et al. (2011). A *Myxococcus xanthus* bacterial tyrosine kinase, BtkA, is required for the formation of mature spores. *Journal of Bacteriology* 193(20):5853-5857.





Fruiting bodies of Myxobacteria

Isolated the genus *Sorangium* 



# Research Area: Life Science & Biotechnology Research Specialization : Enzyme Engineering Name: SAKURABA, Haruhiko

Keywords: hyperthermophiles, archaea, thermostable enzyme, x-ray crystal structure analysis

### **Recent Research**

# 1. Structure-based creation of a novel D-amino acid dehydrogenase

*meso*-Diaminopimelate dehydrogenase (DAPDH) acts stereoselectively on the D-center of substrate. Thus, the enzyme is expected to be useful for one-step production of D-amino acids, which are often utilized as source materials for industrial production of medicines and agrochemicals. However, its high substrate specificity for DAP and instability have proved to be the major disadvantage for the practical application of DAPDH. Recently, we determined the crystal structure of thermostable DAPDH and estimated the amino acid residues related to substrate binding. We used site-directed mutagenesis to identify the residues responsible for determining the enzyme's substrate specificity and found that a single mutation (D94A) caused a striking change in the substrate specificity of the enzyme. The mutant exhibited extremely high activity for reductive amination of phenylpyruvate. This is a major advantage of using the D94A mutant for one-step synthesis of D-phenylalanine.

# 2. Unique coenzyme binding mode of hyperthermophilic dehydrogenase

Homoserine dehydrogenase (HseDH) is a key enzyme in the biosynthetic pathway for the synthesis of methionine, threonine, and isoleucine in plants and microorganisms. HseDH is thought to be a potential target for the structure-based design of antibiotics, as the enzyme is not present in mammals. We recently determined the crystal structure of HseDH from the hyperthermophile and found that this enzyme exhibits a new variation on cofactor binding to a dehydrogenase: very strong NADP binding that acts as an obstacle to NAD(P)-dependent dehydrogenase catalytic activity. More recently, we observed another variation on a structural basis for cofactor preference using hyper-thermophilic *sn*-glycerol-1-phosphate dehydrogenase. Our studies indicate that the molecular basis for the cofactor preference in NAD(P)-dependent dehydrogenases may have much more variation than expected.

# 

cover image

- Hayashi, J., *et al.* (2017) Structure-based engineering of an artificially generated NADP-dependent D-amino acid dehydrogenase. *Applied and Environmental Microbiology*, in press.
- Hayashi, J., *et al.* (2017) Crystal structure of the novel amino-acid racemase isoleucine 2-epimerase from *Lactobacillus buchneri*. *Acta Crystallographica*, D73, 428-437.
- Wakamatsu, T., *et al.* (2016) Structural insights into L-tryptophan dehydrogenase from a photoautotrophic cyanobacterium *Nostoc punctiforme*. *Applied and Environmental Microbiology*, in press.
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- Ohshida, T., *et al.* (2016) First characterization of extremely halophilic 2-deoxy-D-ribose-5-phosphate aldolase. *Protein Expression and Purification*, 126, 62-68.
- Hayashi, J., *et al.* (2015) Crystal structures of a hyperthermophilic archaeal homoserine dehydrogenase suggest a novel cofactor binding mode for oxidoreductases. *Scientific Reports*, 5, 11674.
- Sakuraba, H., *et al.* (2012) Crystal structure of novel dye-linked L-proline dehydrogenase from hyperthermophilic archaeon *Aeropyrum pernix*. *The Journal of Biological Chemistry*, 287, 20070-20080.



Research Area: Life Science & Biotechnology Research Specialization: Molecular and Cellular Biology Name: SUEYOSHI, Noriyuki

KeyWords: Protein Kinase, Protein Phosphatase, Zebrafish

#### **Recent Research**

# 1. Functions of protein kinases and phosphatases on zebrafish embryogenesis.

ontrol Protein kinases and phosphatases are known to play pivotal roles in various signaling pathways and to participate in diverse cellular processes including proliferation. development and differentiation. To investigate protein kinases and phosphatases involved in the developmental processes in zebrafish embryogenesis, we are performing functional gene knockdown experiments in zebrafish using antisense morpholino-modified oligonucleotides that is targeted to the 5'-noncoding sequences of various protein kinases and phosphatases.

#### 2. Regulation of PPM family protein phosphatases.

Ca<sup>2+</sup>/CaM-dependent protein kinase phosphatase (CaMKP) is a Ser/Thr protein phosphatase that belongs to the PPM family. In our laboratory, we focus on the regulation mechanisms of PPM family phosphatases, especially of CaMKP and CaMKP-N, a nuclear isoform of CaMKP.

- Senga and Akizuki *et al.* High-performance CaMKI: A highly active and stable form of CaMKIδ produced by high-level soluble expression in *Escherichia coli. Biochem. Biophys. Res. Commun.* 475, 277-282, 2016
- Onouchi *et al.* Regulation of Ca<sup>2+</sup>/calmodulin-dependent protein kinase phosphatase (CaMKP/PPM1F) by Protocadherin- $\gamma$ C5(Pcdh- $\gamma$ C5). *Arch. Biochem. Biophys.* 585, 109-120, 2015
- Senga *et al.* Expression and gene knockdown of zebrafish Ca<sup>2+</sup>/calmodulin-dependent protein kinase Iδ-LL. *Arch. Biochem. Biophys.* 540, 41-52, 2013
- Sueyoshi *et al.* Functional processing of nuclear Ca<sup>2+</sup>/calmodulin-dependent protein kinase phosphatase (CaMKP-N): evidence for a critical role of proteolytic processing in the regulation of its catalytic activity, subcellular localization and substrate targeting *in vivo. Arch. Biochem. Biophys.* 517, 43-52, 2012
- •Senga *et al.* Knockdown of two splice variants of Ca<sup>2+</sup>/calmodulin-dependent protein kinase Iδ causes developmental abnormalities in zebrafish, *Danio rerio. Arch. Biochem. Biophys.* 517, 71-82, 2012
- •Onouchi *et al.* Phosphorylation and activation of nuclear Ca<sup>2+</sup>/calmodulin-dependent protein kinase phosphatase (CaMKP-N/PPM1E) by Ca<sup>2+</sup>/calmodulin-dependent protein kinase I (CaMKI). *Biochem. Biophys. Res. Commun.* 422, 703-709, 2012
- Baba *et al.* Regulation of Ca<sup>2+</sup>/calmodulin-dependent protein kinase phosphatase (CaMKP) by oxidation/reduction at Cys-359. *Arch. Biochem. Biophys.* 526, 9-15, 2012





Research Area: Life Science & Biotechnology Research Specialization: Applied Microbiology Name: TANAKA, Naotaka

Keywords: fission yeast, Golgi apparatus, secretory pathway, agmatine, glycoprotein

# **Recent Research**

1. Transport control of glycoproteins by lectin-like protein

Secreted proteins and membrane proteins are entrapped in transport vesicles in the endoplasmic reticulum, passed through the Golgi apparatus, and transported to the cell surface and suitable organelles. It is known that glycosylation, which is one of posttranslational modifications, is essential for quality control of secreted proteins and packaging in appropriate vesicles. It has been clarified that lectin-like proteins are involved in protein sorting and transport by binding to specific sugar chains, but there are many unclear points about the mechanism that distinguishes cargo proteins and the localization mechanism of lectin-like proteins. We analyze the function of lectin - like protein by analyzing the phenotype indicated by the deficient strain of fission yeast lectin-like protein.

2. Analysis of the Golgi apparatus formation mechanism in fission yeast

The Golgi apparatus of fission yeast is scattered in the form of about 10 to 20 dots in the cell. There is a clear layer in each Golgi apparatus, but not only the layer formation mechanism, but also the mechanisms involved in determining the number of Golgi apparatus and position within the cell are not well understood. We focus on the peripheral proteins of the Golgi apparatus and analyze the relationship to the Golgi apparatus function.

3. Analysis of polyamine biosynthetic pathway via agmatine in fission yeast

Polyamines are involved in cell function in various organisms. Ornithine-mediated pathways are well known, but the physiological role of agmatine-mediated polyamine biosynthesis pathway is relatively unknown. We are trying to clarify the involvement in cell function by inhibiting the metabolic pathway of agmatine.



Click here for more information.

- Suzuki, S. et al. (2010) Characterization of two different types of UDP-glucose/-galactose 4-epimerase involved in galactosylation in fission yeast. Microbiology 156: 708–718.
- Tanaka, N., et al. (2001) Functional characterization of Gms1p/UDP-galactose transporter in *Schizosaccharomyces pombe*. Yeast 18: 745-757.



# Research Area: Applied Life Science Research Specialization: Applied Molecular Cell Biology Name: TABUCHI, Mitsuaki

KEYWORDs: Yeast, eisosome, Stress response, Pathogen effector

# 1. Plasma Membrane Stress Response in Yeast

Plasma membrane is constitutively damaged from various physical stresses such as heat stress, osmotic stress, membrane tension, which causes plasma membrane stresses. For example, in humans, myocytes are constantly damaged by exposure to physical membrane stress by repeating expansion and contraction as they move intensely. However, cells have a mechanism to repair such damage. In animal cells, it is known that a structure called "Caveolae" plays an essential role in repairing membrane damage, and caveolae abnormality is known to be a cause of cardiomyopaty.

Yeast is a unicellular eukaryote and has contributed to the elucidation f various intracellular mechanisms such as autophagy as a model eukaryotic cell. Mechanisms for sensing and repairing membrane stress are also present in yeast cells, and "Eisosome" are known as organelles corresponding to caveolae of animal cells. Our laboratory focus on cellular function of eisosome and is interested in the downstream signaling pathway that activates the membrane repair during plasma membrane stress.

# 2. Functional Analysis of Pathogen Effector Proteins in Yeast

Numerous bacterial pathogens of plants and animals inject virulence proteins, so-called effetors, directory into the host cell cytoplasm through specialized secretion apparatuses, such as the type III

secretion system. The translocated effector proteins manipulate diverse host cellular processes to promote the perturbation of host immune system and ultimately cause infection.

In our laboratory, we are conducting yeast system to uncover the molecular function of effectors from *Ralstonia solanacarum*, which is the most destructive bacterial pathogen in plant. If the effector inhibits the yeast counterpart of the host effector target and then give us a phenotype, such as growth inhibition of yeast. This effector-induced growth inhibition allows us to understand the molecular function of effectors. Recently, by using the yeast system, we found one of the *R. solanacearum* **effector**, RipAY functions as a glutathione degradation enzyme to inhibit host immune response (1, 2).

#### R. solanacearum Effector proteins inhibit host causes lethal bacterial cellular functions, which causes wilt of more than 200 infection plant species Development of new method Expression of effector proteins in veast cell for plant disease control Uncovering the molecular function of effectors Effector protein

Pathogenic Bacterium

# **Publications**

- 1. Popa C., <u>Tabuchi M</u>. and Valls M. Modification of bacterial effector proteins inside eukaryotic host cells. *Front. Cell. Infect. Microbiol.*, **6**, 73, 2016.
- Fujiwara S., Kawazoe T., Ohnishi K., Kitagawa T., Popa C., Valls M., Genin S., Nakamura K., Kuramitsu Y., Tanaka N. and <u>Tabuchi M.</u> RipAY, a plant pathogen effector protein, exhibits robust γ-glutamyl cyclotransferase activity when stimulated by eukaryotic thioredoxins. *J. Biol. Chem.*, **291** (13), 6813-6830, 2016.
- 3. <u>Tabuchi M.</u>, Audhya A., Parsons A.B., Boone C., Emr S.D. The phosphatidylinositol 4,5-biphosphate and TORC2 binding proteins Slm1 and Slm2 function in sphingolipid regulation. *Mol. Cell Biol.* **15**, 5861-75, 2006.









Pathogen effector proteins

Host plant cell


#### Research Area: Applied Life Science Research Specialization: Microbial Biochemistry Name: WATANABE, Akira

Keywords: Microorganism, Basidiomycetous mushroom, Enzyme, Protein, Gene, Autophagy

#### **Recent Research**

### 1. Studies on biological characteristics in basidiomycetous mushroom

Basidiomyctes develop a fruiting body (called mushroom) as a large organ that produces many basidiospores (right figures). Fruiting body is formed from the aggregation of mycelia by proper environmental factors. However molecular mechanisms of fruiting body development in basidiomycetes are still unclear. In addition, basidiomycetes are very useful species for application. Because, they include edible, medicinal, and wood-degrading species.

In this laboratory, we study the biological characteristics of basidiomycteous mushrooms.

### 2. Studies on autophagy of basidiomycetous mushroom

Autophagy is a conserved intercellular degradation system in eukaryotes, which mediates the turnover of cytoplasmic proteins. Recent studies indicate that autophagy is involved not only in response to nutritional starvation but also in various biological phenomena such as cell developmental processes, intracellular clearance and organelle metabolism.

In this laboratory, we study the physiological role of autophagy in basidiomycetous mushroom.

### 3. Development of molecular biological tools in basidiomycetous mushroom

We also attempt to develop the efficient molecular tools (the gene / protein level) of basidiomycetous mushroom.





Fruiting body formation of Flammulina velutipes



*Coprinopsis cinerea*(left), *Lentinula edodes* (right)



Fluorescence microscopic observation

Transformation of basidiomycetous mushroom

#### Publications

Cesur, A. *et al.* (2022) Relationship between fruiting body development and extracellular laccase production in the edible mushroom *Flammulina velutipes*, Biochemistry and Biophysics Reports, 29: 101204.

Nitheranont, T. *et al.* (2018) Molecular cloning of cDNA encoding a major laccase isozyme from *Grifola frondosa* and its expression in *Pichia pastoris*, Mushroom Science and Biotechnology, 25: 134-140.

Nitheranont, T. *et al.* (2017) Heterologous expression of two minor laccase isozyme cDNAs from the edible mushroom Grifola frondosa, Bioscience, Biotechnology, and Biochemistry, 81: 2367-2369.

Chairin, T. *et al.* (2014) Purification and characterization of the extracellular laccase produced by *Trametes polyzona* WR710-1 under solid state fermentation, Journal of Basic Microbiology 54: 35-43.

#### Research Area: Life Science & Biotechnology Research Specialization: Rare Sugar Enzymology Name: KATO, Shiro

Keywords: Rare sugar, Microbial enzyme, Bacteria, Isomerase

#### **Recent Research**

#### 1. Rare sugar production using microbial enzyme

Rare sugar is defined as monosaccharides and its derivatives that exist in the natural world only in a minute amount. Ongoing studies in our laboratory aim to develop production system of rare sugars using microbial enzymes.

#### 2. Biochemical analysis of isomerases

Chiral compounds such as sugars and amino acids exert various physiological effects. However, their origins and functions have not been completely explained yet. Our researches focus on isomerization reactions catalyzed by various isomerases.



- Kato S, Oikawa T (2017) Genome Sequence of *Lactobacillus sakei* LK-145 Isolated from a Japanese Sake Cellar as a High Producer of D-Amino Acids, Genome Announcements, accepted for publication.
- Kato S, Oikawa T (2017) Genome Sequence of *Leuconostoc mesenteroides* LK-151 Isolated from a Japanese Sake Cellar as a High Producer of D-Amino Acids, Genome Announcements, accepted for publication.
- Washio T, *et al.* (2016) Molecular cloning and enzymological characterization of pyridoxal 5'-phosphate independent aspartate racemase from hyperthermophilic archaeon *Thermococcus litoralis* DSM 5473, Extremophiles, 20, 711-721.
- Kato S, *et al.* (2015) Enantioselective analysis of D- and L-amino acids from mouse macrophages using high performance liquid chromatography, Journal of Pharmaceutical and Biomedical Analysis, 116, 101-104.
- Kato S, *et al.* (2012) Lysine racemase from a lactic acid bacterium, *Oenococcus oeni*: Structural basis of substrate specificity, The Journal of Biochemistry, 152(6), 505-508.
- Kato S, *et al.* (2011) Simultaneous determination of D-amino acids by the coupling method of D-amino acid oxidase with high-performance liquid chromatography, Journal of Chromatography B, 879, 3190-3195.



Research Area: Applied Life Science Research Specialization: Animal cell biology Name: SUGIYAMA, Yasunori

Keywords: signal transduction, type 2 diabetes, methodology

#### **Recent Research**

### 1. Analysis of molecular mechanisms of glucotoxicity in type 2 diabetes

Type 2 diabetes caused by poor lifestyle and genetic factor. Glucotoxicity (impaired insulin secretion and insulin resistance) induced by chronic hyperglycemia accelerates the progression of diabetes. In this laboratory, phosphorylation signaling of glucotoxicity in type 2 diabetes is analyzed by using diabetes model cells and mouse. In our previous studies, we analyzed gene expression in glucotoxicity by microarray, and found the changes in the expression of various genes. In addition, we investigate effects of rare sugars on glucotoxicity.

### 2. Development of new analysis methods for phosphorylation signaling

Protein phosphorylation is crucial role in cellular signal transduction. Protein kinase catalyzes phosphorylation of proteins. In the human genome, 518 protein kinase genes were identified, and the enzymes regulate diverse biological phenomena. Previous studies, we developed monoclonal antibodies, designated Multi-PK antibodies, that can recognize multiple protein kinases. Furthermore, the new methods for analysis of phosphorylation signaling were developed.

#### Click here for more information

#### **Publications**

- Sugiyama. Y., *et al.* (2005) Generation and application of a monoclonal antibody that detects a wide variety of protein tyrosine kinases. Analytical Biochemistry 347:112-120.
- Sugiyama. Y., et al. (2006) Two-dimensional expression pattern analysis of protein kinases after separation by MicroRotofor/SDS–PAGE. Analytical Biochemistry 359:271-273.
- Sugiyama. Y., *et al.* (2010) The DNA-binding activity of mouse DNA methyltransferase 1 is regulated by phosphorylation with casein kinase  $1\delta/\epsilon$ . Biochemical journal 427:489-497.
- Sugiyama. Y., *et al.* (2011) Calcium/calmodulin-dependent protein kinase IV involvement in the pathophysiology of glucotoxicity in rat pancreatic β-cells. Metabolism 60:145-153.
- Kon. N., *et al.* (2014) CaMKII is essential for the cellular clock and coupling between morning and evening behavioral rhythms. Genes & Development 28:1101-1110.
- Sugiyama. Y., *et al.* (2015) Expression and phosphorylation state analysis of intracellular protein kinases using Multi-PK antibody and Phos-tag SDS-PAGE. MethodsX 2:469-474.





### Signal pathway in pancreatic $\beta$ cells



Type 2 diabetes mode cells

#### Key ward: Enzyme, Sugar, Fungi, Yeast, Metagenome

#### **Recent Research**

#### 1. Plant Polysaccharides Degradation-Related Enzymes

Plants produce many kinds of polysaccharides and microorganisms produce plant polysaccharides degradation-related enzymes to assimilate plant polysaccharides. Each enzyme has distinct functions in the degradation, and cooperative actions of these enzymes are crucial to degrade plant polysaccharides.

Our study focus on the identification and characterization of novel plant polysaccharides degradation-related enzymes from microorganisms, including fungi and metagenome.



#### 2. Evolution of Enzymes

Random and site-direct mutagenesis can change enzymatic properties, such as thermal stability and substrate specificity. We use the mutagenesis techniques to improve the enzymatic activities and to elucidate the molecular mechanism of enzymatic reactions.

#### **Publications**

Matsuzawa, T., *et al.* (2021) Enzymatic degradation of xyloglucans by *Aspergillus* species: a comparative view of this genus. *Appl. Microbiol. Biotechnol.* 105:2701–2711

Matsuzawa, T., et al. (2020) Identification and characterization of two xyloglucan-specific endo-1,4-glucanases in Aspergillus oryzae. Appl. Microbiol. Biotechnol. 104:8761–8773

Matsuzawa, T., *et al.* (2020) Identification and characterization of  $\alpha$ -xylosidase involved in xyloglucan degradation in *Aspergillus oryzae*. *Appl. Microbiol. Biotechnol.* 104:201–210

Matsuzawa, T., *et al.* (2019) Cooperation between  $\beta$ -galactosidase and an isoprimeverose-producing oligoxyloglucan hydrolase is key for xyloglucan degradation in *Aspergillus oryzae*. *FEBS J.* 286:3182–3193

Matsuzawa, T., *et al.* (2019) Crystal structure and substrate recognition mechanism of *Aspergillus oryzae* isoprimeverose-producing enzyme. *J. Struct. Biol.* 205:84–90

Matsuzawa, T., *et al.* (2016) Identification of the gene encoding isoprimeverose-producing oligoxyloglucan hydrolase in *Aspergillus oryzae. J. Biol. Chem.* 291:5080–5087

Matsuzawa, T., *et al.* (2016) Crystal structure and identification of a key amino acid for glucose tolerance, substrate specificity, and transglycosylation activity of metagenomic  $\beta$ -glucosidase Td2F2. *FEBS J.* 283:2340–2353

#### (3) Food Safety and Nutraceutical Science Center Advanced research for food safety and functional food

Based in the Faculty of Agriculture, the Food Safety and Nutraceutical Science Center conducts joint research with local, national and overseas institutions. The center focuses on food safety topics, such as detection of pathogenic microorganisms and mycotoxins, reduction of agrochemical usage, and pesticide residue analysis, as well as nutritional evaluation of foods, texture and bioactive compound analysis, aimed at developing functional and safe foods. We support the development of food industry through continuous efforts to solve contemporary problems related to health and food safety. Our mission is to contribute to human health and wellbeing by the development of foods with improved safety and function.



Members	Specialized fields
KATO, Hisashi	Plant Biochemistry
SAKURABA, Haruhiko	Enzyme Engineering
SUEYOSHI, Noriyuki	Molecular and Cellular Biology
TANAKA, Naotaka	Applied Microbiology
TABUCHI , Mitsuaki	Applied Molecular Cell Biology

#### About each research center

WATANABE, Akira	Microbial Biochemistry
OGAWA, Masahiro	Food Protein Functionalities
KAWAMURA, Osamu	Food Hygiene
TAKATA, Goro	Applied Microbiology & Enzyme Chemistry
MATSUO Tatsuhiro	Food Nutrition
MORIMOTO, Kenji	Applied Enzymology
YONEKURA, Lina	Food chemistry and functionality
YOSHIHARA, Akihide	Applied enzymology
ISHII, Toya	Food Processing

Research Area: Bioresource & Functional Chemistry **Research Specialization: Plant Biochemistry** Name: KATO Hisashi

Keywords : Allelopathy, Allelochemical, Tropical and subtropical plants



The negative impacts of commercial herbicide use on the environment make it desirable to diversify weed management options. Many investigations have been attempted to exploit allelopathy of plants for weed control purposes in a variety of agricultural settings, since allelopathy is regarded as the direct influence of an organic chemical released from one living plant on the growth and development of other plants.

We have been extensively studied with respect to plant allelopathy as part of a strategy for sustainable weed management. We have already found and isolated several allelopathic substances from various plant sources and determined their chemical structures. We also try to understand the mode of action of the allelopathic substances for their biological activities by biochemical techniques such as protein, enzyme and gene levels.







barnyard grass



One of our students got the best oral presentation award on International Conference.

- $\geq$ Kato-Noguchi, H., Nakamura, K., Ohno, O., Suenaga, K., and Okuda, N.: Asparagus decline: autotoxicity and autotoxic compounds in asparagus rhizomes. Journal of Plant Physiology 213: 23–29 (2017).
- Kato-Noguchi, H.: Allelopathic chemical interaction of bryophytes with vascular plants. Mini-Reviews in Organic  $\geq$ Chemistry 13: 422 - 429 (2016).
- Kato-Noguchi, H., Saito, Y., Ohno, O. and Suenaga, K.: A phytotoxic active substance in the decomposing litter of the  $\geq$ fern Gleichenia japonica. Journal of Plant Physiology 176: 55-60 (2015).
- Kato-Noguchi, H., Salam, M.A., Ohno, O. and Suenaga, K.: Nimbolide B and nimbic acid B, phytotoxic substances in  $\geq$ neem leaves with allelopathic activity. Molecules 19: 6929-6940 (2014).
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- $\geq$ Kato-Noguchi, H. and Peters, R.J.: The role of momilactones in rice allelopathy. Journal of Chemical Ecology 39:175-185 (2013).



#### Research Area: Life Science & Biotechnology Research Specialization : Enzyme Engineering Name: SAKURABA, Haruhiko

Keywords: hyperthermophiles, archaea, thermostable enzyme, x-ray crystal structure analysis

#### **Recent Research**

#### 1. Structure-based creation of a novel D-amino acid dehydrogenase

*meso*-Diaminopimelate dehydrogenase (DAPDH) acts stereoselectively on the D-center of substrate. Thus, the enzyme is expected to be useful for one-step production of D-amino acids, which are often utilized as source materials for industrial production of medicines and agrochemicals. However, its high substrate specificity for DAP and instability have proved to be the major disadvantage for the practical application of DAPDH. Recently, we determined the crystal structure of thermostable DAPDH and estimated the amino acid residues related to substrate binding. We used site-directed mutagenesis to identify the residues responsible for determining the enzyme's substrate specificity and found that a single mutation (D94A) caused a striking change in the substrate specificity of the enzyme. The mutant exhibited extremely high activity for reductive amination of phenylpyruvate. This is a major advantage of using the D94A mutant for one-step synthesis of D-phenylalanine.

#### 2. Unique coenzyme binding mode of hyperthermophilic dehydrogenase

Homoserine dehydrogenase (HseDH) is a key enzyme in the biosynthetic pathway for the synthesis of methionine, threonine, and isoleucine in plants and microorganisms. HseDH is thought to be a potential target for the structure-based design of antibiotics, as the enzyme is not present in mammals. We recently determined the crystal structure of HseDH from the hyperthermophile and found that this enzyme exhibits a new variation on cofactor binding to a dehydrogenase: very strong NADP binding that acts as an obstacle to NAD(P)-dependent dehydrogenase catalytic activity. More recently, we observed another variation on a structural basis for cofactor preference using hyper-thermophilic *sn*-glycerol-1-phosphate dehydrogenase. Our studies indicate that the molecular basis for the cofactor preference in NAD(P)-dependent dehydrogenases may have much more variation than expected.

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cover image

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- Hayashi, J., *et al.* (2017) Crystal structure of the novel amino-acid racemase isoleucine 2-epimerase from *Lactobacillus buchneri*. *Acta Crystallographica*, D73, 428-437.
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- Hayashi, J., *et al.* (2016) Unique coenzyme binding mode of hyperthermophilic archaeal *sn*-glycerol-1-phosphate dehydrogenase from *Pyrobaculum calidifontis. Proteins*, 84, 1786-1796. (This study was selected as the cover image.)
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- Hayashi, J., *et al.* (2015) Crystal structures of a hyperthermophilic archaeal homoserine dehydrogenase suggest a novel cofactor binding mode for oxidoreductases. *Scientific Reports*, 5, 11674.
- Sakuraba, H., *et al.* (2012) Crystal structure of novel dye-linked L-proline dehydrogenase from hyperthermophilic archaeon *Aeropyrum pernix*. *The Journal of Biological Chemistry*, 287, 20070-20080.



Research Area: Life Science & Biotechnology Research Specialization: Applied Microbiology Name: TANAKA, Naotaka

Keywords: fission yeast, Golgi apparatus, secretory pathway, agmatine, glycoprotein

#### **Recent Research**

1. Transport control of glycoproteins by lectin-like protein

Secreted proteins and membrane proteins are entrapped in transport vesicles in the endoplasmic reticulum, passed through the Golgi apparatus, and transported to the cell surface and suitable organelles. It is known that glycosylation, which is one of posttranslational modifications, is essential for quality control of secreted proteins and packaging in appropriate vesicles. It has been clarified that lectin-like proteins are involved in protein sorting and transport by binding to specific sugar chains, but there are many unclear points about the mechanism that distinguishes cargo proteins and the localization mechanism of lectin-like proteins. We analyze the function of lectin - like protein by analyzing the phenotype indicated by the deficient strain of fission yeast lectin-like protein.

2. Analysis of the Golgi apparatus formation mechanism in fission yeast

The Golgi apparatus of fission yeast is scattered in the form of about 10 to 20 dots in the cell. There is a clear layer in each Golgi apparatus, but not only the layer formation mechanism, but also the mechanisms involved in determining the number of Golgi apparatus and position within the cell are not well understood. We focus on the peripheral proteins of the Golgi apparatus and analyze the relationship to the Golgi apparatus function.

3. Analysis of polyamine biosynthetic pathway via agmatine in fission yeast

Polyamines are involved in cell function in various organisms. Ornithine-mediated pathways are well known, but the physiological role of agmatine-mediated polyamine biosynthesis pathway is relatively unknown. We are trying to clarify the involvement in cell function by inhibiting the metabolic pathway of agmatine.



Click here for more information.

- Suzuki, S. et al. (2010) Characterization of two different types of UDP-glucose/-galactose 4-epimerase involved in galactosylation in fission yeast. Microbiology 156: 708–718.
- Tanaka, N., et al. (2001) Functional characterization of Gms1p/UDP-galactose transporter in *Schizosaccharomyces pombe*. Yeast 18: 745-757.



#### Research Area: Applied Life Science Research Specialization: Applied Molecular Cell Biology Name: TABUCHI, Mitsuaki

KEYWORDs: Yeast, eisosome, Stress response, Pathogen effector

#### 1. Plasma Membrane Stress Response in Yeast

Plasma membrane is constitutively damaged from various physical stresses such as heat stress, osmotic stress, membrane tension, which causes plasma membrane stresses. For example, in humans, myocytes are constantly damaged by exposure to physical membrane stress by repeating expansion and contraction as they move intensely. However, cells have a mechanism to repair such damage. In animal cells, it is known that a structure called "Caveolae" plays an essential role in repairing membrane damage, and caveolae abnormality is known to be a cause of cardiomyopaty.

Yeast is a unicellular eukaryote and has contributed to the elucidation f various intracellular mechanisms such as autophagy as a model eukaryotic cell. Mechanisms for sensing and repairing membrane stress are also present in yeast cells, and "Eisosome" are known as organelles corresponding to caveolae of animal cells. Our laboratory focus on cellular function of eisosome and is interested in the downstream signaling pathway that activates the membrane repair during plasma membrane stress.

#### 2. Functional Analysis of Pathogen Effector Proteins in Yeast

Numerous bacterial pathogens of plants and animals inject virulence proteins, so-called effetors, directory into the host cell cytoplasm through specialized secretion apparatuses, such as the type III

secretion system. The translocated effector proteins manipulate diverse host cellular processes to promote the perturbation of host immune system and ultimately cause infection.

In our laboratory, we are conducting yeast system to uncover the molecular function of effectors from *Ralstonia solanacarum*, which is the most destructive bacterial pathogen in plant. If the effector inhibits the yeast counterpart of the host effector target and then give us a phenotype, such as growth inhibition of yeast. This effector-induced growth inhibition allows us to understand the molecular function of effectors. Recently, by using the yeast system, we found one of the *R. solanacearum* **effector**, RipAY functions as a glutathione degradation enzyme to inhibit host immune response (1, 2).

#### R. solanacearum Effector proteins inhibit host causes lethal bacterial cellular functions, which causes wilt of more than 200 infection plant species Development of new method Expression of effector proteins in veast cell for plant disease control Uncovering the molecular function of effectors Effector protein

Pathogenic Bacterium

#### **Publications**

- 1. Popa C., <u>Tabuchi M</u>. and Valls M. Modification of bacterial effector proteins inside eukaryotic host cells. *Front. Cell. Infect. Microbiol.*, **6**, 73, 2016.
- Fujiwara S., Kawazoe T., Ohnishi K., Kitagawa T., Popa C., Valls M., Genin S., Nakamura K., Kuramitsu Y., Tanaka N. and <u>Tabuchi M.</u> RipAY, a plant pathogen effector protein, exhibits robust γ-glutamyl cyclotransferase activity when stimulated by eukaryotic thioredoxins. *J. Biol. Chem.*, **291** (13), 6813-6830, 2016.
- 3. <u>Tabuchi M.</u>, Audhya A., Parsons A.B., Boone C., Emr S.D. The phosphatidylinositol 4,5-biphosphate and TORC2 binding proteins Slm1 and Slm2 function in sphingolipid regulation. *Mol. Cell Biol.* **15**, 5861-75, 2006.









Pathogen effector proteins

Host plant cell



#### Research Area: Applied Life Science Research Specialization: Microbial Biochemistry Name: WATANABE, Akira

Keywords: Microorganism, Basidiomycetous mushroom, Enzyme, Protein, Gene, Autophagy

#### **Recent Research**

### 1. Studies on biological characteristics in basidiomycetous mushroom

Basidiomyctes develop a fruiting body (called mushroom) as a large organ that produces many basidiospores (right figures). Fruiting body is formed from the aggregation of mycelia by proper environmental factors. However molecular mechanisms of fruiting body development in basidiomycetes are still unclear. In addition, basidiomycetes are very useful species for application. Because, they include edible, medicinal, and wood-degrading species.

In this laboratory, we study the biological characteristics of basidiomycteous mushrooms.

### 2. Studies on autophagy of basidiomycetous mushroom

Autophagy is a conserved intercellular degradation system in eukaryotes, which mediates the turnover of cytoplasmic proteins. Recent studies indicate that autophagy is involved not only in response to nutritional starvation but also in various biological phenomena such as cell developmental processes, intracellular clearance and organelle metabolism.

In this laboratory, we study the physiological role of autophagy in basidiomycetous mushroom.

### 3. Development of molecular biological tools in basidiomycetous mushroom

We also attempt to develop the efficient molecular tools (the gene / protein level) of basidiomycetous mushroom.





Fruiting body formation of Flammulina velutipes



*Coprinopsis cinerea*(left), *Lentinula edodes* (right)



Fluorescence microscopic observation

Transformation of basidiomycetous mushroom

#### Publications

Cesur, A. *et al.* (2022) Relationship between fruiting body development and extracellular laccase production in the edible mushroom *Flammulina velutipes*, Biochemistry and Biophysics Reports, 29: 101204.

Nitheranont, T. *et al.* (2018) Molecular cloning of cDNA encoding a major laccase isozyme from *Grifola frondosa* and its expression in *Pichia pastoris*, Mushroom Science and Biotechnology, 25: 134-140.

Nitheranont, T. *et al.* (2017) Heterologous expression of two minor laccase isozyme cDNAs from the edible mushroom Grifola frondosa, Bioscience, Biotechnology, and Biochemistry, 81: 2367-2369.

Chairin, T. *et al.* (2014) Purification and characterization of the extracellular laccase produced by *Trametes polyzona* WR710-1 under solid state fermentation, Journal of Basic Microbiology 54: 35-43.

#### Research Area: Food Science Laboratory: Food Protein Functionalities Name: OGAWA, Masahiro

Keywords: Olive, rare sugar, egg protein, and polyphenol

#### **Recent Researches**

#### 1. Quality Improvement of Food Products by Olive Leaf Polyphenols

Olive leaf contains a large amount of polyphenols (ca5%). The most abundant polyphenol is oleuropein, which exists only in olive. In Kagawa prefecture, olive leaf powder containing high concentration of polyphenol is fed to aquaculture fish yellowtail. In our laboratory, we evaluate the biochemical (protein-level) and mechanical (breaking force) properties of aquaculture fishes (yellowtail and red sea bream) grown with a feed containing olive leaf powder.

#### 2. Effects of Rare Sugars on Food Processing Properties

D-Allulose (Alu), also known as D-Psicose, is the C-3 epimer of D-fructose. It is a rare sugar that exists in small amounts in nature. Alu is a non-caloric sweetener (0.39 kcal  $g^{-1}$ ) with a suppressive effect on postprandial blood glucose elevation. In our laboratory, we investigate the effects of Alu used as a food additive on various food processing properties (mechanical properties of gels including heat induced egg gel, sausage, and surimi gel, and the effects on lactic fermentation and acetic fermentation).

#### 3. Biological Functions of Egg White Proteins of Chicken Eggs

Chicken egg is a capsule containing all components participating in its embryo development. Some of egg white proteins have antimicrobial activity against bacteria. These antimicrobial proteins prevent bacteria from invading into egg yolk. However, the physiological function of many other proteins except antimicrobial proteins still remain unknown. In our laboratory, we investigate the physiological functions of an egg white protein, called "ovalbumin-related protein X (OVAX)" in chicken egg development.



- Preservation Science, 45: 261-270
- Ilhan et al. (2020): Role of 'D-allulose' in a starch based composite gel matrix,
- Akazawa et al. (2019): Migration of chicken egg-white protein ovalbumin-related protein X and its
- ➢ alteration in heparin-binding affinity during embryogenesis of fertilized egg, Poultry Science, 98巻: 5100-5108
- Oyama et al. (2019): Features of water-soluble compounds in muscle of yellowtail Seriola quinqueradiata fed a diet supplemented with powdered olive Olea europaea leaves, Japanese Journal of Food Chemistry and Safety 26: 99-105
- Arsyad et al. (2018): Effects of olive leaf powder supplemented to fish feed on muscle protein of red sea bream, 44: 1299-1308



Microscopic images of baked meringue containing rare sugar D-allulose

Sperm egg after Seven days incubation(left)

Tertiary structure of ovalbumin-related protein X (Right)



#### Keywords: Mycotoxin, Microcystine (algae toxin), Monoclonal antibody, Immunoaffinity column, ELISA, Food safety

#### **Recent Research**

Mycotoxins, the toxic second metabolites of some fungi, give healthy damage to humans and domestic animals. These fungi are infected with the crops in farms, and the fungi grow on per-/post-harvest crops and produce mycotoxins. Mycotoxins remain on the crops after extinction of the fungi, and are heat stable compounds, therefore after cooking, remain in foods. Furthermore, it is known that mycotoxins in animal feeds are transferred to livestock products. A variety of food is contaminated with small quantity of mycotoxins. By an intake of these food for a long term, it is thought that mycotoxins cause the chronic poisoning such as "cancers".

#### The mycotoxins remains Rice cleaning, sorting The mycotoxins (<150 °C) The mycotoxins (<150 °C) The mycotoxins (<150 °C) The mycotoxins (<150 °C) The mycotoxins and the healthy damage

## 1. Production of monoclonal antibodies against mycotoxins, Development of the immunochemical analysis for mycotoxins in foods, Food contamination investigation, and Risk evaluation

To make clear contamination level of mycotoxins in foods, a simple and easy and high sensitive analysis methods are necessary. Now, the immunochemical methods using antibody are frequently used. We made monoclonal antibodies (mAb) against major mycotoxins, and established immunoaffinity column-HPLC methods using these mAb.

We analyzed mycotoxins in commercial foods, such as rice, coffee, cocoa, and ramen from Japan and Asian countries and feeds for chicken from Brazil and Indonesia We performed behavior experiments of mycotoxin during food processing. We obtained some new findings.

#### 2. Occurrence of algae toxin (microcystine) in fresh water

Microcystin-Leu-Arg (MC-LR) is one of toxic cyanotoxins, which are contaminated with eutrophicated lakes and ponds. We established a sensitive ELISA method for detection of MC-LR in environmental water using our novel mAb. Our best MC.5-3 mAb was a little strongly reacted to MC-RR than MC-LR, and almost same to MC-YR. The sensitivity of our ELISA was 80 pg/mL of MC-LR. This value was less than 1/10 of the regulation level (1 ng/mL) in drinking water by WHO. Using this ELISA, we perform the pollution investigation into reservoirs of Kagawa.



- A. T. Ishikawa, <u>O. Kawamura</u> et. al.; Exposure Assessment of Infants to Aflatoxin M<sub>1</sub> through Consumption of Breast Milk and Infant Powdered Milk in Brazil. *Toxins*, 8, 246-256 (2016)
- Y. Tbuchi & <u>O. Kawamura</u>; Occurrence of microcystins in Oima Pond and Hirata Pond which were reservoirs in the suburbs of Faculty of Agriculture, Kagawa University. (2014), Technical Bulletin of Faculty of Agriculture, Kagawa University, 69, 23-26 (2017).
- Y. Mistumoto & <u>O. Kawamura</u>; Decrease of ochratoxin A during cooking of Sanuki udon and occurrence of ochratoxin A in commercial Sanuki udon and somen, Technical Bulletin of Faculty of Agriculture, Kagawa University, 69, 27-30 (2017).





Keywords: sugar and sugar related enzyme, rare sugar, oligosaccharide

#### **Recent Research**

#### 1. Study on production of rare sugar and rare oligosaccharide

Rare sugar defined as sugar not abundant in nature. Some rare sugars such as Dpsicose and D-allose shows various functionalities. These sugars have potential applications for functional foods and medical materials. Our laboratory is now studying on development of novel oligosaccharide production containing both rare sugar and oligosaccharide. This research is a specific research because we have the technical development of rare sugar production.

#### 2. Study on production of functional oligosaccharide

Production of functional oligosaccharides derived from unused resources using enzyme reaction are investigated. Oligosaccharides have various functionalities such as prebiotic effects. We are studying on efficient and low cost production of these sugars using enzymatic reaction.

#### 3. Molecular and structural study of sugar related enzyme

Molecular improvement and catalytic mechanism of enzyme, which is used for production of rare sugar or oligosaccharide are investigated. By gene recombination and X-ray crystal analysis, we are trying to know the catalytic functions, substrate specificities and environmental tolerant. Furthermore, to optimize the enzyme structure by introducing mutagenesis, we are trying to enhance the efficiency of productivity.

#### **<u>Click here for more information</u>**

#### **Publications**

Uechi, K., <u>Takata, G.</u>, Yoneda, K., Ohshima, T., Sakuraba, H. (2014) Acta Crystallographica Section F:Structural Biology Communications, 70 (7), 890-895.

Yoshida, H., Yoshihara, A., Teraoka, M., Terami, Y., <u>Takata, G.</u>, Izumori, K., Kamitori, S. (2014) FEBS Journal, 281 (14),3150-3164.

Uechi, K., Sakuraba, H., Yoshihara, A., Morimoto, K., <u>Takata, G.</u> (2013) Acta Crystallographica Section D: Biological Crystallography, 69 (12),2330-2339.

Uechi, K., <u>Takata, G.</u>, Fukai, Y., Yoshihara, A., Morimoto, K. (2013) Bioscience, Biotechnology and Biochemistry, 77 (3), 511-515.

Morimoto, K., Shimonishi, T., Miyake, S., <u>Takata, G.</u>, Izumori, K. (2013) Bioscience, Biotechnology and Biochemistry, 77 (2), pp. 253-258.

Morimoto, K., Terami, Y., Maeda, Y.-I., Yoshihara, A., <u>Takata, G.</u>, Izumori, K. (2013) Journal of Bioscience and Bioengineering, 115 (4), pp. 377-381.

Takata, G., Uechi, K., Taniguchi, E., Kanbara, Y., Yoshihara, A., Morimoto, K., Izumori, K. (2011) Bioscience, Biotechnology and Biochemistry, 75 (5), pp. 1006-1009.

Kimura, Y., Yoshimi, M., <u>Takata, G.</u> (2011) Journal of Bacteriology, 193 (8), pp. 2053-2057.

Keywords : carbohydrate and fat metabolism, nutrient, rare sugar



#### Recent Research 1. Study of functions of rare sugar D-allulose and rare sugar syrup

D-Allulose (D-psicose), a C-3 epimer of D-fructose, is a rare sugar present in small quantities in nature. We have since demonstrated that D-allulose is a sweet carbohydrate that provides no energy and that it causes little toxic effect in rats. In addition, we suggested that D-allulose controls postprandial hyperglycemia in normal and diabetic animals. Thus, D-allulose may be useful in preventing obesity and diabetes in patients. On the other hand, rare sugar syrup (RSS) containing about 6% D-allulose has been sold as a less expensive sweetener in markets. We also showed that RSS reduced abdominal fat accumulation compared to high-fructose corn syrup. We will study mechanisms about the functionality of D-allulose and other rare sugars except D-allulose in future.

#### 2. Effects of very low carbohydrate diet on body fat and metabolism

Recently, a very low carbohydrate diet (VLCD) become the topic through media as a diet cure of diabetes and have been already adopted at some hospitals. It is reported that the VLCD improves a hyperglycemia of the diabetic dramatically in a short term, but is doubted about effects and safety for long-term (several years - life). In addition, it is hardly reported the effect by the long-term intake of the VLCD using the laboratory animals. We have monitored the condition of rats and lifespan using the Goto-Kakizaki (GK) rats that were hereditary type-2 diabetes model rats, but were not able to confirm the life extension effect. We will examine the effects of VLCD using normal Wistar rats equally in future.

#### 3. Study of functional food ingredients

Anti-obesity and anti-diabetes effects of functional food ingredients were studied as follows: (1) fermented brown rice, (2) soy germ protein, (3) egg white and egg white hydrolysate, (4) bonito fish protein, (5) dried Itea plant powder, (6) others.

#### Publications

•Ochiai M, et al. (2017) Comparison of anti-obesity effect between two types of syrup containing rare sugars in Wistar rats. J Nutr Sci Vitaminol 63: 207-212.

• Yamada T, et al. (2014) Dietary D-sorbose decreases serum insulin levels in growing Sprague-Dawley rats. J Nutr Sci Vitaminol 60: 297-299.

<sup>•</sup>Ochiai M, et al. (2017) Egg white hydrolysate can be a low-allergenic food material to suppress ectopic fat accumulation in rats fed an equicaloric diet. J Nutr Sci Vitaminol 63: 111-119.

<sup>•</sup> Hjossain A, et al. (2015) Rare sugar D-allulose: Potential role and therapeutic monitoring in maintaining obesity and type 2 diabetes mellitus. Pharmacl Ther 155: 49-59.

<sup>•</sup>Ochiai M, et al. (2015) Dietary protein derived dried bonito fish improves type-2 diabetes mellitus-induced bone frailty in Goto-Kakizaki rats. J Food Sci 80, H848-H856.

Research Area: Food Science Research Specialization: Applied Enzymology Name: MORIMOTO, Kenji

Keywords: Rare sugar, enzyme, separation of rare sugar

#### **Recent Research**

### 1. Screening novel enzyme for rare sugar-producing enzyme

Many enzyme are utilized for rare sugar production as right figure, Izumoring. Each enzyme is derived from microbe and screened from soil. We aim to obtain novel enzymes and more efficient enzyme than existing enzymes.

### 2. Separation of rare sugar by boronic acid

We are developing novel separation system with boronic acid. Boronic acid has strong affinity with cis-diol structure in sugar molecule under the alkaline pH. This affinity strength is depend on a number of cis-diol structure and its location in molecule, resulting that retention time of rare sugars are different.

#### 3. Hexose synthesis by aldol reaction

Some hexoses can be synthesized from two kinds of trioses by aldol reaction. We study production of novel rare sugars using this reaction.

#### **Publications**

• Morimoto, K., Terami, Y., Maeda, Y., Yoshihara, A., Takata, G., Izumori, K.: Cloning and characterization of the L-ribose isomerase gene from Cellulomonas parahominis MB426. Journal of Bioscience and Bioengineering, **115**(4), 377-381, 2013.

Izumoring (Hexose)

Ketose D-Tagatose 3-epimerase

Dxidoreductase

Aldos

of D- and L-series sugars Entrance to L-world from D-world

Aldose isomerase

Aldose reductase

• Shompoosang, S., Yoshihara, A., Uechi, K., Asada, Y., Morimoto, K.: Enzymatic production of three 6-deoxy-aldohexoses from L-rhamnose. Bioscience Biotechnology, and Biochemistry, **78**(2), 317-325, 2014.

• Morimoto, K., Yoshihara, A., Furumoto, T., Takata, G.: Production and application of a rare disaccharide using sucrose phosphorylase from Leuconostoc mesenteroides. Journal of Bioscience and Bioengineering, **119(6)**, 652-656, 2015.

• Shompoosang, S., Yoshihara, A., Uechi, K., Asada, Y., Morimoto, K.: Novel process for producing 6-deoxy monosaccharides from L-fucose by coupling and sequential enzymatic method. Journal of Bioscience and Bioengineering, **121(1)**, 1-6, 2016.





#### Division Food Sciences Research Field Food chemistry and functionality Name YONEKURA, Lina

Keywords: Digestion, absorption, bioactive compounds, polyphenols, carotenoids

• Recent research topics

### Table olive processing methods for preservation of bioactive compounds

Kagawa prefecture is the largest producer of olives in Japan.

Olive fruits contain important bioactive compounds such as the oleuropein aglycones 3,4-DHPEA-EA and 3,4-DHPEA-EA, which have strong antiallergic effects in addition to their antioxidant properties. However, most of those bioactive compounds are lost with the intensive chemical treatment and rinsing during the process to make table olives. We are studying methods to improve the stability of bioactives in table olives, while reducing the environmental impact of the process by reducing the use of water.

#### Digestion and absorption of carotenoids and polyphenols

Carotenoids and polyphenols are important food bioactives. Carotenoids are red, orange and yellow pigments that are radical scavengers, antioxidants and have an important role in the prevention of cancer, and age-related macular degeneration. Catechins are also important modulators of the body's redox status.

Carotenoids are poorly absorbed. Only 10-20% of the ingested amount is actually absorbed. The bioavailability of catechins is even lower. We are investigating the effect of dietary factors that can improve the absorption of carotenoids and catechins.

#### Selected publications

- Soukoulis C, Tsevdou M, Yonekura L, et al. (2017). Does kappa-carrageenan thermoreversible gelation affect β-carotene oxidative degradation and bioaccessibility in o/w emulsions? <u>Carbohydrate Polymers</u> 167, 259-269.
- Yonekura L, Martins CA, et al. (2016). Bioavailability of catechins from guarana (Paullinia cupana) and its effect on antioxidant enzymes and other oxidative stress markers in healthy human subjects. Food & Function 7, 2970-2978.
- Kano S., Komada H, Yonekura L, et al. (2016). Absorption, Metabolism, and Excretion by Freely Moving Rats of 3,4-DHPEA-EDA and Related Polyphenols from Olive Fruits (Olea europaea). <u>Journal of Nutrition and Metabolism</u> 2016, 10.
- Kotake-Nara E, Yonekura L, and Nagao A. (2015). Glyceroglycolipids Affect Uptake of Carotenoids Solubilized in Mixed Micelles by Human Intestinal Caco-2 Cells. <u>Lipids</u> 50, 847-860.
- Yonekura L, et al. (2016). Vitamina A (retinol) e carotenoides In <u>Biodisponibilidade de Nutrientes</u> (5a EDIÇÃO revisada e atualizada), S.M.F. Cozzolino, ed. (Barueri: Manole), pp. 293-340.







Research Area: Food Science Research Specialization: Applied enzymology Name: YOSHIHARA, Akihide

Keywords: Rare sugar, Microbe, Enzyme

#### **Recent Research**

### 1. Screening of microbe and enzyme. Production of rare sugars using their microbe and enzymes

Microbe produces a lot of enzymes. Rare sugars are produced by their biotransformation. Therefore many researchers isolate microbe for effective production of rare sugars. In this research, We screen various rare sugar producing microbes and enzymes. Optimum condition determine for rare sugar production, We try to produce a lot of rare sugars

### 2. Production of rare sugar derivatives using microbial and enzymatic reaction

Rare sugars are produced by several enzymes. These enzymes are able to classify into following four groups.

Isomerase catalyzes reversible aldose-ketose isomerization

Epimerase catalyzes reversible ketose C-3 epimerization.
Dehydrogenase catalyzes the oxidation of polyols to

corresponding ketoses.

•Oxidase catalyzes the oxidation of polyols to corresponding aldoses.

In this research, we try to produce novel rare sugar derivatives that have physiologically activity by microbial and enzymatic reactions. We focus to deoxy monosaccharides, azido monosaccharides and their derivatives.

#### **Publications**

Akihide Yoshihara et al. (2013) Isomerization of deoxyhexoses: green bioproduction of 1-deoxy-D-tagatose from L-fucose and of 6-deoxy-D-tagatose from D-fucose using *Enterobacter agglomerans* strain 221e, Tetrahedron: Asymmetry, 19 (6), 739-745

Pushpakiran Gullapalli et al. (2010) Conversion of L-rhamnose into ten of the sixteen 1- and 6-deoxyketohexoses in water with three reagents: D-tagatose-3-epimerase equilibrates C3 epimers of deoxyketoses, *Tetrahedron Letters*, 51 (6), 895-898 Devendar Rao et al. (2009) A concise approach to the synthesis of all twelve 5-deoxyhexoses: D-tagatose-3-epimerase—a reagent that is both specific and general, Tetrahedron Letters, 50 (26), 3559-3563

Andreas F. G. Glawar et al. (2013) An approach to 8 stereoisomers of homonojirimycin from D-glucose via kinetic & thermodynamic azido-γ-lactones, *Organic & Biomolecular Chemistry*, 11 (40), 6886-6899









Key word: emulsion, foam, gel, powder, microgel, oil body

#### **Recent Research**

Many food products consist of two or more immiscible phases (e.g., oil and water for emulsions, air and water for foams). The dispersed states of these phases in food stuffs closely relate to the product quality such as appearance and texture. We are studying various food ingredients and processing techniques to control the dispersed states of various food systems for designing the desired product quality. Our

another research interest is the assessment and application of plantbased food ingredients towards the sustainable food production.

#### 1. Stabilization of colloidal systems based on edible particles

Food emulsions and foams are usually created and stabilized based on surface-active molecules such as proteins and small-molecule surfactants, whereas recent studies clarified that particles can irreversibly adsorb on oil-water and air-water interface to efficiently stabilize emulsions and foams. We are investigating emulsifying properties of various edible particles, e.g. vegetable powders and microgels.

#### 2. Functions of plant oil bodies (oleosomes) as food ingredients

Plant oil bodies (OBs) are an oil-storing organelle composed of the neutral lipid core, phospholipid monolayer, and oleosin proteins. We are investigating emulsifying and foaming properties of OBs extracted from soybean seeds under various conditions relating to the food processing.

On the other hand, OB-related lipophilic components, e.g., phospholipids and oleosin proteins can be also obtained from defatted soybean meal as a newly-fractionated protein fraction, named soybean lipophilic protein (LP). We are also focusing on the application of LP to food emulsion and foam systems.

lipid phospholipid

Fig. The schematic image of plant oil bodies



Fig. The structure of foams stabilized by the different soybean protein fractions.

#### **Publication**

- H. Ho, <u>T. Ishii</u>, K. Matsumiya, M. Iwasa, & Y. Matsumura. Utilization of dried Japanese apricot and avocado fruit powders as an emulsifying agent: The importance of the powder-dispersed phase in emulsification. *J Food Eng.*, 294, 110411, 2021.
- J. Sirison, <u>**T. Ishii**</u>, K. Matsumiya, M. Samoto, M. Kohno, & Y. Matsumura. Comparison of surface and foaming properties of soy lipophilic protein with those of glycinin and β-conglycinin. *Food Hydrocoll.*, 112, 106345, 2021.
- **T. Ishii**, K. Matsumiya, & Y. Matsumura. Combinational effects of acid and salt addition on colloidal, interfacial, and emulsifying properties of purified soybean oil bodies. *Food Hydrocoll.*, 111, 106213, 2021.
- T. Ishii, K. Matsumiya, M. Aoshima, & Y. Matsumura. Microgelation imparts emulsifying ability to surface-inactive polysaccharides—bottom-up vs top-down approaches, *NPJ Sci. Food*, 2, 15, 2018.
- **T. Ishii**, K. Matsumiya, Y. Nambu, M. Samoto, M. Yanagisawa, & Y. Matsumura. Interfacial and emulsifying properties of crude and purified soybean oil bodies, *Food Structure*, 12, 64–72, 2017.





composed of two immiscible phases.

Fig. Schemes of the system



microgel stabilized emulsion.

Fig. The microstructure of the

oleosin

#### (4)Agricultural Resources and Technology Center Advanced research for development of new agro-technology and resources

In Kagawa University Faculty of Agriculture, various types of researches are in progress for promoting a technological development of new production, usage and breeding of field and horticultural crops with regional characteristics fitting with environment of Kagawa prefecture. Our achievements have been widely used and contributed in society; such as development of strawberry bed cultivation system, floral induction technology of house mandarin orange, development and dissemination of sake rice new varieties, development of brewing grape varieties following commercialization of University-brand wine, and new variety development through breeding of wild chrysanthemum native to Shikoku island. We will continue these achievements and aim to promote development of new agro-technology and resources for the next generation by unity of our research personnel with international, national and regional collaborators.



Members	Specialized fields
TOYOTA, Masanori	Crop Ecophysiology
MATSUMOTO, Yoshiki	Animal Science
MUTO, Yukio	Agricultural Economics
MOROKUMA, Masahiro	Crop Husbandry
MIZUTA, Keisuke	Crop Science

#### About each research center

OKUDA, Nobuyuki	Vegetable Crop Science
TAKAMURA, Takejiro	Horticultural breeding
BEPPU, Kenji	Pomology
MOCHIOKA, Ryosuke	Pomology
ONO, Kentaro	Pomology

Research Area: Bioresource Production Science Research Specialization: Crop Ecophysiology Name: TOYOTA, Masanori

Keywords: Crop ecophysiology, Field crop, High yielding, Light quality Water-saving culture, Yield determination.

#### **Recent Research**

### 1. Controlling shade avoidance response as an avenue to increase potential crop yield

A lowered ratio of red and far-red irradiance (R:FR) is a warning signal for future competition, triggering plant morphogenic responses for shade avoidance, seen as increased apical dominance and reduced axillary bud growth. The growth and survival of tillers in wheat controlled by R:FR determines the final number of spike which is a major component of wheat yield. This study tests the hypothesis whether the reducing shade avoidance response increase the number of tiller in wheat or branch in soybean, and therefore increase yield potential.

#### 2. Rice cultivation with drip irrigation for watersaving and greenhouse gas mitigation

Drip irrigation system, which has been developed for watersaving in arid land agriculture, is now applied to large-scale upland rice cultivation in some countries. In this study, drip rice cultivation in upland field is performed to estimate how much water could be saved compared to paddy, and to evaluate the emission reduction of methane: because paddy rice is one of the major source of methane emission in agriculture.

### 3. Ecophysiological approaches to maximize potential yield of Japanese soybean

Japanese soybean yield (1.6 t/ha) is only half of that in advanced cultivation country. This study aimed to optimize light interception and radiation use efficiency in soybean canopy using different cultivars or environment to improve cultivar and cultivation technologies, and to maximize potential yield of Japanese soybean.

- Laaorthip, M. and Toyota, M. (2017) A high seed yield and associated attributes of dry matter production achieved by recent Japanese soybean cultivars, Plant Production Science, 20(2), 193-204.
- Toyota, M., *et al.* (2017) Changes in radiation interception and R:FR over time and with canopy depth of two soybean cultivars with different branching characteristics. Plant Production Science, 20(2), 205-214.
- Toyota, M., et al. (2014) Tillering responses to high Red: Far-red ratio of four Japanese wheat cultivars. Plant Production Science 17, 124-130.
- Toyota, M., et al. (2010) Effects of reduction in plant height induced by chlormequat on radiation interception and radiationuse efficiency in wheat in southwest Japan. Plant Production Science 13, 67-73.
- Toyota, M., *et al.* (2001) Initiation and development of spikelets and florets in wheat as influenced by shading and nitrogen supply at the spikelet phase. Plant Production Science 4, 283-290.









Research Area: Bioresource Production Science Research Specialization: Animal Science Name: MATSUMOTO, Yoshiki

Keywords: Neuroscience, Intestinal Villus, Microbiota, Trapping Device, Red mite, Feeding environment and Poultry health.

#### **Research Interests**

Our primary interest is the development of functional animal production sciences, widely anatomy and physiology, statistically detecting the target molecule expression and localization, quantitative absorbed amino acid using new imaging methods on an intestine villus tips and contributing for future industrial sustainability. Particularly, this is very topical and important for safety animal products, poultry health that has affected by intestinal microbiota, and in understanding and developing theoretical principles of feeding environment.

#### 1. Gut mucosal functions and health in poultry

The poultry industry needs more stable production which will require an improved environment for layers and better health controls. One major health and environmental concern is caused by feeding related microbiota on intestinal villus. Fourier Transform Infrared imaging (FT-IRI) is possible to analyze collagen maturity, crystallization and calcification, and to estimate organic compound by analyzing atomic bonds (-C=O -O-H -PO<sub>4</sub><sup>3-</sup> etc.) in tissues. FT-IRI and MALDI-TOF MSI are establishing new methods to evaluate certain nutrient absorption in terms of morphology and physiology combined with SEM and light microscope.





HE staining

MALDI-TOF MSI

#### 2.Epidemiological research and developing ectoparasite trap devices

Dermanyssus gallinae (De Geer, 1778), known as the "red mite," is a hematophagia ectoparasite, commonly found in laying hens and is one of the most important epidemiological and economic problems. We have developed an electrostatic charged device (*i*-Trap®, Kondo-Electric Co., Ltd.) which can attract and capture red mites without the use chemicals or insecticides. This device has an electrical charge from static electricity that is created by the polyurethane composition of the material. This allows for quantification of the red mite infestation population from which the contamination level forred mites can

be determined.



#### Publications

• Egg collagen content is increased by a diet supplemented with wood charcoal powder containing wood vinegar liquid, British Poultry Science, 57, 601-611, 2016, Yamauchi K, Matsumoto Y and Yamauchi KE.

 Increased collagen accumulation in eggshell membrane after feeding with dietary wood charcoal powder and vinegar, Connective Tissue Research, 54(6), 416-25, 2013. Yamauchi K, Manabe N, Matsumoto Y, Yamauchi KE.

• Red mite population: Increase has a direct correlation to a decrease in egg production, **17th AAAP** 1070-1074,

(2016). Imade Y., Kondo T., KayaharaY., Yamauchi K., Lutes P., Matsumoto Y.

 New parameters and evaluation: Collagen-related molecules in chicken intestine, 17th AAAP ANIMAL SCIENCE CONGRESS 156-160, (2016). Takagi R, Yamauchi KE Matsumoto Y.

Pest-accumulating device and pest-accumulating method, Patent Number: JP 5690986, US9510583,

TH1501000949, CN201380043196. Matsumoto Y, .Kondo T, Yamauchi K and Yamauchi KE.



#### Research Area: Bioresource Production Science Course Research Specialization: Agricultural Economics Name: MUTO, Yukio

Keywords: managerial resources, managerial strengths, constraints on farm management growth

#### Research issue: Economic Analysis on the Growth Processes of Farm Managements in Japan

In agriculture, farmers can obtain managerial resources by appropriate investment and employment using financial resources, and by acquiring skills with "learning by doing" on their farms. We can envision an ideal growth model of farm managements, where, accumulating and using managerial resources, farmers realize managerial strengths to earn more profits (see the figure below). In Japan, however, most farmers cannot follow such a model, thus failing to grasp growth opportunities.

In our laboratory, using economic theory and econometric tools, we analyze how socio-economic constraints lead Japanese farmers to give up following the ideal growth model, and what should be done to alleviate those constraints.



#### **Publications**

•Yukio Muto, "Fault-Responsibility-Dilemmas and Distortions in Pest Control Advising". *The Japanese Journal of Rural Economics* **3**, 1-14 (2001).

• Yukio Muto, "The Use of Non-linear Tariffs for Irrigation Water in Agricultural Drainage Control". *The Journal of Rural Economics* 81(3), 179-192 (2009), in Japanese.

• Yukio Muto, "Consideration of the Tendencies in Employment Management and Human Resource Development in Farm Organizations". *The Journal of Rural Problems* 53(2), 99-107 (2017), in Japanese.



Research Area: Bioresource Production Science Research Specialization: Crop Husbandry Name: MOROKUMA, Masahiro

Keywords:crop, rice, unused resources, sustainable agriculture, organic cultivation.

#### **Recent Research**

#### 1. Studies on Organic Cultivation of Crops

Organic cultivation without chemical fertilizers and agrochemicals are sustainable agriculture which reduce stress to environment depending on crop production and use resources effectively. And it will be benefit for sell because of additional values.

In our laboratory, we started organic cultivation of rice at 2005 in paddy field of University Farm. We study the effect of organic materials on weeds and rice yield. We simultaneously study organic cultivation of upland crops for crop rotation in paddy field. Table Effect of organic materials on dry weight of monochoria during heading time(2016).

Materials	Dry weight(g/m <sup>2</sup> )
Rice Bran 100	58.6
Rapeseed Meal 100	5.6
Camellia Meal 100	83.3
Camellia Meal 500	5.3
control	102.3

#### 2. Studies on water saving cultivation of paddy rice

It will be important for the future concerned water shortage to accumulate the information about water saving cultivation of rice.

In our laboratory, we introduce drip irrigation system to paddy field of University Farm, and study the effect of this system on water saving extent and rice yield.

#### 3. Studies on water saving cultivation of paddy rice

There are many unused resources, for example, food waste, waste from vegetable shipping adjustment, etc., We study to use them to crop production as organic materials. Now, we investigate to use sake cake and food waste to rice or vegetable production.

#### **Publications**

Combined application of oil cake and rice bran reduced the number of weeds and increased the yield of paddy rice in a paddy field incorporated with white clover, 9<sup>th</sup> Asian Crop Sci. Association Conference, 287, 2017, Sugimoto, H., Araki T., **Morokuma, M.** and Hossain S.T.

The use of desalinated-dried jellyfish and rice bran for controlling weeds and rice yield, Journal of organic systems, 8, 28-37, 2013, Hossain, S.T., Sugimoto, H., Asagi, N., Araki, T., Ueno, H., **Morokuma, M.** and Kato H.

Role of dried jellyfish as an innovative means of organic rice production, Proceeding of the 6th international weed science congress, Hangzhou, China, 92, 2012, Sugimoto, H., Hossain, S.T., Asagi, N., Ueno, H., Araki, T., **Morokuma, M.** and Kato, H.





#### Research Area: Food Production Research Specialization: Crop Science Name: MIZUTA, Keisuke

Key words: Wheat, High yield, High quality grain, Lodging, Growth diagnosis

#### **Recent Research**

1. Development of wheat cultivation method to achieve high yield and grain quality

In wheat cultivation, it is important to achieve both yield and grain quality at a high level. Wheat for bread and durum wheat, whose production has increased in recent year in western Japan, are required high grain protein content in quality evaluation. However, The grain protein content is negatively correlated with yield. In this laboratory, we are verifying cultivation methods which can achieve high yield and grain quality by improving the fertilizer application method and sowing system.

2. Study on light competition and lodging in wheat

If lodging occurs, it reduces the yield and grain quality of wheat. Previous studies have reported that nitrogen application during stem elongation stage increase the risk of lodging by lengthened stem length. However, when plant growth before stem elongation stage was limited and increasing light interception, the stem length was not lengthened even though nitrogen was applied more than three times during stem elongation stage. In this laboratory, we hypothesize and verify that the direct cause of lodging is not nitrogen application but light competition during stem elongation stage.

3. Development of stable wheat production method by growth diagnosis

To achieve high yield and grain quality while preventing lodging in wheat, it is considered that the variable-rate nitrogen application method based on the growth diagnosis must be effective. However, special equipment such as a spectroradiometer or laser sensor is required for plant growth diagnosis. In this laboratory, we are verifying whether the growth can be diagnosed by image analysis without using special equipments.

#### Publications

• Effect of intensive nitrogen fertilization during stem elongation (INFSE) on grain yield and grain protein content in bread wheat cultivar "Minaminokaori". Jpn. J. Crop Sci. 86: 319-328. 2017. K. Mizuta, H. Araki, K. Nakamura, H. Matsunaka, K. Tanno and T. Takahashi.

• Enhancement of yield and quality of grains thorough intensive nitrogen fertilization during stem elongation in the highyield wheat cultivar for bread "Setokirara". Jpn. J. Crop Sci. 88: 98-107. 2019. K. Mizuta, H. Araki and T. Takahashi.

• Shifting timing of intensive nitrogen topdressing later to the stem-elongation phase reduced lower internodes length and lodging risk of wheat. Plant Production Science 23: 427-435. 2020. Keisuke Mizuta, Hideki Araki and Tadashi Takahashi.

• The verification of variable-rate fertilization in response to the tiller number of a wheat cultivar for bread "Setokirara". Jpn. J. Crop Sci. 89: 299-306. 2020. K. Mizuta, H. Araki and T. Takahashi.







#### Research Area: Horticultural Science Research Specialization: Vegetable Crop Science Name: OKUDA, Nobuyuki

Keywords: vegetable crops, growth and development regulation, raising of superior strain

#### **Recent Research**

### 1. Morphogenetic control of the lettuce under the high temperature condition

On the high temperature condition, a lettuce forms a flower buds and elongates a stem. Therefore, the morphological product value of the lettuce decrease remarkably by global warming. We study the morphogenetic control technology of the lettuce under the high temperature condition to resist global warming.

#### 2. Ecological characteristic of a asparagus

In West Japan, a asparagus is harvested from spring to the autumn, we cannot harvest a asparagus spears from October to February. Furthermore one major problem of the asparagus cultivation is the asparagus decline. In our laboratory, it is studied the reduction of asparagus growth in the low temperature period and measures for the asparagus decline.

#### 3. Production of high functional vegetables

The quality of vegetables greatly varies according to an environmental condition. We study the cultivation condition to produce high functional vegetables which attract attention.

#### 4. Raising of superior strain of Brassica vegetable

Generally, the flower vegetable has high nutritive value, and these consumptions increase. We study raising of superior strain of Brassica flower vegetables.

- Okuda, N. et al. (2017) Effects of end of day lighting after night chilling treatment on the growth and development of lettuce. Environmental Control in Biology, **55**, 7-11.
- Okuda, N. et al. (2015) Effect of glutathione blended fertilizer on the growth and development of garlic. Hort. Res.(Japan) 14 (SUPPL.2).483.
- Okuda, N. et al. (2014) Effect of end-of-day light irradiation using LED light sources on the growth of lettuce under a high temperature. Environmental Control in Biology, **52**, 73-77.
- Okuda, N. et al. (2014) Effects of end-of-day irradiation after short-day and night-cooling treatment on the growth and development of lettuce. The International Conference on Plant Factory 2014, abstract p.30. Kyoto, Japan
- Okuda, N. et al. (2012) Effects of bamboo charcoal to reduce growth inhibition caused asparagus, Hort. Res.(Japan) 11, (Suppl.2), 463.
- Okuda, N. et al. (2011) Effects of glutathione application on the flower bud formation of choy sum, Hort. Res.(Japan) **10**(Suppl.2), 478.
- Okuda, N. et al. (2009) Studies on flower bud and seed formation of Chinese kale: a Brassica adjusted in the tropics, Research for tropical Agriculture 2, 52-55.









#### Research Area: Horticultural Science Research Specialization: Horticultural breeding Name: TAKAMURA, Takejiro

Keywords: Flower color, Flower pigment, inter-specific hybridization, polyploidy breeding

#### **Recent Research**

### 1. Genetic control and improvement of flower-color expression in cyclamen

Although petal color of cyclamen was red, purple, white, and their neutral tint until the 1980s, the yellow-flowered cultivars and individuals with bluish-violet petals in cyclamen are available now. We have clarified the main factor causing the bluish-violet petals and inheritance of the yellow-flowered characteristics. We presently study the mechanism of cyclamen petals becoming bluish color, expression of anthocyanin and flavonoid synthesis-related gene in cyclamen, and so on.

### 2. Interspecific hybridization and polyploidy breeding in the genus *Cyclamen*

The genus *Cyclamen* contains more than 20 species. Almost all the species, except *C. persicum*, have not contributed to the breeding of horticultural cyclamen cultivars, whereas some *Cyclamen* species have useful characteristics. In our laboratory, interspecific hybridization by using such *Cyclamen* species is studied. Polyploidy breeding by using such species and interspecific hybrids is also researched.

### 3. Analyses of mechanisms of pigmentation and flower-color expression in some ornamental plants

Mechanisms of flower-color expression and pigmentation in some ornamental plants (e.g., Persian buttercup and glory lily) are studied. Effects of environmental factors on the flower-color expression and pigmentation in some ornamental plants are also researched.

#### Publications

Takamura, T. : Cyclamen, Flower Breeding & Genetics: Issues, challenges, and opportunities for the 21st century (N. O. Anderson ed.), 459-478, Springer-Verlag, Dordrecht (2006).

Takamura, T. *et al.*: Ploidy levels of degenerated embryos in the crosses between diploid and tetraploid cyclamen, Acta Horticulturae 855 ,261-266 (2010).

Takamura, T. *et al.*: Effects of CO<sub>2</sub> enrichment on in vitro plant regeneration through somatic embryogenesis in cyclamen (*Cyclamen persicum* Mill.) Technical Bulletin of Faculty of Agriculture, Kagawa University 62, 1-4 (2010). Takamura, T. *et al.*: Effect of carbon source on *in vitro* plant regeneration in anther culture of cyclamen (*Cyclamen persicum* Mill.). Acta Horticulturae 923: 129-134 (2011).









Research Area: Horticulture Research Specialization: Pomology Name: BEPPU, Kenji

#### Keywords: peach, plum, cherry, reproductive physiology, breeding

#### **Recent Research**

#### 1. Breeding and utilization of lower-chilling peach

Forcing culture of peach under plastics is intended to ensure early shipping of production aimed at obtaining a higher market value. Usually, peach growers start forcing after attaining 900 chilling hours required by the high-chill cultivars. For achieving earlier forcing, lower-chilling peach with a high tasting quality would be necessary. Therefore, we bred a new cultivar named 'KU-PP1' and 'KU-PP2' that contained these traits by crossing Japanese high-chill and introduced low-chill cultivars. Currently we investigate effective methods for forcing culture with these cultivars.

#### 2. Breeding of self-compatible Japanese plum

Most commercial cultivars of Japanese plum are self-incompatible and cross-compatible cultivars need to be interplanted. Thus, information about S-haplotypes of each cultivar is important. We demonstrated that the S-RNase genes of Japanese plum could be amplified effectively by S-RNase gene-specific primers, and identified 14 different S-RNase genes. In addition, we revealed the involvement of the S<sup>e</sup>-haplotype in self-compatibility in Japanese plum and developed the S<sup>e</sup>-RNase gene-specific primer. Currently, we are breeding self-compatible Japanese plum cultivars with good fruit quality.

#### 3. Regulation of fruit set of sweet cherry in warm region

Poor fruit set due to physiological fruit drop have become serious problems preventing stable production of sweet cherry in warm regions. Under controlled conditions, we demonstrated that the fruit set rate decreased markedly due to the rapid degeneration of the embryo sacs when the trees were exposed to high temperatures above  $20 \,^{\circ}$ C, and that gibberellin may be involved in this phenomenon. We also found that extremely high temperature in summer reduced photosynthesis and cause early defoliation, which result in low fruit set rate in the following spring. We have developed several methods to improve fruit set in warm regions.







- Beppu, K. and Kataoka, I. (2016) Registration of new plant varieties, Peach, 'KU-PP2' (*Prunus persica* (L.) Batsch), Registration number: 24983.
- Beppu, K. et al. (2014) Examination of time of heating in forcing culture of lower-chilling peach selection HKH×FLP3, Acta Hort. 1059, 201-204.
- Beppu, K. et al. (2012) Molecular and genetic analyses of the S-haplotype of the self-compatible Japanese plum (*Prunus salicina* Lindl.) 'Methley', J. Hort. Sci. Biotech. 87, 493-498.
- Beppu, K. and Kataoka, I. (2011) Studies on pistil doubling and fruit set of sweet cherry in warm climate, J. Jap. Soc. Hort. Sci. 80, 1-13.

Research Area: Horticultural Science Research Specialization: Pomology Name: MOCHIOKA, Ryosuke

Key words: Vitis, wild grape, breeding, coloration

#### Recent research

### 1. Research of habitat, collection, and evaluation of wild grapes native to Japan

15 wild grapes are identified in Japan, but just *Vitis coignetiae* was utilized. We have been collecting various wild grapes over the different places in Japan, and researching their characteristics. As a result, we clarified some of them have some good characteristics (e.g. no bud dormancy, good skin coloration even under the high temperature, high sugar content, ever-bearing).

# 2. Breeding the new grape cultivars with good skin coloring even under high temperatures in the warm areas

Plant pigments can express with sugars. Therefore, low sugar contents in fruits make fruit skin color worse. As the climate of Seto Inland Sea shows many hot summer days and tropical nights in summer, there are some serious problems about skin coloring there. We bred the new wine cultivar 'Kadainou R-1' using with *Vitis ficifolia* var. *ganebu* distributed in the subtropical regions in Japan. 'Kadainou R-1' has a lot of functional substances (anthocyanin, polyphenol, flavonol etc.), and the taste of wine made from this cultivar is not bitter but light.







#### Publications

•Polyphenolic diversity and characterization in the red-purple berries of East Asian wild *Vitis* species. Phytochemistry 134: 78-86. 2017. Koyama, K., H. Kamigakiuchi, K. Iwashita, **R. Mochioka**, N. Goto-Yamamoto.

• Distribution of *Vitis flexuosa* Thunb., wild grape native to Japan, in Okinawa Island. Journal of ASEV Japan27 : 105-110. 2016. **Mochioka,R.** 

• The registration of the new red wine cultivar 'Kadainou R-1'. Technical Bulletin of Faculty of Agriculture, Kagawa University66: 5-16. 2014. **Mochioka, R.** 

- 'KadainouR-2'. The registered wine grape cultivar of MAFF. No/25537. 2016. Mochioka, R.
- 'KadainouR-1'. The registered wine grape cultivar of MAFF. No.13646. 2006. Mochioka, R.





Key ward: kiwifruit, environmental stores, Prunus, self-incompatibility, pollen

#### **Recent Research**

#### 1. Environmental stress tolerance in Actinidia species

There are increasing requirements for crop tolerance to the environmental stress. Some of the wild *Actinidia* species are known for their resistance to high or low temperature, drought, and flooding. We will analyze the mechanism of these traits to develop their use in kiwifruit production.

#### 2. Self-incompatibility in *Prunus* species.

Some of the *Prunus* species exhibit selfincompatibility, which is an obstacle in efficient fruit production. We are investigating the underlying molecular mechanism of this trait and trying to develop the artificial control method.

#### **3** . Molecular manipulation of pollen and its utilization to the fruit tree breeding

The breeding of fruit tree is an intensive work. We attempt to apply molecular techniques like selection and genome editing to pollen of fruit trees. We aim to develop the labor-saving breeding method and its use in fruit tree species.



(Molecular treatment before pollination)

#### **Publications**

- Ono, K. et al (2018) Genome re-sequencing of diverse sweet cherry (*Prunus avium*) individuals reveals a modifier gene mutation conferring pollen-part self compatibility. Plant Cell Physiol. 59: 1265-1275.
- Ono, K. et al (2021) Characterization of a gene coexpression network associated with *MGST*, the pollen modifier gene of gametophytic self incompatibility in sweet cherry (*Prunus avium* L.). Acta Hortic. 1312, 9-16
- Ono, K. et al (2021) Antisense suppression of the expression of the pollen-part genes to breakdown self-incompatibility in *Prunus*. Hort. Res. (Japan) 20 (Suppl. 1): 56.
- Ono, K. et al (2021) Characterization of the molecular function of MGST, the pollen-part modifier of the *Prunus* specific self-incompatibility. Hort. Res. (Japan) 20 (Suppl. 2): 265.

Stress on Temperature, Light, Water ...



