# 2.3 DIVISION OF APPLIED LIFE SCIENCES

Division of Applied Life Sciences was established in 1997 by merging Department of Agricultural Chemistry (founded in 1924), Department of Food Science and Technology (founded in 1967), and a part of Pesticide Research Institute (founded in 1963). In 2001, it was divided into the two current divisions; Division of Applied Life Sciences and Division of Food Science and Biotechnology.

The present division focuses on sciences and technologies concerning microorganisms, animals, and plants, both from basic and applied aspects. Educational and research programs in the fields of physical chemistry, organic chemistry, biochemistry and molecular biology are given.

Professor Takaaki Nishioka of Laboratory of Biofunction Chemistry, Chair of Bioorganic and Biophysical Chemistry, retired on 31 March, 2008.

# Chair of Applied Biochemistry

# 2.3.1 Laboratory of Cellular Biochemistry

Staff Professor : Ueda, Kazumitsu, Ph.D. Associate Professor: Kioka, Noriyuki, Ph.D. Assistant Professor : Matsuo, Michinori, Ph.D. Assistant Professor : Kimura, Yasuhisa, Ph.D. Postdoctoral fellow : Kodan Atsushi, Ph. D. Students and research fellows Doctor's program: (6) Master's program: (13)

# A. Research activities (2007.4-2008.3) A-1. Main subjects

Undergraduate : (5)

a) ABC proteins: their physiological functions and molecular mechanisms

ATP-binding cassette superfamily proteins (ABC proteins) are membrane protein family, which have two highly conserved ATP binding domains in a molecule. ABC proteins are important for various cellular functions, which are involved in host defense mechanisms, glucose homeostasis, and lipid homeostasis. ABC proteins have divergent functions and can be classified as transporters, channels, and receptors, although their predicted secondary structures are very much alike. We are studying physiological functions of ABC proteins and molecular mechanisms of their functional diversity.

 b) Molecular mechanism of xenobiotic efflux pumps MDR1, MRP1, and MRP2 MDR1/P-glycoprotein is a physiologically important ABC protein in limiting the uptake of toxic compounds from the gastrointestinal tract, stimulating their excretion from the liver, kidney, and intestine, and moreover protecting the brain by functioning as a blood-brain barrier. MRP1 and MRP2 are also physiologically important ABC proteins, which extrude xenobiotics after conjugated with glutathione and glucuronate. To understand the mechanism of drug efflux by these ABC proteins and to overcome multidrug resistance of cancer cells by preventing their function, we are studying molecular mechanisms how these ABC proteins transport a wide variety of compounds and how they carry their substrates across membranes by using the energy of ATP hydrolysis.

#### c) Molecular mechanism of ATP-sensitive potassium channels

Pancreatic 6-cell ATP-sensitive potassium (K<sub>ATP</sub>) channels play an important role in the regulation of glucose-induced insulin secretion. The6-cell K<sub>ATP</sub> channel comprises two subunits, the sulfonylurea receptor SUR1, a member of ABC proteins, and Kir6.2, a channel pore subunit. We have analyzed properties of the two NBFs of SURs and proposed that SUR1 is not a transporter but a switch, like a G-protein, and is a sensor monitoring changes in intracellular ADP concentration. We are analyzing ATP hydrolysis properties of SURs and comparing with those of other ABC proteins to reveal how K<sub>ATP</sub> channels are regulated by intracellular ATP and ADP concentrations.

d) ABC proteins involved in fatty acid and cholesterol homeostasis

Many ABC proteins are involved in lipid homeostasis. ABCA1 mediates release of cellular cholesterol and phospholipids to form high density lipoprotein (HDL). Cholesterol is not catabolized in the peripheral cells and therefore mostly released and transported to the liver for conversion to bile acids to maintain cholesterol homeostasis. Although it is clear that ABCA1 plays a critical role in HDL generation, the molecular mechanism of ABCA1 remains unclear. We are analyzing ATP hydrolysis properties and post-transcriptional regulation of ABC proteins involved in lipid homeostasis to reveal physiological roles of ABC proteins in lipid homeostasis.

e) Functional analysis of focal adhesion proteins on cell migration, cell proliferation and tumor metastasis.

Cell adhesion to extracellular matrix regulates various cellular events, including cell proliferation, survival, differentiation, and migration, in a coordinated manner with growth factor signalings. We have shown that a focal adhesion protein vinexin is involved in regulation of cell adhesion, cytoskeletal organization, and anchorage-dependent cell signaling. Our goal is to understand this coordination of cell adhesion and growth factor signalings using methods of molecular biology and cell biology.

### A-2. Publication and presentations

#### a) Publications

### **Original papers**

- Sano, O., A. Kobayashi, K. Nagao, K. Kumagai, N. Kioka, K. Hanada, K. Ueda and M. Matsuo. Sphingomyelin-dependence of cholesterol efflux mediated by ABCG1. J Lipid Res 48(11); 2377-2384, 2007
- Tachibana, S., M. Hirano, T. Hirata, M. Matsuo, I. Ikeda, K. Ueda and R. Sato. Cholesterol and Plant Sterol Efflux from Cultured Intestinal Epithelial Cells Is Mediated by ATP-Binding Cassette Transporters. Biosci Biotechnol Biochem 71(8); 1886-1895, 2007

Morita, S.-y., A. Kobayashi, Y. Takanezawa, N. Kioka, T. Handa, H. Arai, M. Matsuo and K. Ueda.

Bile Salt-Dependent Efflux of Cellular Phospholipids Mediated by ATP Binding Cassette Protein B4. Hepatology 46; 188-199, 2007

- Nagao, K., K. Takahashi, K. Hanada, N. Kioka, M. Matsuo and K. Ueda. Enhanced apoA-I-dependent cholesterol efflux by ABCA1 from sphingomyelin-deficient CHO cells. J Biol Chem 282; 14868-14874, 2007
- Mitsushima, M., K. Ueda and N. Kioka. Involvement of phosphatases in the anchorage-dependent regulation of ERK2 activation. Exp Cell Res 313; 1830-1838, 2007
- Kimura, Y., N. Kioka, H. Kato, M. Matsuo and K. Ueda. Modulation of drug-stimulated ATPase activity of human MDR1/P-glycoprotein by cholesterol. Biochem J 401; 597-605, 2007
- Momma, K., Y. Masuzawa, N. Nakai, M. Chujo, A. Murakami, N. Kioka, Y. Kiyama, T. Akita and M. Nagao. Direct interaction of Cucurbitacin E isolated from Alsomitra macrocarpa to actin filament. Cytotechnology 56; 33-39, 2008

#### Reviews

- Kimura, Y., A. Kodan, M. Matsuo and K. Ueda. Cholesterol fill-in model mechanism for substrate recognition by ABC proteins. J Bioenerg Biomembr 39(5-6); 447-452, 2007
- Kimura, Y., S.-y. Morita, M. Matsuo and K. Ueda. Mechanism of multi-drug recognition by MDR1/ABCB1. Cancer Sci 98; 1303-1310, 2007
- Matsuo, M. and K. Ueda. Function of ABCA1 and ABCG1 in cholesterol homeostasis. Membrane 32; 240-246, 2007
- b) Conference and seminar paper presented
- The 2nd FEBS Special Meeting "ATP-Binding Cassette (ABC) Proteins: From Multidrug Resistance Genetic Diseases" : 1 plenary lecture, 6 papers

2007 FASEB Summer Research Conferences: invited lecture

The 30th annual meeting BMB 2007:1 symoisium, 9 papers

- The 2008 Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry: 6 papers
- The 128th Annual Meeting of the Pharmaceutical Society of Japan: 1 symposium, 2 papers
- The 47th Annual Meeting of American Society for Cell Biology: 2 papers

The 65th Annual Meeting of Japanese Canceer Association: 1 paper

The 29th annual meeting of The Membrane Society of Japan: invited lecture

### A-3. Off-campus activities

### Memberships of Academic Societies

- Ueda, K.: The Japan Society for Biosceince, Biotechnology, and Agrochemistry (a member of the board of directors)
- Ueda, K.: The Japanese Cancer Association (Councilor)

Ueda, K.: The Japanese Biochemical Society (Councilor)

#### Research grants

Monbukagakusho research grants: Developmental Scientific Research (B)(2): Study on molecular mechanism of ABC proteins involved in cholesterol homeostasis (Ueda, K.) Creative Scientific Research: Molecular basis of novel transporter proteins (Ueda, K.) The Bio-oriented Technology Research Advancement Institution: Regulation of lipid transporters by high-functional food (Ueda, K.) The Pharmaceutical and Medical Devices Agency: Basic Research Promotion Project (Ueda, K.) Priority Area: Studies of focal

adhesion proteins and the regulation of cancer cell adhesion and migration. (Kioka, N.) Scientific Research (B): Functional roles of a novel membrane cytoskeletal protein vinexin. (Kioka, N.) Grant-in-Aid for Young Scientists (B): Functional analysis of ABC proteins involved in cholesterol efflux (Matsuo, M.) Grant-in-Aid for Young Scientists (B): Structual analysis of the receptor of the sulfonylurea drugs. (Kimura, Y.)

# A-4. International cooperations and overseas activities

# International meetings (roles)

Ueda, K.: 2nd FEBS Special Meeting on ABC Proteins (Vice Organazer, invited lecture)

2007 FASEB Summer Research Conferences: invited lecture

Kioka, N.: ASCB 47th Annual Meeting (presentation)

Matsuo, M.: 2nd FEBS Special Meeting on ABC Proteins (presentation)

# Membership in international academic societies

Ueda, K.: Active member of American Association for Cancer Research

# B. Educational Activities (2007.4-2008.3)

# B-1. On-campus teaching

a) Course given

Undergraduate level: New Strategy of Agricultural Biotechnology (Ueda, K.), Biochemistry I (Ueda, K), Molecular cellular Biology I (Ueda, K. Kioka, N), Molecular Biology II (Ueda, K. Kioka, N), Introductory lecture and laboratory course in Molecular Biology (Kioka, N. Matsuo, M.Kimura,Y.)

Graduate level: advaced Molecular Biology (Ueda, K.), Biochemistry Seminar (Ueda, K. Kioka, N), Experimental Course of Biochemistry (Ueda, K. Kioka, N).

# C. Other remarks.

Matsuo, M.: The Japan Bioscience, Biotechnology and Agrochemistry Award for the Encouragement of Young Scientists from the Japan Society for Bioscience, Biotechnology, and Agrochemistry

# 2.3.2 Laboratory of Biomacromolecular Chemistry

Staff Professor : Ueda, Mitsuyoshi, Dr. Engineering Assistant Professor : Kato-Murai, Michiko, Dr. Agric. Sci. Assistant Professor : Kuroda, Kouichi, Dr. Engineering

Students and research fellows

Doctor's program	: (6)	Research fellow : (1)
Master's program	: (15)	Research student : (1)
Undergraduate	: (5)	

# A. Research Activities (2007.4-2008.3)

#### A-1. Main subjects

a) Creating the life sciences of the future through exploration and analysis of fundamental biological phenomena

Biological phenomena are among the most important and fascinating research themes in the life sciences. We approach our research from the perspective of biochemistry, both basic and applied, which means that we take a chemically based view of biological phenomena and attempt to explain them in chemical terms. Our aim is to uncover the essence of the diverse and complex phenomena observed in humans and other high-level eukaryotic organisms. To do this we use the latest methods to systematically investigate the genes and proteins enclosed in the cellular envelope which are the vehicles of life, the intracellular transmission of various kinds of biological data, and the mechanisms involved in interactions between cells, proteins, and genes. We are also active in applied biotechnology research, which seeks to advance the development and wellbeing of humankind by rapidly converting basic research findings into practical uses.

b) Using genomic information and the latest techniques to analyze complex biological phenomena at molecular level

Biological data transmission systems, which in high-level eukaryotic organisms underpin biological phenomena such as morphogenesis and development, rely on an interdependent series of complex physical and chemical processes involving huge numbers of molecules. Introducing new and systematic analytical techniques alongside conventional biochemical methodology, we attempt to elucidate complex biological processes at molecular level by studying cells from yeasts, *Arabidopsis thaliana*, zebra fish, mouse, and other model eukaryotic organisms in which genomic decoding is advancing.

c) Expanding biological functions through bio- and nano-technology

In order to exploit the functions of living organisms in a wide range of fields, we undertake research which utilizes an understanding of the basic principles of bio-phenomena to modify genomic information and thereby access latent capabilities in living organisms or endow them with novel functions. We led the world in the development of cell-surface engineering, a relevant technique which makes use of the address (signal sequence) information contained in proteins and whose revolutionary approach has allowed the creation of many new cell types. This development has continued with the establishment of a completely new field in biochemistry known as combinatorial bioengineering and through fusion with nanotechnology and other fields to create the concept of nano-biotechnology. Through these, we look forward to creating new bioactive proteins and cells which transcend the limitations of known genomic information.

### A-2. Publications and presentations

a) Publications

#### Original papers

- Fukada, H., J. Mima, M. Nagayama, M. Kato and M. Ueda : Biochemical analysis of the yeast proteinase inhibitor (I<sup>c</sup>) homolog I<sup>c</sup> and its comparison with I<sup>c</sup>. Biosci Biotechnol Biochem 71(2); 472-480, 2007
- Kato, M., J. Fuchimoto, T. Tanino, A. Kondo, H. Fukuda and M. Ueda: Preparation of a whole-cell biocatalyst of mutated *Candida antarctica* lipase B (mCALB) by a yeast molecular display system and its practical properties. Appl Microbiol Biotechnol 75(3); 549-555, 2007
- Shibasaki, S., A. Kawabata, J. Ishii, S. Yagi, T. Kadonosono, M. Kato, N. Fukuda, A. Kondo and M. Ueda: Construction of a novel synergistic system for production and recovery of secreted recombinant proteins by the cell surface engineering. Appl Microbiol Biotechnol 75(4); 821-828, 2007
- Seong, T. K., Y. Katakura, K. Ninomiya, C. Moukamnerd, A. Kondo, M. Ueda and S. Shioya: Enhancement of ethanol production by promoting surface contact between starch granules and arming yeast in direct ethanol fermentation. J Biosci Bioeng 103(1); 95-97, 2007
- Kadonosono, T., M. Kato and M. Ueda: Substrate specificity of rat brain neurolysin disclosed by molecular display system and putative substrates in rat tissues. Appl Microbiol Biotechnol 75(6); 1353-1360, 2007
- Kadonosono, T., M. Kato and M. Ueda: Metallopeptidase, neurolysin, as a novel molecular tool for analysis of properties of cancer-producing matrix metalloproteinases-2 and -9. Appl Microbiol Biotechnol 75(6); 1285-1291, 2007
- Fukuda, N., J. Ishii, S. Shibasaki, M. Ueda, H. Fukuda and A. Kondo: High-efficiency recovery of target cells using improved yeast display system for detection of protein-protein interactions. Appl Microbiol Biotechnol 76(1); 151-158, 2007
- Sakakura, M., S. Kajiyama, M. Tsutsumi, J. Si, E. Fukusaki, Y. Tamaru, S. Akiyama, K. Miura, K. Hirao and M. Ueda: Femtosecond pulsed laser as a micro-scalpel for microdissection and isolation of specific sections from biological samples. J J Appl Physics 46(9A); 5859-5864, 2007
- Fukuda, T., M. Kato-Murai, S. Suye and M. Ueda : Development of high-throughput screening system by single cell reaction using microchamber array chip. J Biosci Bioeng 104(3); 241-243, 2007
- Fukuda, T., M. Kato-Murai, T. Kadonosono, H. Sahara, Y. Hata, S. Suye and M. Ueda: Enhancement of substrate recognition ability by combinatorial mutation of beta-glucosidase displayed on the yeast cell surface. Appl Microbiol Biotechnol 76(5); 1027-1033, 2007
- Fukuda, T., D. Isogawa, M Takagi, M. Kato-Murai, H. Kimoto, H. Kusaoke, M. Ueda and S. Suye: Yeast cell surface expression of chitosanase from *Paenibacilus fukuinensis*. Biosci Biotechnol Biochem 71(11); 2845-2847, 2007
- Okochi, N., M. Kato-Murai, T. Kadonosono and M. Ueda : Design of a serine protease-like catalytic

triad on an antibody light chain displayed on the yeast cell surface. Appl Microbiol Biotechnol 77(3); 597-603, 2007

b) Conference and seminar papers presented

Annual Meeting of the Society for Biotechnology, Japan 2007: 3 reports

International Annual Meeting of the JBS and MBSJ: 3 reports

Annual Meeting of Japan Society for Bioscience, Biotechnology and Agrochemistry 2008: 14 reports

7<sup>th</sup> Annual Meeting of Protein Science Society of Japan: 2 reports

The 128th Annual Meeting of the Pharmaceutical Society of Japan: 1 report

The 18<sup>th</sup> conference of the Society for Chromatographic Sciences: 1 report

### A-4. International cooperations and overseas activities

#### International meetings (roles)

Ueda, M.: International Conference of Combinatorial Bioengineering (President)

### B. Educational Activities (2007.4-2008.3)

### B-1. On-campus teaching

a) Courses given

Undergraduate level: General Biomacromolecular Chemistry (Ueda), Structure and Function of Biomacromolecules (Ueda), Reading of Literatures I (Ueda), Reading of Literatures II (Ueda), Introduction to Applied Life Sciences III (Ueda), Applied Life Sciences (Ueda), Experiments of Biomacromolecular Chemistry (Ueda, Kato-Murai and Kuroda)

Graduate level: Biomacromolecular Chemistry (Ueda), Experiments of Biomacromolecular Chemistry (Ueda, Kato-Murai and Kuroda)

# 2.3.3 Laboratory of Bioregulation Chemistry

Staff Professor : Miyagawa, Hisashi, Dr. Agric. Sci. Associate Professor: Nakagawa, Yoshiaki, Dr. Agric. Sci.

Assistant Professor : Miyashita, Masahiro, Dr. Agric. Sci.

Students and research fellows

Research fellow :(1)	Doctor's program: (3)
Master's program: (5)	Undergraduate :(4)

# A. Research Activities (2007.4-2008.3)

### A-1. Main subjects

a) Metabolism of Plant Hormone Auxin

Indole-3-acetic acid (IAA) is a plant hormone auxin that plays an important regulatory role in plant growth and development. In order to elucidate the species differences in the metabolic pathways of IAA among gramineous plants, IAA metabolite profile in maize was compared with that of rice. Quantitative analysis of metabolites using LC-MS/MS showed that IAA mainly underwent successive oxidation at C-2 and C-7 of IAA, followed by the glucosylation at the resulting 7-hydroxy group in maize seedlings. Major metabolites in rice seedlings such as N-glucosyl IAA and its aspartate- and glutamate-conjugates were detected only in a trace amount, giving contrast between the metabolite profiles in these two plants.

b) Structure-Activity Relationships of Ecdysone Agonists

Insect molting is regulated by a steroidal hormone, 20-hydroxyecdysone in vivo. In plants a number of ecdysone agonists are identified, and ponasterone A is known as the most potent ecdysone agonist. Ponasterone A analogs with various steroid moiety were chemically synthesized and their binding activity to ecdysone receptor of Drosophila cells (Kc cells) was measured. Introduction of functional groups such as hydroxyl and oxo groups to A and B-rings of steroid enhanced the ligando-receptor binding. Non steroidal ecdysone agonists such as *N*-benzoyl tetrahydroquinoline and oxazoline-type analogs were chemically synthesized and their binding acyivity to Lepidoptera and Diptera ecdysone receptors.

c) Chemistry of bioactive peptides.

i) Plants induce various defense responses when they are attacked by pathogens. These defense responses are triggered by a variety of molecules (elicitors). In order to search for new elicitors, random hexapeptide libraries were prepared using a technique of combinatorial chemistry, and the active compounds were screened by an assay based on the response in cultured tobacco cells. Consequently, active peptides,YGIHTH, were obtained. Structure-activity relationship study showed that the *N*-terminal 4 residues were essential for the activity.

ii) An insecticidal peptide was isolated from the venom of the Japanese scorpion *Isometrus maculatus*, and its amino acid sequence was determined by Edman degradation of the intact and fragment peptides obtained by partial enzymatic digestion. Since deduced amino acid sequence had homology with the antimicrobial scorpion toxins, the antimicrobial activity of the peptides was examined. As a result, the peptide showed the significant antimicrobial activity in addition to the insect toxicity.

### A-2. Publications and presentations

#### a) Publications

#### Reviews

- Nakagawa, Y.: Molting hormone analogs, In Hormone Handbook. eBook. Nankoudou, III-B-7-b, 2007 (in Japanese)
- Nakagawa, Y.: Structure-activity relationship and mode of action study of insect growth regulators. J Pestic Sci 32, 143-150, 2007 (in Japanese)
- Nakagawa, Y., and H. Miyagawa: New Insecticides in "Shokubutsu-wo-mamoru", 123-150, 2008 (in Japanese)

#### Original papers

- Dubouzet, J. G., A. Ishihara, F. Matsuda, H. Miyagawa, H. Iwata and K. Wakasa: Integrated metabolomic and transcriptomic analyses of high-tryptophan rice expressing a mutant anthranilate synthase alpha subunit, J Exp Bot 58; 3309-3321, 2007
- Ishihara, A., F. Matsuda, H. Miyagawa and K. Wakasa: Metabolomics for metabolically manipulated plants: effects of tryptophan overproduction, Metabolomics 3; 319-334, 2007
- Kai, K., S. Nakamura, K. Wakasa and H. Miyagawa: Facile preparation of deuterium-Labeled standards of indole-3-acetic acid (IAA) and its metabolites to quantitatively analyze the

disposition of exogenous IAA in Arabidopsis thaliana, Biosci Biotechnol Biochem 71; 1946-1954, 2007

- Kai, K., K. Wakasa and H. Miyagawa: Metabolism of indole-3-acetic acid in rice: Identification and characterization of N-6-D-glucopyranosyl indole-3-acetic acid and its conjugates, Phytochemistry. 68; 2512-2522, 2007
- Kai, K., J. Horita, K. Wakasa and H. Miyagawa: Three oxidative metabolites of indole-3-acetic acid from Arabidopsis thaliana, Phytochemistry 68; 1651-1663, 2007
- Matsuda, F., K. Wakasa and H. Miyagawa: Metabolic flux analysis in plants using dynamic labeling technique: Application to tryptophan biosynthesis in cultured rice cells, Phytochemistry 68; 2290-2301, 2007
- Matsushita, N., M. Miyashita, A. Sakai, Y. Nakagawa and H. Miyagawa: Purification and characterization of a novel short-chain insecticidal toxin with two disulfide bridges from the venom of the scorpion *Liocheles australasiae.* Toxicon 50; 861-867, 2007
- Minakuchi, C., T. Ogura, H. Miyagawa and Y. Nakagawa.: Effects of the structures of ecdysone receptor (EcR) and ultraspiracle (USP) on the ligand-binding activity of the EcR/USP heterodimer. J Pest Sci 32; 379-384, 2007
- Miyashita, M., J. Otsuki, Y. Hanai, Y. Nakagawa and H. Miyagawa: Characterization of peptide components in the venom of the scorpion *Liocheles australasiae* (Hemiscorpiidae). Toxicon 50; 428-437, 2007
- Nakagawa, Y., A. Sakai, F. Magata, T. Ogura, M. Miyashita and H. Miyagawa: Molecular cloning of the ecdysone receptor and the retinoid X receptor from the scorpion *Liocheles australasiae.* FEBS J 274; 6191-6203, 2007
- Tamaki, H., A. Shimada, Y. Ito, M. Ohya, J. Takase, M. Miyashita, M. Miyagawa, H. Nozaki, R. Nakayama and H. Kumagai: LPT1 encodes a membrane-bound O-acyltransferase involved in the acylation of lysophospholipids in the yeast Saccharomyces cerevisiae, J Biol Chem 282, 34288-34298; 2007

### **Proceedings and Reports**

- Nakagawa, Y.: Structure-activity relationship and mode of action study of insect growth regulators. J Pestic Sci 32, 135-136, 2007
- Miyagawa, H., and Ueyama, I., Summary of Scientific Programs in 11th IUPAC International Congress of Pesticide Chemistry, in Pesticide Chemsitry, Ohkawa, H., Miyagawa, H., and Lee, P. W., Eds., Wiley-VCH, Weinheim, pp. 459-478, 2007
- b) Conferences and seminar papers presented

The 33rd Annual Meeting of Pesticide Science Society of Japan: 9 reports

- Annual Meeting of the Japan Society for Bioscience, Biotechnology, and Agrochemistry 2008: 6 reports
- Japan Society for Bioscience, Biotechnology, and Agrochemistry (Kansai-Chubu Branch Meeting): 1 report
- The 44<sup>th</sup> Peptide Meeting: 2 report
- The 55th Annual Conference on Mass Spectrometry: 1 report

# A-3. Off-campus activities

#### Membership in academic societies (roles)

Miyagawa, H.: Japan Society for Pesticide Science (chief editor), Japan Society for Bioscience,

Biotechnology, and Agrochemistry (councilor of Kansai branch). The 33rd Annual Meeting of Pesticide Science Society of Japan (organizing comitee)

- Nakagawa, Y.: The Division of Structure-Activity Studies, The Pharmaceutical Society of Japan (board member, treasurer), Japan Society for Pesticide Science (editorial board member, councilor), Japan Society for Bioscience, Biotechnology, and Agrochemistry (editorial board member), Japan Society for Bioscience, Biotechnology, and Agrochemistry-Kansai Branch (general officer), The 33rd Annual Meeting of Pesticide Science Society of Japan (organizing comitee)
- Miyashita, M.: The Mass Spectrometry Society of Japan (training planning committee member), The 33rd Annual Meeting of Pesticide Science Society of Japan (organizing comitee)

### Research grants

- Monbukagakusho Research Grant: Encouragement of Young Scientists (B): Screening for plant defense activating peptides from combinatorial peptide Libraries (Miyashita).
- Others: Development of the highly sensitive mass spectrometer and the analysis of endocrine disruptor (Miyashita, member). The 21st century COE program for Innovative Food and Environmental Studies Pioneered by Entomomimetic Sciences, from the Ministry of Education, Culture, Sports, Science and Technology of Japan (Nakagawa, Miyagawa, member).

# A-4. International cooperations and overseas activities

### International joint researches, overseas research surveys

Nakagawa, Y.: Structure-activity Relationship of Ecdysone Agonists (Belgium, Greece, USA)

### B. Educational Activities (2007.4-2008.3)

### B-1. On-campus teaching

a) Courses given

- Undergraduate level: Bioorganic Chemistry I (Miyagawa, Nakagawa), Organic Reaction Mechanism II (Nakagawa), Laboratory Course in Bioorganic Chemistry (Miyagawa, Nakagawa, Miyashita), Structure Analysis of Organic Compounds (Miyagawa), Food Safety II (Miyagawa), Experimental Course in Division of Applied Life Sciences (Nakagawa, Miyashita)
- Graduate level: Bioregulation Chemistry Seminar (Miyagawa, Nakagawa, Miyashita), Experimental Course in Bioregulation Chemistry (Miyagawa, Nakagawa, Miyashita).

### B-2. Off-campus teaching, etc.

#### Part-time lecturer

Miyagawa, H.: Graduate School of Bioresource & Bioenvironmental Scienes, Kyushu University (Special lecture on bioregulation chemistry), Graduate School of Engineering, Fukuoka Institute of Technology (Special lecture on functional materials engineering), School of Life and Environmental Sciences, Osaka Prefecture University (Molecular design)

Nakagawa, Y.: Faculty of Agriculture, Kyoto Prefectural University (Industrial organic chemistry)

### C. Other Remarks

Miyagawa, H.: Chief Manager of Radio Isotope Experiments at College of Agriculture; Member of

# 2.3.4 Laboratory of Chemical Ecology

Staff Professor : Nishida, Ritsuo, D. Agric. Sci. Associate Professor: Mori, Naoki, D. Agric. Sci. Assistant Professor : Ono Hajime, D. Agric. Sci. Postdoctoral fellows: Ayako Katsumata, D. Agric. Naoko Yoshinaga, D. Agric. Sci.

Students and research fellows

Doctor's program: (2) Master's program: (10) Undergraduate : (3)

# A. Research Activities (2007.4-2008.3) A-1. Main subjects

a) Flavonol glycosides as probing stimulants of pest aphids

A series of flavonoid glycosides including kaempferol 7-O- $\alpha$ -rhamnopyranoside were characterized from leaves of the broad bean, *Vicia faba*, as a major phytochemical cue stimulating probing behavior in a pest aphid, *Aphis craccivora*, which infest selectively on plants in the family Fabaceae, causing serious damages on bean crops. A solution of the glycosides induced a specific probing response equivalent to that of a crude extract from *V. faba*. Several related flavonoid glycosides having quercetin aglycone, however, exhibited much less activity, suggesting the structural selectivity in the process of the settling on the host plants in these aphid species.

b) Fatty acid amides, found in the cricket and fruit fly larvae

Fatty acid amides (FAAs) are known elicitors that induce plants to release volatile compounds that, in turn, attract foraging parasitoids. Since the discovery of volicitin  $[N^{-}(17\text{-hydroxylinolenoyl})\text{-L-glutamine}]$  in the regurgitant of larval *Spodoptera exigua*, a series of related FAAs have been identified in several other species of lepidopteran caterpillars. We screened 13 non-lepidopteran insects for the presence of FAAs and found that these compounds were present in adults of two closely related cricket species, *Teleogryllus taiwanemma* and *T. emma* (Orthoptera: Gryllidae), and larvae of the fruit fly, *Drosophila melanogaster* (Diptera: Drosophilidae). Although these FAAs were not found in ten of the insects studied here, their identification in two additional orders of insects suggests that FAAs are more common than previously reported and may have physiological roles in a wide range of insects besides caterpillars.

c) Developmental timing regulated by insect hormones in *Drosophila melanogaster* 

In insects, developmental transitions, such as molting and metamorphosis, are regulated by the insect steroid hormones, 20-hydroxyecdysone (20E). The neuropeptide PTTH is a key regulator of 20E production and release from the prothoracic gland in insects. Cell ablation and loss-of-function analysis show that PTTH directs proper temporal progression through larval and pupal stages and contributes to the determination of final body size by regulating the timing of 20E production through control of gene expressions of several ecdysone biosynthesis enzymes in *Drosophila*.

# A-2. Publications and presentations

a) Publications

# Book

Nishida, R.: Arms race and harmonization between insects and plants. 21 Century Agriculture based on Bioresources (Vol. 3) How to protect plants (edited by S. Sakuma), pp.83-122, Kyoto University Press, Kyoto, 2007 (in Japanese)

# Original papers

- Tan, K.H. and R. Nishida: Zingerone in floral synomone of *Bulbophyllum baileyi* (Orchidaceae) attracts *Bactrocera* fruit fly during pollination. Biochem Syst Ecol 35; 334-341, 2007
- Wee, Suk Ling, K.H. Tan and R. Nishida: Pharmacophagy of methyl eugenol by males enhances sexual selection of *Bactrocera carambolae* (Diptera: Tephritidae). J Chem Ecol 33; 1272-1282, 2007
- Yoshinaga, N., T. Aboshi, C. Ishikawa, M. Fukui, M. Shimoda, R. Nishida, C. G. Lait, J. H. Tumlinson and N. Mori: Fatty acid amides, previously identified in caterpillars, found in the cricket *Teleogryllus taiwanemma* and fruit fly *Drosophila melanogaster* larvae. J Chem Ecol 33; 1376-1381, 2007
- Kuwahara, Y., N. Mori and T. Tanabe: Detection of a neotropical frog alkaloid spiropyrrolizidine 236 from a Japanese polyzoniid millipede *Kiusiozonuim okai* as a major defense component together with polyzonimine and nitropolyzonamine. Jpn J Entomol Zool 18; 91-95, 2007
- Noge, K., M. Kato, N. Mori, M. Kataoka, C. Tanaka, Y. Yamasue, R. Nishida and Y. Kuwahara: Geraniol dehydrogenase, the key enzyme in biosynthesis of the alarm pheromone, from the astigmatid mite *Carpoglyphus lactis* (Acari: Carpoglyphidae). FEBS J 275; 2807-2817, 2008
- McBrayer, Z., H. Ono, M.J. Shimell, J.P. Parvy, R.B. Beckstead, J.T. Warren, C.S. Thummel, C. Dauphin-Villemant, L.I. Gilbert and M.B. O'Connor: Prothoracicotropic hormone regulates developmental timing and body size in *Drosophila*. Dev Cell 13; 857-871, 2007

### Review

- Yoshinaga, N. and N. Mori: Insect-produced elicitor, volicitin Physiological functions in insects - . Kagaku to Seibutsu 45: 411-418, 2007 (in Japanese)
- b) Conference and seminar papers presented

The 23rd ISCE Annual Meeting, Yena, Germany (2007): 2 papers

The 4<sup>th</sup> Asia-Pacific Conference on Chemical Ecology, Tsukuba, Japan (2007): 11 papers

The annual meeting for Environmental Entomology and Zoology (2007): 1 paper

The 52th The Japanese Society of Applied Entomology and Zoology (2008): 1 paper

Japan Society for Bioscience, Biotechnology, and Agrochemistry (2008): 4 papers

The 8th Japanese Drosophila Research Conference, Awaji, Japan: 1 paper

# A-3. Off-campus activities

# Membership in academic societies (roles)

Ritsuo Nishida: Japanese Society of Applied Entomology and Zoology (councilor,)

Naoki Mori: Japanese Society of Applied Entomology and Zoology (editor), Japanese Society of Environmental Entomology and Zoology (managing editor), The Acarological Society f Japan (executive committee, editor)

# Research grants

- Monbukagakusho Research Grant: Integrated Research (B) (2), (Nishida: representative). Integrated Research (B) (2), Molecular chemical ecological approach for coevolutionary process between insects and plants (Nishida: representative). Research Grant: Integrated Research (C) (2), Insect-produced elicitor, volicitin Physiological functions in insects . (Mori: representative). Research Grant: Integrated Research (C) (2), Molecular basis of counter-adaptation by agricultural pests against chemical defense of host plants. (Mori: member). Grant-in-Aid for Young Scientists (Start-up) (Ono: representative).
- 21st Century COE program: COE for Innovative Food and Environmental Studies Pioneered by Entomomimetic Sciences (Nishida, Mori)

# A-4. International cooperations and overseas activities

# Membership in international academic societies (roles)

Nishida, R.: Asia-Pacific Association of Chemical Ecologists (President), The 4th Asia-Pacific Conference on Chemical Ecology (Vice chairman of the organizing committee), "Biochemical Systematics and Ecology" (Editorial advisory board), "Chemoecology" (Editorial advisory board), "Applied Entomology and Zoology" (Editorial board)

### International cooperation

- Nishida, R.: Chemical ecology on fruit fly attractants (Malaysia, Thailand, Laos, Papua New Guinea, USA)
- Mori, N.: Biosynthesis of insect-derived elicitors (USA), Physiological changes induced by insect-derived elicitors in plants (New Zealand)

# B. Educational Activities (2007.4-2008.3)

# B-1. On-campus teaching

- Undergraduate level: Bioorganic chemistry III (Nishida, Mori), Organic Reaction Mechanisms I (Mori), Structure analyses of organic compounds (Nishida), Laboratory Course in Bioorganic Chemistry (Mori, Ono).
- Graduate level: Chemical Ecology (Advanced Course) (Nishida, Mori), Laboratory Course in Chemical Ecology (Nishida, Mori, Ono), Chemical Ecology Seminar (Nishida, Mori, Ono)

# B-2. Off-campus teaching

### Part-time lecturer

Nishida, R.: University of Tsukuba (Graduate School of Life and Environmental Sciences)

# Chair of Molecular and Cellular Sciences

# 2.3.5 Laboratory of Plant Nutrition

Staff Professor : Matoh, Toru, Dr. Agric. Sci. Associate Professor : Kobayashi, Masaru, Dr. Agric. Sci. Research Associate : Ochiai, Kumiko, Dr. Agric. Sci.

Students and research fellows

Doctor's program : (3) Master's program : (3) Undergraduate : (4) Research student: (2)

# A. Research Activities (2007.4-2008.3)

### A-1. Main subjects

a) Functions of inorganic constituents in plant cell walls.

Boron and calcium are the major inorganic elements in cell walls, and they are likely to contribute to cell wall integrity. We have demonstrated that B cross-links two pectic chains at the rhamnogalacturonan II (RG-II) regions, and that Ca strengthens the bonding together. We will study the function of cell walls in terms of the function of inorganic elements which are localized there.

Tolerance mechanism of rice plants toward excessive B in soils is also our subject. Genetic difference between rice varieties tolerant to and sensitive to excessive B has been examined.

b) Nitrogen-use efficiency of rice plants.

We have studied the mechanism underlying difference of the nitrogen use efficiency among rice varieties to breed an efficient variety which is suitable for sustainable agriculture.

c) Sustainable agriculture.

We are trying to find out a suitable chemical fertilizer to develop sustainable, low-input and consumer-conscious farming. We also try to establish a method to evaluate the quality of fermented manure.

### A-2. Publications and presentations

a) Publications

### **Original** papers

- Matsuda, A., K. Ohno, T. Nagakubo and T. Matoh: Labeling of food industry sludge compost with <sup>15</sup>N and its availability to Italian ryegrass (*Lolium multiflorum* L.) plants. Jpn J Soil Sci Plant Nutr 78; 317-321, 2007
- Matsuda, A., K. Yamazaki and T. Matoh: Evaluation of nitrogen availability of animal manure composts based on chemical analysis and pot experiments. Jpn J Soil Sci Plant Nutr 78; 476-485, 2007

### Books and review articles

Matoh, T.: Chemical fertilizers versus composted manure. Kagaku to Seibutsu 45; 426-429, 2007

- Ochiai, K.: Mechanism for excessive B operating in crop plants. Kagaku to Seibutsu 45; 449-450, 2007
- Matoh, T.: "Nitrogen cycling and food production" in "Agriculture in the 21th century", Chapter 5, Y. Yamasue editor, Kyoto University Press, pp.131-152, (2007)
- b) Conference and seminar papers presented
- Annual Meeting of the Japanese Society of Plant Physiologists, 2008: 2 reports

Annual Meeting of Japanese Society of Soil Science and Plant Nutrition, 2007: 4 reports

# A-3. Off-campus activities

# Membership in academic societies (roles)

- Matoh, T.: Japanese Society of Soil Science and Plant Nutrition (Board member, Chairperson of the 4th Committee, Editor)
- Kobayashi, M.: Japanese Society of Plant Physiologists (Treasurer)

# Research grants

- Matoh, T.: General Scientific Research (B) (2) Environmental evaluation of the export-oriented farming in the tropical countries, General Scientific Research (B) (2) Molecular breeding of an excessive-B tolerant rice.
- Kobayashi, M.: Grant-in-Aid for Young Scientists (B), Analysis of physiologyical responses to boron deprivation in plants. Grant-in-Aid for Scientific Research on Priority Areas (Plant Nutrition and Transport), Role for Plant Cell Walls in Nutrient Uptake (research member).

# A-4. International cooperations and overseas activities

### International meetings (roles)

Matoh, T.: Organizing Committee for International Boron Symposium 2009

Kobayashi, M.: Gordon Research Conference on Plant Cell Walls, Biddeford, Maine USA, July 2006.

### International joint researches, overseas research surveys

Matoh, T.: Grant-in-Aid for International Scientific Research, Sustainable development of Chaopraya delta farming (Kasetsart University), Studies of sustainable development in the mountain area of Laos.

# B. Educational Activities (2007.4-2008.3)

# B-1. On-campus teaching

a) Course given

- Undergraduate level: Biochemistry 2 (Matoh), Plant Nutrition (Matoh), Plant Biochemistry (Matoh, Kobayashi), Laboratory Course in Plant Biochemistry (Matoh, Kobayashi), Stress Physiology in Plants (Matoh)
- Graduate level: Advanced Cource in Plant Biochemistry (Matoh, Kobayashi), Experimental Course in Plant Nutrition (Matoh, Kobayashi), Plant Nutrition Seminar (Matoh, Kobayashi)

# B-2. Off-campus teaching. etc.

# Part-time lecturer

Matoh, T.: Faculty of Agriculture, Kyoto Prefectural University (Plant Nutrition 1,2), Graduate School of Agriculture, Shimane University (Plant Nutrition)

# C. Other Remarks

Matoh, T.: Advisory member for Commitee for Promoting Sustainable Agriculture, Survey Commitee for Dioxins (Kyoto City), Technical advisor of the Kyoto Organic Farmers' Association

# 2.3.6 Laboratory of Molecular Microbiology

Staff Professor : Kita, Keiko, Dr. Agric. Sci. Associate Professor: Inoue, Yoshiharu, Dr. Agric. Sci. Assistant Professor : Izawa, Shingo, Dr. Agric. Sci. Postdoctoral fellow : Nagao, Jun-ichi, Dr. Agric. Sci.

Students

Doctor's program: (2) Master's program: (3) Undergraduate : (3)

# A. Research Activities (2007.4-2008.3) A-1. Main subjects

a) Structure and function analysis of restriction endonucleases

Restriction endonuclease EcoT38I isolated from *Escherichia coli* TH38 was found to cleave sequences which are similar, but not identical, to their defined recognition sequences under nonoptimal conditions. X-ray structure of native EcoT38I was solved to 2Å resolution and MAD data with heavy atom derivatives were collected. A mutant enzyme of EcoO109I restriction endonuclease of *Escherichia coli* H709c, constructed on the basis of structural information of the DNA-protein complex, shows high-fidelity activity under non-standard conditions. In order to clarify structure-function relationship, EcoO109I mutant enzyme was cocrystallized with cognate DNA and native data were collected.

b) Green tea polyphenols function as prooxidants to activate oxidative stress-responsive transcription factors in yeasts

Epigallocatechin gallate (EGCG) is the most abundant polyphenolic flavonoid in green tea. Catechin and its derivatives, including EGCG, are widely believed to function as antioxidants. Here we demonstrate that both EGCG and green tea extract (GTE) cause oxidative stress-related responses in the budding yeast *Saccharomyces cerevisiae* and in the fission yeast *Schizosaccharomyces pombe* under weak alkaline conditions in terms of the activation of the oxidative stress-responsive transcription factors. GTE as well as EGCG induced the nuclear localization of Yap1 in *S. cerevisiae*, which was repressed by addition of catalase but not of superoxide dismutase. The same phenomena were observed for the nucleocytoplasmic localization of Msn2 in *S. cerevisiae* and Pap1, a Yap1 homologue in *S. pombe*. The formation of intramolecular disulfide bonds has been proposed to be crucial for the  $H_2O_2$ -induced nuclear localization of Yap1, and we verified the importance of cysteine residues of Yap1 in the response to EGCG and GTE. Additionally, we show that EGCG and GTE produce  $H_2O_2$  in a weak alkaline medium. Finally, we conclude that tea polyphenols is able to act as prooxidants to cause a response to oxidative stress in yeasts under certain conditions.

c) Quality improvement of microorganisms by soy peptides

The physiological functionality of soy peptides in mammal has been well investigated. It has been reported that soy peptides show various potencies such as scavenging oxidative stress, reducing serum lipid levels, and anti-fatigue effects in mammalian cells. Therefore, soy peptides have been attracting worldwide attention as functional food ingredients. In contrast to the recent progress in the study on mammalian physiology, effects of soy peptides on industrial microorganisms remain to be solved. To promote more effective use of soy peptides in different ways, we examined their validity as ingredients of culture media to improve the quality of food microorganisms. In this study, we show that the cultivation of yeast cells in media containing soy peptides can improve tolerance to freeze-thaw stress, indicating that soy peptides are suitable ingredients of culture media to provide high-quality yeast cells for frozen-dough technology. The baked bread using yeast cells cultured with soy peptides was of better quality in terms of texture and volume than the bread using cells with casein peptone. We further investigated the mechanisms of the improved tolerance to freeze-thaw stress by soy peptides. Compared with yeast cells cultured with casein peptone, cells cultured with soy peptides showed higher levels of intracellular proline, valine, and aspartic acid. Contrary, the cultivation with soy peptides resulted in decreased levels of intracellular alanine and arginine. Additionally, yeast cells cultured with soy peptides showed the decreased number and size of lipid particles.

### A-2. Publications and presentations

a) Publications

#### Original papers

- Maeta, K., W. Nomura, Y. Takatsume, S. Izawa and Y. Inoue: Green tea polyphenols function as prooxidants to activate oxidative stress-responsive transcription factors in yeasts. Appl Environ Microbiol 73(2); 572-580, 2007
- Inoue, Y., W. Nomura, Y. Takeuchi, T. Ohdate, S. Tamasu, A. Kitaoka, Y. Kiyokawa, H. Masutani, K. Murata, Y. Wakai, S. izawa and J. Yodoi: Efficient extraction of thioreodxin from *Saccharomyces cerevisiae* with ethanol. Appl Environ Microbiol 73(5); 1672-1675, 2007
- Izawa, Y., K. Ikeda, N. Takahashi and Y. Inoue: Improvement of tolerance to freeze-thaw stress of baker's yeast by cultivation with soy peptides. Appl Microbiol Biotechnol 75(3); 533-537, 2007
- Takeuchi, Y., W. Nomura, T. Ohdate, S. Tamasu, H. Masutani, K. Murata, S. Izawa, J. Yodoi and Y. Inoue: Release of thioredoxin from *Saccharomyces cerevisiae* with environmental stimuli: solubilization of thioredoxin with ethanol. Appl Microbiol Biotechnol 75(6); 1393-1399, 2007
- Izawa, S., T. Kita, K. Ikeda, T. Miki and Y. Inoue: Formation of the cytoplasmic P-bodies in *sake* yeast during Japanese *sake* brewing and wine making. Biosci Biotechnol Biochem 71(11);

2800-2807, 2007

- Izawa, S. K. Ikeda, T. Ohdate and Y. Inoue: Msn2p/Msn4p-activation is essential for the recovery from freezing stress in yeast. Biochem Biophys Res Commun 352(3); 750-755, 2007
- Takatsume, Y., S. Izawa and Y. Inoue: Modulation of Spc1 stress-activated protein kinase activity by methylglyoxal through inhibition of protein phosphatase in the fission yeast *Schizosaccharomyces pombe.* Biochem Biophys Res Commun 363(4); 942-947, 2007
- b) Conference and seminar papers presented

The 9th Meeting of RNA Society: 1 paper

Joint Meeting of the Japanese Society of Developmental Biologist & the Japan Society for Cell Biology: 1 paper

The 40th Meeting of Yeast Genetics and Molecular Biology, Japan: 6 papers

The Annual Meeting of the Society for Biotechnology 2007, Japan: 4 papers

Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry 2008: 5 papers The 17th Meeting of the Japan Maillard Reaction Society: 2 papers

BMB2007: 4 papers

# A-3. Off-campus activities

### Membership in academic societies

- Kita, K.: Japan Society of Bioscience, Biotechnology, and Agrochemistry (Councilor of Nation-wide, Councilor of Kansai branch)
- Inoue, Y.: Yeast Society (Committee Member), The Society for Biotechnology, Japan (Committee Member of Kansai Branch)

### Research grants

Research Grants from Ministry of Education, Culture, Sports, Science, and Technology: Grant-in-Aid for Scientific Research (B) Metabolic signaling: physiological role and mechanism of signal transduction by glycolytic intermediate. (Inoue, Y.) Grant-in-Aid for Young Scientist (B) (2) Function of thioredoxin in alcoholic fermentation and its transport. (Izawa, S.)

### A-4. International cooperations and overseas activities

### Internaional meetings (roles)

Kita, K.: The 14th Japanese-German Workshop on Enzyme Technology, Kanazawa (invited speaker).

# B. Educational Activities (2007.4-2008.3)

### B-1. On-campus teaching

a) Courses given

- Undergraduate level: Applied Life Sciences (Kita), Applied Microbiology II (Kita), Laboratory Course in Biochemistry (Kita, Inoue, Izawa), Applied Microbiology IV (Inoue), Introduction to Applied Life Sciences III (Inoue)
- Graduate level: Cellular Bioenergy Conversion Seminar (Kita, Inoue, Izawa), Experimental Course of Cellular Bioenergy Conversion (Kita, Inoue, Izawa)

# C. Other Remarks

Inoue, Y.: Committee on Redox Life Science, Japan Society for the Promotion of Science (Member) Izawa, S.: Editorial board member of *Applied Microbiology and Biotechnology*.

# Chair of Applied Microbiology

# 2.3.7 Laboratory of Fermentation Physiology and Applied Microbiology

Staff	Professor :	Shimizu, Sakayu, Dr. Agric. Sci.
	Associate Professor:	Kataoka, Michihiko, Dr. Agric. Sci.
	Assistant Professor :	Ogawa, Jun, Dr. Agric. Sci.
	Assistant Professor :	Sakuradani, Eiji, Dr. Agric. Sci.
	Postdoctoral fellows:	Shimada, Yoshimi, Dr. Agric. Sci.
		Urano, Nobuyuki, Dr. Agric. Sci.

Students and research fellows

Doctor's program	: (1)
Master's program	: (19)
Undergraduate	: (5)
Research fellow	: (2)
Foreign research fellow	: (1)
Educational assistant	: (1)

# A. Research Activities (2007.4-2008.3) A-1. Main subjects

#### a) Microbial production of useful lipids

We have found that mycelia of the fungus *Mortierella alpina*, which was isolated from soil of Kyoto, are rich source of a polyunsaturated fatty acid, arachidonic acid. Furthermore, we succeeded in the selective production of various polyunsaturated fatty acids, for example, dihomo- $\gamma$ -linolenic acid and EPA, by controlling of the culture conditions and breeding of the mutant producers. These strains are now under investigation at practical level with 10,000-liter jar fermenter. We are doing enzymatic and genetic analysis of the filamentous fungus and trying to establish novel transformation system for the fungus. We are making further research in microorganisms for the production of novel functional lipids, and found that lactic acid bacteria produce conjugated fatty acids. Further development of conjugated linoleic acid production by lactic acid bacteria is on going.

b) Microbial production of optically active compounds

Reactions catalyzed by enzymes display for greater specificities than more conventional forms of organic reactions. Among these specificities, stereospecificity is one of the most excellent properties. To overcome the disadvantage of a conventional synthetic process of optically active compounds (amino acids, vitamins and so on), i.e., the troublesome resolution of a racemic mixture, microbial transformations with enzymes possessing stereospecificities (carbonyl reductases, lactonase, aldolase, etc.) have been applied to the asymmetric synthesis of them. Studies on enzyme and protein chemistry of the enzymes involved in these reactions are also carried out.

#### c) Functional analysis and application of novel microbial enzymes

Microbial transformations of nucleic acid-related compounds are studied. The enzymes involved in these transformations are applied for followings: 1) dihydropyrimidinase, which functions in nucleic acid-base degradation, is applied for D-amino acids production from DL-5-monosubstituted hydantoins, 2) the enzymes involved in creatinine metabolism are applied to clinical diagnosis of renal dysfunction. A variety of microbial oxidases, such as peroxidases and laccases, are screened and its applicabilities are evaluated. Peroxidases from filamentous fungi are now under development as bleaching agents in clothes washing and as analytical tools for diagnosis. Laccases from basidiomycete are examined as potential tools for bioremediation, novel bio-control reagents and dyeing/bleaching reagents.

d) Microbial nitrile degradation and its application

Nitriles are widely manufactured and extensively used by chemical industries. They are very toxic and are generally bio-undegradable compounds. However, some microorganisms have the ability to utilize nitriles as carbon and/or nitrogen sources. The microbial degradation of nitriles has been found to proceed through two enzymatic pathways. Nitrilase catalyzes the direct cleavage on nitriles to the corresponding acids and ammonia. In the second pathway, nitriles are catabolized in two stages, via conversion to the corresponding amides by nitrile hydratase and then the acids plus ammonia by amidase. These nitrile-converting enzymes are expected to have great potential as catalysts in organic chemical processing, because of the mild conditions, quantitative yields, absence of by-products and in some cases enantio- or regioselectivity. Thus, we established the process for the industrial production of acrylamide, an important chemical commodity, from acrylonitrile using the *Rhodococcus rhodochrous* J1 nitrile hydratase in 1991.

### A-2. Publications and presentations

a) Publications

#### Books

- Isobe, K., A. Iwasaki, S. Kawano, Y. Yasohara, J. Hasegawa, M. Kataoka, J. Ogawa, S. Shimizu: Chapter 3 Screening of regioslective enzymatic oxidation and stereoselective enzymatic reduction. Novel Production Technology that uses Microorganism Function –Minimum Genome Factory and System Biology- (edited by Shimizu et al.), pp.138-153, CMC Press, Tokyo, 2007 (in Japanese)
- Shimizu, S.: For the development of industrial bioprocess. Bioprocess Handbook, pp.695-701, NTS Press, Tokyo, 2007 (in Japanese)
- Shimizu, S.: Introduction "For the development of biological production process". Novel Production Technology that uses Microorganism Function –Minimum Genome Factory and System Biology- (edited by Shimizu et al.), pp.1-8, CMC Press, Tokyo, 2007 (in Japanese)

### **Original** papers

Isobe, K., A. Kato, J. Ogawa, M. Kataoka, A. Iwasaki, J. Hasegawa and S. Shimizu: Characterization of alcohol oxidase from *Aspergillus ochraceus* AIU 031. J Gen Appl Microbiol 53; 177-183, 2007

- Isobe, K., A. Kato, Y. Sasaki, S. Suzuki, M. Kataoka, J. Ogawa, A. Iwasaki, J. Hasegawa and S. Shimizu: Purification and characterization of a novel alcohol oxidase from *Paenibacillus* sp. AIU 311. J Biosci Bioeng 104(2); 124-128, 2007
- Ogawa, J., H. Yamanaka, J. Mano, Y. Doi, N. Horinouchi, T. Kodera, N. Nio, S.V. Smirnov, N.N. Samsonova, Y.I. Kozlov and S. Shimizu: Synthesis of 4-hydroxyisoleucine by the aldolase-transaminase coupling reaction and basic characterization of the aldolase from *Arthrobacter simplex* AKU 626. Biosci Biotechnol Biochem 71(7); 1607-1615, 2007
- Shibata, N., Y. Tamimoto, T. Hanamura, R. Yamamoto, M. Ueda, Y. Ueda, N. Mizuno, H. Ogata, H. Komori, Y. Shomura, M. Kataoka, S. Shimizu, J. Kondo, H. Yamamoto, A. Kikuchi and Y. Higuchi: Crystallization and preliminary X-ray crystallographic studies of the axin DIX domain. Acta Cryst F63; 529-531, 2007
- Smirnov, S.V., N.N. Samsonova, A.E. Novikova, N.G. Matrosov, N.Y. Rushkevich, T. Kodera, J. Ogawa, H. Yamanaka and S. Shimizu. A novel strategy for enzymatic synthesis of 4-hydroxyisoleucine: identification of an enzyme possessing HMKP (4-hydroxy-3-methyl-2-ketopentanoate) aldolase activity. FEMS Microbiol Lett 273(1); 70-77, 2007
- Ukitsu, H., T. Kuromori, K. Toyooka, Y. Goto, K. Matsuoka, E. Sakuradani, S. Shimizu, A. Kamiya, Y. Imura, M. Yuguchi, T. Wada, T. Hirayama and K. Shinozaki: Cytological and ciochemical analysis of *COF1*, an *Arabidopsis* mutant of an ABC transporter gene. Plant Cell Physiol 8(11); 1524-1533, 2007
- Yamamoto, M., C. Zhu, L. Yi, Z. Rong, Y. Miura, M. Izumi, S. Nakajima, K. Tanamoto, S. Shimizu and N. Baba: Synthesis of lipid derivatives of pyrrole polyamide and their biological activity. Biosci Biotechnol Biochem 71(4); 1078-1082, 2007
- Zhang, S., E. Sakuradani and S. Shimizu: Identification of a sterol Δ7 reductase gene involved in desmosterol biosynthesis in *Mortierella alpina* 1S-4. Appl Environ Microbiol 73(6); 1736-1741, 2007
- Zhang, S., E. Sakuradani, K. Ito and S. Shimizu: Identification of a novel bifunctional Δ12/Δ15 fatty acid desaturase from a basidiomycete, *Coprinus cinereus* TD#822-2. FEBS Lett 81(2); 315-319, 2007

### Reviews

- Asano, Y. and S. Shimizu: Preface on the special issue of "Directed evolution of biocatalysts and thier uses in industry". Seibutsu Kogaku 85(9); 393, 2007 (in Japanese)
- Shimizu, S.: Microbial factory. Microbiol Cult Coll 23(1); 23-28, 2007 (in Japanese)
- Kataoka, M.: New function of old yellow enzyme as asymmetric hydrogenation catalyst. Vitamin 81(12) ; 631-633, 2007 (in Japanese)
- Kataoka, M., K. Honda, K. Sakamoto and S. Shimizu: Microbial enzymes involved in lactone compound metabolism and their biotechnological applications. Appl Micriobiol Biotechnol 75(2); 257-266, 2007
- Kataoka, M., N. Urano and S. Shimizu: Application of directed evolution to chiral technology. Seibutsu Kogaku 85(9) ; 400-402、2007 (in Japanese)
- b) Conference and seminar papers presented
- Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry 2007: 21 reports

98th American Oil Chemists' Society Annual Meeting and Expo: 2 reports
28th Symposium of Bioactive Society in Okayama: 1 report
4th Frontiers Research Symposium: 1 report
BioTrans 2007: 2 reports
9th Mini Symposium of 21st Century COE Program, Okayama: 1 report
Annual Meeting of the Society for Industrial Microbiology 2007: 2 reports
1st Seminar of Applied Microbiology Institute, Sojo University: 1 report
Annual Meeting of the Society for Fermentation and Bioengineering, Japan 2007: 6 reports
13th European Congress on Biotechnology: 1 report
2nd International Congress on Conjugated Linoleic Acid: 2 reports
The 14th Japanese-German Workshop on Enzyme Technology: 2 reports
Meeting of Kansai & Chubu Branches of Japan Society for Bioscience, Biotechnology, and

Agrochemistry 2007: 2 reports Special Symposium of Nagase Foundation: 1 report

Kyoto Wellness Industry Developing Meeting Health Business Seeds Searching Seminar: 1 report

7th Conference of Fungal Molecular Biology Society of Japan: 2 reports

Autumn Seminar of Japan Society for Lactic Acid Bacteria 2007: 1 report

33rd Meeting of Enzyme Application Association: 1 report

The 2nd Kyoto-U.-Korea U. Joint Symposium on Microbiology and Biotechnology: 2 reports

2007 International Symposium on Biocatalysis and Biotechnology: 1 report

453rd Meeting of Kansai Branches of Japan Society for Bioscience, Biotechnology, and Agrochemistry: 1 reports

Symposium of Target Protein Program 2007: 1 report

Symposium of Vitamin B Comittie 2007: 1 report

6th Lipid Reserch Seminar: 3 reports

16th Japanese Society for Bioscience, Biotechnology and Agrochemistry Frontiers Symposium: 1 report

### A-3. Off-campus activities

#### Membership in academic societies (roles)

- Shimizu, S.: Japan Society for Bioscience, Biotechnology, and Agrochemistry (director, vice president); The Society for Fermentation and Bioengineering, Japan (councilor); The Japanese Biochemical Society (councilor); The Vitamin Society of Japan (councilor); The Society of Enzyme Engineering (committieman); Japan Bioindustry Association (director, vice president, editor), Japan Applied Microbiology Society (director); The Society of Fermentation and Metabolism (president)
- Kataoka, M.: The Society of Enzyme Engineering (secretary); The Vitamin Society of Japan (topics editor); The Society for Fermentation and Bioengineering, Japan (secretary of IT-driven microbiology group)
- Ogawa, J.: The Society for Fermentation and Bioengineering, Japan (chairman of lipid technology group); Japanese Society for Bioscience, Biotechnology and Agrochemistry (Member of Industry-Government-Academy young person interchange society)

#### Research grants

Monbukagakusho Research Grant: Scientific Research (A) Creation of novel functional lipids by

using multi-use of microbial functions (Shimizu, Kataoka, Ogawa, Sakuradani), Scientific Research (B) Development of microbial production process for the aymmetric synthesis of nitrogen-containing chiral compounds (Kataoka, Shimizu, Ogawa, Sakuradani), Specific Field Research Biocatalytic module development for non-invasive cellular lipid analysis (Ogawa, Sakuradani), Young Scientist Research (A) Production of useful compounds by molecular breeding of oleaginous microbes (Sakuradani)

- Research project funded by New Energy and Industrial Technology Development Organization (NEDO): The Project for Development of a Technological Infrastructure for Industrial Bioprocesses (Shimizu, Kataoka, Ogawa, Sakuradani), Next Generation Fuel Battery Development (Ogawa), Microbial production of functional lipids (Sakuradani)
- Target Protein Research program: Structural analysis and modification of enzymes useful for chiral compound production (Shimizu, Kataoka, Ogawa, Sakuradani)
- 21st Century COE program: COE for Microbial-Process Development Pineering Future Production Systems (Shimizu, Kataoka, Ogawa)

### A-4. International cooperations and overseas activities

### International meetings (roles)

Shimizu, S.: Annual Meeting of the Society for Industrial Microbiology 2007, USA (invited speaker); 13th European Congress on Biotechnology, Spain (invited speaker); The 14th Japanese-German Workshop on Enzyme Technology, Kanazawa (organizer)

Kataoka, M.: BioTrans 2007, Spain (speaker)

Ogawa, J.: 98th American Oil Chemists' Society Annual Meeting and Expo, Canada (invited speaker), BioTrans 2007, Spain (speaker), Annual Meeting of the Society for Industrial Microbiology 2007, USA (invited speaker); 2nd International Congress on Conjugated Linoleic Acid, Italy (speaker); The 14th Japanese-German Workshop on Enzyme Technology, Kanazawa (invited speaker); The 2nd Kyoto-U.-Korea U. Joint Symposium on Microbiology and Biotechnology, Korea (Invited speaker); 2007 International Symposium on Biocatalysis and Biotechnology, Taiwan (invited speaker)

#### Membership in international academic societies

- Shimizu, S.: American Oil Chemists' Society (member of comittie on biotechnology section), Journal of American Oil Chemists' Society (editor), Journal of Molecular Catalyst B: Enzymatic (editor)
- Kataoka, M.: Journal of Bioscience and Bioengineering (editor), Recent Patents on Biotechnology (editor)

#### International joint researchers, overseas research surveys

- Shimizu, S.: Development of thermotolerant microbial resources and their applications in Thailand and Japan (Thailand)
- Kataoka, M.: Development of thermotolerant microbial resources and their applications in Thailand and Japan (Thailand); Overseas research grant by MEXT (Germany)
- Ogawa, J.: Development of thermotolerant microbial resources and their applications in Thailand and Japan (Thailand)

# B. Educational Activities (2007.4-2008.3)

# B-1. On-campus teaching

a) Courses given

- Undergraduate level: Outline of Applied Life Sciences II (Shimizu), Applied microbiology III (Shimizu), Applied microbiology IV (Shimizu, Kataoka), Laboratory course in applied microbiology (Kataoka, Ogawa, Sakuradani), Biotechnology (Shimizu)
- Graduate level: Fermentation physiology and applied microbiology seminar (Shimizu, Kataoka, Ogawa, Sakuradani), Experimental course of fermentation physiology and applied microbiology (Shimizu, Kataoka, Ogawa, Sakuradani)

b) Seminars

21st Century COE seminar (organizer, 18 times)

# B-2. Off-campus teaching, etc.

# Part-time lecturer

Shimizu, S.: Shiga Prefectural University (Utilization of microorganism)

# Open lecture organizer

9th Mini Symposium of 21st Century COE Program, Okayama; 10th Mini Symposium of 21st Century COE Program, Kyoto

# B-3. Overseas teaching

# Students and research fellows from abroad

Reserch fellows: Unesco biotechnology post-graduate school (1) (Indonesia)

# Lacture in abroad

Kataoka, M.: Muenster University (Germany), Stuttgart University (Germany), Greifswald University (Germany), Lonza (Switzerland), ETH (Switzerland), RWTH Aachen University (Germany), University of Dortmund (Germany)

Ogawa, J.: l'Institut National de la Recherche Agronomique (France)

# 2.3.8 Laboratory of Microbial Biotechnology

Staff Professor : Sakai, Yasuyoshi, Dr. Agric. Sci. Associate Professor: Yurimoto, Hiroya, Dr. (Agric. Sci.) Postdoctoral fellow: Yamashita, Shun-ichi, Dr. (Agric. Sci.) (2007.6.–)

Students and research fellows Doctor's program: (5)

> Master's program: (12) Undergraduate : (4) Research student: (1)

# A. Research Activities (2007.4-2008.3) A-1. Main subjects

a) Molecular and cellular biology for efficient production of heterologous proteins

We have developed the field of "C1 fermentation", in which methanol is used as the raw material for microbial cultivation and chemical synthesis. We have noticed methylotrophs that grow on C1 compounds as a useful biocatalyst and a protein production system. In our studies, a new heterologous gene expression system using the methylotrophic yeast has been established. This is widely noticed as a system for production of various eucaryotic proteins.

b) Development of novel metabolic functions of microbes

For the application of the heterologous gene expression system and the metabolic function of the methylotrophic yeast, many genes that participate in methanol metabolism were cloned and we tried to clarify the metabolic pathway at the molecular level. We have found the genes encoding formaldehyde fixation pathway, which has been well characterized in methylotrophic bacteria, in nonmethylotrophic bacteria and archaea. We study on the physiological role and its application of these enzymes. We focus on methane, methanol, and short-chain alkanes as the future natural resources, and clarify the cellular and metabolic function of microorganisms, which utilize these resources, from the aspect of biochemistry, molecular biology and intracellular structure.

c) Development of technology to monitor intracellular redox potential

It has been recognized that reactive oxygen species (ROS) attack various biomolecules resulting in aging and many diseases. For the prevention of diseases and control of aging, evaluation and control of oxidative stress *in vivo* may become essential. However, it has been difficult to monitor oxidative stress in a living cell and in real time. We have developed a new molecular probe that can detect intracellular oxidative stress non-invasively using methylotrhophic yeasts and mammalian cells as model cells.

### A-2. Publications and presentations

# a) Publications

### Books

Sakai, Y.: Peroxisome. Koubo No Subete (edited by Ohsumi, Y. and C. Shimoda), pp.83-86, Springer Japan, Tokyo, 2007 (in Japanese)

Yurimoto, H. and Y. Sakai: Production of heterologous proteins. Koubo No Subete (edited by

Ohsumi, Y. and C. Shimoda), pp.315-319, Springer Japan, Tokyo, 2007 (in Japanese)

### Original papers

- Yamashita, S., M. Oku and Y. Sakai.: Functions of PI4P and sterol glucoside necessary for the synthesis of a nascent membrane structure during pexophagy. Autophagy 3; 35-37, 2007
- Kotani, T., H. Yurimoto, N. Kato and Y. Sakai: A novel acetone metabolism in a propane-utilizing bacterium *Gordonia* sp. strain TY-5. J Bacteriol 189; 886-893, 2007
- Sasano, Y., H. Yurimoto and Y. Sakai: Gene-tagging mutagenesis in the methylotrophic yeast *Candida boidinii*. J Biosci Bioeng 104; 86-89, 2007
- Orita, I., N. Sakamoto, N. Kato, H. Yurimoto and Y. Sakai: Bifunctional enzyme fusion of 3-hexulose-6-phosphate synthase and 6-phospho-3-hexuloisomerase. Appl Microbiol Biotechnol 76; 439-445, 2007

#### Reviews

- Yurimoto, H. and Y. Sakai: C1-microorganisms aiming at efficient utilization of natural resources. Kagaku Kogyo 58(1); 21-26, 2007 (in Japanese)
- b) Conference and seminar papers presented
- Annual meeting of the Japan Society for Bioscience, Biotechnology, and Agrochemistry 2008: 11 reports
- Yeast Genetics and Molecular Biology News Japan No. 40: 5 reports

Biochemistry and Molecular Biology 2007: 5 reports

Annual meeting of the Society for Biotechnology, Japan 2007: 4 reports

- The 17th annual meeting of Japanese Society of Plant Microbe Interactions: 2 reports
- Joint Meeting of the Japanese Society of Developmental Biologists and the Japan Society for Cell Biology: 1 reports

The 7th Annula Meeting of the Protein Science Society of Japan: 1 report

- Joint Meething of Kansai Branch and Chubu Branch of the Japan Society for Bioscience, Biotechnology, and Agrochemistry: 1 report
- The 86th Research Institute for Sustainable Humanosphere Seminar: 1 report

AIST Symposium "Glycobiology and its application in yeasts": 1 report

The 449th meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry Kansai Branch: 1 report

### A-3. Off-campus activity

#### Membership in academic societies (roles)

Sakai, Y.: Japan Society for Bioscience, Biotechnology, and Agrochemistry (Councilor., Kansai Branch). Yeast Genetics Society of Japan (Administrator). Japan Bioindustry Association; Academic Society for Biotransformations with New Resources (Standing Director).

Yurimoto, H: Japan Bioindustry Association (Topics)

#### Research grants

Monbukagakusho Scientific research on priority areas: Mechanism of selective intracellular degradation by autophagy (Sakai), Scientific research on priority areas: Analysis of higher cellular fuction of pexophagy by monitoring organelle and Atg proteins (Sakai), Scientific research (B): Cellular function of C1-microorganisms aiming at reducing green house gas (Sakai), Young Scientists (B): Molecular basis of one-carbon metablism of microorganisms and regulatory mechanism of gene expression (Yurimoto)

Other Research grant: Japan Science and Technology Agency, CREST, Metabolism-based regulation of organelle homeostasis and cell function (Sakai), The Asahi Glass Fundation Resarch grant: Molecular and cellular biology of microorganisms aiming at efficient utilization of C1 compounds (Sakai).

#### A-4. International cooperation and overseas activities

#### International meetings (roles)

- Sakai, Y.: Keystone Symposia on Autophagy in Health and Disease, USA (invited speaker). Gordon Resarch Conference on Autophagy in Stress, Development and Disease, USA (poster).
- Yurimoto, H.: JSPS-NRCT Concluding Joint Seminar on Development of Thermotolerant Microbial Resources and Their Applications, Thailand (invited speaker).
- Yamashita, S.: Gordon Resarch Conference on Autophagy in Stress, Development and Disease, USA (poster).

### Membership in international academic societies

Sakai, Y.: Biosci Biotechnol Biochem (Editor), Autophagy (Editor)

#### International joint researches, overseas research surveys

- Sakai, Y.: JSPS-NRCT Core University Program between Kasetsart University and Yamaguchi University on Development of thermotolerant microbial resources and their application in Thailand and Japan
- Yurimoto, H.: JSPS-NRCT Core University Program between Kasetsart University and Yamaguchi University on Development of thermotolerant microbial resources and their application in Thailand and Japan

#### Scholars from abroad

Invited foreign scholars (1) (University of Michigan, USA, Professor)

### B. Educational Activities (2007.4-2008.3)

### B-1. On-campus teaching

### a) Courses given

- Undergraduate level: Applied Microbiology I (Sakai), Applied Microbiology IV (Yurimoto), Laboratory Course in Applied Microbiology (Yurimoto), Introduction to Applied Life Sciences II (Sakai), Seminar in Applied Life Sciences, Part I and II (Sakai, Yurimoto), Pocket Seminar (Sakai, Yurimoto)
- Graduate level Basis of Microbial Biotechnology (Sakai, Yurimoto), Microbial Biotechnology Seminar (Sakai, Yurimoto), Experimental Course of Microbial Biotechnology (Sakai, Yurimoto)

### B-2. Off-campus teaching, etc.

#### Open lectures

Sakai, Y.:Sanseido Science Cafe in Kyoto (lecturer)

Yurimoto, H.: Bioscience Seminar of the Society for Biotechnology, Japan "Bio-cafe and labo 2007" (lecturer)

### B-3. Overseas teaching

### Students and reseach fellows from abroad

Foreign students: Master's program (1) (Peru), Research student (1) (Vietnam)

# C. Other Remarks

- Sakai, Y.:. Member of the Advisory Committee of Technological Research for the Verification of Feasibility on CO<sub>2</sub> Fixation and Effective Utilization in Research Institute of Innovative Technology for the Earth. Member of the Advisory Committee of International Center for Environmental Technology Transfer.
- Yurimoto, H.: Assistant Chief of Radioisotope Managing Committee at Graduate School of Agriculture, Kyoto University.

# Chair of Bioorganic and Biophysical Chemistry

# 2.3.9 Laboratory of Bio-Analytical and Physical Chemistry

Staff	Professor	: Kano, Kenji, Dr. Agric.	Sci.
	Associate Professor	: Shirai, Osamu, Dr. Sci	
	Assistant Professor	: Tsujimura, Seiya, Dr.	Agric. Sci.
	Postdoctoral fellows	:Wang, Yong Fu, Dr.	
		: Santo, Ryoko, Dr. Sci.	
Student	ts and research fellows		
	Doctor's program : (1)		
	Master's program: (10)	Undergraduate	: (4)
	Research fellow : (1)	Foreign research fe	ellow:(1)

# A. Research Activities (2007.4-2008.3)

### A-1. Main subjects

a) Fundamental analysis of oxidation-reduction reactions relevant to biological phenomena.

Structure and function of histamine dehydrogenase from *Antinomycetes* (molecular cloning, structural analysis of active site of histamine dehydrogenase, thermochemical and dynamic properties, etc.). Single mutation of multicopper oxidaze and its function analysis. Interaction between the enzymes and various electrode materials.

b) Fundamental study of bioenergy conversion system and its application to biofuel cell.

Multi-copper oxidases as very efficient catalysts for electrocatalytic reduction of dioxygen to water based on mediated and direct electron transfer mechanisms. Bioelectrocatalytic reduction of saccharide using dehydrogenase. Bioelectrocatalytic reduction of saccharide using saccharide dehydrogenase (Mediator-type and Direct Electron transfer-type bioelectrocatalysis). Multiple oxidation process using enzymes of the TCA cycle. Electron transfer at an enzyme-adsorbed and modified carbon electrode. Development of biofuel cell using enzymes and microbes.

c) Construction of electrochemical biosensing systems.

Development of a method of complete electrolysis micro-coulometry for multi-purpose use. Development of histamine sensor.

d) Fundamental study on charge (ion and electron) transfers across biomembranes

Electrochemical analysis on ion transport across planar lipid bilayers in the presence of hydrophobic ions and ionophores. Function of ion channels using planar bilayer lipid membranes (Effect of coexisting ions, Reaction mechanism of accelerator and inhibitor).

e) Fundamental study of bioenergy conversion system and signal transmission processes.

Uncoupling mechanism of hydrophobic weak acids. Coupling mechanism between electron transport system and ion transport system using enzymes, ionophores and hydrophobic ions. Consumption and excretion of heavy metal ions in biocells. Ion transport across liposomal membranes. Identification of transferred ions across planar lipid bilayers using radioisotopes.

### A-2. Publications and presentations

a) Publications

#### Original papers

- Kamitaka,Y, S. Tsujimura, K. Kataoka, T. Sakurai, T. Ikeda and K. Kano: Effects of Axial Ligand Mutation of the Type I Copper Site in Bilirubin Oxidase on Direct Electron Transfer-type Bioelectrocatalytic Reduction of Dioxygen. J Electroanal Chem 601(1/2); 119-124, 2007
- Murakami, Y., N. Yoshimoto, N. Fujieda, K. Ohkubo, K. Kano, S. Fukuzumi and I. Itoh: Model Studies of 6,7-Indolequinone Cofactors of Quinohemoprotein Amine Dehydrogenases. J Org Chem 72(9); 3369-3380, 2007
- Kamitaka, Y., S. Tsujimura, N. Setoyama, T. Kajino and K. Kano: Fructose/Dioxygen Biofuel Cell based on Direct Electron Transfer-type Bioelectrocatalysis. Phys Chem Chem Phys 9(15); 1793-1801, 2007 ··· Cover Image of the Issue
- Kamitaka, Y., S. Tsujimura and K. Kano: High Current Density Bio-electrolysis of D-Fructose at Fructose Dehydrogenase-adsorbed and Ketjen Black-modified Electrodes without a Mediator. Chem Lett 36(2); 218-219, 2007
- Miura, Y., S. Tsujimura, Y. Kamitaka, S. Kurose, K. Kataoka, T. Sakurai and K. Kano: Bioelectrocatalytic Reduction of O<sub>2</sub> Catalyzed by CueO from *Escherichia coli* Adsorbed on a Highly Oriented Pyrolytic Graphite Electrode. Chem Lett 36(1); 132-133, 2007
- Ozaki, S., O. Shirai, S. Kihara and K. Kano: Voltammetric Study on the Mechanism of Ion Transfer Caused by Weak Acids in the Bilayer Lipid Membrane. Electrochem Commun 9(9); 2266-2270, 2007
- Tsujimura, S., Y. Kamitaka and K. Kano: Diffusion-controlled Oxygen Reduction on Multi-copper Oxidase-adsorbed Carbon Aerogel Electrodes without Mediator. Fuel Cells 7(6); 463-469, 2007
- Tsujimura, S., T. Abo, Y. Ano, K. Matsushita and K. Kano: Electrochemistry of D-Gluconate 2-Dehydrogenase from *Gluconobacter frateurii* on Indium Tin Oxide Electrode Surface. Chem Lett 36(9); 1164-1165, 2007
- Wang, Y.-F., S. Tsujimura, S.-S. Cheng and K. Kano: Self-excreted Mediator from Escherichia coli K-12 for Electron Transfer to Carbon Electrodes. Appl Microbiol Biotechnol 76(6); 1439-1446, 2007
- Kataoka, K., H. Komori, Y. Ueki, Y. Konno, Y. Kamitaka, S. Kurose, S. Tsujimura, S. Higuchi, K.

Kano, D. Seo and T. Sakurai: Structure and Function of the Engineered Multicopper Oxidase CueO from *Escherichia coli* - Deletion of the Methionine-Rich Helical Region Covering the Substrate Binding Site. J Mol Biol 373(1); 141-152, 2007

- Uehara, A., O. Shirai, T. Nagai, T. Fujii and H. Yamana: Spectroelectrochemistry and electrochemistry of Europium Ion in Alkali Chloride Melts. Zeit Natur 62a; 191-196, 2007
- Ishii, A., S. Tsujimura and K. Kano: Dependence of Steady-state Catalytic Currents on the Thickness of an Enzyme-mediator-immobilized Layer Fabricated by Layer-by-layer Method. Bunseki Kagaku 56(6); 419-424, 2007 (in Japanese)
- Shirai, O.: Electrochemical Study on Ion Trasnfports Accross a Bilayer Lipid Membrane in the Presence of Hydrophobic Ions and Ionophores. 56(7); 547-560, 2007 (in Japanese)
- Onishi, J., K. Kano and O. Shirai: Electrochemical Study on Facilitated Ion Transport Across a Bilayer Lipid Membrane in the Presence of Nonactin. Bunseki Kagaku 56(11); 965-971, 2007 (in Japanese)
- Shirai, O., T. Nagai, A. Uehara and H. Yamana: Electrochemical properties of the Ag<sup>+</sup>|Ag and other reference electrodes in the LiCl-KCl eutectic melts. J Alloys Comp 456(1/2); 498-502, 2008
- Uehara, A., M. Kasuno, T. Okugaki, Y. Kitatsuji, O. Shirai, Z. Yoshida and S. Kihara: J Electroanal Chem 604; 115-124, 2007
- Tsujimura, S., Y. Miura and K. Kano: CueO-immobilized Porous Carbon Eelectrode Exhibiting Improved Performance of Electrochemical Reduction of Dioxygen to Water. Electrochimica Acta 53(18); 5716-5720, 2008
- Tsutsumi, M., N. Fujieda, S. Tsujimura, O. Shirai and K. Kano: Thermodynamic Redox Properties Governing Half-reduction Characteristics of Histamine Dehydrogenase from *Nocardioides simplex.* Biosci Biotechnol Biochem 72(3); 786-796, 2008
- Tsujimura, S., A. Ishii, T. Abo and K. Kano: Mediated Bioelectrocatalytic Reaction Using Monolayered Redox Polymer on a Glassy Carbon Electrode Surface and Effect of the Ionic Strength on the Catalytic Current. J Electroanal Chem 614(1/2); 67-72, 2008

### Reviews and others

- Ikeda, T, H. Tatsumi, H. Katano and K. Kano: Bioelectrochemistry Development of Biosensor · Bioelectrochemical Cell- (edited by T. Ikeda), Chapters 1-4, CMC Press; pp.1-54, 2007 (in Japanese)
- Tsujimura, S. and K. Kano: Direct Electron Transfer Type Bioelectrochemical Cell. The Practice of Bioelectrochemistry- Practical Development of Biosensor · Bioelectrochemical Cell-(edited by T. Ikeda), Chapter 18, CMC press; 290-302, 2007 (in Japanese)
- Ikeda, T. and K. Kano: Amperometry. Jikkenkagakukoza 5th edition. Vol. 20-1 Bunsekikagaku (edited by the Chemical Society of Japan), Chapter 5 Electrochemical Analysis, Maruzen; 179-209, 2007 (in Japanese)
- Shirai, O.: Topic-Overview of Recent Progress on The Working Mechanism of Voltage-gated Ion Channels. Bunseki 7; 405-406, 2007 (in Japanese)
- Tsujimura, S.: Application of Redox Enzyme to Electrode Catalyst. Kagaku to Seibutsu 45(1); 7-9, 2007 (in Japanese)
- Tsujimura, S. and K. Kano: Next Generation of Energy Conversion System by use of Biological Function. Kagakukougyo 58(1); 33-38, 2007 (in Japanese)

- b) Conference and seminar papers presented.
- The Joint Annual Meeting of Kansai Branch and Chubu Branch of Japan Society for Bioscience, Biotechnology, and Agrochemistry in 2007: 2 reports
- The 450th Kansai Branch Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry: 1 report
- The Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry in 2008: 6 reports
- The 22th Meeting of Division of Biofunctional Chemistry, The Chemical Society of the Japan: 2 reports

Biochemistry and Molecular Biology 2007: 1 report

The 75th Electrochemical Society Meeting: 7 reports

The Autumn Meeting of the Electrochemical Society of Japan in 2007: 4 reports

The 57th Annual Meeting of Japan Society of Coordination Chemistry: 1 report

The 33th Annual Symposium of The Japan Bioenergitics Group: 1 report

The 56th Annual Meeting of the Japan Society for Analytical Chemistry: 6 reports

The 53th Annual Meeting on Polarography and Electroanalytical Chemistry: 3 reports

Workshop on Bioelectrochemical Cell: 1 report

Workshop of the Society of Polymer Science, Japan: 1 report

The 37th Laboratory Course of Kansai Branch of the Electrochemical Society of Japan: 1 report

The 1st Joint Workshop of chemical sensor, biosensor and techniques: 1 report

Talk Shower in Kyushu, Kyushu Branch of the Electrochemical Society of Japan: 1 report

The seminar on the electrochemical all, The Kinki Chemical Society: 1 report

### A-3. Off-campus activities

### Membership in academic societies (roles)

- Kano K.: The Japan Society for Analytical Chemistry (a council member); The Electrochemical Society of Japan (a director); The Japan Society for Bioscience, Biotechnology, and Agrochemistry (a director); The Polarographic Society of Japan (a director), Analytical Biochemistry (an editorial board member), Journal of Electroanalytical Chemistry (a council member of editorial board).
- Shirai O.: The Japan Society for Analytical Chemistry (an accountant secretary of Kinki Branch); The Polarographic Society of Japan (an accountant secretary)

Tsujimura S.: The Polarographic Society of Japan (a general secretary)

#### Research grants

Grant-in-Aids for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan (Kano), NEDO (Kano, Tsujimura)

### A-4. International cooperation and overseas activities

#### International meetings (roles)

Kano, K.: Analytical Biochemistry (Editor), Journal of Analytical Biochemistry (Advisory Board) Scholars from abroad

Collaborative researcher from Taiwan (2)

# B. Educational Activities (2007.4-2008.3)

# B-1. On-campus teaching

### a) Courses given

- Undergraduate level: Biophysical Chemistry I (Kano), Biophysical Chemistry II (Kano), Introduction to Applied Life Science I (Kano), Analytical Chemistry (Shirai), Laboratory Course in Analytical Chemistry (Shirai, Tsujimura), Laboratory Course in Biophysical Chemistry (Shirai, Tsujimura), New Strategies in Agricultural Sciences (Kano and others), Applied Life Science (Kano and others), Pocket seminar (Kano), Introduction of Agriculture II (Kano and others)
- Graduate level: Grant-in-Aids for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan (Kano, Shirai), Bio-Analytical and Physical Chemistry (advanced course) (Kano, Shirai, Tsujimura), Experimental Course of Bio-Analytical and Physical Chemistry (Kano, Shirai, Tsujimura).

### B-2. Off-campus teaching, etc.

### Part-time lecturer

Kano, K.: Tokyo Institute of Technology (Electrochemistry)

# 2.3.10 Laboratory of Biofunction Chemistry

Staff Professor : Nishioka, Takaaki, Dr. Agric. Sci. Associate Professor: Miyoshi, Hideto, Dr. Agric. Sci. Assistant Professor : Ishihara, Atsushi, Dr. Agric. Sci. Postdoctoral fellow : Abe, Masato, Dr. Agric. Sci.

Students and research fellows

Doctor's program: (2) Master's program: (8) Undergraduate : (4)

# A. Research Activities (2007.4-2008.3) A-1. Main subjects

a) Studies on insect sex-pheromone receptors

Male moths discriminate conspecific female-emitted sex pheromones. Although the chemical components of sex pheromones have been identified in more than 500 moth species, only three components in *Bombyx mori* and *Heliothis virescens* have had their receptors identified. We identified receptors for the main sex pheromone components in three moth species, *Plutella xylostella, Mythimna separata*, and *Diaphania indica*. We cloned putative sex pheromone receptor genes *PxOR1, MsOR1*, and *DiOR1* from *P. xylostella, M. separata*, and *D. indica*, respectively. Each of the three genes was exclusively expressed with an Or83b orthologous gene in male olfactory receptor neurons (ORNs) that are surrounded by supporting cells expressing pheromone binding protein genes (PBPs). By two-electrode voltage clamp recording, we tested

the ligand specificity of *Xenopus* oocytes co-expressing PxOR1, MsOR1, or DiOR1 with an OR83b family protein. Among the seven sex pheromone components of the three moth species, the oocytes dose-dependently responded only to the main sex pheromone component of the corresponding moth species. In our study, PBPs were not essential for ligand specificity of the receptors. On the phylogenetic tree of insect olfactory receptors, the six sex pheromone receptors identified in the present and previous studies are grouped in the same subfamily but have no relation with the taxonomy of moths. It is most likely that sex pheromone receptors have randomly evolved from ancestral sex pheromone receptors before the speciation of moths and that their ligand specificity was modified by mutations of local amino acid sequences after speciation. b) Development of a comprehensive and high-throughput chemical analysis for metabolites.

Metabolome is defined as all the metabolites in a cell or a tissue. Most of the metabolites are such ionic or highly polar substances as metabolic intermediates in central carbon metabolism, amino acids, and nucleotides. These metabolites are not analyzed by conventional analytical methods such as LC-MS and GC-MS without any chemical modifications before analysis. We successfully developed capillary electrophoresis coupled to mass spectrometry (CE-MS) as a tool of metabolome analysis. CE-MS does not require any chemical modification. We applied CE-MS to *Escherichia coli* and *Bacillus subtilis* to analyze how environmental and genetic perturbations affect their metabolite profiles. We are accumulating the experimental data that metabolism is a system where genomic information interacts with environmental perturbations. c) Bioorganic chemical study for elucidating mitochondrial complex I.

Proto-translocating NADH-ubiquinone oxidoreductase (complex I) is the first complex of the mitochondrial respiratory chain. It couples the transfer of two electrons from NADH to ubiquinone to the translocation of four protons across the inner mitochondrial membrane. The enzyme is composed of at least 46 different subunits with a total molecular mass of approximately 1 MDa. Because of the complexity of the enzyme, our knowledge about the molecular structure and the catalytic mechanism is still highly limited. The aim of our research is to get insights into the structural and functional features of complex I through the syntheses of various molecular probes and the mode of action studies for them. We have been carrying out i) structure-activity study of natural product acetogenins, the most potent inhibitor of complex I and ii) identification of inhibitor and ubiquinone binding site(s) through a photo-affinity labeling study.

d) Bioorganic chemical study for helminth mitochondrial respiratory system.

Parasitic helminth have exploited a variety of energy transducing systems in their adaptation to peculiar habitats in their hosts. Parasitic nematode, *Ascaris suum*, resides in the host small intestine where oxygen tensions are low, and has exploited a unique anaerobic respiratory chain to adapt to its microaerobic habitat. *A. suum* uses both ubiquinone and rhodoquinone as a respiratory substrate, whereas the biosynthetic pathways of these quinones are still not known. We have been carrying out i) structure-activity study of potent inhibitors of helminth respiratory enzymes, ii) examination of the biosynthetic pathway of rhodoquinone, and iii) identification of inhibitor and ubiquinone binding site(s) through a photo-affinity labeling study.

e) Bioorganic chemical study on the functions and regulation of plant secondary metabolism.

Plants defend themselves by multiple mechanisms. One of these mechanisms is the utilization of secondary metabolites. Plants respond to pathogenic infection by rapid accumulation of toxic secondary metabolites. We have been analyzing biosynthesis of those metabolites in gramineous species and Arabidopsis thaliana.

In addition, we have found that the metabolism of secondary metabolites is important in the plant defense. For example, in response to pathogenic attack, rice leaves accumulate serotonin, which is metabolized and deposits in the cell walls. The serotonin deposition results in the reinforcement of cell walls. We can elucidate sophisticated defense mechanisms of plants by investigating "where they have been and where they are going".

### A-2. Publications and presentations

a) Publications

### Books

Nishioka, T.: Metabolomics – Chemical analysis of cells reveals interactions between genomes and environment, Toward the perceive the life on the view point of how genomes determine the life (edited by A Team of Grants-in-Aids for Priority Areas Research on Genome), pp.43-55, Kubapro, Tokyo, 2007 (in Japanese).

#### **Original papers**

- Dubouzet, J. G., A. Ishihara, F. Matsuda, H. Miyagawa, H. Iwata and K. Wakasa: Integrated metabolomic and transcriptomic analyses of high-tryptophan rice expressing a mutant anthranilate synthase alpha subunit. J Exp Bot 58; 3309-3321, 2007
- Hattori, Y., H. Konno, M. Abe, H. Miyoshi, T. Goto and H. Makabe: Synthesis, determination of the absolute configuration of tonkinelin, and inhibitory action with bovine heart mitochondrial complex I. Bioorg Med Chem 15; 3026-3031, 2007
- Heinzle, E., F. Matsuda, H. Miyagawa, K. Wakasa and T. Nishioka: Estimation of metabolic fluxes, expression levels and metabolite dynamics of a secondary metabolic pathway in potato using label pulse feeding experiments combined with kinetic network modeling and simulation. Plant J 50; 176-187, 2007
- Horiuchi, J., A. Muroi, J. Takabayashi and T. Nishioka: Exposing Arabidopsis seedlings to borneol and bornyl acetate affects root growth: Specificity due to the chemical and optical structures of the compounds. J Plant Interactions 2; 101–104, 2007
- Ichimaru, N., N. Yoshinaga, T. Nishioka and H. Miyoshi: Effect of stereochemistry of Δlac-acetogenins on the inhibition of mitochondrial complex I (NADH-ubiquinone oxidoreductase). Tetrahedron 63; 1127-1139, 2007
- Ishii, N., K. Nakahigashi, T. Baba, M. Robert, T. Soga, A. Kanai, T. Hirasawa, M. Naba, K. Kenta Hirai, A. Hoque, P. Y. Ho, Y. Kakazu, K. Sugawara, S. Igarashi, S. Harada, T. Masuda, N. Sugiyama, T. Togashi, M. Hasegawa, Y. Takai, K. Yugi, K. Arakawa, N. Iwata, Y. Toya, Y. Nakayama, T. Nishioka, K. Shimizu, H. Mori and M. Tomita: Multiple high-throughput analyses monitor the response of *E. coli* to perturbations. Science 316; 593-597, 2007
- Matsumoto, J., K. Sakamoto, N. Shinjo, Y. Kido, N. Yamamoto, K. Yagi, H. Miyoshi, N. Nonaka, K. Katakura, K. Kita and Y. Oku: Anaerobic NADH-fumarate reductase system is predominant in the respiratory chain of *Echinococcus multilocularis*, providing a novel target for the chemotherapy of alveolar echinococcosis. Antimicrob Agents Chemother 52, 164-170, 2007
- Murai, M., A. Ishihara, T. Nishioka, T. Yagi and H. Miyoshi: The ND1 subunit constructs the inhibitor binding domain in bovine heart mitochondrial complex I. Biochemistry 46; 6409-6416, 2007

- Nomura, T., A. Ishihara, H. Iwamura and T. R. Endo: Molecular characterization of benzoxazinone-deficient mutantion in A-genome diploid wheat. Phytochemistry 68; 1008-1016, 2007
- Nomura, T., A. Ishizuka, K. Kishida, A. K. M. R. Islam, T. R. Endo, H. Iwamura, and A. Ishihara: Chromosome arm location of the genes for the biosynthesis of hordatines in barley. Gene Genet Syst 82; 455-464, 2007
- Okazaki, Y., A. Ishizuka, A. Ishihara, T. Nishioka and H. Iwamura: New dimeric compounds of avenanthramide phytoalexin in oats. J Org Chem 72; 3830-3839, 2007
- Tebayashi S., Y. Horibata, E. Mikagi, T. Kashiwagi, D. B. Mekuria, A. Dekebo, A. Ishiahra, C. Kim: Induction of resistance against leafminer, Liriomyza trifolii, by jasmonic acid, in sweet pepper. Biosci Biotechnol Biochem 71; 1521-1526, 2007
- Yamashita, T., E. Nakamaru-Ogiso, H. Miyoshi, A. Matsuno-Yagi and T. Yagi: Roles of bound quinone in the single subunit NADH-quinone oxidoreductase (Ndi1) from Saccharomyces cerevisiae. J Biol Chem 282; 6012-6020, 2007
- Yang, K., J. Zhang, A. S. Vakkasoglu, R. Hielscher, J. P. Osborne, J. James Hemp, H. Miyoshi, P. Hellwig and R. B. Gennis: Glutamate 107 in subunit I of the cytochrome bd quinol oxidase from *Escherichia coli* is protonated and is near the heme d/heme b<sub>595</sub> binuclear center. Biochemistry 46; 3270-3278, 2007
- Yoshida, T., M. Murai, M. Abe, N. Ichimaru, T. Harada, T. Nishioka and H. Miyoshi: Crucial structural factors and mode of action of polyene amides as inhibitors for mitochondrial NADH-ubiquinone oxidoreductase (complex I). Biochemistry 46; 10365-10372, 2007

#### Reviews

- Mitsuno, H., T. Sakurai and T. Nishioka: Studies on the insect odorant receptors. Annual Report of Chemical Research Institute of Sugiyama Foundation 2007; 33-51, 2007
- Ishihara, A., F. Matsuda, H. Miyagawa and K. Wakasa: Metabolomics for metabolically manipulated plants: effects of tryptophan overproduction. Metabolomics 3; 319-334, 2007
- b) Conference and seminar papers presented
- The 51st Annual Meeting of the Japanese Society of Applied Entomology and Zoology: 1 report.
- The 55th Annual Meeting of the Mass Spectrometry Society of Japan (Workshop): 1 report.
- The 2nd Symposium on Metabolomics: 1 report (Invited lecture).
- The Symposium on Informatics of Chemistry and Biology: 1 report (Invited lecture).
- Annual Meeting of Japanese Society of Bioscience, Biotechnology, and Agrochemistry 2007: 5 reports.
- The 33nd Annual Meeting of the Japan Bioenergetics Group: 1 report.

The 32th Annual Meeting of Pesticide Science Society of Japan: 1 report.

### A-3. Off-campus activities

#### Membership in academic societies

Nishioka, T.: The Japanese Society of Bioscience, Biotechnology, and Agrochemistry (The Board Member of Exceptive Committee), The Chemical Society of Japan (The Board Member of Exceptive Committee, Division of Chemical Informatics).

Miyoshi, H.: Pesticide Science Society of Japan (councilor, editorial board member)

### Research grants

Monbu-Kagakusho Research Grant: Grants-in-Aids for Scientific Research (B), Metabolomics

analysis of the global management of primary metabolites for the secondary metabolism in plants (Nishioka, head); Grants-in-Aids for Priority Areas Research, Development of analytical method for metabolomics and predictions of metabolic pathways (Nishioka, member); Exploratory Research, Development of conductive inhibitor-modified electrodes toward pin-point analysis of the electron transfer in respiratory enzymes (Miyoshi, head); Grant-in-Aids for Scientific Research (C) Metabolic fates of sencondary metabolites in plant defense (Ishihara, head). Grants-in-Aids for Scientific Research (B), Metabolomics analysis of the global management of primary metabolites for the secondary metabolism in plants (Ishihara, member)

# A-4. International cooperatons and overseas activities

### International joint researches, overseas research surveys

Miyoshi, H.: Functional analysis of membrane domain subunits of mitochondrial complex-I through photoaffinity labeling study. (USA)

### B. Educational Activities (2007.4-2008.3)

### B-1. On-campus teaching

a) Courses given

- Undergraduate level: Bioorganic chemistry II (Nishioka, Miyoshi), Biotechnology Strategy of agricultural sciences (Nishioka), Introduction of applied life sciences I (Nishioka), Introduction of applied life sciences IV (Miyoshi), Laboratory course in bioorganic chemistry (Miyoshi, Ishihara)
- Graduate level: Chemistry of biologically active compounds (Nishioka), Biofunction chemistry seminar (Nishioka, Miyoshi), Experimental course of biofunctional chemistry (Nishioka, Miyoshi).

### B-2. Off-campus teaching, etc.

### Part-time lecturer

- Nishioka, T.: Part-time professor, Graduate School of Media and Governance, Keio University; Invited professor, Institute of Natural Medicine, Toyama University; Part-time lecturer, Graduate School of Informatics, Nara Institute of Science and Techonology.
- Ishihara, A.: Part-time lecturer, Faculty of Human Life and Science, Doshisha Women's College of Liberal Arts.

# 2.3.11 Laboratory of Applied Structural Biology

Staff Professor : Mikami Bunzo, Dr. Agric. Sci. Associate Professor: Aibara, Shigeo, Dr. Agric. Sci. Assistant Professor : Takahashi, Nobuyuki, Dr. Agric. Sci. Assistant Professor : Mizutani, Kimihiko, Dr. Agric. Sci. Students and research fellows

> Master's program : (2) Undergraduate : (3) Research student :(1)

# A. Research Activities (2007.4-2008.3) A-1. Main subjects

a) Structure Determination of Proteins and Enzymes

Using X-ray crystallographic analysis, we have determined 3D structures of many proteins (Egg white proteins, plant seed proteins, lectins, and so on) and enzymes (amylase, pullulanase, polysaccharide lyase, and so on). Furthermore, proteins forming good crystals such as ovotransferrin could be applied for sub-atomic resolution X-ray crystallography and neutron crystallography to determine the positions of hydrogen atoms.

b) Functional Analysis and Protein Engineering based on Structure Analysis

Industrially utilized enzymes such as  $\beta$ -amylase and pullulanase are trying to be improved on their enzymatic functions by protein engineering based on their structural analyses. The optimal pH and product specificity of  $\beta$ -amylase are modified by site-directed mutagenesis of a few amino acid residues around the catalitic site including a flexible loop of the enzyme based on their crystallographic models. The product specificity of pullulanase is proved to be engineered by site-directed mutagenesis on the loop adjacent to its active site. Furthermore, ovalbumin, a majour component of egg white is going to be modified by rational design based on its 3D structure: The protein does not have inhibitory activity, although it belongs to a superfamily of serine proteinase inhibitors, which exert physiologically important roles in vertebrate by a conformational change called loop-insertion. The crystallographic data along with successful productions for mutants with an increased loop-insertion rate strongly suggested that the acquisition of the serpin inhibitory activity is possible for ovalbumin. As another object, the structure of ovotransferrin and its mutant was studied in detail. Transferrin is a iron transporter protein derivering iron from blood to target cells. On the target cells, transferrin-iron complex binds with a specific receptor, internalized into the cell, and then release iron by a domain opening mechanism. To find the anion-dependent iron binding mechanism, the structure of ovotransferrin was studied by X ray crystallographic analysis at sub-atomic resolution.

c) Protein crystal growth using the microgravity environment

The effects of microgravity on protein crystal growth and the mechanism of the crystal growth were studied on the basis of the results of crystallographic analysis of single crystals prepared in space. Although protein single crystals of good diffraction quality were obtained in space, the crystal growth proceeded by the same mechanism just as on the ground. In space, however, fluctuation of solution was less than on the ground and the migration rate of protein molecules was controlled to the diffusion transport. We explained that it was a factor in growing single crystals of good diffraction quality.

### A-2. Publications and presentations

### a) Publications

### Original papers

- Shimada, N., B. Mikami, S. Watanabe and K. Makino: Preliminary crystallographic analysis of L-2-keto-3-deoxyarabonate dehydratase, an enzyme involved in an alternative bacterial pathway of L-arabinose metabolism. Acta Crystallograph F 63 (Pt 5); 393-395, 2007
- Terasaki, S., Y. Tanaka, T. Nagakura, T. Hayashi, S. Shibayama, K. Muroi, T. Okazaki, D. Garboczi, B. Mikami, T. Honjo and N. Minato: Specific and high-affinity binding of tetramerized PD-L1 extracellular domain to PD-1-expressing cells: possible application to enhance T cell function. Int Immunol 19(7); 881-890, 2007
- Cui, Z., Y. Maruyama, B. Mikami, W. Hashimoto and K. Murata: Crystal structure of glycoside hydrolase family 78 alpha-L-Rhamnosidase from *Bacillus* sp. GL1. J Mol Biol 374(2); 384-398, 2007
- Ochiai, A., T. Itoh, Y. Maruyama, A. Kawamata, B. Mikami, W. Hashimoto and K. Murata: A novel structural fold in polysaccharide lyases: *Bacillus subtilis* family 11 rhamnogalacturonan lyase YesW with an eight-bladed beta-propeller. J Biol Chem 282(51); 37134-37145, 2007
- Hashimoto, W., T. Itoh, Y. Maruyama, B. Mikami and K. Murata: Hydration of vinyl ether groups by unsaturated glycoside hydrolases and their role in bacterial pathogenesis. Int Microbiol 10(4); 233-243, 2007
- Maruyama, Y., M. Momma, B. Mikami, W. Hashimoto and K. Murata: Crystal structure of a novel bacterial cell-surface flagellin binding to a polysaccharide. Biochemistry 47(5) 1393-1402, 2008
- Yoshikane, Y., N. Yokochi, M. Yamasaki, K. Mizutani, K. Ohnishi, B. Mikami, H. Hayashi and T. Yagi: Crystal structure of pyridoxamine-pyruvate aminotransferase from *Mesorhizobium loti* MAFF303099. J Biol Chem 283(2); 1120-1127, 2008
- Murata, K., S. Kawai, B. Mikami and W. Hashimoto: Superchannel of bacteria: biological significance and new horizons. Biosci Biotechnol Biochem 72(2) 265-277, 2008
- Lin, D.Y., Y. Tanaka, M. Iwasaki, A.G. Gittis, H.P. Su, B. Mikami, T. Okazaki, T. Honjo, N. Minato and D.N. Garboczi: The PD-1/PD-L1 complex resembles the antigen-binding Fv domains of antibodies and T cell receptors. Proc Natl Acad Sci USA 105(8); 3011-3016 2008
- Aibara, S., K. Mizutani, A. Suzuki, H. Horiuchi, K. Hashimoto and T. Yamane: Comparison of Molecular Packing between two kinds of hen egg-white lysozyme orthorhombic crystals. J Jpn Soc Microgravity Appl 25(2); 82-88 2008

#### Reports

- Mikami, B., A. Tanabe, T. Itoh and M. Yamasaki, T: X-ray crystallographic analysis of V99N beta-amylase/maltose complex. SPring-8 User Experiment Report 2007A1437, 2007
- Mikami, B., T. Fukuda, T. Itoh, K. Park. N. Maruyama and S. Utsumi: X-Ray Crystallographic Analysis of Pea Proglobulin. SPring-8 User Experiment Report 2007A1439, 2007
- Mikami B., S. Takehara, M. Yamasaki, N. Takahashi nd M. Onda: X-Ray crystallographic analysis of neuroserpin SPring-8 User Experiment Report 2007A1685, 2007
- Mikami, B., T. Saito, A. Tanabe, M. Adachi and S. Utsumi: X-ray crystallographic analysis of

G97A beta-amylase: The role of a flexible loop in the active site. SPring-8 User Experiment Report 2007B1418, 2007

- Mikami, B., T. Fukuda, T. Itoh, K. Park. N. Maruyama and S. Utsumi: X-Ray crystallographic analysis of rapeseed procruciferin. SPring-8 User Experiment Report 2007B1490, 2007
- b) Conference and seminar papers presented
- The 2008 Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry : 8 papers

The 80th Annual Meeting of The Japanese Biochemical Society: 2 papers

The 2007 Annual Meeting of The Society for Biotechnology, Japan: 1 papers

The 454th Kansai Branch Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry: 2 reports

# A-3. Off-campus activities

### Membership in academic societies

Mikami, B.: The Japanese Society of Applied Glycoscience (an editoryal board member)

Aibara, S.: The 169 committee of Japan society for the promotion of science (General secretary)

### Research grants

National Project on Target Proteins (Mikami, shared with Hashimoto)

Grants-in-Aid for Scientific Research From the Ministry of Education, Science, Sports and Culture of Japan: General Scientific Research (B) (Mikami). General Scientific Research (C) (Takahashi).

### A-4. International cooperations and overseas activities

### International joint researchers, overseas research surveys

Mikami, B.: Tertiary structure of bacterial enzymes (Seoul University, Korea)

Mizutani, K.:Structure determination of membrane proteins by X-ray crystallography (Imperial College London, England)

# B. Educational Activities (2007.4-2008.3)

### B-1. On-campus teaching

a) Courses given

- Undergraduate level: Laboratory Course in Biological Chemistry (Mikami, Aibara, Takahashi, Mizutani), Chemistry of Biological Catalysis (Mikami)
- Graduate level: Applied Structural Biology Seminar (Mikami, Aibara, Takahashi, Mizutani), Experimental Course of Applied Structural Biology (Mikami, Aibara, Takahashi, Mizutani)

### B-2. Off-campus teaching, etc.

### Part-time lecturer

- Mikami, B.: Department of Agricultural Sciences; Kobe University, Faculty of Agriculture, Department of Agricultural Sciences; Kyoto Prefectural University, Faculty of Agriculture
- Aibara, S.: Mukogawa Women's University; Dep. of Food Sci. and Nutr., School of Human Environ. Sci. (Biochemistry)

# Chair of Molecular Biofunction (Institute for Chemical Research)

# 2.3.12 Laboratory of Chemistry of Molecular Biocatalysts

Staff Professor

Associate Professor: Hiratake, Jun, Dr. Agric. Sci. Assistant Professor : Mizutani, Masaharu, Dr. Agric. Sci. Assistant Professor : Shimizu, Bun–ichi, Dr. Agric. Sci. Postdoctoral fellow: Han Liyou Students and research fellows

> Doctor's program : (2) Master's program : (10)

# A. Research Activities (2007.4-2008.3)

### A-1. Main subjects

a) A new diglycosidase family in plant kingdom

A  $\beta$ -primeverosidase from tea plants (*Camellia sinensis*) is a unique disaccharide-specific diglycosidase, which hydrolyses  $\beta$ -primeverosides (6- $O\beta$ -D-xylopyranosyl- $\beta$ -D-glucopyranosides) to liberate primeverose and various aroma alcohols as aglycons.  $\beta$ -Primeverosidase is classified in glycosyl hydrolase family 1. In order to clarify the molecular mechanism by which the diglycosidases specifically recognize disaccharide-glycosides, the recombinant  $\beta$ -primeverosidase was produced in insect cells using a baculovirus expression system, and was purified with a novel affinity column with our original inhibitor  $\beta$ -primeverosylamidine as ligand. We succeeded in crystallization of  $\beta$ -primeverosidase, and its crystal structure at 1.8 Å resolution was obtained to identify the key residues recognizing the diglycoside. The co-crystallization with  $\beta$ -primeverosylamidine and its crystal structure at 1.8 Å resolution was also obtained.

b) Active-site mapping of  $\gamma$ -glutamyltranspeptidases by transition-state analogue inhibitors

 $\gamma$ -Glutamyltranspeptidase (GGT) is a key enzyme in glutathione metabolism. A series of electrophilic  $\gamma$ -phosphonate diester analogues of glutamate were designed and synthesized as transition-state analogue inhibitors of *E. coli* and human GGTs. These phosphonate diesters served as highly potent and selective mechanism-based inhibitors of GGT that reacted covalently with the catalytic Thr residue to inactivate GGT. These inibitors were used successfully for probing the active-site geometries of both GGTs. Human GGT has distinct substrate specificity with respect to the acceptor site, and accordingly the phosphonate inhibitors with an appropriate functional group at a specific site served as extremely potent inhibitor of human GGT. On the other hand, *E. coli* GGT was inhibited by any structural analogues of phosphonates, according solely on their chemical reactivity, which agrees well with broad substrate specificity of this enzyme. The phosphonate diesters thus served successfully as chemical probes for active-site mapping of GGTs to understand the mechanism of substrate recognition.

c) Directed evolution of *Pseudomonas* lipase:

A *Pseudomonas* lipase was subjected to directed evolution for improved amide-hydrolyzing activities. A library of mutant lipases was made by whole-gene random mutagenesis and

saturation mutagenesis at specific sites. The CAST-P program was used to identify the active-site residues that interact directly with the substrate. After five rounds of random mutagenesis combined with saturation mutagenesis, a mutant lipase with 20-time higher molecular activity was obtained for the hydrolysis of oleoyl  $\beta$ -naphthylamide. The kinetic analyses of the mutant and wild-type lipases suggested that the increase in amide-hydrolyzing activities was due to the increase in the leaving group protonation during the collapse of the tetrahedral intermediate.

d) Mechanism of the activation/inactivation processes of plant hormones

The physiological functions of plant hormones are regulated by the concerted processes of biosynthesis, catabolism and translocation in the responsive organs. Therefore, identification and characterization of enzymes involved in these process are very important in understanding how they regulate the plant life cycle from germination to flowering. In this study, we have characterized cytochrome P450 monooxygenases (P450) involved in the biosynthesis of brassinosteroids (BRs). We characterized the biochemical properties of a C-22 hydroxylase and a C-23 hydroxylase, and found novel shortcut routes of BR biosynthetic pathway. In addition, we have identified the *Arabidopsis* CYP710A family as sterol C-22 desaturases involved in the final reaction of plant sterol biosynthesis.

e) Coumarin biosynthesis in plants

The coumarin contents in wild-type and a mutant of Arabidopsis were deretemined to find that the roots of Arabidopsis accumulate a significant amount of scopolin (a  $\beta$ -glucoside of scopoletin). The mutations of several genes coding the enzymes of the phenylpropanoid pathway caused severe decrease in scopolin contents. Functional analysess of these genes with the recombinant proteins successfully identified the enzymes catalyzing methylation and oxidation steps of scopoletin biosynthesis in Arabidopsis. We also identified UGT71C1 (At2g29750) as a glucosyltransferase catalyzing the glucosylation step of scopoletin.

# A-2. Publications and presentations

### a) Publications

#### Reviews

- Mizutani, M.: Molecular evolution of cytochrome P450s and the origin of chemical diversity of plant metabolites. Protein Nucleoic Acid and Enzyme 52; 1454-1464, 2007
- Mizutani, M.: Enzyme chemical studies on cytochrome P450s involved in the metabolism of abscisic acid and plant steroids. Plant Growth Regulations 42; 260-268, 2007.

### **Original papers**

- Han, L., J. Hiratake, A. Kamiyama and K. Sakata: Design, synthesis and evaluation of γ-phosphono diester analogues of glutamate as highly potent inhibitors and active site probes of γ-glutamyl transpeptidase. Biochemistry 46; 1432-1447, 2007
- Nakagawa, Y., A. Hasegawa, J. Hiratake, and K. Sakata: Engineering of *Pseudomonas aeruginosa* lipase by directed evolution for enhanced amidase activity: Mechanistic implication for amide hydrolysis by serine hydrolases. Protein Engineering, Design and Selection 20; 339-346, 2007
- Shimomura, H., H. Etoh, M. Mizutani, N. Hirai and Y .Todoroki: Effect of the minor ABA metabolite 7'-hydroxy-ABA on Arabidopsis ABA 8'-hydroxylase CYP707A3. Bioorg Med Chem Lett 17; 4977-4981, 2007

- Ueno, K., H. Yoneyama, M. Mizutani, N. Hirai and Y. Todoroki: Asymmetrical ligand binding by abscisic acid 8'-hydroxylase. Bioorg Med Chem 15; 6311-6322, 2007
- Ahn, Y.O, H. Saino, M. Mizutani, B. Shimizu and K. Sakata: Vicianin hydrolase is a novel cyanogenic β-glycosidase specific to β-vicianoside (6-O-α-L-arabinopyranosyl-β-Dglucopyranoside) in seeds of Vicia angustifolia. Plant Cell Physiol 48; 938-947, 2007
- Cho, J-Y, M. Mizutani, B. Shimizu, T. Kinoshita, M. Ogura, K. Tokoro, M-Li. Lin and K. Sakata: Gene expression profiling and chemical profiling during the manufacturing process of Taiwan oolong tea "Oriental Beauty". Biosci Biotechnol Biochem 71; 1476-1486, 2007
- b) Conference and seminar papers presented
- The 2007 Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry: 10 papers
- The 49th Annual Meeting of the Japanese Society for Plant Physiologists: 6 papers
- The 2<sup>nd</sup> Annual Meeting of Japan Society for Chemical Biology: 1 paper
- K · I Chemical Research Institute Co., Ltd., Seminar: 1 paper
- Kumiai Chemical Industry Co., Ltd. Seminar: 1 paper
- 19th International Conference on Plant Growth Substances: 3 papers
- The 25th Annual Meeting of Japanese Society for Plant Cell and Molecular Biology: 1 paper
- 2nd International Symposium on Diffraction Structural Biology 2007: 1 paper
- The 2007 Joint Meeting of Kansai and Chubu Branches of Japan Society for Bioscience, Biotechnology, and Agrochemistry: 1 papers
- The 59th Annual Meeting of The Society for Biotechnology, Japan: 1 paper
- Gordon Research Conference: 1 paper
- Kyoto University, Graduate School of Agriculture Symposium: 1 paper
- The 42<sup>nd</sup> Annual Meeting of The Japanese Society for Chemical Regulation of Plants: 3 papers
- Shizuoka University, Department of Agriculture, Lecture and Seminar: 2 papers
- Toyama University, Institute for Natural Medicine Special Seminar: 1 paper
- The 2007 Annual Spring Meeting of The Japanese Society for Horticultural Science: 1 paper
- The 113th Symposium of Japanese Society of Breeding: 1 paper
- The 10th Biocatalyst Chemistry Symposium: 1 paper
- Bio-related Chemistry Joint Symposium: 2 papers

### A-3. Off-campus activities

### Membership in academic societies (roles)

Hiratake, J.: Japan Society for Bioscience, Biotechnology, and Agrochemistry Kansai Branch (councillor)

### Research grants

Research Grants from Ministry of Education, Culture, Sports, Science and Technology and Japan Society for the Promotion of Science:

Grant-in-Aid for Scientific Research (B), Field studies on mechanism of aroma formation of high-quality Darjeeling tea and development of novel black tea production (Sakata K); Grant-in-Aid for Scientific Research (B), Development chemicals for controlling glutathione metabolism and oxidative stress for use in chemical biology (Hiratake J) Grant-in-Aid for Scientific Research (C) (2) Construction of plant oxygenase library and its functional characterization (Mizutani M).

# A-4. International cooperation and overseas activities

# International joint researches, overseas research surveys

Hiratake, J.: Design and Synthesis of Asparagine Synthetase Inhibitors for Development of Chemotherapeutics for Acute Lymphoblastic Leukemia (USA)

# B. Educational Activities (2007.4-2008.3)

# B-1. On-campus teaching

a) Courses given

Undergraduate level: Pocket Seminar (Let's touch the heart of live Organic Chemistry) (Hiratake) Graduate level: Seminar in Molecular Biocatalysts (Hiratake, Mizutani, Shimizu), Laboratory Course in Molecular Biocatalysts (Hiratake, Mizutani, Shimizu)

# B-2. Off-campus teaching, etc.

# Part-time lecturer

Hiratake, J.: Shizuoka University (Graduate School of Agriculture, Division of Applied Bioligical Chemistry)

# An extension lecture etc.

Hiratake, J.: Super-Science High School (SSH) sponsored by the Ministry of Education, Culture, Sports, Science and Technology, Special lecture for students of Zeze high school, Shiga (2007. 6. 14), Super-Science High School (SSH) sponsored by the Ministry of Education, Culture, Sports, Science and Technology, Special lecture for students of Rakuhoku High School, Kyoto (2007. 10. 11), Science-Partnership Project (SPP) sponsored by the Ministry of Education, Culture, Sports, Science and Technology, Special lecture at Momoyama High School, Kyoto (2007. 11. 10 and 11. 24), Special lecture of Rakuhoku Science Program for junior high school students of Rakuhoku High School, Kyoto (2007. 11. 13)

# 2.3.13 Laboratory of Molecular Microbial Science (Institute for Chemical Research)

Staff Professor : Esaki, Nobuyoshi, Dr. Agric. Sci. Associate Professor: Kurihara, Tatsuo, Dr. Eng. Assistant Professor : Mihara, Hisaaki, Dr. Agric. Sci. Postdoctoral fellows: Yoshida, Masahiro, Dr. Eng. Abe, Katsumasa, Dr. Agric. Sci Kawamoto, Jun, Dr. Agric. Sci.

Students and research fellows Doctor's Program : (7) Master's Program: (12)

### A. Research Activities (2007.4-2008.3)

### A-1. Main subjects

a) Function of polyunsaturated fatty acid in the cell membrane of a psychrotrophic bacterium

An Antarctic psychrotrophic bacterium, Shewanella livingstonensis Ac10, produces cis 5,8,11,14,17-eicosapentaenoic acid (EPA), a long-chain polyunsaturated fatty acid (LPUFA), as a component of membrane phospholipids at low temperatures. The EPA-less mutant generated by disruption of the EPA synthesis gene becomes cold-sensitive. We studied whether the cold sensitivity could be suppressed by supplementation of various LPUFAs. The EPA-less mutant was cultured at 6°C in the presence of synthetic phosphatidylethanolamines (PEs) that contained oleic acid at the sn-1 position and various C20 fatty acids with different numbers of double bonds from zero to five or cis 4,7,10,13,16,19 docosahexaenoic acid (DHA) at the sn-2 position. Mass spectrometric analyses revealed that all these fatty acids became components of various PE and phosphatidylglycerol species together with shorter partner fatty acids, indicating that large-scale remodeling followed the incorporation of synthetic PEs. As the number of double bonds in the sn-2 acyl chain decreased, the growth rate decreased, and the cells became filamentous. The growth was restored to the wild-type level only when the medium was supplemented with phospholipids containing EPA or DHA. We found that about a half of DHA was converted into EPA. The results suggest that intact EPA is best required for cold adaptation of this bacterium. b) Mechanism of biosynthesis of selenocysteyl-tRNA<sup>Sec</sup>

Selenium, a cognate element of sulfur, is an essential trace element for many organisms including mammals and plays various physiological function as a selenocysteine residue of proteins. Selenocysteine residue is inserted into a polypeptide chain in the translation system using selenocysteyl-tRNA<sup>Sec</sup>. It has been thought that the synthesis of *O*-phosphoseryl-tRNA<sup>Sec</sup> from seryl-tRNA<sup>Sec</sup> by *O*-phosphoseryl-tRNA<sup>Sec</sup> kinase (MJPSTK) was indispensable in the process of selenocysteyl-tRNA<sup>Sec</sup> biosyntheses in archaea. We performed the *in vitro* analysis of SelD, MJPSTK, and selenocysteine synthase (MJSecS) from a methanogenic archaeon *Methanocaldococcus jannaschii* and found that selenocysteyl-tRNA<sup>Sec</sup> can be synthesized through two independent pathways: one involves MJPSTK and SelD, and the other depends on MJSecS and SelD.

### A-2. Publications and presentations

a) Publications

### **Original Papers**

- Fujita, M., H. Mihara, S. Goto, N. Esaki and M. Kanehisa: Mining prokaryotic genomes for unknown amino acids: a stop-codon-based approach. BMC Bioinformatics 8; 1-11, 2007
- Kawamoto, J., T. Kurihara, M. Kitagawa, I. Kato and N. Esaki: Proteomic studies of an Antarctic cold-adapted bacterium, *Shewanella livingstonensis* Ac10, for global identification of cold-inducible proteins. Extremophiles 11; 819-826, 2007
- Omi, R., K. Jitsumori, T. Yamauchi, S. Ichiyama, T. Kurihara, N. Esaki, N. Kamiya, K. Hirotsu and I. Miyahara: Expression, purification and preliminary X-ray characterization of DL-2-haloacid dehalogenase from *Methylobacterium* sp. CPA1. Acta Crystallograph Sect F Struct Biol Cryst Commun 63; 586-589, 2007
- Miyake, R., J. Kawamoto, Y.-L. Wei, M. Kitagawa, I. Kato, T. Kurihara and N. Esaki: Construction of a low-temperature protein expression system using a cold-adapted bacterium, *Shewanella* sp. strain Ac10, as the host. Appl Environ Microbiol 73; 4849-4856, 2007
- Yoshida, M., T. Oikawa, H. Obata, K. Abe, H. Mihara and N. Esaki: Biochemical and genetic analysis of the y-resorcylate (2,6-dihydroxybenzoate) catabolic pathway in *Rhizobium* sp. strain MTP-10005: Identification and functional analysis of its gene cluster. J Bacteriol 189; 1573-1581, 2007
- Yamanishi, Y., H. Mihara, M. Osaki, H. Muramatsu, N. Esaki, T. Sato, Y. Hizukuri, S. Goto and M. Kanehisa: Prediction of missing enzyme genes in a bacterial metabolic network reconstruction of the lysine-degradation pathway of *Pseudomonas aeruginosa*. FEBS J 274; 2262-2273, 2007
- Saito, M., K. Nishimura, Y. Hasegawa, T. Shinohara, S. Wakabayashi, T. Kurihara, M. Ishizuka and Y. Nagata: Alanine racemase from *Helicobacter pylori* NCTC 11637: Purification, characterization and gene cloning. Life Sci 80; 788-794, 2007
- Saito, M., K. Nishimura, S. Wakabayashi, T. Kurihara and Y. Nagata: Purification of branched-chain amino acid aminotransferase from *Helicobacter pylori* NCTC 11637. Amino Acids 33; 445-449, 2007
- Takahata, M., T. Tamura, K. Abe, H. Mihara, S. Kurokawa, Y. Yamamoto, R. Nakano, N. Esaki and K. Inagaki: Selenite assimilation into formate dehydrogenase H depends on thioredoxin reductase in *Escherichia coli*. J Biochem 143; 467-473, 2008

# Reviews

- Kurihara, T. and N. Esaki: Bacterial hydrolytic dehalogenases and related enzymes: Occurrences, reaction mechanisms, and applications. Chem Rec 8; 67-74, 2008
- Kurihara, T. and N. Esaki: Proteomic studies of psychorophilic microorganisms. Psychrophiles: From Biodiversity to Biotechnology 333-343, 2008
- b) Conference and seminar papers presented
- The 59th Annual Meeting of the Vitamin Society of Japan: 2 papers
- The 18th Annual Meeting of the Japan Society for Biomedical Research on Trace Elements: 4 papers
- Annual Meeting of the Society for Biotechnology, Japan 2007: 1 paper

Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry 2008: 6 papers

Biochemistry and Molecular Biology 2007: 6 papers

The 8th Annual Meeting of the Japanese Society for Extremophiles: 3 papers

International Symposium on Metallomics 2007: 1 paper

International Conference on Biotechnology Engineering (ICBioE '07): 1 paper

The 41st IUPAC World Chemistry Congress: 1 paper

TRACE ELEMENTS in DIET, NUTRITION, & HEALTH: Essentiality and Toxicity : 2 papers Gordon Research Conference: Molecular and Cellular Biology of Lipids : 1 paper

# A-3. Off-campus activities

# Membership in academic societies

- Esaki, N.: The Japanese Biochemical Society (Councilor and a Member of International Exchange Committee), The Japan Trace Nutrients Research Society (Director), The Japan Society for Bioscience, Biotechnology and Agrochemistry (Councilor), The Society for Biotechnology, Japan (Councilor), The Vitamin Society of Japan (Councilor), Japan Society for Biomedical Research on Trace Element (Councilor)
- Kurihara, T.: The Society for Biotechnology, Japan (Editorial Board), The Japanese Biochemical Society (Kinki Branch Councilor, Secretary)

# Research grants

Research Grants from Japan Society for the Promotion of Science: Grant-in-Aid for Scientific Research (B); Structure-function analysis of selenium-specific chemical transformation system and cotranslational selenium insertion into protein (N. Esaki), Grants from Japan Society for the Promotion of Science: Grant-in-Aid for Scientific Research (B); Exploration of organisms with a unique selenium metabolic activity and its application to bioremediation (N. Esaki), Grant-in-Aid for Scientific Research (B); Bioconversion of organofluorine compounds with microbial enzymes: analysis of reaction mechanisms and application for production of useful compounds and remediation of environments (T. Kurihara), Grant-in-Aid for Scientific Research (B); Exploration of novel cold-adapted microorganisms to develop a system for the production of useful compounds at low temperatures (T. Kurihara), Grant-in-Aid for Young Scientists (B); Mechanism of insertion of sulfur and selenium into the wobble base of tRNA anticodon (H. Mihara), A Grant for Research for Promoting Technological Seeds form JST; Development of novel lysine α-oxidase and application to L-pipecolate production (H. Mihara)

# A-4. International cooperations and overseas activities

# International meetings (roles)

Esaki, N.: International Conference on Biotechnology Engineering (ICBioE '07) (speaker), The 41st IUPAC World Chemistry Congress (speaker), TRACE ELEMENTS in DIET, NUTRITION, & HEALTH: Essentiality and Toxicity (speaker)

Kurihara, T.: Gordon Research Conference: Molecular and Cellular Biology of Lipids (speaker)

Mihara, H.: TRACE ELEMENTS in DIET, NUTRITION, & HEALTH: Essentiality and Toxicity (speaker)

# Membership in international academic societies

Esaki, N.: The International Society for Extremophiles (editorial board)

# B. Educational Activities (2007.4-2008.3)

# B-1. On-campus teaching

a) Courses given

Graduate level : Molecular Microbial Science Seminar (Esaki, Kurihara and Mihara), Experimental Course of Molecular Microbial Science (Esaki, Kurihara and Mihara), Molecular Microbial Science (Esaki and Kurihara)

# B-2. Off-campus teaching, etc.

### Part-time lecturer

Esaki, N.: Faculty of Science, Osaka Prefecture University (Biological science) Kurihara, T.: Nagahama Institute of Bio-Science and Technology (Bioreaction engineering)

# B-3. Overseas teaching

### Students and research fellows from abroad

Foreign students: Master course student 2 (China, Korea), Guest Research Associate 1 (Korea)

Division of Diagnostics and Control of Humanospere (Research Institute for Sustainable Humanosphere)

# 2.3.14 Laboratory of Plant Gene Expression

Staff	Professor : Yazaki, Kazufumi, Dr. Pharm. Sci.		
	Associate Professor: Hayashi, Takahisa, Dr. Agric. Sci.		
	Lecturer :	Kuroda, Hiroyuki, Dr. Agric. Sci.	
	Program-Specific Assistant Professor (METI) : Shitan, Nobukazu, Dr. Agric. Sci.		
	Postdoctoral fellows:	Masuno-Nakanishi, Tsugumi	
		Kaku, Tomomi	
		Kaida, Rumi	
	Philip J. Linley		
Student	s and research fellows		
	Doctor's program: (4)	Research students: (2)	
	Master's program: (5)		

# A. Research Activities (2007.4-2008.3) A-1. Main Subjects

We are studying on the characterization of plant genes including woody plants which are involved in biosyntheses and transport of various valuable metabolites, e.g. secondary products, in plants, and also studying on the regulatory mechanism of the expression of those genes. The molecular breeding using those genes to establish novel woody plants, for instance phytoremediators to be applied for environmental biotechnology, is also our research targets. Individual research activity is as follows. a) Molecular and cellular biology of secondary metabolism in higher plants.

We are studying on the characterization of plant genes involved in biosyntheses of various secondary metabolites, e.g. isoprenoids and polyphenols, and elucidating the regulatory mechanism of the expression of those genes. 1) Structures and functions of prenyltransferases accepting aromatic substrates such as flavonoids are investigated, i. e. subcellular localization, and the molecular mechanism of their functional diversities, such as substrate specificity. 2) Shikonin is a red naphthoquinone pigment occurring only in Boraginaceous plant species, which are used modern and traditional medicines. Molecular mechanism on the regulation of shikonin biosynthesis is investigated in *Lithospermum erythrorhizon* cell cultures and in the hairy root cultures as well. 3) Dark-inducible genes responsible for the production of secondary metabolites are isolated and characterization of these genes is carried out. 4) Engineering of ubiquinone biosynthesis. Biosynthetic engineering of ubiquinone, the representative electron carrier in respiratory chain of mitochondria, is carried out with yeast and plant as host organisms. In particular, environmental stress tolerance e.g. anti-oxidative stress of high ubiquinone-producing plants are studied.

b) Molecular biology of ABC proteins in plants.

Arabidopsis thaliana contains 123 members of (ATP-binding cassette) ABC proteins. Some of them are reported to function as molecular pump for xenobiotics. 1) Plant ABC proteins, particularly members of multidrug-resistance protein (ABCB)-subfamily and ABCA ortholog in plant is selected to analyze their biochemical functions, i.e. transport of substrates, and physiological role in plant body. In particular, we are focusing on the ABC transporter involved in auxin transport in Arabidopsis. 2) Transport properties of endogenous alkaloid are analyzed with model plant cell cultures, *Coptis japonica and Thalictrum minus* (both Ranunculaceae), and transporter molecules for their main alkaloid, berberine, are cloned to be characterized. Recently we are characterizing MATE-type transporters in plants 3) Isolation and characterization of cDNAs from woody plants, e.g. isoprene synthase: One of the aim is to characterize cDNAs involved in the biosynthesis of secondary metabolites and is to design the genes to good use. The others are to discover unique genes and the expression that are characteristic in woody plants. c) Cell wall and cellulose biosynthesis.

 Cell wall loosening: This study focuses on the structure and function of endo-1,4-β-glucanase.
 Biosynthesis of cellulose in higher plants and in Acetobacter xylinum: Molecular and cell biology of cellulose biosynthesis in higher plants and Acetobacter xylinum.

d) Metabolic and transport engineering of native plant functions and phytoremediation.

By introducing heterologous genes from various organisms into host plants, their functions are altered, e.g. producing a large amount of useful phytochemicals. 1) Genes of prenyltransferase accepting aromatic substrates are cloned from various organisms, such as yeast, E. coli, as well as higher plants, and transgenic medicinal plants that produce high amount of secondary metabolites. 2) Isoprene synthase gene is introduced into Arabidopsis to engineer their terpene metabolism to study the physiological role of isoprene emission from many tree species. 3) Establishment of novel phytoremediation technique by use of ABC transporter genes that are capable of transporting cadmium or arsenate attempted aiming toward clean up the heavy metal-contaminated soil environment.

### e) Molecular biology of intrinsic cDNA clones from woody plants

We are focusing on the cDNAs involved in pathogen-resistant traits, some of which are

related to secondary metabolism and water stress in woody plants. Their translates and transcripts are respectively studying for the molecular machines and for making a diagnosis of the forest biosphere possible.

# A-2. Publications and presentations

a) Publications

### Books

- Yazaki, K. and K. Yamamoto: Shikonin production by *Lithospermum erythrorhizon* cell cultures: biosynthesis, regulation, and bioprocess development. Molecular Plant Cell-Based Bioprocessing (Ed. Wei Zhang), Springer-Verlag, in press
- Shitan, N. and K. Yazaki: Chapter 12. Membrane transport of plant secondary metabolites. In: Plant Membrane and Vacuolar Transporters (Ed. Pawan K. Jaiwal, Rana P. Singh, Om Parkash Dhankher), CAB International, Oxfordshire, UK, pp.283-300, 2008

#### **Original** papers

- Takanashi, K., N. Shitan, A. Sugiyama, Y. Kamimoto, M. Hamamoto, T. Iwaki, K. Takegawa and K. Yazaki: Galactinol synthase gene of *Coptis japonica* involved in berberine tolerance. Biosci Biotech Biochem 72(2); 398-405, 2008
- Sugiyama, A., N. Shitan and K. Yazaki: Involvement of a soybean ATP-binding cassette-type transporter in the secretion of genistein, a signal flavonoid in legume-*Rhizobium* symbiosis. Plant Physiol 144(4); 2000-2008, 2007
- Sasaki, K., T. Saito, M. Lämsä, K-M. Oksman-Caldentey, M. Suzuki, K. Ohyama, T. Muranaka, K. Ohara and K. Yazaki, K.: Plants utilize isoprene emission as a thermotolerance mechanism. Plant Cell Physiol 48(9); 1254-1262, 2007
- Watanabe, T., N. Shitan, T. Umezawa, K. Yazaki, M. Shimada and T. Hattori: Involvement of FpTRP26, a thioredoxin-related protein, in oxalic acid-resistance of the brown-rot fungus *Fomitopsis palustris.* FEBS Lett 581(9); 1788-1792, 2007
- Alonso-Simon, A., P. Garcia-Angulo, AE. Encina, JM. Alvarez, JL. Acebes and T. Hayashi: Increase in XET activity in bean (*Phaseolus vulgaris* L.) cells habituated to dichlobenil. Planta 226; 765-771, 2007
- Nishikubo, N., T. Awano, A. Banasiak, V. Bourquin, F. Ibatullin, R. Funada, H. Brumer, TT. Teeri,
  T. Hayashi, B. Sundberg and EJ. Mellerowicz: Xyloglucan *Endo*-transglycosylase (XET)
  Functions in Gelatinous Layers of Tension Wood Fibers in Poplar—A Glimpse into the
  Mechanism of the Balancing Act of Trees. Plant Cell Physiol 48; 843-855, 2007
- Urbanowicz, BR., AB. Bennett, E. del Campillo, C. Catalá, T. Hayashi, B. Henrissat, H. Höfte, SJ. McQueen-Mason, SE. Patterson, O. Shoseyov, TT. Teeri and JKC. Rose: Structural organization and a standardized nomenclature for plant endo-1,4-8-glucanases (cellulases) of glycosyl hydrolase family 9. Plant Physiol 144;1693-1696, 2007
- Hayashi, T., YW. Park, A. Isogai and T, Nomura: Cross-linking of plant cell walls with dehydrated fructose by smoke-heat treatment. J Wood Sci 54; 90-93, 2008

#### Reviews

- Yazaki, K., A. Sugiyama, M. Morita and N. Shitan: Secondary transport as an efficient membrane transport mechanism for plant secondary metabolites. Phytochem Review, in press
- Sugiyama, A., N. Shitan and K .Yazaki: Signaling from soybean roots to rhizobium, an ATP-binding cassette-type transporter mediates genistein secretion. Plant Signaling &

Behavior 3(1); 38-40, 2008

- Yazaki, K.: ABC proteins involved in plant growth and development and stress responses. The Medical Frontline 62(11); 2447-2453, 2007 (review in Japanese)
- Yazaki, K.: Plant ABC proteins and auxin transport. Regulation of Plant Growth & Development 42(1); 45-53, 2007 (review in Japanese)
- Kuroda, H.: Biology around the pine wilt., Seisonken-Kenkyu (Japanese Bulletin of Research Institute of Sustainable Humanosphere) 3; 1-9, 2007 (review in Japanese)

### Patents

- Yazaki, K.: Membrane-bound prenyltransferases. Submitted by Kyoto University and Api Co. Ltd. April 15th, 2007.
- b) Conference and seminar papers presented
- 49th Annual Meeting of Japanese Society for Plant Physiologyists : 5 reports

Annual Meeting of Japan Wood Society 2007: 3 reports

Annual Meeting of Japanese Society for Bioscience, Biotechnology and Agrochemistry 2007 : 5 reports

The 24th Annual Meeting of Japanese Society for Plant Cell and Molecular Biology : 10 reports

The 128th Annual Meeting of Pharmaceutical Society: 1 report

54<sup>th</sup> Annual Meeting of Pharmacognosy: 2 report

# A-3. Off-campus activities

### Membership in academic societies (roles)

- Yazaki, K. : The Japanese Society for Plant Cell and Molecular Biology (Secretary, Editor), The Japanese Society for Plant Physiologist (Secretary, Editor), Japan Society for Bioscience, Biotechnology, and Agrochemistry (Board member), METI Plant Project Comittee (Board member), The Japanese Bioindustry Association (Editorial Board), Association of Bio Quinone (Executive Board).
- Hayashi, T.: Japan Society for Carbohydrate (Board Member), National Institute of Science and Technology Policy (Researcher), Ministry of Agriculture, Forestry and Fisheries biological environmental assessment (Member)
- Kuroda, H.: The Japan Wood Research Society (sub-coordinator)

### Research grants

- Monbusho Research Grant: Priority Areas, Alkaloid transport by MATE-type transporter localized to vacuolar membrane and the sink function (Yazaki, Head), Scientific Research (B) Structural and functional analyses of prenyltransferase accepting aromatic substrates (Yazaki, Head), Exploratory Research, Creation of cadmium non-absorbing crop plants by proteinase in hibitor BBI, Scientific Research (B) Tension wood formation (Hayashi, Head), Scientific Research (B) A study on upper limit value evaluation of the atmosphere / flood disaster harm external force by a tropical cyclone becoming gigantic (Hayashi), Scientific Research (B) Expression genes involved in pine wilt diseases (Kuroda, Head).
- Others: MEXT Plant Project, Plant metabolic engineering with prenyltransferase genes (Yazaki, Head), Kirin Holdings, Molecular genetic studies on secondary metabolites in hop flowers (Yazaki); Research grant for Sustainable Humanosphere for Mission 1, Increment of plant mass by isoprene synthase gene expression (Yazaki), Grant from Institute of Sustainability Science Hoga research, Molecular mechanism of plant-insect interactions

mediated by plant volatile compounds (Yazaki, Head), Research grant for Sustainable Humanosphere for Hoga Mission, Analysis of signal network via 'green flavor' in transgenic plants (Yazaki), Grant from Institute of Sustainability Science Hoga research, Morphological and functional developments of root hair as an absorbing unit of inorganic nutrients (Yazaki). Program for the Promotion of Basic Research Activities for Innovative Biosciences: Plant cell walls (Hayashi, head), Research grant for Sustainable Humanosphere for Mission 1: Tests of transgenic trees (Hayashi), Research grant from Institute of Sustainability Science: Reforestation — A reformation scenario from deforestation (Hayashi, head), Grant from Kato Memorial Bioscience Research Promoting Foundation: High production of prenylated aromatic compounds by analyzing transport mechanism (Shitan).

### A-4. International cooperations and overseas activities

### International meetings (roles)

- Yazaki, K.: PSE Congress, Plants for human health in the post genome era (Helsinki, Invited speaker), Japan-Korea Joint Seminar, Plant metabolism: from biosynthesis to signal transduction (Incheon, Korea, Invited speaker), SBS-RISH Workshop (Penan, Malaysia, Invited speaker), 2<sup>nd</sup> FEBS Special Meeting, ABC 2008 (Innsbruck, Austria, Invited speaker)
- Hayashi, T.: Xyloglucan creates tensile stress in the secondary wall. XI Cell Wall Meeting (Copenhagen, Denmark)

### International Joint Researches, overseas research sruveys

Yazaki, K.: Biochemical analyses of plant ABC protein functions (Cadarache Institute, France), Characterization and application of alkaloid transporter genes of plant cells (Leiden University, Netherland), Transport mechanism of auxin in arabidopsis (Zurich University, Switzerland), Alkaloid transport by MATE-type transporter in tobacco (Ghent University, Belgium), Statistical analysis of cell size and numbers in isoprene-emitting transgenic plants (VTT Technical Research Center, Finland)

#### Editorial work for international journals (roles)

Yazaki, K.: Plant & Cell Physiology (Editor), Plant Biotechnology (Editor)

Hayashi, T.: Cellulose (Editoral board)

### Scholars from abroad

Ph D scientists (1) (England)

# B. Educational Activities (2007.4-2008.3)

### B-1. On-campus teaching

a) Courses given

- Undergraduate level: Science of Sustainable Humanosphere (Shiotani, Tsuda, Yazaki), KSI lecture Science for Diagnostics and Control of Humanosphere (Shiotani, Hashiguchi, Horinouchi, Yazaki, Honda, Umezawa, Sugiyama)
- Graduate level: Plant Gene Expression (Yazaki), Laboratory Course in Plant Gene Expression (Yazaki, Hayashi, Kuroda), Seminar in Plant Gene Expression (Yazaki, Hayashi, Kuroda)

# B-2. Off-campus teaching, etc.

### Part-time Lecturer

Yazaki, K.: Kobe Pharmaceutical University, Natural Product Course (Undergraduate level), Special seminar in Institute of Natural Medicine

#### Open seminar, etc.

Yazaki, K.: 16<sup>th</sup> Meeting on dolichol and isoprenoids, Organizer (Uji), 86<sup>th</sup> Symposium in Research Institute for Sustainable Humanosphere, Organizer (Uji).

Hayashi, T.: Tropical trees in Southeast Asia - A reformation scenario from deforestation, Plant Science and Genetics seminar, the Hebrew University of Jerusalem (Israel)

Kuroda, H.: 79<sup>th</sup> Symposium in Research Institute for Sustainable Humanosphere, Organizer (Uji).

### B-3. Overseas teaching

### Lectures and seminars

Yazaki, K.: Lecture in Phytochemical Society of Europe (Helsinki, Finland), Lecture in Japan-Korea Joint Seminar (Hyndai, Korea), Lecture in Malaysia SBS (Penan, Malaysia), Lecture in 2<sup>nd</sup> FEBS Metting (Innsbruck, Austria).

# C. Other remarks.

Shitan, N.: Best Poster Award from Japan Transporter Research Association (JTra)

# 2.3.15 Laboratory of Metabolic Science of Forest Plants and Microorganisms

Staff Professor : Umezawa, Toshiaki, Dr. Agric. Sci. Assistant Professor : Hattori, Takefumi, Dr. Agric. Sci. Postdoctoral fellow : Yamamoto, Naoki, Dr. Life Sci. Students and research fellows Doctor's Program: (3)

Master's Program: (3)

# A. Research Activities (2007.4-2008.3) A-1. Main subjects

a) Integrated mechanisms for wood formation

It is obvious that we need to move from the fossil resource based society to the renewable resource dependant society. Among renewable biomass resources, it is wood biomass that the most abundantly accumulated is. Therefore, mechanisms for wood formation provide us the basic knowledge for tree biotechnology and cell-wall metabolic engineering. Lignin is one of the major components of plant cell wall, and much attention has been focused on the regulation of its biosynthesis from the standpoints of postharvest, cellulose-based wood processing for fiber, chemical, and bioethanol production. We are working on elucidating the integrated control mechanisms, including isolation of transcription factors, for the biosynthesis of lignin and other cell wall components by gene-coexpression network analysis and by comprehensive metabolite analysis.

b) Biosynthetic mechanisms for lignans produced by woody plants

Many lignans are isolated from various parts of plants, *e.g.* heartwoods, and known to have various biological activities. Lignans are optically active and their biosyntheses involve enantioselective processes. However, little has been known about biosynthetic mechanisms of lignans. We have been working on elucidating the stereochemical mechanisms for dibenzylbutyrolactone lignan biosyntheses and the biosynthetic mechanisms for antitumor lignans.

c) Biosynthetic mechanisms for norlignans produced by woody plants

Norlignans are compounds which cause heartwood coloration in important woods such as *Cryptomeria japonica* and *Chamaecyparis obtusa*. However, little has been known about biosynthetic mechanisms of norlignans. We have isolated cDNAs encoding a norlignan synthase (hinokiresinol synthase, HRS) for the first time. We are working on elucidating the reaction mechanisms for HRS cagtalyzed reactions and its gene expression mechanisms.

d) Molecular breeding of trees suitable for sustainable societies

It is extremely important to establish systems for the sustainable production of renewable biomass recources, mostly wood biomass. In our laboratory, we are working on molecular breeding of trees which are suitable for sustainable societies with respect to commercial benefits such as improved resistance to wood-rotting fungi and high production of industrial raw materials and bioethanol based on knowledges of biosynthetic mechansisms for wood components.

e) Mechanisms for organic acid metabolism of wood-rotting fungi and ectomycorrhizal fungi

Biodegradation of wood components by wood-rotting (WR) fungi including white- and brown-rot basidiomycetes is important as a first process leading to humus production, which in turn contributes greatly to sustainable forest ecosystems. On the other hand, ectomycorrhizal (ECM) fungi, symbiont of some woody plants, serve as phosphate supplying biofertilizers for host plants, which help trees in growing well in forest. Oxalate excreted from WR and ECM fungi play a wide variety of roles in these process. The purpose of this study is to elucidate regulatory mechanisms for metabolism of organic acid including oxalate in WR and ECM fungi for comprehensive understanding of possible role of the two fungi in forest at molecular level.

# A-2. Publications and presentations

### a) Publications

### Books

Umezawa, T.: Lignan and Norlignan Biosynthesis and Biotechnology, In "Biotechnology and Sustainable Agriculture 2006 and Beyond" (Eds. Z. Xu, J. Li, Y. Xue, W. Yand), Springer, pp.341-343, 2007

### **Original** papers

- Suzuki, S., M. Yamamura, T. Hattori, T. Nakatsubo and T. Umezawa: The subunit composition of hinokirsinol synthase controls geometrical selectivity in norlignan formation. Proc Natl Acad Sci USA 104; 21008-21013, 2007
- Watanabe, T., N. Shitan, T. Umezawa, K.Yazaki, M. Shimada and T. Hattori: Involvement of FpTRP26, a thioredoxin-related protein, in oxalic acid-resistance of the brown-rot fungus *Fomitopsis palustris*. FEBS Lett 581; 1788-1792, 2007

- Kuroda, K., T. Ahitani, K. Fujita and T. Hattori: Thermal behavior of β-1 subunits in lignin: pyrolysis of 1,2-diarylpropane-1,3-diol-type lignin model compounds. J Agric Food Chem 55; 2770-2778, 2007
- Noguchi, A., Y. Fukui, A. Iuchi-Okada, S. Kakutani, H. Satake, T. Iwashita, M. Nakao, T. Umezawa and E. Ono: Sequential glucosylation of a furofuran lignan, (+)-sesaminol, by Sesamum indicum UGT71A9 and UGT94D1 glucosyltransferases, Plant J 54; 415-427, 2008

#### Reviews

- Hattori, T.: The differences in mechanisms for carbon metabolism in between wood-rotting and ectomycorrhizal fungi. Lichenology 6; 150-151, 2007
- Suzuki, S. and T. Umezawa: Biosynthesis of lignans and norlignans, J Wood Science 53; 273-284, 2007
- Suzuki, S. and T. Umezawa: Recent advances of tree biotechnology in Acacia mangium (Fabaceae), Seizonken Kenkyu 3; 41-42, 2007
- Umezawa, T. and S. Suzuki: Chemical components of Acacia mangium and *Acacia auriculiformis*, Seizonken Kenkyu 3; 43-47, 2007
- Umezawa, T., S. Wada, M. Yamamura, N. Sakakibara, T. Nakatubo, S. Suzuki, T. Hattori and M. Koda: Protocols for lignin analysis for Forest Biomass Analytical System of RISH, Kyoto University, Seizonken Kenkyu 3; 73-75, 2007
- b) Conference and seminar papers presented
- 57th Annual Meeting of Japan Wood Res. Soc. (Hiroshima): 3 papers
- 58th Annual Meeting of Japan Wood Res. Soc. (Tuskuba): 2 papers
- The 25th Annual Meeting of the Japanese Society of Plant Cell and Molecular Biology (Chiba): 1 paper
- 52nd Lignin Symposium, (Utsunomiya): 1 paper

2nd Transporter meeting (Tokyo): 1 paper

- ICOB-5 & ISCNP-25 IUPAC International Conference on Biodiversity and Natural Products (Kyoto): 1 paper
- Annual meeting of Japan Society of Bioscience, Biochemistry and Agrochemistry 2007: 1 paper
- The 6th conference on fungal genetics and molecular biology, (Osaka): 1 paper
- The 11th International Association for Plant Tissue Culture and Biotechnology Congress (Beijing): 1 paper

### A-3. Off-campus activities

#### Membership in academic societies (roles)

Umezawa, T.: International Academy of Wood Science (Fellow), The Japan Wood Research Society (Committee Member of Future Planning, Committee Member of International Academic Exchange, Chair of working group of Extractives and Wood Utilization)

Hattori, T.: The Japan Wood Research Society (Editorial Board)

#### Research grants

Monbukagakusho Research Grants: Grant-in-Aid for Scientific Research (B) (2): Basic studies towards elucidation of heartwood formation mechanisms. (Head Investigator: Umezawa, T.). Grant-in-Aid for Exploratory Research: Identification of genes controlling wood formation. (Principal Investigator: Umezawa, T.). Institute of Sustainability Science,

Kyoto University, *Grant-in-Aid for Exploratory Research* (Principal Investigator: Umezawa, T.). Support for a conference jointly organized with a partner institute under the MOU (Principal Investigator: Umezawa, T.). New Energy and Industrial Technology Development Organization, High Efficiency Bioenergy Conversion Project / Development of Preparatory Basic Bioenergy Technologies (Genetic Modification of Rice Cell Walls for Efficient Saccharification) (Principal Investigator: Umezawa, T.). R&D Project of Industrial Science and Technology Frontier Program supported by New Energy and Industrial Technology Development Organization (Umezawa, T.), Grant-in-Aid for Scientific Research (C): Elucidation of the mechanisms for solubilization of insoluble phosphate salt by mycorrhizal fungi. (Head Investigator: Hattori, T.).

# A-4. International cooperations and overseas activities

### International meetings (roles)

Umezawa, T.: The 10th ICBPPI, Madison, USA (Oral presentation, Program Committee Member),
 Tree Biotechnology of Tropical Acacia - The 92<sup>nd</sup> RISH Symposium, Towards
 Establishment of Sustainable Humanosphere, Cibinong, Indonesia (Originizing
 Committee Member, Invited lecture). The 11th International Congress of Biotechnology
 in the Pulp and Paper Industry (program committee).

#### Oral presentation

Umezawa, T.: The 10th ICBPPI, Madison, USA (Oral presentation, Program Committee Member), PSNA 2007, St. Louis, USA (Oranl presentation), JSPS- Sweden (SU)/Japan (NAIST) Colloquium on Frontiers of Plant Biotechnology, Stockhorm, Sweden (Invited lecture), Royal Institute of Technology Seminar, Royal Institute of Technology, Stockhorm, Sweden (Invited lecture), The First Kyoto University – LIPI Southeast Asian Forum: Sustainable Humanosphere in Indonesia, Jakarta, Indonesia (Invited lecture), Tree Biotechnology of Tropical Acacia - The 92<sup>nd</sup> RISH Symposium, Towards Establishment of Sustainable Humanosphere, Cibinong, Indonesia (Invited lecture).

### International Joint Researches, overseas research surveys

Umezawa, T.: International collaboration of phenylpropanoid biosynthesis (North Carolina StateUniversity), Field study of Acacia mangium breeding (Perusahaan Kosinar, Malaysia), Field study of Acacia mangium breeding (PT Musi Hutan Persada, Indonesia), International collaboration of Acacia mangium biotechnology (Indonesian Institute of Sciences, Indonesia), International collaboration of antitumor lignan biosynthesis (Duesserdorf University, Germany), Sustainable Production of Tropical Forest Reservces for Establishment of Recycling-based Society (Indonesian Institute of Sciences, Indonesia)

#### Scholars from abroad

- Bambang Subiyanto: Collaborative research work on "Sustainable Production of Tropical Forest Reservces for Establishment of Recycling-based Society" November 5 (2007)
- Endang Sukara: Collaborative research work on "Sustainable Production of Tropical Forest Reservces for Establishment of Recycling-based Society" March 11 (2008)

# B. Educational Activities (2007.4-2008.3)

# B-1. On-campus teaching

Undergraduate level: Cyclic Utilization of Bio-based Resources of the Humanosphere (Umezawa), Introduction to mushroom science (Hattori), Muschroom science (Hattori)

Graduate level: Metabolic Science of Forest Plants and Microorganisms (Advanced Course) (Umezawa), Experimental Course of Metabolic Science of Forest Plants and Microorganisms (Umezawa and Hattori), Seminar on Metabolic Science of Forest Plants and Microorganisms (Umezawa and Hattori), Science for Diagnostics and Control of Humanosphere (Umezawa)

# B-2. Off-campus teaching

# Open seminar, etc.

- Umezawa, T.: Transgenic tree biotechnology for prevention of global warming (Tsukuba,July 17, 2007) (Invited speaker), 1<sup>st</sup> Interdisciprinaly Workshop –Southeast Asian Plantation Forest for Bioethanol Production- (Uji, July 31, 2007)(Invited speaker), 81<sup>st</sup> RISH Symposium Interuniversity and International Collaboration Studies (Uji, December 6, 2007)(Invited speaker), 90<sup>th</sup> RISH Symposium Tree Biotechnology Initiative (Yokohama, February 18, 2008)(Organizer, Invited speaker), Biomass Symposium –Collection, strage, and pretreatment of harvaceous biomass- (Tokyo, March 12, 2008)(Invited speaker), Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry 2008 (Nagoya, March 29, 2008)(Invited speaker, Chairperson)
- Hattori, T.: 2007 Biodeterioration meeting in spring Adaptation for environmental adaptation of insects and microorganisms (April 6, 2007, Uji, invited speaker), The 6<sup>th</sup> Japan Soceity for Lichenology Symposium "Diversity of the Symbiosis of All Organisms" (July 7, 2007, Suita, invited speaker)

# 2.3.16 Laboratory of Biomass Conversion

 Stuff
 Professor
 : Watanabe, Takashi, Dr. Agric. Sci.

 Associate Professor: Honda, Yoichi, Dr. Agric. Sci.
 Assistant Professor : Watanabe, Takahito, Dr. Agric. Sci.

 Postdoctoral fellows: Ohashi, Yasunori, Dr. Sci.
 Oyadomari, Masafumi, Dr. Agr. Sci.

 Sato, Shin, Dr. Agr. Aci.
 Rudiant, Amirta, Dr. Agr. Sci.

 Liu, Jian, Dr. Eng.
 Pradeep, Verma, Dr. Microbiol.

Students and research fellows

Doctor's program: (3)Research fellow: (2)Master's program: (8)

### A. Research Activities (2007.4-2008.3)

### A-1. Main subjects

a) Conversion of wood biomass to energy and functional materials by microorganisms and enzymatic reactions

Wood biomass and its components are converted to energy and useful materials including ethanol, chemicals, feedstuff and others by using microorganisms and their enzymes. The research subjects include pretreatments of wood by selective white rot fungi, solvolysis and milling. The research includes enzymatic decomposition of inhibitors for ethanol fermentation, and analysis of physiological response of alcohol-producing microorganisms to the inhibitors of ethanol fermentation.

#### b) Molecular biological characterization of white rot fungi

Extracellular enzymes are isolated from the culture of white rot basidiomycetes and genes encoding these enzymes are cloned and characterized. Regulation of gene expression, overexpression with gene engineering techniques, a reaction mechanism of the enzymes, and their application in degradation of polymers are studied. New strategies for transformation and gene-targeting system are under development.

c) Development of efficient biocatalysts for wood biomass conversion

Isolation of biocatalysts for efficient conversion of wood biomass is aimed by modifying microorganisms including bacteria, yeasts, and lignin-degrading basidiomycetes with gene engineering techniques. These include construction of basidiomycetes with higher and more selective ligninolytic activities, and alcohol-producing microorganisms with higher tolerance to the fermentation inhibitors.

#### d) Analysis and application of free radical-regulating systems of selective white rot fungi

Ligninolytic systems of selective white rot fungi including functions of key metabolites in the selective lignolysis are studied. Molecular cloning and expression of the genes encoding enzymes responsible for the biosynthesis of key metabolites are also studied. Gene-engineered white rot fungi and biomimetic lignin-degrading reactions are applied to the degradation of organopollutants and pretreatments for enzymatic saccharification and fermentation of wood

# A-2. Publifications and presentations

a) Publifications

### Books

- Watanabe, T.: Pretreatments using biological functions of microbes. In Industrial Technologies of Bio-Refinery for Automobile Biofuel, CMC Publishing, Tokyo, pp.207-222, 2008
- Watanabe, T.: Pretreatments using biological functions of microbes. In Frontiers of Cellulose Utilization Technologies, CMC Publishing, Tokyo, pp.334-349, 2008.

#### Reviews

- Watanabe, T.: Biomass conversion by selective white rot fungi and lipid-related factors controlling radical reactions, Mokuzaihozon, 33, 102-116, 2007
- Watanabe, T. and T. Watanabe: Biorefinery of tropical biomass, Seizonken-Kenkyu, 3, 65-71, 2007
- Fackler, K., C. Gradinger, M. Schmutzer, C. Tavzes, I. Burgert, M. Schwanninger, B. Hiterstoissr,
  T. Watanabe and K. Messner: Molecular mechanisms of biotechnological wood modification with selective white-rot fungi. Food Tecnol Biotechnol 45(3); 269-276, 2007

#### Original papers

- Sakai S., Y. Tsuchida, S. Okano, O. Ichihashi, H. Kawaguchi, T. Watanabe, M. Inui and H. Yukawa: Effect of lignocellulose-derived inhibitors on growth of and ethanol production by growth-arrested *Corynebacterium glutamicum*<sup>R</sup>. Appl Environ Micorbiol 73; 2349-2353, 2007
- Kohzu, A., T. Miyajima, T. Tateishi, T. Watanabe, M. Takahashi, and E. Wada: Dynamics of <sup>15</sup>N natural abundance in wood decomposing fungi and their ecophysiological implications, Isotopes in Environmental and Health Studies. 43; 83-94, 2007
- b) Conference and seminar papers presented
- Annual meeting of Japan Society of Bioscience, Biochemistry and Agrochemistry 2008: 1 presentaion
- The 14th Annual meeting of The Japan Institute of Energy: 1 presentation

Annual meeting of the Society for Bioscience and Bioengineering: 1 presentation

The 57th Annual Meeting of Japan Wood Research Society: 4 presentations

The 58th Annual Meeting of Japan Wood Research Society: 5 presentations

The 52th Lignin Symposium: 1 presentation

The 11th Annual meeting of Japanese Society of Mushroom Science and Biotechnology: 2 presentations

The 50th anniversary meeting of The Mycological Society of Japan: 1 presentation

The 10th International Congress on Biotechnology in the Pulp and Paper Industry: 4 presentations

### A-3. Off-campus activities

#### Membership in academic societies

Watanabe, Takashi: Japan Society of Bioscience, Biochemistry and Agrochemistry (council of Kansai branch), Japan Tappi (Committee member of Wood Sci.), Japanese Society of Mushroom Science and Biotechnology (Council member), Japan Wood Research Society (Committee member) Honda, Y.: Japan Wood Research Society (Secretary of the Institute.), Japanese Society of Mushroom Science and Biotechnology (Council member and boad)

# Research grants

- Monbukagakusho Research Grants: Grant-in-Aid for Scientific Research (B), Elucidation of structure, functions and biosynthetic pathway of hydrophobic metabolites produced by white rot fungi (Takashi Watanabe), Grant-in-Aid for Scientific Research (C), Molecular breeding of white rot fungi suitable for highly effective sccahrification of wood biomass (Honda), Grant in Aid for Young Scientists (B), Elucidation of the biosynthetic pathway of lipid-related metabolites produced by selective lignin-degraders (Takahito Watanabe)
- Others: Grant: NEDO Grant for Frontier Research and Technology of biomass energy, Pretreatments of wood for enzymatic saccharification by combination of selective white rot fungi and microwave solvolysis (Takashi Watanabe), RITE Research grant for advanced research, Aanaysis and molecular breeding of selective white rot fungi for the production of ethanol (Takashi Watanabe)

# A-4. International cooperations and overseas activities

# International meetings (roles)

- Watanabe, Takashi: The 10th International Symposium on Emerging Technologies of Pulping and Papermaking (ICBPPI), Madison, (program committee member, moderator, presentation), The first Kyoto University- LIPI South East Asian Forum: Sustainable Humanosphere in Indonesia (moderator, presentation), Research Institute for Sustainable Humanosphere (Kyoto University) - School of Biological Sciences (Universiti Sains Malaysia) Seminar (presentation), University of Indonesia-Special Seminar (presentation), LIPI Biotechnology Center -Workshop (presentation), G-COE In Search of Sustainable Humanosphere in Asia and Africa: The First International Conference (commentator)
- Honda, Y.: The 10th International Symposium on Emerging Technologies of Pulping and Papermaking (ICBPPI), Madison, (participant)

### International joint researches, overseas research surveys

Watanabe, Takashi: G-COE program, Survey of Biomass utilization in Indonesia

# B. Educational Activities (2007.4-2008.3)

# B-1. On-campus teaching

- a) courses given
- Undergraduate level: Science of Humanosphere –Conversion of Solar Energy- (Takashi Watanabe, Honda), Mushroom Biology Seminar (Honda), Mushroom Science (Honda)
- Graduate level: Sminar on Chemistry of Wood Biomass Conversion (Takashi Watanabe, Honda, Takahito Watanabe), Experimental Course in Chemistry of Wood Biomass Conversion (Takashi Watanabe, Honda, Takahito Watanabe), Science for diagnostics and control of the Humonosphere (Honda)

# B-2. Off-campus teaching etc.

# Part-time lecturer

Watanabe, T.: The University of Tokyo

# Open seminar, etc

Watanabe, Takashi: Shobara Biomass Forum 2007, Japan Material Society, The 30<sup>th</sup> Material Seminar, Special Lecture in Iwate Biotechnology Research Center, The 4th Energy Recycling Symposium - Biomass conversion and solar power satellite, NTS Seminar, The Mycological Society of Japan, 2007 West Japan Branch Meeting, G-COE Paradigm Seminar, Institute of Sustainability Science, The first Interdisciplinary Workshop –Forest plantation of South East Asia as Bioethanol Production Site.

Honda, Y.; The 4th Energy Recycling Symposium - Biomass conversion and solar power satellite *Students and research fellows from abroad* 

Doctor course: 2 Cooperative research fellows: 1

# B-3. Overseas teaching

### Students and research fellows from abroad

JSPS PD fellow: Rudianto Amirta (Indonesia) JSPD PD fellow: Liu Jian (China) JSPS PD fellow: Pradeep Verma (India)