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 two 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.

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.
Students and research fellows
Doctor's program: (6)
Master's program: (11)
Undergraduate : (5)

A. Research activities (2006.4-2007.3)

A-1. Main subjects

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 ß-cell ATP-sensitive potassium (K_{ATP}) channels play an important role in the regulation of glucose-induced insulin secretion. Theß-cell K_{ATP} 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_{ATP} 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

Books

Abe-Dohmae, S., K. Ueda and S. Yokoyama: ABCA7, a molecule with unknown function, FEBS Lett 580; 1178-1182, 2006

Original papers

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

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

Abe-Dohmae, S., K. H. Kato, Y. Kumon, W. Hu, H. Ishigami, N. Iwamoto, M. Okazaki, C. A. Wu, M. Tsujita, K. Ueda and S. Yokoyama: Serum amyloid A generates high density lipoprotein with cellular lipid in an ABCA1- or ABCA7-dependent manner. J Lipid Res

47; 1542-1550, 2006

Kobayashi, A., Y. Takanezawa, T. Hirata, Y. Shimizu, K. Misasa, N. Kioka, H. Arai, K. Ueda, and M. Matsuo: Efflux of sphingomyelin, cholesterol and phosphatidylcholine by ABCG1. J Lipid Res 47; 1791-1802, 2006

Matsumura, Y., N. Ban, K. Ueda and N. Inagaki: Characterization and classification of ATP-binding cassette transporter ABCA3 mutants in fatal surfactant deficiency. J Biol Chem 281; 34503-34514, 2006

Mitsushima, M., T. Sezaki, R. Akahane, K. Ueda, S. Suetsugu, T. Takenawa and N. Kioka: Protein kinase A-dependent increase in WAVE2 expression induced by the focal adhesion protein vinexin. Genes Cells 11; 281-292, 2006

Mitsushima, M., K. Ueda and N. Kioka: Vinexin beta regulates the phosphorylation of epidermal growth factor receptor on the cell surface. Genes Cells 11; 971-82, 2006

Mitsushima, M., H. Takahashi, T. Shishido, K. Ueda and N. Kioka N: Abl kinase interacts with and phosphorylates vinexin. FEBS Lett 580; 4288-95, 2006

Mutoh, K., J. Mitsuhashi, Y. Kimura, S. Tsukahara, E. Ishikawa, K. Sai, S. Ozawa, J. Sawada, K. Ueda, K. Katayama and Y. Sugimoto: A T3587G germ-line mutation of the MDR1 gene encodes a nonfunctional P-glycoprotein. Mol Cancer Ther 5; 877-84, 2006

Nagao, S., K. Murao, H. Imachi, W. M. Cao, X. Yu, J. Li, K. Matsumoto, T. Nishiuchi, R. A. Ahmed, N. C. Wong, K. Ueda and T. Ishida: Platelet derived growth factor regulates ABCA1 expression in vascular smooth muscle cells. FEBS Lett 580; 4371-4376, 2006

Takahashi, K., Y. Kimura, N. Kioka, M. Matsuo and K. Ueda: Purification and ATPase Activity of Human ABCA1. J Biol Chem 281; 10760-10768, 2006

Yazaki, K, N. Yamanaka, T. Masuno, S. Konagai, N. Shitan, S. Kaneko, K. Ueda and F. Sato: Heterologous Expression of a Mammalian ABC Transporter in Plant and its Application to Phytoremediation. Plant Molecular Biology 61; 491-503, 2006

b) Conference and seminar paper presented

The first FEBS Special Meeting “ATP-Binding Cassette (ABC) Proteins: From Multidrug Resistance Genetic Diseases” : 6 papers, Chair

The 20th IUBMB International Congress of Biochemsitry and Molecular Biology : 6 papers

The 65th Annual Meeting of Japanese Cancer Association, symposium, 3 paper

The 28th Annual Meeting of Japan Society for Molecular Biology, 2 papers

The 2007 Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry, 6 papers

The 49th Annual Meeting of Japan Society of Lipid Biochmistry, 1 paper

The 45th Annual Conference of Japan Society for Medical and Biological Engeering, symposium, 1 paper

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 (Start-up): Structual analysis of the receptor of the sulfonylurea drugs. (Kimura, Y.)

A-4. International cooperations and overseas activities

International meetings (roles)

Ueda, K.: 1st FEBS Special Meeting on ABC Proteins (Vice Organazer, invited lecture)
Gordon Reseach Conference, Membrane Transport Protein (invited lecture)
MDR1 20th Anniversary Symposium (invited lecture)
FEBS Special Meeting European Lipidomics Initiative (presentation)

Kioka, N.: ASCB 46th Annual Meeting (presentation)

Matsuo, M.: 20th IUBMB International Congress of Biochemestry and Molecular Biology (presentation)

Membership in international academic societies

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

B. Educational Activities (2006.4-2007.3)

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. and Matsuo, M.)

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

2.3.2 Laboratory of Biomacromolecular Chemistry

Staff *Professor* *: Ueda, Mitsuyoshi, Dr. Engineering*
Assistant Professor : Kato, Michiko, Dr. Agric. Sci.
Assistant Professor : Kuroda, Kouichi, Dr. Engineering (2006.5～)
Students and research fellows
Doctor's program :(5)
Master's program :(12)
Undergraduate :(4)
Research fellow :(6)

A. Research Activities (2006.4-2007.3)

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

Books

Ye, K. and M Ueda (Edited): Combinatorial Bioengineering Protein Display and Its Development. Biotechnol. Progress 22(4); 2006

Original papers

Shibasaki, S., K. Kuroda, H. D. Nguyen, T. Mori, W. Zou, and M. Ueda: Detection of protein-protein interactions by a combination of a novel cytoplasmic membrane targeting system of recombinant proteins and fluorescence resonance energy transfer (FRET). Appl Microbiol Biotechnol 70(4); 451-457, 2006

Kuroda, K. and M. Ueda: Effective display of metallothionein tandem repeats on the bioadsorption of cadmium ion. Appl Microbiol Biotechnol 70(4); 458-463, 2006

Hata, K., H. Morisaka, K. Hara, J. Mima, N. Yumoto, Y. Tatsu, M. Furuno, N. Ishizuka and M. Ueda: Two-dimensional HPLC on-line analysis of phosphopeptides using titania and monolithic columns. Anal Biochem 350(2); 292-297, 2006

Matsui, K., T. Hirayama, K. Kuroda, K. Shirahige, T. Ashikari and M. Ueda: Screening for candidate genes involved in tolerance to organic solvents in yeast. Appl Microbiol Biotechnol 71(1); 75-79, 2006

Kato, M., Y. Kuzuhara, H. Maeda, S. Shiraga and M. Ueda: Analysis of a processing system of yeast vacuolar proteases using cell surface engineering: Conversion of precursor of proteinase A to active proteinase A. Appl Microbiol Biotechnol 72(6); 1229-1237, 2006

Takayama, K., S. Suye, K. Kuroda, M. Ueda, T. Kitaguchi, K. Tsuchiyama, W. Chen and A. Mulchandani: Surface display of organophosphorus hydrolase on Saccharomyces cerevisiae. Biotechnol. Prog., 22(4), 939-943 (2006)

Tamaru, Y., M. Ohtsuka, K. Kato, S. Manabe, K. Kuroda, M. Sanada, and M. Ueda: Application of the arming system for the expression of the 380R antigen from red sea bream iridovirus (RSIV) on the surface of yeast cells: a first step for the development of an oral vaccine. Biotechnol Prog 22(4); 949-953, 2006

Fukuda, T., S. Shiraga, M. Kato, S. Suye and M. Ueda: Construction of cultivation system of a yaset single cell in a cell chip chamber. Biotechnol Prog 22(4); 944-948, 2006

Fukuda, T., T. Ishikawa, M. Ogawa, S. shiraga, M. Kato, S. Suye and M. Ueda: Enhancement of cellulase activity by the clones selected from the combinatorial library of the cellulose-binding domain by cell surface engineering. Biotechnol Prog 22(4); 933-938, 2006

Nakamura, Y., T. Matsumoto, F. Nomoto, M. Ueda, H. Fukuda and A. Kondo: Enhancement of activity of lipase-displaying yeast cells and their application to optical resolution of (*RS*)-1-benzyloxy-3-chloro-2-propyl succinate. Biotechnol Prog 22(4); 998-1002, 2006

Morisaka, H., K. Hata, J. Mima, T. Tanigawa, M. Furuno, N. Ishizuka, N. Tanaka and M. Ueda: Enhanced sequence coverage in tryptic fragment analysis by two-dimensional HPLC/MS using a monolithic silica capillary column. Biosci Biotechnol Biochem 70(9); 2154-2159, 2006

Seong, K., Y. Katakura, K. Ninomiya, Y. Bito, S. Katahira, A. Kondo, M. Ueda and S. Shioya: Effect of flocculation on performance of arming yeast in direct ethanol fermentation. Appl Microbiol Biotechnol 73(1); 60-66, 2006

Mima, J., H. Fukada, M. Nagayama and M. Ueda: Specific membrane binding of the carboxypeptidase Y inhibitor I^c, a phosphatidylethanolamine-binding protein family member. FEBS J 273; 5374-5383, 2006

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

Reviews

Kuroda, K., M. Kato, J. Mima, and M. Ueda: Systems for the detection and analysis of protein-protein interactions. Appl. Microbiol. Biotechnol., 71(2), 127-136 (2006)

b) Conference and seminar papers presented

Annual Meeting of the Society for Biotechnology, Japan 2006: 8 reports

International Annual Meeting of the JBS and MBSJ: 11 reports

Annual Meeting of Japan Society for Bioscience, Biotechnology and Agrochemistry 2006: 12 reports

Japan Science Society of Biological Macromolecules 2006: 1 report

A-4. International cooperations and overseas activities

International meetings (roles)

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

B. Educational Activities (2006.4-2007.3)

B-1. On-campus teaching

a) Courses given

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

Graduate level: Biomacromolecular Chemistry (Ueda), Experiments of Biomacromolecular Chemistry (Ueda, Kato 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 : (2) *Doctor's program: (3)*
Master's program: (5) *Undergraduate : (4)*

A. Research Activities (2006.4-2007.3)

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 novel metabolic pathways of IAA in plants, new metabolites were searched for in Arabidopsis and rice plant using MS/MS analysis. Several hitherto-unknown compounds were identified, including a conjugate of 2-oxo-IAA with glucose, and conjugates of 6-hydroxy-IAA with valine and phenylalanine from Arabidopsis; N-glucosyl IAA and its aspartate- and glutamate-conjugates from rice plant. Quantitative analysis revealed that some of these metabolites are involved in the major inactivation pathways of IAA in plants.

b) Structure-Activity Relationships of Ecdysone Agonists

While the insect molting is regulated in vivo by a steroidal hormone, 20-hydroxyecdysone, it has been demonstrated that several types of non-steroidal compounds also have molting-hormonal activity. Structural similarity between *N*-benzoyl tetrahydroquinolines and dibenzoylhydrazines, typical non-steroids with molting hormonal activity, was examined using computer-based 3D molecular modeling techniques to extract some structural factors that are likely to be favorable for the activity. Synthetic methods to modify the tetrahydroquinoline moiety to introduce those factors into the molecule were also investigated.

c) Peptide Chemistry

i) Plants induce various defense responses when they are attacked by pathogens. These defense responses are triggered by a variety of molecules (elicitors) derived from pathogenic microorganisms, including peptides derived from bacterial flagellin. In order to search for new elicitor-active peptides, a series of libraries comprised of peptides with random 3 to 6 amino acid sequences 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, two active peptides with high sequence novelty as elicitors were obtained. ii) An insecticidal peptide was isolated from the venom of the Japanese scorpion *Liocheles australasiae,* and its amino acid sequence was determined by Edman degradation and MS/MS analysis of fragments obtained by partial enzymatic digestion. The deduced amino acid sequence had no homology with the hitherto known scorpion toxins. The presence of two disulfide bridges in the molecule was also determined by MS.

A-2. Publications and presentations

a) Publications

Review papers

Fujita, T. and Y. Nakagawa: QSAR and mode of action studies of insecticidal ecdysone agonists. SAR QSAR Environ Res 18; 77-78, 2007

Original papers

Suzuki, J., I. Tanji, Y. Ohta, K. Toda, and Y. Nakagawa: QSAR of 2,4-diphenyl-1,3-oxazolines for ovicidal activity against the two-spotted spider mite *Tetranychus urticae*. J Pestic Sci 31; 409-416, 2006

Minakuchi, C., J. Suzuki, K. Toda, M. Akamatsu and Y. Nakagawa: Estimation of the hydrophobicity of 2,4-diphenyl-1,3-oxazoline analogs and QSAR analysis of their ovicidal activity against *Tetranychus urticae*. Bioorg Med Chem Lett 16; 4080-4084, 2006

Yamamoto, S., B. Watanabe, J. Otsuki, Y. Nakagawa, M. Akamatsu and H. Miyagawa: Synthesis of 26,27-bisnorcastasterone analogs and analysis of conformation-activity relationship for brassinolide-like activity. Bioorg Med Chem 14; 1761-1770, 2006

Wheelock, C. E., Y. Nakagawa, T. Harada, N. Oikawa, M. Akamatsu, G. Smagghe, D. Stefanou, K. Iatrou and L. Swevers: High throughput screening of ecdysone agonists using a reporter gene assay followed by 3-D QSAR analysis of the molting hormonal activity. Bioorg Med Chem 14; 1143-1159, 2006

Ishihara, A., Y. Asada, Y. Takahashi, N. Yabe, Y. Komeda, T. Nishioka, H. Miyagawa and K. Wakasa: Metabolic changes in *Arabidopsis thaliana* expressing the feedback-resistant anthranilate synthase alpha subunit gene *OASA1D*. Phytochemistry 67; 2349-2362, 2006

Wakasa, K., H. Hasegawa, H. Nemoto, F. Matsuda, H. Miyazawa, Y. Tozawa, K. Morino, A. Komatsu, T. Yamada, T. Terakawa and H. Miyagawa: High-level tryptophan accumulation in seeds of transgenic rice and its limited effects on agronomic traits and seed metabolite profile. J Exp Bot 57; 3069-3078, 2006

Ohnishi, T., T. Nomura, B. Watanabe, D. Ohta, T. Yokota, H. Miyagawa, K. Sakata and M. Mizutani: Tomato cytochrome P450 *CYP734A7* functions in brassinosteroid catabolism. Phytochemistry 67; 1895-1906, 2006

Miyata, K., M. Miyashita, R. Nose, Y. Otake and H. Miyagawa: Development of a colorimetric assay for determining the amount of H_2O_2 generated in tobacco cells in response to elicitors and its application to study of the structure-activity relationship of flagellin-derived peptides. Biosci Biotechnol Biochem 70; 2138-2144, 2006

Kawakatsu, M., Y. Yamamoto and H. Miyagawa: Pyranonaphthoquinone pigment from cultured lichen mycobiont of *Haematomma* sp. Lichenology 5; 31-36, 2006

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 modelling and simulation. Plant J 50; 176-187, 2007

Miyashita, M., Y. Otake, M. Oda and H. Miyagawa: Development of a high-throughput screening method using a cell-based, lawn format assay for the identification of novel plant defense activators from combinatorial peptide libraries. J Agric Food Chem 55; 806-811, 2007

Proceedings and Reports

Miyagawa, H.: Eleventh IUPAC International Congress of Pesticide Chemistry, Kobe, Japan,

2006 - From crop protection to crop production and beyond. Pest Manag Sci 62; 687-689, 2006

b) Conferences and seminar papers presented

The 32nd Annual Meeting of Pesticide Science Society of Japan: 5 reports

Annual Meeting of the Japan Society for Bioscience, Biotechnology, and Agrochemistry 2007: 9 reports

Japan Society for Bioscience, Biotechnology, and Agrochemistry (Kansai Branch Meeting): 1 report

The 43rd Peptide Meeting: 1 report

The 48th Annual Meeting of Plant Physiology: 1 report

The 34th Symposium of Structure-Activity Relationship: 1 report

The 41st Meeting of Regulation of Plant Growth and Development: 2 reports

The 54th Annual Conference on Mass Spectrometry: 1 report

The 11th IUPAC Congress of Pesticide Chemistry: 12 reports

The 16th International Ecdysone Workshop: 5 reports

Meiji University International Symposium on Plant Immunity: 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)

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)

Miyashita, M.: The Mass Spectrometry Society of Japan (training planning committee member)

Research grants

Monbukagakusho Research Grant: Encouragement of Young Scientists (B): Screening for plant defense activating peptides from combinatorial peptide Libraries (Miyashita).

Others: Core Research for Evolutional Science and Technology (CREST), Regulation and utilization of tryptophan-related primary/secondary metabolism (Miyagawa, member). 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 meetings (roles)

Miyagawa, H.: 2006 IUPAC International Congress of Pesticide Chemistry (Organizing and Program Committee).

Nakagawa, Y.: 2006 IUPAC International Congress of Pesticide Chemistry (Organizing Committee). The 16th International Ecdysone Workshop (Organizing Committee)

Miyashita, M.: 2006 IUPAC International Congress of Pesticide Chemistry (Organizing

Committee)

International joint researches, overseas research surveys

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

B. Educational Activities (2006.4-2007.3)

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), Exercises in Information Processing Basics (Nakagawa)

Graduate level: Bioregulation Chemistry (Advanced Course) (Miyagawa, Nakagawa), 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 Natural Science and Technology (Special lecture on science for bioresources)

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 the Advisory Committee of Radio Isotope Center, Kyoto University.

Nakagawa, Y.: Member of the Advisory Committee of the Environmental Preservation Center, Kyoto University.

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.
Students and research fellows
Doctor's program: (0)
Master's program: (11)
Undergraduate : (4)

A. Research Activities (2006.4-2007.3)

A-1. Main subjects

a) Identification of larval feeding stimulants of swallowtail butterflies in their host plants.

Larvae of a swallowtail butterfly, *Papilio xuthus*, feed exclusively on the plant family Rutaceae, including *Citrus* crops. The larvae were strongly stimulated to feed a strip of tissues impregnated with ethanolic extracts of host plant leaves. The feeding stimulant in *Citrus unshiu* leaves was found to be composed of multiple chemical factors including sugar components [glucose, fructose and sucrose], a betaine [stachydrine], a cyclic peptide [citrusin I], a polymethoxyflavone [isosinensetin] and lipids [1-linoleoylglycerol, 1-linolenoylglycerol and 1,2-dilinolenoyl-3-galactosyl-sn-glycerol (MGDG)]. The larvae consumed test paper strip when subsets of these components were mixed together, indicating that the larval host recognition is controlled by multiple components of a specific chemical composition. Larvae of a primitive pipevine swallowtail butterfly, *Sericinus montela* (tribe Zerynthiini), was found to utilize aristolochic acids and MGDG in addition to sugar components contained in the host plant, *Aristolochia debilis*.

b) Absolute configuration of volicitin from the regurgitant of lepidopteran caterpillars and biological activity of volicitin-related compounds

Volicitin [*N*-(17-hydroxylinolenoyl)-L-glutamine] and *N*-linolenoyl-L-glutamine are known as insect-produced plant volatile elicitors. The absolute configuration of the hydroxylinolenoyl moiety of volicitin from three noctuid species, *Helicoverpa armigera*, *Mythimna separata* and *Spodoptera litura*, was determined to be all 17*S* in high enantiomeric excess. When treated with 30 pmol of (17*S*)- and (17*R*)-volicitin, corn seedlings were induced to release volatiles, there being no significant difference in the amount released between the two isomers. On the other hand, *N*-linolenoyl-L-glutamine was only about 30% as active as volicitin. Among several synthesized *N*-linolenoylamino acid conjugates, only the L-glutamine conjugate induced the emission of volatile organic compounds. These results show that the L-glutamine moiety of volicitin played a more critical role than the hydroxyl moiety, although both moieties affected the elicitor activity inducing the release of volatiles.

c) Identification of crinosterol from astigamatid mites

A 24-alkylsterol, crinosterol [(24*S*)-24-methylcholesta-5,22(*E*)-dien-3β-ol] has been isolated from sea-living animals, protists and plants. Here we identified crinosterol from 9 species of mites (Acari). The compound was identified by using ^{1}H-NMR analysis and GCMS spectral data along with the HPLC retention time by comparing with those of the synthesized compound. As far as we

know, this is the first report on the identification of crinosterol from arthropods. Furthermore, after *Rhizoglyphus robini* were fed on artificial diets with d_3-methionine, d_2-crinosterol was detected from the mite's extracts. The incorporation of two deuterium atoms into the sterol indicated that a d_3-methyl group was introduced into the C24 of the side chain to form crinosterol. Although the details of the biosynthesis of crinosterol remain unknown, the discovery of crinosterol in the mites implies the existence of interesting sterol metabolisms in the animals.

A-2. Publications and presentations

a) Publications

Original papers

Ohta, N., M. Mori, Y. Kuwahara and R. Nishida: A hemiterpene glucoside as a probing deterrent of the bean aphid, *Megoura crassicauda*, from a non-host vetch, *Vicia hirsuta.* Phytochemistry 67; 584-588, 2006

Takemura, M., Y. Kuwahara and R. Nishida: Chemical basis of feeding behavior of a bean aphid, *Megoura crassicauda*, controlled by primary and secondary substances in the host *Vicia angustifolia.* Entomol Exp Appl 121; 51-57, 2006

Tan, K.H. and R. Nishida: Zingerone in floral synomone of *Bulbophyllum baileyi* (Orchidaceae) attracts *Bactrocera* fruit fly during pollination. Biochem Syst Ecol 32; 245-252, 2007

Tan, K. H., L. T. Tan and R. Nishida: Floral phenylpropanoid cocktail and architecture of *Bulbophyllum vinaceum* orchid in attracting fruit flies for pollination. J Chem Ecol 32: 2429-2441, 2006

Takeyama, K., N. Mori and Mh. Osakabe: Effect of cytochrome P450 inhibitor, piperonyl butoxide, on survival of *Panonychus citri* (McGregor)(Acari: Tetranychidae) on citrus leaves. Appl Entomol Zool 41; 487-491, 2006

Sawada, Y., N. Yoshinaga, K. Fujisaki, R. Nishida, Y. Kuwahara and N. Mori: Absolute configuration of volicitin from the regurgitant of lepidopteran caterpillars and biological activity of volicitin-related compounds. Biosci Biotechnol Biochem 70; 2185-2190, 2006

Murakami, K., K. Noge, N. Mori and Y. Kuwahara: Chemical ecology of astigmatid mites LXXX. γ-Acaridial (3-Hydroxybenzene-1,2-dicarbaldehyde) as a female sex pheromone from an alarm-pheromone-emitting unidentified *Rhizoglyphus* mite (Acari: Acaridae). Jpn J Entomol Zool 17; 99-105, 2006

Shimizu, N., A. Mizoguchi, K. Murakami, K. Noge, N. Mori, R. Nishida and Y. Kuwahara: Synthesis of (+)-(S)-isorobinal together with its antipode, a cyclic monoterpene functioning as the sex pheromone of *Rhizoglyphus setosus* and its distribution among Astigmata. J Pestic Sci 31; 311-315, 2006

Murakami, K., B. Watanabe, R. Nishida, N. Mori and Y. Kuwahara: Identification of crinosterol from astigamatid mites. Insect Biochem Mol Biol 37; 506-511, 2007

Maruno, G., N. Mori, R. Nishida and Y. Kuwahara: Chemical ecology of astigmatid mites LXXXII. β-Acaridial as a female sex pheromone of the mold mite *Tyrophagus putrescentiae* (Acari: Acaridae). Jpn J Entomol Zool 16; 167-173, 2006

Aboshi, T., N. Yoshinaga, K. Noge, R. Nishida and N. Mori: Efficient incorporation of unsaturated fatty acids into volicitin-related compounds in *Spodoptera litura* (Lepidoptera: Noctuidae). Biosci Biotechnol Biochem 71; 607-610, 2007

Ono, H., K. F. Rewitz, T. Shinoda, K. Itoyama, A. Petryk, R. Rybczynski, M. Jarcho, J. T. Warren,

G. Marqués, M. J. Shimell, L. I. Gilbert and M. B. O'Connor: Spook and Spookier code for stage-specific components of the ecdysone biosynthetic pathway in Diptera. Dev Biol 298; 555-570, 2007.

Review

Kuwahara, Y. and N. Mori: Unexpected matters on mite chemistry. Chemistry 61: 37-40, 2006 (in Japanese)

b) Conference and seminar papers presented

The 51th The Japanese Society of Applied Entomology & Zoology (2007): 6 papers

Japan Society for Bioscience, Biotechnology, and Agrochemistry (2007): 5 papers

Japan Society for Bioscience, Biotechnology, and Agrochemistry Kansai branch office meet (2007): 1 paper

11th IUPAC International Congress on Pesticide Chemistry, Kobe, Japan: 5 papers

Insect workshop (2006): 1 paper

A-3. Off-campus activities

Membership in academic societies (roles)

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

Mori, Naoki: Japanese Society of Enviromental Entomology and Zoology (managing editor)

Research grants

Monbukagakusho Research Grant: Integrated Research (B) (2), Evolutionary process of domestication in the household pest cockroaches: chemical and ecological analyses (Nishida: representative). Integrated Research (B) (2), (Nishida: representative). Integrated Research, Chemical ecological study on mutual interactions between fruit flies and orchids via pollination in Southeast Asia (Nishida: representative). Exploratory Research, Pharmacologically active principles sequestered in the butterfly wings (Nishida: 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 (vice-president). Biochemical Systematics and Ecology (editorial advisory board), Chemoecology (editorial advisory board), Applied Entomology and Zoology (editorial board). Journal of Chemical Ecology (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), DIMBOA biosynthesis induced by insect-derived elicitors (New Zealand)

B. Educational Activities (2005.4-2006.3)

B-1. On-campus teaching

Undergraduate level: Bioorganic chemistry III (Nishida, Mori), Organic Reaction Mechanisms I

(Nishida, Mori), Structure analyses of organic compounds (Nishida), Laboratory Course in Bioorganic Chemistry (Mori).

Graduate level: Laboratory Course in Chemical Ecology (Nishida, Mori), Chemical Ecology Seminar (Nishida, Mori)

B-2. Off-campus teaching

Part-time lecturer

Nishida, R.: Kobe University, Kyoto University of Education

Chair of Molecular and Cellular Sciences

2.3.5 Laboratory of Plant Nutrition

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

Students and research fellows
Doctor's program : (4)
Master's program : (5)
Undergraduate : (4)
Research student: (1)

A. Research Activities (2006.4-2007.3)

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.

b) Salt damage on plants.

We have studied the mechanism underlying the salt damage on higher plants, paying special attention to how do the salts intrude into plants. We use naturally occuring salt-tolerant plants, halophyte, for a comparative study with crop plants regarding to salt sensitivity.

c) Sustainable agriculture.

We are trying to find out a suibable 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

Inamura, T., T. Chin, T. Matoh, S. Inoue and Y. Yamasue: Reduction of low-level cadmium contaimination in brown rice by the methane-fermented manure liquid. Jpn J Crop Sci 75; 273-280, 2006

Matsuda, A., R. Watanabe, K. Ochiai and T. Matoh: Quality evaluation of organic amendments based on their CO_2 evolution rates sold at local markets around Kyoto city. Jpn J Soil Sci Plant Nutr 77; 387-393, 2006

Kohorn, B.D., M. Kobayashi, S. Johansen, J. Riese, L.F. Huang, K. Koch, S. Fu, A. Dotson and N. Byers: An Arabidopsis cell wall-associated kinase required for invertase activity and cell growth. Plant J 46; 307-316, 2006

Kohorn, B.D., M. Kobayashi, S. Johansen, H. P. Friedman, A. Fischer and N. Byers: Wall-associated kinase 1 (WAK1) is crosslinked in endomembranes, and transport to the cell surface requires correct cell-wall synthesis. J Cell Science 119; 2282-2290, 2006

b) Conference and seminar papers presented

Annual Meeting of the Japanese Society of Plant Physiologists, 2007: 2 reports

Annual Meeting of Japanese Society of Soil Science and Plant Nutrition, 2006: 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)

Research grants

Matoh, T.: General Scientific Research (B) (2) Environmental evaluation of the export-oriented farming in the tropical countries.

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 (2006.4-2007.3)

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.

Students
Doctor's program: (3)
Master's program: (8)
Undergraduate : (4)

A. Research Activities (2006.4-2007.3)

A-1. Main subjects

a) Structure and function analysis of proteins involved in restriction-modification system

Restriction endonuclease and cognate methyltransferase of *Escherichia coli* TH38 are regulated by a multifunctional transcriptional factor, C.EcoT38I. In *E. coli* TH38 cell, two kinds of C.EcoT38I, in which the molecular weight differs, were produced and it was clarified that their recognition specificity and affinity for DNA were different. X-ray structure of the low molecular weight C.EcoT38I was solved to 1.95Å resolution and the molecular model was constructed. Although there is no significant similarity between C.EcoT38I and C.BclI, a transcriptional factor of restriction-modification system of *Bacillus caldolyticus*, nearly the entire length of C.EcoT38I can be superimposed on the structure of C.BclI.

A mutant enzyme of EcoO109I restriction endonuclease of Escherichia coli H709c was constructed on the basis of structural information of the DNA-protein complex, and the biochemical analysis was carried out. Recognition specificity and catalytic activity of mutant enzyme was the same as those of wild type enzyme under the standard conditions. It was shown that under non-standard conditions such as presence of organic solvents or low metal ion concentration, wild type EcoO109I cleaved noncanonical sites which are similar but not identical to the defined recognition sequence, while lowering of the specificity was remarkably suppressed in the mutant enzyme. These results may provide new insight into the interaction between restriction endonucleases and DNA.

b) Methylglyoxal as a signal Initiator for activation of p38 MAP kinase cascade in the fission yeast *Schizosaccharomyces pombe*

Methylglyoxal (MG) is a typical 2-oxoaldehyde derived from glycolysis. We have found that MG activates transcription factors such as Yap1 and Msn2, and triggers a Hog1 mitogen-activated protein kinase cascade in *Saccharomyces cerevisiae.* To gain further insight into the role of MG as a signal initiator, here we analyze the response of *Schizosaccharomyces pombe* to extracellular MG. Spc1, a stress-activated protein kinase (SAPK), was phosphorylated following the treatment with MG. No phosphorylation was observed in a *wis1*Δ mutant. The His-to-Asp phosphorelay system consisting of three histidine kinases (Phk1, Phk2 and Phk3), a phosphorelay protein (Spy1) and a response regulator (Mcs4) exists upstream of the Spc1-SAPK pathway. The phosphorylation of Spc1 following MG treatment was observed in *phk1*Δ*phk2*Δ*phk3*Δ and *spy1*Δ cells, but not in *mcs4*Δ cells. These results suggest that *S. pombe* has alternative module(s) that direct the MG signal to the SAPK pathway *via* Mcs4. Additionally, we found that the transcription factor Pap1 is concentrated in the nucleus in response to MG, independent of the Spc1-SAPK pathway.

c) Unique effects of ethanol on 3'-processing and nuclear export of *HSP* mRNAs in *Saccharomyces cerevisiae*.

Under conditions of heat shock at 42˚C, mRNAs of heat shock protein (HSP) genes are exported out of the nucleus, whereas bulk poly(A)+ mRNA shows a nuclear accumulation in Saccharomyces cerevisiae. Such a selective mRNA export seems a smart way to adapt rapidly to stress. Ethanol stress (10% v/v) as well as heat shock blocks the export of bulk poly(A)+ mRNA. However, little is known about mRNA transport in ethanol-treated cells. Ethanol stress induced transcriptional activation of a subset of yeast HSP genes, but most of such transcripts were held in a hyperadenylated state in the nucleus and, as a consequence, were not translated into HSPs. Additionally, ethanol stress enhanced the formation of cytosolic P-bodies. Elimination of ethanol resulted in the rapid shortening of the poly(A) tails of HSP mRNAs, loss of their nuclear retention, and coincidental synthesis of the respective HSPs. These results indicate that cells respond differently to ethanol stress and heat shock in the 3'-processing and transport of HSP mRNAs. Furthermore, these results suggest that cells may use hyperadenylation and nuclear retention of mRNAs as a means to control gene expression under stressed conditions.

A-2. Publications and presentations

a) Publications

Book

Izawa, S. and Y. Inoue: Ethanol tolerance and response of yeast during the brewing process.

Hakko・Jouzoshokuhin no saishin gijutsu to kinousei. (edited by K. Kitamoto) , pp. 37-46, CMC press, Tokyo, 2006 (in Japanese)

Original papers

Izawa, S., T. Kita, K. Ikeda and Y. Inoue: Asr1, an alcohol-responsive factor of *Saccharomyces cerevisiae*, is dispensable for alcoholic fermentation. Appl Microbiol Biotechnol 72(3); 560-565, 2006

Takatsume, Y., S. Izawa and Y. Inoue: Methylglyoxal as a signal initiator for activation of the stress-activated protein kinase cascade in the fission yeast *Schizosaccharomyces pombe.* J Biol Chem 281(14); 9086-9092, 2006

Review

Maeta, K., S. Izawa and Y. Inoue: Signal transduction system via modification of cysteine residue of protein in yeast: activation by methylglyoxal. Kagaku to Seibutsu 44(6); 361-363, 2006

Patent

Yodoi, J., Y. Inoue, S. Izawa, H. masutani, K. Murata and S. Tamasu: Process for producing thioredoxin. 2006.9.15 (PCT/JP2006/31841)

S. Izawa, Y. Inoue, K. Ikeda, S. Kitagawa, Y. Furukawa: Yeast cells with hyper tolerance to freezing stress 2006. 10. 18 (2006-283233)

b) Conference and seminar papers presented

The 6th Annual Meeting of the Protein Science Society of Japan: 2 papers

The 39th Meeting of Yeast Genetics and Molecular Biology, Japan: 4 papers

The Annual Meeting of the Society for Biotechnology 2006, Japan: 1 paper

Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry 2007: 4 paper

The 1st Meeting of the Biochemical Society for Stress Response: 1 paper

The Molecular Biology Society of Japan Forum 2006: 1 paper

FASEB Summer Research Conferences “Nucleic Acid Enzymes”: 1 paper

20th IUBMB International Union of Biochemistry and Molecular Biology and 11th FAOBMB Congress: 4 papers

A-3. Off-campus activities

Membership in academic societies

Kita, K.: Japan Society of Bioscience, Biotechnology, and Agrochemistry (Councilor of Kansai branch), The Society for Biotechnology, Japan (Editorial Board of Journal of Bioscience and Biotechnology)

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) (2) Structural analysis of recognition mechanism of endonucleases and its application to alteration of specificity. (Kita, K.) 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.: 10th Japanease-Swiss Meeting on Biotechnology and Bioprocess Development, Kanazawa (invited speaker).

B. Educational Activities (2006.4-2007.3)

B-1. On-campus teaching

a) Courses given

Undergraduate level: Applied Life Sciences (Kita), Applied Microbiology II (Kita), Introduction to Applied Life Sciences III (Kita), Laboratory Course in Biochemistry (Kita, Inoue, Izawa), Applied Microbiology IV (Inoue)

Graduate level: Cellular Bioenergy Conversion (Kita), 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.*

Students and research fellows

Postdoctral research fellow (COE) : *(2)*
Research fellow : *(1)*
Foreign research fellow : *(2)*
Doctor's program : *(4)*
Master's program : *(18)*
Undergraduate : *(5)*
Research student : *(2)*

A. Research Activities (2006.4-2007.3)

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-γ-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

Sakuradani, E. and S. Shimizu: Chapter 3 Production of functional lipids by fatty acid fermentation. Frotier Technologies and Functions of Fermentative Foods (4) (edited by Kitamoto, K.). CMC Press, Tokyo, pp. 210-225 (2006) (in Japanese).

Sakuradani, E., S. Takeno, T. Abe, J. Ogawa and S. Shimizu: Arachidonic acid-producing *Mortierella alpina*: Molecular breeding of mutants and creation and application of a host-vector system. Biocatalysis and Biotechnology for Functional Foods and Industrial Products (edited by Hou, C.T. and J.-F. Shew), CRC Press, New York, USA, pp. 267-282 (2006).

Shimizu, S.: Cottage cheese and natto, Chicken and Tofu Soup and Asiatic Roast Pork. Science meets Cooking (edited by G. Antranikian), TuTech Innovation GmbH, Hamburg, pp. 82-83 (2006).

Ogawa, J., S. Kishino, S. Shimizu: Production of conjugated fatty acids by microorganisms. Biocatalysis and Biotechnology for Functional Foods and Industrial Products (edited by Hou, C.T. and J.-F. Shew), CRC Press, New York, USA, pp. 121-136 (2006).

Original papers

Abe, T., E. Sakuradani, T. Asano, H. Kanamaru and S. Shimizu: Functional characterization of Δ9 and ω9 desaturase genes in *Mortierella alpina* 1S-4 and its derivative mutants. Appl Microbiol Biotechnol 70 (6); 711-719, 2006

Chen, R., K. Matsui, M. Ogawa, M. Oe, M. Ochiai, H. Kawashima, E. Sakuradani, S. Shimizu, M. Ishimoto, M. Hayashi, Y. Murooka and Y. Tanaka: Expression of Δ6, Δ5 desaturase and GLELO elongase genes from *Mortierella alpina* for prodution of arachidonic acid in soybean [*Glycine max* (L.) Merril]seeds. Plant Science 170 (2); 399-406, 2006

Hashimoto, K., H. Kawasaki, K. Akazawa, J. Nakamura, Y. Asakura, T. Kudo, E. Sakuradani, S. Shimizu and T. Nakamatsu: Changes in composition and content of mycolic acids in glutamate-overproducing *Corynebacterium glutamicum*. Biosci Biotechnol Biochem 70 (1); 22-30, 2006

Horinouchi, N., J. Ogawa, T. Kawano, T. Sakai, K. Saito, S. Matsumoto, M. Sasaki, Y. Mikami and S. Shimizu: Biochemical retrosynthesis of 2'-deoxyribonucleosides from glucose, acetaldehyde, and a nucleobase. Appl Microbiol Biotechnol 71 (5); 615-621, 2006

Horinouchi, N., J. Ogawa, T. Kawano, T. Sakai, K. Saito, S. Matsumoto, M. Sasaki, Y. Mikami and S. Shimizu: Efficient production of 2-deoxyribose 5-phosphate from glucose and acetaldehyde by coupling of the alcoholic fermentation system of baker's yeast and deoxyriboaldolase-expressing *Escherichia coli*. Biosci Biotechnol Biochem 70 (6); 1371-1378, 2006

Horinouchi, N., J. Ogawa, T. Kawano, T. Sakai, K. Saito, S. Matsumoto, M. Sasaki, Y. Mikami and S. Shimizu: One-pot microbial synthesis of 2'-deoxyribonucleoside from glucose, acetaldehyde, and a nucleobase. Biotechnol Lett 28 (12); 877-881, 2006

Kataoka, M., A. Hoshino-Hasegawa, R. Thiwthong, N. Higuchi, T. Ishige and S. Shimizu: Gene cloning of an NADPH-dependent menadione reductase from *Candida macedoniensis*, and its application to chiral alcohol production. Enzyme Microb Technol 38 (7); 944-951, 2006

Kataoka, M., Y. Nakamura, N. Urano, T. Ishige, G. Shi, S. Kita, K. Sakamot and, S. Shimizu: A

novel $NADP^+$-dependent *l*-1-amino-2-propanol dehydrogenase from *Rhodococcus erythropolis* MAK154: a promising enzyme for the production of double chiral aminoalcohols. Lett Appl Microbiol 43 (4); 430-435, 2006

Kojima, Y., E. Sakuradani and S. Shimizu: Different specificity of two types of *Pseudomonas* lipases for C20 fatty acids with Δ5 unsaturated double bond and their application for selective concentration of fatty acids. J Biosci Bioeng 101 (6); 496-500, 2006

Kojima, Y., E. Sakuradani and S. Shimizu: Acidolysis and glyceride synthesis reactions using fatty acids with two *Pseudomonas* lipases having different substrate specificities. J Biosci Bioeng 102 (3); 179-183, 2006

Mano, J., J. Ogawa and S. Shimizu: Microbial production of optically active β-phenylalanine through stereoselective degradation of racemic β-phenylalanine. Biosci Biotechnol Biochem 70 (8); 1941-1946, 2006

Ogawa, J., J. Mano and S. Shimizu: Microbial production of optically active β-phenylalanine ethyl ester through stereoselective hydrolysis of racemic β-phenylalanine ethyl ester. Appl Microbiol Biotechnol 70 (6); 663-669, 2006

Ogawa, J., CL. Soong, S. Kishino, QS. Li, N. Horinouchi and S. Shimizu: Screening and industrial application of unique microbial reactions involved in nucleic acid and lipid metabolisms. Biosci Biotechnol Biochem 70 (3); 574-582, 2006

Zhang, S., E. Sakuradani and S. Shimizu: Identification and production of n-8 odd-numbered polyunsaturated fatty acids by a Δ12 desaturation-defective mutant of *Mortierella alpina* 1S-4. Lipids 41 (6); 623-626, 2006

Reviews

Kataoka, M. and S. Shimizu: Re-discovery of Old Yellow Enzyme. Bioscience & Industry 64 (5); 277-278, 2006 (in Japanese)

Ogawa, J., N. Horinouchi and S. Shimizu: Industrial production of 2'-deoxyribonucleosides and DNA. KAGAKU to SEIBUTSU 44 (9); 582-584, 2006 (in Japanese)

b) Conference and seminar papers presented

Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry 2006: 23 reports

MECP06: 1 report

97th American Oil Chemists' Society Annual Meeting and Expo: 3 reports

404th Meeting of Vitamin B Research Comittie: 1 report

biocat 2006 Symposium: 2 reports

Annual Meeting of the Society for Fermentation and Bioengineering, Japan 2006: 6 reports

Japanese-Swiss Meeting on Biotechnology and Bioprocess Development: 1 report

Meeting of Kansai Branch of Japan Society for Bioscience, Biotechnology, and Agrochemistry 2006: 7 reports

56th Meeting of Japan Society of Enzyme Engineering: 1 reports

9th Japan-China-Korean Joint Symposium on Enzyme Engineering: 2 reports

The 8th Kyoto University International Symposium: 3 reports

406th Meeting of Vitamin B Research Comittie: 1 report

32nd Meeting of Enzyme Application Association: 1 report

International Symposium on Biocatalysis and Bioenergy: 2 reports

5th Lipid Reserch Seminar: 5 reports

A-3. Off-campus activities

Membership in academic societies (roles)

Shimizu, S.: Japan Society for Bioscience, Biotechnology, and Agrochemistry (director, chairman of Kansai branch); 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 (councilor, editor), Japan Applied Microbiology Society (director); The Society of Fermantation and Metabolism (president)

Kataoka, M.: The Society of Enzyme Engineering (secretary); Japan Society for Bioscience, Biotechnology, and Agrochemistry (secretary of Kansai branch); 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)

Sakuradani, E.: The Society for Fermentation and Bioengineering, Japan (member of young scientist group, editor of Biomedia)

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 th aymmetric synthesis of nitrogen-containing chiral compounds (Kataoka, Shimizu, Ogawa, Sakuradani), Exploratory Research Development of single cell oil (Shimizu, Kataoka, Ogawa, Sakuradani), Young Scientist Research (A) Frontier of Anaerobiotechnology (Ogawa) Young Scientist Research (B) Development and application of gene recombinat technology in oleaginous fungi (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), Microbial production of 1-propanol (Kataoka), Microbial production of nucleosides (Ogawa), Microbial production of functional lipids (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.: 10th Japanese-Swiss Meeting on Biotechnology and Bioprocess Development, Kanazawa (organizer, invited speaker); 9th Japan-China-Korean Joint Symposium on Enzyme Engineering, Otsu (Keynote Lecturer)

Kataoka, M.: Biocat 2006 Symposium, Germany (speaker), 9th Japan-China-Korean Joint Symposium on Enzyme Engineering, Otsu (speaker), International Symposium on Biocatalysis and Bioenergy, Taiwan (invited speaker)

Ogawa, J.: 97th American Oil Chemists' Society Annual Meeting and Expo, USA (invited speaker), MECP (Multi-step Enzyme Catalyzed Processes) 06, Austria (invited speaker), biocat 2006 Symposium, Germany (speaker)

Sakuradani, E.: 97th American Oil Chemists' Society Annual Meeting and Expo, USA (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.: Applied Miciobiology and Biotechnology (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)

Ogawa, J.: Development of thermotolerant microbial resources and their applications in Thailand and Japan (Thailand)

Scholars from abroad

Research fellow (2) (Germany, Indonesia)

B. Educational Activities (2006.4-2007.3)

B-1. On-campus teaching

a) Courses given

Undergraduate level: Outline of Applie Life Sciences II (Shimizu), Applied microbiology III (Shimizu), Applied microbiology IV (Shimizu, Kataoka), Laboratory course in applied microbiology (Kataoka, Ogawa, Sakuradani), Biotechnology (Shimizu), Pocket Seminar-Applied Microbiology (Kataoka)

Graduate level: Fermentation physiology and applied microbiology (Advanced course) (Shimizu, Kataoka, Ogawa, Sakradani), Fermentation physiology and applied microbiology seminar (Shimizu, Kataoka, Ogawa, Sakuradani), Experimental course of fermentation physiology and applied microbiology (Shimizu, Kataoka, Ogawa, Sakuradani)

b) Seminars

Special Symposium of Graduate School of Agriculture, Kyoto University (organizer, invited speaker), 4th Symposium of Graduate School of Agriculture, Kyoto University (invited speaker), 21st Century COE seminar (organizer, 13 times)

B-2. Off-campus teaching, etc.

Part-time lecturer

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

Open lecture organizer

7th Mini Symposium of 21st Century COE Program, Sendai; 8th Mini Symposium of 21st Century COE Program, Kyoto

B-3. Overseas teaching

Students and research fellows from abroad

Foreign students: Doctor's program (1) (China), Master's program (1) (China)

Lacture in abroad

Kataoka, M.: Univeristy of Dortmund (Germany)

Ogawa, J.: l'Institut National de la Recherche Agronomique (France), Yakult Europe Reseearch Center (Bergium), Karlsruhe University (Germany), Stuttgart University (Germany), l'Institut National des Sciences Appliquees de Toulouse (France), Universite Paul Cezanne - Universite d'Aix-Marseille III (France)

C. Other Remarks

Ogawa, J.: "Research Grant 2006" by JSBBA, "Award for young scientists 2006" by Japanese Society of Enzyme Engineering, "Award for young scientists 2006" by the Foundation of Agricultural Sciences of Japan

2.3.8 Laboratory of Microbial Biotechnology

Staff *Professor* *: Sakai, Yasuyoshi, Dr. Agric. Sci.*

Associate Professor: Yurimoto, Hiroya, Dr. (Agric. Sci.)

Students and research fellows

Doctor's program: (7)

Master's program: (13)

Undergraduate : (4)

A. Research Activities (2006.4-2007.3)

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, long-chain alkanes, 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

Kato, N. and H. Yurimoto: Isolation of microorganisms. Jikken Kagaku Kouza 29 Basic technology in Biotechnology (edited by Nihon Kagaku-kai), pp.1-18, Maruzen, Tokyo, 2006 (in Japanese)

Sakai, Y., M. Oku and H. Mukaiyama: Observation of microorganisms. Jikken Kagaku Kouza 29 Basic technology in Biotechnology (edited by Nihon Kagaku-kai), pp.19-33, Maruzen, Tokyo, 2006 (in Japanese)

Original papers

Nakagawa, T., A. Inagaki, T. Ito, S. Fujimura, T. Miyaji, H. Yurimoto, N. Kato, Y. Sakai and N. Tomizuka: Regulation of two distinct alcohol oxidase promoters in the methylotrophic yeast *Pichia methanolica*. Yeast 23; 15-22, 2006

Kajikawa, M., K. T. Yamato, Y. Sakai, H. Fukuzawa, K. Ohyama and T. Kohchi: Isolation and functional characterization of fatty acid D5-elongase gene from the liverwort *Marchantia polymorpha* L. FEBS Lett 580; 149-154, 2006

Kajikawa, M., K. T. Yamato, Y. Kohzu, S. Shoji, K. Matsui, Y. Tanaka, Y. Sakai and H. Fukuzawa: A front-end desaturase from *Chlamydomonas reinhardtii* produces pinolenic and coniferonic acids by w13 desaturation in methylotrophic yeast and Tobacco. Plant Cell Physiol 47; 64-73, 2006

Kawaguchi, K., Y. Shinoda, H. Yurimoto, Y. Sakai and N. Kato: Purification and characterization of benzoate-CoA ligase from *Magnetospirillum* sp. strain TS-6 capable of aerobic and anaerobic degradation of aromatic compounds. FEMS Microbiol Lett 257; 208-213, 2006

Orita, I., T. Sato, H. Yurimoto, N. Kato, H. Atomi, T. Imanaka and Y. Sakai: The ribulose monophosphate pathway substitutes for the missing pentose phosphate pathway in the archaeon *Thermococcus kodakaraensis*. J Bacteriol 188; 4698-4704, 2006

Yamashita, S., M. Oku, Y. Wasada, Y. Ano and Y. Sakai: PI4P-signaling pathway for the synthesis of a nascent membrane structure in selective autophagy. J Cell Biol 173; 709-717, 2006

Kotani, T., Y. Kawashima, H. Yurimoto, N. Kato and Y. Sakai: Gene structure and regulation of alkane monooxygenases in propane-utilizing *Mycobacterium* sp. TY-6 and *Pseudonocardia* sp. TY-7. J Biosci Bioeng 102; 184-192, 2006

Oku, M., T. Nishimura, T. Hattori, Y. Ano, S. Yamashita and Y. Sakai: Role of Vac8 in formation of the vacuolar sequestering membrane during micropexophagy. Autophagy 2; 272-279, 2006

Reviews

Kato, N., H. Yurimoto and R. K. Thauer: The physiological role of the ribulose monophosphate pathway in bacteria and archaea. Biosci Biotechnol Biochem 70; 10-21, 2006

van der Klei, I. J., H. Yurimoto, Y. Sakai and M. Veenhuis: The significance of peroxisomes in

methanol metabolism in methylotrophic yeast. Biochim Biophys Acta 1763; 1453-1462, 2006

Sakai, Y., M. Oku, I.J. van der Klei and J.A.K.W. Kiel: Pexophagy: autophagic degradation of peroxisomes. Biochim Biophys Acta 1763; 1767-1775, 2006

Yamashita, S. and Y. Sakai: Pexophagy and regulation of membrane dynamics by Atg proteins. Protein, Nucleic Acid, and Enzyme 51(10); 1474-1479, 2006 (in Japanese)

Sakai, Y.: Intracellular dynamics of yeast micropexophagy. Kenbikyo 41(2); 81-83, 2006 (in Japanese)

Reports

Yurimoto, H.: Molecular mechanism of strategy for minimization of the intracellular formaldehyde level in microorganisms and its application. Report on the Noda Institute for Scientific Research 50; 77-78, 2006

Yurimoto, H.: Reports on "Annual Meeting of The Society for Biotechnology, Japan 2006". Bioscience & Industry 64(12); 697, 2006 (in Japanese)

b) Conference and seminar papers presented

Annual meeting of the Japan Society for Bioscience, Biotechnology, and Agrochemistry 2006: 13 reports

Yeast Genetics and Molecular Biology News Japan No. 39: 7 reports

19th Annual meeting of Japan Society for Archaea: 1 report

20th IUBMB International Congress of Biochemistry and Molecular Biology and 11th FAOBMB Congress: 6 reports

4th Interational Symposium on Autophagy: 4 reports

17th Joint Symposium on Yeasts: 1 report

19th Annual and International Meeting of the Japanese Association for Animal Cell Technology : 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 on priority areas: Peroxisomal proteins: molecular mechanism of their biogenesis and degradation (Sakai)

Other Research grant: Japan Science and Technology Agency, CREST, Metabolism-based regulation of organelle homeostasis and cell function (Sakai).

A-4. International cooperation and overseas activities

International meetings (roles)

Sakai, Y.: Gordon Resarch Conference on Molecular Basis of Microbial One-Carbon Metabolism, UK (poster). *Hansenula polymorpha* Worldwide Network (HPWN) Meeting, The Netherlands (invited speaker). The 5th JSPS-NRCT Joint Seminar on Development of Thermotolerant Microbial Resources and Their Applications, Thailand (invited speaker).

Yurimoto, H.: Gordon Resarch Conference on Molecular Basis of Microbial One-Carbon Metabolism, UK (oral and poster).

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 (3) (Max-Planck Institute, Germany, Group leader; UCSD, USA, Professor; Kasetsart University, Thailand, Associate Professor)

B. Educational Activities (2006.4-2007.3)

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)

Graduate level: Microbial Biotechnology Seminar (Sakai, Yurimoto), Experimental Course of Microbial Biotechnology (Sakai, Yurimoto)

B-2. Off-campus teaching, etc.

Part-time lecturer

Sakai, Y.: Nara Institute of Science and Technology (Special Lecture on Biotechnology)

B-3. Overseas teaching

Students and reseach fellows from abroad

Foreign students: Master's program (1) (Peru)

Lecture in abroad

Sakai, Y., Ruhr-Universitat Bochum, Germany (invited seminar)

C. Other Remarks

Sakai, Y.: Assistant Chief of Radioisotope Managing Committee at Graduate School of Agriculture, Kyoto University.

Yurimoto, H.: Japan Bioscience, Biotechnology and Agrochemistry Society Award for the Encouragement of Young Scientists (2007).

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, Ms. Agric. Sci.*

Students and research fellows

PD fellow : *(2)*
Research fellow : *(1)*
Foreign research fellow : *(1)*
Doctor's program : *(2)*
Master's program : *(8)*
Undergraduate : *(4)*

A. Research Activities (2006.4-2007.3)

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 transmmision processes across biomembranes.

Uncoupling mechanism of hydrophobic weak acids. Coupling mechanism between electron transport system and ion transport system using enzymes, ionophores and hydrophobic ions. Comsumption 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

Tsujimura, S., S. Kojima, K. Kano, T. Ikeda, M. Sato, H. Sanada and H. Omura: Novel FAD-dependent Glucose Dehydrogenase for a Dioxygen-insensitive Glucose Biosensor. Biosci Biotechnol Biochem 70 (3); 654-659, 2006

Tsujimura, S., A. Katayama and K. Kano: Osmium Complex Grafted on a Carbon Electrode Surface as a Mediator for a Bioelectrocatalytic Reaction. Chem Lett 35 (11); 1244-1245, 2006

Okumura, N., T. Abo, S. Tsujimura and K. Kano: Electron Transfer Kinetics between PQQ-dependent Soluble Glucose Dehydrogenase and Mediators. Electrochemistry 74 (8); 639-641, 2006

Kamitaka, Y., S. Tsujimura, T. Ikeda and K. Kano: Electrochemical Quartz Crystal Microbalance Study on Adsorption of Bilirubin Oxidase as a Catalyst in Bioelectrocatalytic Reduction of Dioxygen. Electrochemistry 74 (8); 642-644, 2006

Tsujimura, S., S. Kojima, T. Ikeda and K. Kano: Potential-step Coulometry of D-Glucose Using Novel FAD-dependent Glucose Dehydrogenase. Anal Bioanal Chem 386; 645-651, 2006

Wang, Y. -F., S. -S. Cheng, S. Tsujimura, T. Ikeda and K. Kano: Escherichia coli-catalyzed Bioelectrochemical Oxidation of Acetate in the Presence of Mediators. Bioelectrochemistry 69 (1); 74-81, 2006

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

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_2 Catalyzed by CueO from *Escherichia coli* Adsorbed on a Highly Oriented Pyrolytic Graphite Electrode. Chem Lett 36 (1); 132-133, 2007

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

Shirai, O., H. Yamana and Y. Arai: Electrochemical behavior of actinides and actinide nitrides in LiCl-KCl eutectic melts. J Alloys Comp 408-412; 1267-1273, 2006

Yamana, H., B. -G. Park, O. Shirai, T. Fujii, A. Uehara and H. Moriyama: Electrochemically Produced Divalent Neodymium in Chloride Melt. J Alloys Comp 408-412; 66-70, 2006

Yoshida, Y., K. Maeda, O. Shirai and T. Ohnuki: Binding Affinity of a Basic Amino Acids to the Surface of a Neutral Phospholipid Monolayer. Chem Lett 35 (1); 132-133, 2006

Nagai, T., A. Uehara, T. Fujii, O. Shirai and H. Yamana: In-situ measurement of UO_2^{2+} concentration in molten NaCl-2CsCl by differential pulse voltammetry. J Nucl Sci Technol 43 (12); 1511-1516, 2006

Shirai,O., Y. Yoshida and S. Kihara: Voltammetric study on ion transport across a bilayer lipid membrane in the presence of a hydrophobic ion or an ionophore. Anal Bioanal Chem 386 (3); 494-505, 2006

Shirai, O., Y. Yoshida, S. Kihara, T. Ohnuki, A. Uehara and H. Yamana: Ion transport across a bilayer lipid membrane facilitated by gramicidin A - Effect of counter anions on the cation transport. J Electroanal Chem 595 (1); 53-59, 2006

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 membrane thickness of Layer-by-Layer Enzyme-Mediator Modified Electrode on Steady Catalytic Current. Bunseki Kagaku, in press (in Japanese)

Shirai, O.: Voltammetric study on ion transports across a bilayer lipid membrane in the presence of hydrophobic ions and ionophores. Bunseki Kagaku, in press (in Japanese)

Shirai, O., T. Nagai, A. Uehara and H. Yamana: Electrochemical properties of the $Ag^+|Ag$ and other reference electrodes in the LiCl-KCl eutectic melts. J Alloys Comp, in press

Uehara, A., M. Kasuno, T. Okugaki, Y. Kitatsuji, O. Shirai, Z. Yoshida and S. Kihara: J Electroanal Chem 604; 115-124, 2007

Reviews and others

Tsujimura, S. and K. Kano: Next Generation on Electric Power Supply Caused by the Innovation of Electric Cells. S・T・S ipp.; 17-26, 2006 (in Japanese)

Tsujimura S.: Steady-state Bioelectrocatalytic Current. Rev Polarogr 52 (2); 81-88, 2006 (in Japanese)

Tsujimura, S. and K. Kano: Bioelectrochemical Cell. Cell Technol (DenchiGijutsu) 18; 115-164, 2006 (in Japanese)

Ikeda, T., S. Tsujimura and K. Kano: Current Situation and Prospects of of Biofuel Cell. The Latest Technology on Ubiquitous Energy (edited by T. Sakai and T. Kobayashi), CMC Press; 187-193, 2006 (in Japanese)

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)

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)

Shirai, O.: Voltammetry for Ion Transfer Through a Liquid Membrane. Rev Polarogr 52 (2); 93-97, 2006 (in Japanese)

Shirai, O.: Fabrication of Planar Bilayer Lipid Membrane. Rev Polarog r 52 (2) ; 98-101, 2006 (in Japanese)

Uehara, A., T. Fujii, T. Nagai, O. Shirai, N. Sato and H. Yamana: Electrochemical and Spectroscopic Characteristics of Uranium Ions in Hydrate Melt. Recent Advances in Actinide Science. I. May, R. Alvares, N. Bryan Eds. 548-550, 2006

Fujii, T., A. Uehara, T. Nagai, O. Shirai, N. Sato and H. Yamana: Spectroscopic Study for the Oxychloride Precipitation of Neodimium and Uranium in Chloride Melts, Recent Advances in Actinide Science. I. May, R. Alvares, N. Bryan Eds. 551-553, (2006)

Shirai, O.: Topic-Overview of recent progress on the working mechanism of voltage-gated ion channels -. Bunseki, in press (in Japanese)

b) Conference and seminar papers presented.

The Kansai Branch Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry in 2006: 3 report

The 448th 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 2007: 5 reports

The 21th Meeting of Division of Biofunctional Chemistry, The Chemical Society of the Japan: 1 report

The 34th Symposium on Biomolecular: 1 report

The 74th Electrochemical Society Meeting: 6 reports

The Autumn Meeting of the Electrochemical Society of Japan in 2006: 7 reports

The 2nd Workshop of Kansai Branch of the Electrochemical Society of Japan: 1 report.

The 3rd Workshop of Kansai Branch of the Electrochemical Society of Japan: 1 report.

The 30th Symposium of Organic Electrochemistry: 1 report.

The 55 th annual meeting of the Japan Society for Analytical Chemistry: 2 reports.

The 52th Annual Meeting on Polarography and Electroanalytical Chemistry: 3 reports.

5th East Asian Biophysics Symposium & 44th Annual Meeting of the Biophysical Society of Japan: 2 reports.

A-3. Off-campus activities

Membership in academic societies (roles)

Kano, K.: The Japan Society for Analytical Chemistry (a council member, a JIS member, a standing committee member of Kinki Branch); The Electrochemical Society of Japan (a council member, a standing committee member of Kansai Branch); The Japan Society for Bioscience, Biotechnology, and Agrochemistry (a standing committee member of Kansai Branch); The Polarographic Society of Japan (general secretary), Analytical Biochemistry (an editoryal board member), Journal of Electroanalytical Chemistry (a council member of editorial board).

Shirai, O.: The Japan Society for Analytical Chemistry (accountant secretary of Kinki Branch); The Polarographic Society of Japan (accountant secretary)

Research grants

NEDO (Kano), COE (Kano), Grants-in-Aid for Scientific Research From the Ministry of Education, Science, Sports and Culture of Japan: Young Scientific Research (B) (Tsujimura)

A-4. International cooperation and overseas activities

International meetings (roles)

Kano, K.: The 209th ECS Meeting, Denver, Colorado, May (Invited Speaker), German-Italian-Japanese Meeting of Electrochemists, Ludiwig-Maximilian-Universitat, Munich, Germany, September (Invited Speaker), 3rd International Symposium of Environmental Biotechnologies, National Cheng Kung University, Tainan, Taiwan, October (Invited speaker), 2nd International Symposium on Organic Electron Transfer Chemistry, Yokohama, January (Invited Speaker), Analytical Biochemistry (Editor), Journal of Analytical Biochemistry (Advisory Board),

Shirai, O. 210th ECS Meeting, Cancun, Mexico, November (Invited Speaker).

Scholars from abroad

Collaborative researcher from Taiwan (1)

B. Educational Activities (2005.4-2006.3)

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 (Kano, Shirai, Tsujimura), New Strategies in Agricultural Sciences (Kano and others)

Graduate level: Bio-Analytical and Physical Chemistry (advanced course) (Kano, Shirai), Experimental Course of Bio-Analytical and Physical Chemistry (Kano, Shirai, Tsujimura).

B-2. Off-campus teaching, etc.

Part-time lecturer

Kano, K.: Kyoto Institute of Technology (Bioelectrochemistry), Shiga University of Medicinal Science (Chemistry), Osaka City University (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.
Students and research fellows
Doctor's program: (3)
Master's program: (6)
Undergraduate : (4)

A. Research Activities (2006.4-2007.3)

A-1. Main subjects

a) Isolation of sex pheromone receptors in insects

With high sensitivity and ligand specificity, male moths detect the sex pheromones that the conspecific female moths release. Each sex pheromone is composed of a species specific blend of odorants. Sex pheromone components were chemically identified for more than 500 hundreds moth species. Among them, only two have their identified receptors that we identified from silkmoth. Recently we identified another sex pheromone receptor genes from four moth species that are far from each other on the phylogenetic tree of moths. On Xenopus oocytes, each receptor gene was coexpressed with an Or83b ortholog gene isolated from the same species. The oocytes expressing a receptor gene responded only to one of the sex pheromone components of the same species that the gene was isolated. All of the receptors tested showed high ligand specificity and sensitivity.

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.

Benzoxazinones (Bxs) accumulate at high concentrations in young seedlings of graminineous plants including wheat, rye, and maize. Avenanthramides (Avs) have been well characterized as phytoalexins in oats. We have been analyzing the functions and biosynthesis of these defensive secondary metabolites in gramineous plants by using the techniques of bioorganic chemistry and biochemistry.

Anthranilate is a precursor of tryptophan synthesis. In addition, the anthranialte metabolism supplies precursors for various secondary metabolites. To elucidate the regulatory mechanism of anthranilate metabolism, we have been investigating the metabolic changes in mutants of Arabidopsis and rice.

A-2. Publications and presentations

a) Publications

Books

Matsukawa, T., A. Ishihara and H. Iwamura: Phytoalexin and Phytoanticipin. Plantmimetics (edited by S. Kai and H. Morikawa), pp. 424-430, NTS, Tokyo, 2006

Ishihara, A.: Life cycle of Plants. Studies in Agricultural Chemistry (CD-ROM) (edited by Japanese Society of Bioscience, Biotechnology, and Agrochemistry), 2006

Original papers

Baran, R., H. Kochi, N. Saito, M. Suematsu, T. Soga, T. Nishioka, M. Robert and M. Tomita: MathDAMP: a package for differential analysis of metabolite profiles. BMC Bioinformatics 7; 530-538, 2006

Fujita, D., M. Murai, T. Nishioka and H. Miyoshi: Light control of mitochondrial complex I activity by a photoresponsive inhibitor. Biochemistry 45; 6581-6586, 2006

Hattori, Y., Y. Kimura, A. Moroda, H. Konno, M. Abe, H. Miyoshi, T. Goto and H. Makabe: Synthesis of murisolin, (15*R*,16*R*,19*R*,20*S*)-murisolin-A, (15*R*,16*R*,19*S*,20*S*)-16,19-*cis*-murisolin, and their inhibitory action with bovine heart mitochondrial complex I. Chem. Asian J 1; 894-904, 2006

Ichimaru, N., M. Abe, M. Murai, M. Senoh, T. Nishioka and H. Miyoshi: Function of the alkyl side chains of Dlac-acetogenins in the inhibitory effect on mitochondrial complex I (NADH-ubiquinone oxidoreductase). Bioorg Med Chem Lett 16; 3555-3558, 2006

Ishihara, A., Y. Asada, Y. Takahashi, N. Yabe, Y. Komeda, T. Nishioka, H. Miyagawa and K. Wakasa, Metabolic changes in *Arabidopsis thaliana* expressing the feedback-resistant

anthranilate synthase α subunit gene *OASA1D*. Phytochemistry 67; 2349-2362, 2006

Kang, S., K. Kang, G. C. Chung, D. Choi, A. Ishihara, D.-S. Lee and K. Back, Functional analysis of the amine substrate specificity domain of pepper tyramine and serotonin *N*-hydroxycinnamoyltransferases. Plant Physiology 140; 704-715, 2006

Matsumoto, Y., E. Muneyuki, D. Fujita, K. Sakamoto, H. Miyoshi, M. Yoshida and T. Mogi: Kinetic mechanism of quinol oxidation by cytochrome *bd* studied with ubiquinone-2 analogs. J Biochem 139; 779-788, 2006

Matsumoto, Y., M. Murai, D. Fujita, K. Sakamoto, H. Miyoshi, M. Yoshida and T. Mogi: Mass spectrometric analysis of the ubiquinol-binding site in cytochrome *bd* from *Escherichia coli*. J Biol Chem 281: 1905-1912, 2006

Makabe, H., Y. Kimura, M. Higuchi, H. Konno, M. Murai and H. Miyoshi: Synthesis of (4*R*, 15*R*, 16*R*, 21*S*)- and (4*R*, 15*S*, 16*S*, 21*S*)- rollicosin, squamostolide, and their inhibitory action with bovine heart mitochondrial complex I. Bioorg Med Chem 14; 3119-3130, 2006

Mogi, T., S. Akimoto, S. Endo, T. Watanabe-Nakayama, E. Mizuochi-Asai and H. Miyoshi: Probing the ubiquinol-binding site in cytochrome *bd* by site-directed mutagenesis. Biochemistry 45; 7924-7930, 2006

Mogi, T., S. Endou, S. Akimoto, M. Morimoto-Tadokoro and H. Miyoshi: Glutamates 99 and 107 in transmembrane helix III of cytochrome *bd* are critical for binding of the heme b_{595}-*d* binuclear center and enzyme activity. Biochemistry 45; 15785-15792, 2006

Murai, M., N. Ichimaru, M. Abe, T. Nishioka and H. Miyoshi: Mode of inhibitory action of Dlac-acetogenins, a new class of inhibitors of bovine heart mitochondrial complex I. Biochemistry 45; 9778-9787, 2006

Murai, M., N. Ichimaru, M. Abe, T. Nishioka and H. Miyoshi, Synthesis of photolabile Dlac-acetogenin for photoaffinity labeling of mitochondrial complex I. J Pesticide Sci 31; 156-158, 2006

Saito, N., M. Robert, S. Kitamura, R. Baran, T. Soga, H. Mori, T. Nishioka and M. Tomita: Metabolomics approach for enzyme discovery. J Proteome Res 5; 1979-1987, 2006

Shiojiri, K., K. Kishimoto, R. Ozawa, S. Kugimiya, S. Urashimo, G. Arimura, J. Horiuchi, T. Nishioka, K. Matsui and J. Takabayashi: Changing green leaf volatile biosynthesis in plants:a novel approach to improve plant resistance against both herbivores and pathogens. Proc Natl Acad Sci USA 103; 16672-16676, 2006

Soga, T., R. Baran, M. Suematsu, Y. Ueno, S. Ikeda, T. Sakurakawa, Y. Kakazu, T. Ishikawa, M. Robert, T. Nishioka and M. Tomita: Differential metabolomics reveals ophthalmic acid as an oxidative stress biomarker indicating hepatic glutathione consumption. J Biol Chem 281; 16768-16776, 2006

Ui, H., K. Shiomi, H. Suzuki, H. Hatano, H. Morimoto, Y. Yamaguchi, R. Masuda, K. Sakamoto, K. Kita, H. Miyoshi, H. Tomoda and S. Ohmura, Paecilaminol, a new NADH-fumarate reductase inhibitor, produced by Paecilomyces sp. FKI-0550. J Antibiot 59; 591-596, 2006

Reviews

Sakurai, T., Y. Seki, T. Nishioka and R. Kanzaki: Molecular basis of pheromone receptions in insects, Hikaku Seirigaku 23; 11-25, 2006 (in Japanese)

Nishioka, T.: Metabolome mass spectral database. Johokagaku-Bukai-shi 24; 38-42, 2006 (in Japanese)

Horai, H. and T. Nishioka: Mass spectral database for metabolomics. Saibokogaku 25; 1394-1398,

2006 (in Japanese)

Patents

Yabe, N., K. Wakasa, A. Ishihara, M. Tsuchiya: Genes involved in IG biosynthesis and mutant plants accumulating IGs at a high level. No.2006-115813

b) Conference and seminar papers presented

The 54th Annual Meeting of the Mass Spectrometry Society of Japan (Workshop): 1 report.

The 40th Annual Meeting of the Taste and Olfactory Society of Japan: 1 report.

The 55th Annual Meeting of the Analytical Chemistry Society of Japan: 1 report (Invited lecture)

The 31st Annual Meeting of the Medical Mass Spectrometry Society of Japan: 1 report (Invited lecture)

The first Symposium on Metabolomics: 1 report (Invited lecture)

The 29th Symposium of the Chemical Informatics Division of the Chemical Society of Japan: 1 report

Annual Meeting of Japanese Society of Bioscience, Biotechnology, and Agrochemistry 2006: 4 reports

The 78th Annual Meeting of the Japanese Biochemical Society: 1 report

The 32nd Annual Meeting of the Japan Bioenergetics Group: 1 report

The 31th Annual Meeting of Pesticide Science Society of Japan: 3 report

The 5th Symposium "Function and Regulation of Plants", Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency (JST): 2 reports

The 17th Annual Meeting of the Japanese Society for Host Defense Research: 1 report (Invited lecture)

A-3. Off-campus activities

Membership in academic societies

Nishioka, T.: The Board Member of Exceptive Committee of the Japanese Society of Bioscience, Biotechnology, and Agrochemistry

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

Research grants

Monbu-Kagakusho Research Grant: Grants-in-Aids for Priority Areas Research, Applications of metabolic regulatory network in Bacillus subtilis to productions (Nishioka, member); Grants-in-Aids for Priority Areas Research, Development of analytical method for metabolomics and predictions of metabolic pathways (Nishioka, member); 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 Scientific Research (B), Synthetic studies of functional acetogenins toward elucidation of respiratory enzyme complex I (Miyoshi, head); 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)

Others: CREST from JST, Regulation and utilization of tryptophan-related primary/secondary

metabolism (Ishihara, member).

A-4. International cooperatons and overseas activities

International meetings (roles)

Nishioka, T.: The 20th International Conference on Biochemistry and Molecular Biology, 18 - 23 June 2006, Kyoto International Conference Center, Kyoto, Japan (1 report).

Nishioka, T.: The International Conference on Analytical Sciences 2006, 25 - 30 June, 2006, Russian National Academy, Moscow, Russia (Invited lecture, 1).

Nishioka, T.: Biosystems Engineering Workshop 2006, 10 - 15 September, 2006, Brawnbald, Switzerland (1 report).

Nishioka, T.: Genome Informatics 2006, 18 - 20 December, 2006, Yokohama, Japan (1 report).

Ishihara, A.: 4th Annual Meeting of Special Committee on Agrochemical Bioscience in Pesticide Science Society of Japan" - Satellite Symposium for IUPAC ICPC 2006 -(Invited lecture, 1)

International joint researches, overseas research surveys

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

Ishihara, A.: Characterization of rice plants expressing THT gene form pepper (Korea)

B. Educational Activities (2006.4-2007.3)

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), Chemistry of bio-catalist (Nishioka, Shimizu), 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) Others

Nishioka, T.: Head of the Division of The Applied Life Sciences

B-2. Off-campus teaching, etc.

Part-time lecturer

Nishioka, T.: Part-time professor, Graduate school of media and governance, Keio University.

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.
Mizutani, Kimihiko, Dr. Agric. Sci.

Students and research fellows
Master's program: (2)
Undergraduate : (3)
Research student:(1)

A. Research Activities (2006.4-2007.3)

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 β-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 β-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

Mizutani, K., Y. Chen, H. Yamashita, M. Hirose and S. Aibara: Thermostabilization of ovotransferrin by anions for pasteurization of liquid egg white. Biosci Biotechnol Biochem 70(8); 1839-1845, 2006

Malle, D., T. Itoh, W. Hashimoto, K. Murata, S. Utsumi S. and B. Mikami: Overexpression, purification and preliminary X-ray analysis of pullulanase from *Bacillus subtilis* strain 168. Acta Crystallograph F 62 (Pt 4); 381-384, 2006

Itoh, T., W. Hashimoto, B. Mikami and K. Murata: Substrate recognition by unsaturated glucuronyl hydrolase from *Bacillus* sp. GL1. Biochem Biophys Res Commun 344(1); 253-262, 2006

Mikami, B., H. Iwamoto, D. Malle, H. J. Yoon, E. Demirkan-Sarikaya, Y. Mezaki and Y. Katsuya: Crystal structure of pullulanase: evidence for parallel binding of oligosaccharides in the active site. J Mol Biol 359(3); 690-707, 2006

Ochiai, A., M. Yamasaki, T. Itoh, B. Mikami, W. Hashimoto and K. Murata: Crystallization and preliminary X-ray analysis of the rhamnogalacturonan lyase YesW from Bacillus subtilis strain 168, a member of polysaccharide lyase family 11. Acta Crystallograph F 62 (Pt 4); 438-440, 2006

Ochiai, A., M. Yamasaki, B. Mikami, W. Hashimoto and K. Murata: Crystallization and preliminary X-ray analysis of an exotype alginate lyase Atu3025 from Agrobacterium tumefaciens strain C58, a member of polysaccharide lyase family 15. Acta Crystallograph F 62 (Pt 4); 486-488, 2006

Itoh, M., M. Ochiai, B. Mikami, W. Hashimoto and K. Murata: A novel glycoside hydrolase family 105: the structure of family 105 unsaturated rhamnogalacturonyl hydrolase complexed with a disaccharide in comparison with family 88 enzyme complexed with the disaccharide. J Mol Biol 360(3); 573-585, 2006

Itoh, M., R. N. Garcia, M. Adachi, Y. Maruyama, E. M. Tecson-Mendoza, B. Mikami and S. Utsumi: Structure of 8Salpha globulin, the major seed storage protein of mung bean. Acta Crystallograph D 62 (Pt 7); 824-832, 2006

Cui, Z., Y. Maruyama, B. Mikami, W. Hashimoto and K. Murata: Crystallization and preliminary crystallographic analysis of the family GH78 alpha-L-rhamnosidase RhaB from Bacillus sp. GL1. Acta Crystallograph F 62 (Pt 4); 646-648, 2006

Itoh, M., M. Ochiai, B. Mikami, W. Hashimoto and K. Murata: Structure of unsaturated rhamnogalacturonyl hydrolase complexed with substrate. Biochem Biophys Res Commun 347(4); 1021-1029, 2006

Itoh, M., W. Hashimoto, B. Mikami and K. Murata: Crystal structure of unsaturated glucuronyl hydrolase complexed with substrate: molecular insights into its catalytic reaction mechanism. J Biol Chem 281(40); 29807-29816, 2006

Maruyama, Y., B. Mikami, W. Hashimoto and K. Murata: A structural factor responsible for substrate recognition by Bacillus sp. GL1 xanthan lyase that acts specifically on

pyruvated side chains of xanthan. Biochemistry 46(3); 781-791, 2007

Tanaka, Y., H. Kobayashi, T. Terasaki, H. Thoma, A. Aruga, T. Uchiyama, K. Mizutani, B. Mikami, C. T. Morita and N. Minato: Synthesis of pyrophosphate-containing compounds that stimulate Vgamma2Vdelta2 T cells: application to cancer immunotherapy. Med Chem 3(1); 85-99, 2007

Reports

Mikami, B., A. Tanabe, M. Yamasaki, T. Itoh and S. Utsumi: Elucidation of reaction mechanism of amylases by high resolution X-ray crystallographic analysis. SPring-8 User Experiment Report 2006B; 1096, 2006

Mikami, B., T. Fukuda, T. Itoh, N. Maruyama and S. Utsumi: Mechanism of structure-formation of globulins in edible plant seeds. SPring-8 User Experiment Report 2006B; 1652, 2006

Mikami, B., K. Park, T. Fukuda, T. Itoh, N. Maruyama and S. Utsumi: Mechanism of structure-formation of globulins in edible plant seeds. SPring-8 User Experiment Report 2006B; 2652, 2006

b) Conference and seminar papers presented

The 2007 Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry : 7 papers

The 79th Annual Meeting of The Japanese Biochemical Society: 2 papers

The 6 th Annual Meeting of Protein Science Society of Japan: 2 papers

The 2006 Annual Meeting of The Society for Biotechnology, Japan: 2 papers

The 439th 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 Protein Structural and Functional Analyses (Mikami)

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 meetings (roles)

Mikami, B.: 23 rd European Crystallographic Meeting, August 6-11, 2006, Leuven, Belgium (1 poster)

Mikami, B.: 7 th Joint Conference of the Asian Crystallographic Association and the Crystallographic. Society of Japan, November 20-23, 2006, Tsukuba, Japan (Symposium)

International joint researchers, overseas research surveys

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

Aibara, S.: The Committee of The second International Symposium on Diffraction Structural Biology 2007 (Executive Committee)

B. Educational Activities (2006.4-2007.3)

B-1. On-campus teaching

a) Courses given

Undergraduate level: Laboratory Course in Biological Chemistry (Aibara, Takahashi, Mizutani), Introduction to Applied Life Sciences, Part III (Mikami), Chemistry of Biological Catalysis (Mikami)

Graduate level: Applied Structural Biology Seminar (Mikami. Aibara, Takahashi, Mizutani), Experimental Course of Applied Structural Biology (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 : Sakata, Kanzo, Dr. Agric. Sci.*
Associate Professor: Hiratake, Jun, Dr. Agric. Sci.
Assistant Professor : Mizutani, Masaharu, Dr. Agric. Sci.
Assistant Professor : Shimizu, Bun-ichi, Dr. Agric. Sci.

Students and research fellows
Research fellow : (3)
Doctor's program : (3)
Master's program : (12)
Research student : (1)

A. Research Activities (2006.4-2007.3)

A-1. Main subjects

a) Approaches to establish a new diglycosidase family in plant kingdom:

A β-primeverosidase from tea plants (Camellia sinensis) is a unique disaccharide-specific diglycosidase, which hydrolyses aroma precursors of β-primeverosides (6-O-β-D-xylopyranosyl-β-D-glucopyranosides) to liberate a primeverose unit and various aroma compounds. β-Primeverosidase is classified in glycosyl hydrolase family 1. In order to clarify the molecular mechanism by which diglycosidases recognize and bind disaccharide-glycosides, the recombinant β-primeverosidase was produced in insect cells using a baculovirus expression system, and was

purified with a novel affinity column of β-primeverosylamidine that we prepared by ourselves. We succeeded in crystallization of β-primeverosidase, and its crystal structure at 1.8 Å resolution was obtained. The residues involved in enzyme catalysis and glucose recognition are well conserved between the structures of maize β-glucosidase and β-primeverosidase. The co-crystallization with β-primeverosylamidine and its crystal structure at 1.8 Å resolution was also obtained.

b) Synthetic elaboration and applications of β-glycosylamidines as glycosidase inhibitors:

The glycosylamidines were synthesized as selective inhibitiors of glycosidases and were applied for glycosidase studies as research tools. A series of glycosylamidine derivatives with different glycon and aglycon moieties have been synthesized and assayed for inhibition of glycosidases with varying substrate specificities. The glycosylamidines selectively inhibited glycosidases according to their glycon and aglycon substrate specificities. The cyclic glucosylamidines designed to mimic an oxazoline intermediate were synthesized and found to serve as extremely potent and selective inhibitors of family 20 *N*-acetylglucosaminidases. The glycosylamidines thus serve as "tailor-made" inhibitors according to the reaction mechanisms, as well as to the substrate specificities of enzymes. The glycosylamidines were found to bind the glycosidases by electrostatic interaction with the catalytic acid-base in the enzyme active site, and these properties were used successfully as ligand for novel affinity chromatography where the adsorption and desorption of enzyme is controlled by pH change. The utility of this novel affinity chromatography was evidenced by successful purification of diglycoside-specific glycosidases with β-primeverosylamidine as an affinity ligand.

c) Design and synthesis of mechanism-based inactivator of γ-glutamyltranspeptidase:

γ-Glutamyltranspeptidase (GGT) is a key enzyme in glutathione metabolism. According to the enzyme reaction mechanism, a series of γ-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 active-site Thr residue to inactivate GGT. They were used successfully for probing the active-site geometries of both *E. coli* and human GGTs. Human GGT has distinct substrate specificity with respect to the acceptor site, and according to this, 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 uniformly by any structural analogues of phosphonates, depending solely on the leaving group ability, in accordance with broad substrate specificity of this enzyme. The phosphonate diesters thus served successfully as chemical probes for active-site mapping of GGT. The X-ray crystal structure of *E. coli* GGT in complex with the transition-state analogue inhibitor was successfully solved to reveal detailed active-site structure for the recognition of substrate glutathione.

d) 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 was obtained that showed 20-time higher molecular activity for the hydrolysis of oleoyl β-naphthylamide. The kinetic analyses of the mutant and wild-type lipases suggested that the increase in amide-hydrolyzing activities was ascribed to the increase in the leaving group protonation during the collapse of the

tetrahedral intermediate.

e) Mechanism of the activation/inactivation process of plant hormones:

The physiological functions of plant hormones are regulated by the concerted process among their biosynthesis, catabolism and translocation in the responsive organs. Therefore, identification and characterization of enzymes involved in these process are very important to understand how they regulate the plant life cycle from germination to flowering. In this study, we have characterized cytochrome P450 monooxygenases (P450) involved in biosynthesis of brassinosteroids (BRs). We determined biochemical properties of C-22 hydroxylase and 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.

f) Coumarin biosynthesis in plants:

Investigation into the coumarin contents in wild type and the mutants of Arabidopsis was performed, resulting that the roots of Arabidopsis accumulate scopolin (a β-glucoside of scopoletin). The mutations of a several genes coding the enzymes of the phenylpropanoid pathway caused severe decrease in scopolin contents. Functional analysis of these genes with the recombinant proteins revealed 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.

g) Studies on molecular basis of the characteristic aroma formation of the Formosa oolong tea (Oriental Beauty)

Oriental Beauty is a flavor-rich oolong tea produced from tea leaves infested by the tea green leafhopper (*Jacobiasca formosana*) in Taiwan. We have studied to clarify the molecular basis of the characteristic aroma formation of the tea by various approaches such as natural product chemistry, biochemistry, and molecular biology. Oolong tea samples were prepared from tea leaves infeted/noninfested by the insects. Samples were obtained at each step of the manufacturing process and subjected to evaluation tests by professional tea tasters and to GC-MS analysis. The tea produced from tea leaves infested by the insects was found to be superior in the quality and quantity of aroma to that from tea leaves without or with much less the insect attack. Hotrienol and its related compound, 2,6-dimethylocta-3.7-diene-2,6-diol, were confirmed to be responsible for the insect attack. Genes induced in response to the insect attack and the tea manufacturing processes were identified by the differential screening based on the Megasort analysis. These results have revealed that the tea leaves of Oriental Beauty are greatly affected by the stresses of the insect attack and the tea manufacturing processes such as solar withering and turning-over, and these stresses are important factors to increase the production of the aroma compounds characteristic to this characteristic oolong tea. Genes responsible for the characteristic aroma compounds are now under screening.

A-2. Publications and presentations

a) Publications

Reviews

Hiratake, J.: Rational Design of Enzyme Inhibitors for Use as Bioprobes. Chemical Industry 57; 680-686, 2006

Hiratake, J.: β-Glycosylamidines as Inhibitors of β-Glycosidases and Their Use as Ligand for

Affinity Chromatography. Trends in Glycosci Glycotechnol 18; 371-381, 2006

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

Han, L., J. Hiratake, N. Tachi, H. Suzuki, H. Kumagai and K. Sakata: γ-(Monophenyl)phosphono glutamate analogues as mechanism-based inhibitors of γ-glutamyl transpeptidase. Bioorg Med Chem 14; 6043-6054, 2006

Tomiya, N., S. Narang, J. Park, B. Abdul-Rahman, O. Choi, S. Singh, J. Hiratake, K. Sakata, M. J. Betenbaugh, K. B. Palter and Y. C. Lee: Purification, characterization, and cloning of a *Spodoptera frugiperda* SF9 β-*N*-acetylhexosaminidase that hydrolyzes terminal *N*-acetylglucosamine on N-glycan core. J Biol Chem 281; 19545-19560, 2006

Guitierrez, J. A., Y. X. Pan, L. Koroniak, J. Hiratake, M. S. Kilberg and N. G. J. Richards: An inhibitor of human asparagine synthetase suppresses proliferation of an L-asparaginase resistant leukemia cell line. Chem Biol 13; 1339-1347, 2006

Ohnishi, T., S. Bancos, B. Watanabe, S. Fujita, M. Szatmari, C. Koncz, M. Lafos, T. Yokota, K. Sakata, M. Szekeres and M. Mizutani: C-23 hydroxylation by Arabidopsis CYP90C1 and CYP90D1 reveals a new shortcut route in brassinosteroid biosynthesis. Plant Cell 18; 3275-3288, 2006

Morikawa, T., M. Mizutani and D. Ohta: Cytochrome P450 subfamily CYP710A genes encodes sterol C-22 desaturase in plants. Biochemical Society Transactions 34; 1202-1205, 2006

Mizutani, M. and Y. Todoroki: ABA 8'-hydroxylase and its chemical inhibitors. Phytochemistry Review 5; 385-404, 2006

Ohnishi, T., T. Nomura, B. Watanabe, D. Ohta, T. Yokota, H. Miyagawa, K. Sakata and M. Mizutani: Tomato CYP734A7 catalyzes the C-26 hydroxylation of C_{27} and C_{28} brassinosteroids, Phytochemistry 67; 1895-1906, 2006

Ohnishi, T., B. Watanabe, K. Sakata and M. Mizutani: CYP724B2 and CYP90B3 function in the early C-22 hydroxylation steps of brassinosteroid biosynthetic pathway in tomato, Biosci Biotechnol Biochem 70 2071-2080, 2006

Saito, S., M. Okamoto, S. Shinoda, T. Kushiro, T. Koshiba, Y. Kamiya, N. Hirai, K. Sakata, E. Nambara and M. Mizutani: Uniconazole is a potent inhibitor of ABA 8'-hydroxylase in *Arabidopsis*, Biosci Biotechnol Biochem 70; 1731-1739, 2006

Araki, Y., A. Miyawaki, T. Miyashita, M. Mizutani, N. Hirai and Y. Todoroki: A new non-azole inhibitor of ABA 8'-hydroxylase: Effect of the hydroxyl group substituted for geminal methyl groups in the six-membered ring, Bioorg Med Chem Lett 16; 3302-3305, 2006

Morikawa, T., M. Mizutani, N. Aoki, B. Watanabe, H. Saga, S. Saito, A. Oikawa, H. Suzuki, N. Sakurai, D. Shibata, A. Wadano, K. Sakata and D. Ohta: Cytochrome P450 *CYP710A* encodes the sterol C-22 desaturase in plants. Plant Cell 18; 1008-1022, 2006

b) Conference and seminar papers presented

11th IUPAC International Congress of Pesticide Chemistry: 2 papers

ICOB-5 and ISCNP-25 IUPAC: International Conference on Biodiversity and Natural Products: 3 papers

Trends in Plant Hormones (RIKEN Plant Science Center International Symposium): 1 paper

8th International Symposium on Cytochrome P450 Biodiversity and Biotechnology: 4 papers

The 2006 Meeting of Kansai Branch of Japan Society for Bioscience, Biotechnology, and Agrochemistry: 2 papers

The 41th Annual Meetng of Regulation of Plant Growth & Development: 4 papers

The 2006 Forum of the Molecular Biology Society of Japan: 1 paper

The 2007 Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry: 11 papers

The 48th Annual Meeting of the Japanese Society for Plant Physiologists: 6 papers

Kyoto-Newcastle Meeting on Chemical Biology: 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)

Sakata, K.: Japan Society for Bioscience, Biotechnology, and Agrochemistry (councillor); The Japanese Society for Chemical Regulation of Plants (editor and a member of awarding committee); Japan Society for Bioscience, Biotechnology, and Agrochemistry Kansai Branch (councillor); Kyoto Prefecture Tea Association (a member of selection committee of scientific research projects)

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) (2) Studies on Catalytic Mechanism of Disaccharide-Specific Glycosidases and Evolution of Plant β-Glucosidases (Sakata K); Grant-in-Aid for Scientific Research (B) (2) Grant-in-Aid for Scientific Research (B) (2) Bio- and Organic Chemical Studies on Plant Glycosidases by Using β-Glycosylamidine Derivatives as Tools (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 meetings (roles)

Sakata, K.: Member of American Chemical Society (Division of Agricultural Food Chemistry)

Hiratake, J.: Lecture in Free Radical Research Center, Medical College of Wisconsin (USA), Sep. 21, 2006 (Invited lecture)

B. Educational Activities (2006.4-2007.3)

B-1. On-campus teaching

a) Courses given

Undergraduate level: Pocket Seminar (Let's touch the heart of live Organic Chemistry) (Hiratake and Sakata)

Graduate level: Seminar in Molecular Biocatalysts (Shimizu, Mizutani, Hiratake and Sakata), Laboratory Course in Molecular Biocatalysts (Shimizu, Mizutani, Hiratake and Sakata)

B-2. Off-campus teaching, etc.

Part-time lecturer

Sakata, K.: Fukui Prefectural University (Graduate School of Bioscience and Biotechnology; Obama campus)

Sakata, K.: Gifu University (Graduate School of Bioscience and Biotechnology, Division of Biological Resources and Production)

Mizutani, M.: Shizuoka University (Graduate School of Agriculture, Division of Applied Bioligical Chemistry)

An extension lecture etc.

Hiratake, J.: Science-Partnership Program for collaboration in educational between high schools and universities, sponsored by the Ministry of Education, Culture, Sports, Science and Technology, Special lecture at Kyoto Momoyama high school

Hiratake, J.: Super-Science High School, sponsored by the Ministry of Education, Culture, Sports, Science and Technology, Special lecture for students of Zeze high school

Hiratake, J.: Super-Science High School, sponsored by the Ministry of Education, Culture, Sports, Science and Technology, Special lecture for students of Nara Women's University Secondary School

B-3. Overseas teaching

Students and research fellows from abroad

Research Fellow: 2 (Korea, China)

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.

Students and research fellows

Doctor's Program : (9)

Master's Program: (15)

A. Research Activities (2006.4-2007.3)

A-1. Main subjects

a) Analysis of cold-adaptation mechanism of psychrotrophic bacteria

Shewanella livingstonensis Ac10, a psychrotrophic bacterium isolated from Antarctic seawater, grows at a temperature range of 4˚C to 25˚C. The bacterium produces eicosapentaenoic acid (EPA) as a component of phosphatidylglycerol and phosphatidylethanolamine at low temperatures. EPA constitutes about 5% of the total fatty acids of the cells grown at 4˚C. We found that five genes are essential for the production of EPA by targeted disruption of the respective genes. The mutant cells lacking EPA exhibited

significant growth retardation at 4˚C, whereas they grew normally at 18˚C. Microscopic observation revealed that the EPA-deficient strains became filamentous at 4˚C, suggesting that they have a defect in cell division. We analyzed the fluidity of the cell membrane at low temperatures by using pyrene as a fluorescence probe and found that the fluidity of the membrane from the EPA-deficient strain was not significantly different from that of the membrane from the parent strain. The results suggest that EPA has a physiological function other than the function to maintain the membrane fluidity. Proteomic analysis of the membrane proteins revealed that the amounts of six proteins, including outer membrane porin, were decreased and the amounts of two proteins were increased by the absence of EPA. The cold-sensitive phenotype of the EPA-deficient strains may be ascribed to a defect in the function of these membrane proteins.

b) Studies on mechanism of selenoprotein biosynthesis

Selenium, an essential trace element, exists as a selenocysteine residue in the active site of proteins and plays an important role in various biological processes. We found that thioredoxin reductase is crucial for selenoprotein biosynthesis as a selenite-reducing enzyme in HeLa cells. Three-dimensional structure-based analysis of substrate recognition mechanism of selenocysteine lyase, which is essential for the initial step of selenoprotein biosynthesis, revealed that Cys375 of the enzyme specifically interacts with selenocysteine but not cysteine.

A-2. Publications and presentations

a) Publications

Original Papers

Yoshida, M., T. Oikawa, H. Obata, K. Abe, H. Mihara and N. Esaki: Biochemical and Genetic Analysis of the γ-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. FEBS J 274; 2262-2273, 2007

Kudou, D., S. Misaki, M. Yamashita, T. Tamura, T. Takakura, T. Yoshioka, S. Yagi, R. M. Hoffman, A. Takimoto, N. Esaki and K. Nagaki: Structure of the Antitumour Enzyme L-Methionine γ-Lyase from Pseudomonas putida at 1.8 Å Resolution. J Biochem 141; 535-544, 2007

Yow, G.-Y., T. Uo, T. Yoshimura and N. Esaki: Physiological role of D-amino acid-*N*-acetyltransferase of Saccharomyces cerevisiae: detoxification of D-amino acids. Arch Microbiol 185; 39-46, 2006

Yasuda, M., M. Ueda, H. Muramatsu, H. Mihara and N. Esaki: Enzymatic synthesis of cyclic amino acids by *N*-methyl-L-amino acid dehydrogenase from Pseudomonas putida. Tetrahedron: Asymmetry 17; 1775-1779, 2006

Takakura, T., A. Takimoto, Y. Notsu, H. Yoshida, T. Ito, H. Nagatome, M. Ohno, Y. Kobayashi, T. Yoshioka, K. Inagaki, S. Yagi, R. M. Hoffman and N. Esaki: Physicochemical and Pharmacokinetic Characterization of Highly Potent Recombinant L-Methionine γ-Lyase Conjugated with Polyethylene Glycol as an Antitumor Agent. Cancer Res 66; 2807-2814, 2006

Takakura, T., T. Ito, S. Yagi, Y. Notsu, T. Itakura, T. Nakamura, K. Inagaki, N. Esaki, R. M. Hoffman and A. Takimoto: High-level expression and bulk crystallization of recombinant L-methionine γ-lyase, an anticancer agent. Appl Microbiol Biotechnol 70; 183-192, 2006

Papajak, E., R. A. Kwiecien, J. Rudzinski, D. Sicinska, R. Kaminski, L. Szatkowski, T. Kurihara, N. Esaki and P. Paneth: Mechanism of the Reaction Catalyzed by DL-2-Haloacid Dehalogenase As Determined from Kinetic Isotope Effects. Biochemistry 45; 6012-6017, 2006

Muramatsu, H., H. Mihara, M. Yasuda, M. Ueda, T. Kurihara and N. Esaki: Enzymatic Synthesis of L-Pipecolic Acid by Δ1-Piperideine-2-carboxylate Reductase from Pseudomonas putida. Bioscience, Biotechnology and Biochemistry 70; 2296-2298, 2006

Mihara, H., S. Kurokawa, R. Omi, T. Kurihara, K. Hirotsu and N. Esaki: Selenoprotein Biosynthesis and Selenium-Specific Enzymes. Biomed Res Trace Elem 17; 355-359, 2006

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); Dynamics of an essential trace element selenium and molecular basis of selenoprotein biosynthesis in mammals (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 tis application to bioremediation (N. Esaki), National Project on Protein Structural and Functional Analyses; Large-scale preparation of proteins from microorganisms living in extreme environment (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); Screening of novel cold-adapted microorganisms inhabiting the polar regions and development of their useful enzymes (T. Kurihara), A Grant for Research for Promoting Technological Seeds form JST; Construction of a low-temperature protein expression system using cold-adapted bacteria (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-4. International cooperations and overseas activities

International meetings (roles)

Esaki, N.: Selenium 2006 (speaker)

Kurihara, T.: Extremophiles 2006 (speaker)

Mihara, H.: 20th IUBMB (speaker)

Membership in international academic societies

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

Kurihara, T.: Applied Microbiology and Biotechnology (editorial board)

B. Educational Activities (2006.4-2007.3)

B-1. On-campus teaching

a) Courses given

Graduate level : Exercise Course of Microbial Biochemistry (Esaki and Kurihara), Experimental Course of Microbial Biochemistry (Esaki and Kurihara), Molecular Microbial Science (Esaki and Kurihara)

B-2. Off-campus teaching, etc.

Part-time lecturer

Esaki, N.: Faculty of Agriculture, University of the Ryukyus (Bioscience and Biotechnology)

B-3. Overseas teaching

Students and research fellows from abroad

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

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.*

Students and research fellows

Doctor's program: (3) *Post doc research fellow: (6)*

Master's program: (7)

A. Research Activities (2006.4-2007.3)

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 polyketides, and elucidating the regulatory mechanism of the expression of those genes. 1) 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. 2) Dark-inducible genes responsible for the production of secondary metabolites are isolated and characterization of these genes is carried out. 3) 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. 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 129 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 (mdr)-subfamily and ABCA1 ortholog in plant is selected to analyze their biochemical functions, i.e. transport of substrates, and physiological role in plant body. 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. 3) Isolation and characterization of cDNAs from woody plants: 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.

1) Cell wall loosening: This study focuses on the structure and function of endo-1,4-β-glucanase. 2) 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) Limonene synthase gene is introduced into tobacco and Lithospermum erythrorhizon to engineer their terpene metabolism to produce the monoterpene. 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 Yamamoto, K: Shikonin production by *Lithospermum erythrorhizon* cell cultures: biosynthesis, regulation, and bioprocess development. In: Molecular Plant Cell-Based Bioprocessing (edited by Wei Zhang), Springer-Verlag, in press.

Shitan, N. and K. Yazaki: Membrane transport of plant secondary metabolites. In: Plant Genetic Engineering Volume 9, Plant membrane and vacuolar transporters (edited by Pawan K. Jaiwal), Studium Press LLC, Huston, USA, in press.

Hayashi, T.: The Science and Lore of the Plant Cell Wall - Biosynthesis, Structure and Function, BrownWalker Press, Florida USA, 2006

Original papers

Shitan, N., K. Horiuchi, F. Sato and K. Yazaki: Bowman–Birk proteinase inhibitor confers heavy metal and multiple drug tolerance in yeast. Plant Cell Physiol 48 (1); 193-197, 2007

Kato, N., E. Dubouzet, Y. Kokabu, S. Yoshida, Y. Taniguchi, J. G. Dubouzet, K. Yazaki and F. Sato: Identification of a WRKY Protein as a Transcriptional Regulator of Benzylisoquinoline Alkaloid Biosynthesis in *Coptis japonica*. Plant Cell Physiol 48 (1); 8-18, 2007

Shitan, N., M. Tanaka, K. Terai, K. Ueda and K. Yazaki: Human MDR1 and MRP1 recognize berberine as their transport substrate. Biosci Biotech Biochem 71 (1); 242-245, 2007

Sugiyama, A., N. Shitan, S. Sato, Y. Nakamura, S. Tabata and K. Yazaki: Genome-wide analysis of ATP-binding cassette (ABC) proteins in a model legume plant, *Lotus japonicus*: comparison with *Arabidopsis* ABC protein family, DNA Res 13(5); 205-228, 2006

Ohara, K., K. Yamamoto, M. Hamamoto, K. Sasaki and K. Yazaki: Functional characterization of OsPPT1, which encodes *p*-hydroxybenzoate polyprenyltransferase involved in ubiquinone biosynthesis in *Oryza sativa*. Plant Cell Physiol 47 (5); 581-590, 2006

Yazaki, K., N. Yamanaka, T. Masuno, S. Konagai, N. Shitan, S. Kaneko, K. Ueda and F. Sato; Heterologous expression of a mammalian ABC transporter in plant and its application to phytoremediation. Plant Mol. Biol 61(3); 491-503, 2006

Konishi, T., T. Takeda, Y. Miyazaki, M. Ohnishi-Kameyama, T. Hayashi, M.A. O'Neill and T. Ishii: A plant mutase that interconverts UDP-arabinofuranose and UDP-arabinopyranose. Glycobiology 17; 345-354, 2007

Okumura, S., M. Sawada, M. Shimamura, Y-W, Park, T. Hayashi, A. Yamashita, M. Hattori, H. Kanamoto, H. Takase, C. Miyake and K. Tomizawa: A strategy for desert afforestation using plastid transformation technique for CO2 sequestration. J Arid Land Stud 15; 505-508, 2006

Okumura, S., M. Sawada, Y-W, Park, T. Hayashi, M. Shimamura, H. Takase and K. Tomizawa: Transformation of poplar (*Populus alba*) plastids and expression of foreign proteins in tree chloroplasts. Transgenic Res 15; 637-646, 2006

Patents

Horiuchi, K. and K. Yazaki: Creation of plants with high productivity valuable compounds. August 9th, 2006.

Horiuchi, K. and K. Yazaki: Promoter showing strict expression specificity for pericycle of plant roots. December 22nd, 2006.

Reviews

Yazaki, K.: ABC transporters involved in the transport of plant secondary metabolites. FEBS Lett 580 (4); 1183-1191, 2006

Yazaki, K.: Production of tannin by tissue culture of woody plants and tannin biosynthesis. Mokuzai Gakkaishi 52 (2); 67-76, 2006

b) Conference and seminar papers presented

Annual Meeting of Japanese Society for Plant Physiologyists 2006 : 11 reports

Annual Meeting of Japan Wood Society 2006 : 5 reports

Annual Meeting of Japanese Society for Bioscience, Biotechnology and Agrochemistry 2006 : 5 reports

The 24th Annual Meeting of Japanese Society for Plant Cell and Molecular Biology : 8 reports

The Forum of Molecular Biology: 1 report

The 127th Annual Meeting of Pharmaceutical Society: 1 report

Annual Meeting of Japan Forest Science Society: 1 report

A-3. Off-campus activities

Membership in academic societies (roles)

Yazaki, K. : The Japanese Society for Plant Cell and Molecular Biology (Board Member, Associate Editor), The Japanese Society for Plant Physiologist (Board member, Editorial Board),Japan Society for Bioscience, Biotechnology, and Agrochemistry (Board member), The Japan Wood Research Society (Editorial Board), MEXT Plant Project Comitttee (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)

Research grants

Monbusho Research Grant: Priority Areas (2) Molecular mechanism of polar auxin transport by MDR-type ABC transporter in plants (Yazaki, Head), Development of vacuolar function concerning indole metabolites via ABC proteins (Yazaki, Head), Scientific Research (B) Structural and functional analyses of prenyltransferase accepting aromatic substrates (Yazaki, Head), 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), Nitta Corp, Phytoremediation (Yazaki); Research grant for Sustainable Humanosphere for Mission 1, Physiological function of isoprene emission in poplar (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)

A-4. International cooperations and overseas activities

International meetings (roles)

Yazaki, The 53rd NIBB Conference Dynamic Organelles in Plants (June, 2006, Okazaki, Invited speaker), ICOB-5 & ISCNP-25 IUPAC International Conference on Biodiversity and Natural Products (une, 2006, Kyoto, Speaker), The Second International Symposium on Research into Plant Secondary Metabolites and Medicinal Phytocompounds (December, 2006, Taipei, Taiwan, Invited speaker).

Hayashi, T.: Expression of xyloglucanase and cellulase in mangium and sengon (Biotechnology Center, Indonesia), Expression of xyloglucanase and cellulase in Eucalyptus (CBD Technology, Israel), Studies on CGA (Syngenta, Switzerland), Korrigan projects in Europe (INRA, France), Soluble cellulose (Univ of Leon, Spain)

Kuroda : ICOB-5 & ISCNP-25 IUPAC International Conference on Biodiversity and Natural Products (July, Kyoto)

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 alkaloids in isolated vacuoles of plants (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 (Editorial Board), Plant Biotechnology (Associate Editor), J. Wood Sci. (Editorial Board)

Hayashi, T.: Cellulose (Editoral board)

Scholars from abroad

Ph D scientists (3) (England, Korea and Brasil)

Ph D student (1) (Spain)

B. Educational Activities (2006.4-2007.3)

B-1. On-campus teaching

a) Courses given

Undergraduate level: Science of Sustainable Humanosphere (Shiotani, Tsuda, Yazaki)

Graduate level: Plant Gene Expression (Yazaki), Laboratory Course in Molecular Biology in Woody Plants (Yazaki, Hayashi, Kuroda), Seminar in Molecular Biology in Woody Plants (Yazaki, Hayashi, Kuroda)

B-2. Off-campus teaching, etc.

Part-time Lecturer

Yazaki, K.: Kobe Pharmaceutical University, Natural Product Course (December 2005,

Undergraduate level).

Hayashi, T.: Ryukyu University, Agricultural course (May, 2006), Kyushu Univesity, Examinar for Ph.D defense for graduate student (January, 2007)

Open seminar, etc.

Yazaki, K.: 44th Symposium in Research Institute for Sustainable Humanosphere, Organizer (June, 2006, Kyoto),

Kuroda, H.: 3rd Open Seminar in Research Institute for Sustainable Humanosphere, Symposist (October, 2006, Uji).

B-3. Overseas teaching

Lectures and seminars

Yazaki, K.: Special Seminar, ENS Lyon, (February 2007, Lyon, Fance), Special Seminar, Institut des Sciences du Vegetal – CNRS (February 2007, Gif-sur-Yvette, France), Special Seminar, CEA Cadarache (February 2006, Cadarache, France).

Hayashi, T.: Special Lecture: INRA Versailles (April 2006, Versailles, France), Special Lecture: INRA Orleans (April 2006, Orleans, France), Special Lecture: Pontianak University (September 2006, Pontianak, Indonesia)

Kuroda, H.: Special Lecture, LIPI, Indonesian Institute of Science (March 2006, Serpong, Indonesia)

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.

Students and research fellows

Doctor's Program: (3)

Master's Program: (5)

A. Research Activities (2006.4-2007.3)

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

Original papers

Sakakibara, N., T. Nakatsubo, S. Suzuki, D. Shibata, M. Shimada and T. Umezawa: Metabolic Analysis of the Cinnamate/Monolignol Pathway in *Carthamus tinctorius* Seeds by a Stable-Isotope-Dilution Method. Org Biomol Chem 5; 802-815, 2007

Sakai, S., T. Nishide, E. Munir, K. Baba, H. Inui, Y. Nakano, T. Hattori and M. Shimada: Subcellular localization of glyoxylate cycle key enzymes involved in oxalate biosynthesis of wood-destroying basidiomycete *Fomitopsis palustris* grown on glucose. Microbiology 152; 1609-1620, 2006

Takano, T., Y. Tobimatsu, T. Hosoya, T. Hattori, J. Ohnishi, M. Takano, H. Kamitakahara and F. Nakatsubo: Studies on the dehydrogenative polymerizations of monolignol ß-glucosides. Part 1. Syntheses of monolignol ß-glucosides, (*E*)-isoconiferin, (*E*)-isosyringin, and (*E*)-triandrin. Journal of wood chemistry and technology 26; 215-229, 2006

b) Conference and seminar papers presented

56th Annual Meeting of Japan Wood Res. Soc. (Akita): 2 papers

The 24th Annual Meeting of the Japanese Society of Plant Cell and Molecular Biology (Tsukuba): 1 papers

51st Lignin Symposium, (Sapporo): 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 Award Selection, Chair of working group of Extractives and Wood Utilization)

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.). Subsidy from Presidential Fund (Principal Investigator: Umezawa, T.), Institute of Sustainability Science, Kyoto University, Grant-in-Aid for Exploratory Research (Principal Investigator: Umezawa, T.), and Support for a conference jointly organized with a partner institute under the MOU (Principal Investigator: Umezawa, T.)

Others: R&D Project of Industrial Science and Technology Frontier Program supported by NEDO (New Energy and Industrial Technology Development Organization) (Umezawa, T.), Cooperative research for an application of forest microorganism for mycorrhizal remediation supported by Biol. Environ. Inst. (Hattori, T.)

A-4. International cooperations and overseas activities

International meetings (roles)

Umezawa, T.: The 10th International Congress of Biotechnology in the Pulp and Paper Industry (program committee), The 69th RISH Symposium Tropical Tree Biotechnology Initiative (The chair of organizing committee, keynote), Wood Science School 2007 in Cibinong (The chair of organizing committee).

Oral presentation

Umezawa, T.: ICOB-5 & ISCNP-25 IUPAC International Conference on Biodiversity and Natural Products (Kyoto), The 11th International Association for Plant Tissue Culture and Biotechnology Congress (Beijing, China), The 69th RISH Symposium Tropical Tree Biotechnology Initiative (Cibinong, Indonesia, keynote)

International Joint Researches, overseas research surveys

Umezawa, T.: International collaboration of phenylpropanoid biosynthesis (North Carolina

StateUniversity)

Umezawa, T.: Field study of *Acacia mangium* breeding (Perusahaan Kosinar, Malaysia)

Umezawa, T.: Field study of *Acacia mangium* breeding (PT Musi Hutan Persada, Indonesia)

Umezawa, T.: International collaboration of *Acacia mangium* biotechnology (Indonesian Institute of Sciences, Indonesia)

Umezawa, T.: International collaboration of antitumor lignan biosynthesis (Duesserdorf University, Germany)

Umezawa, T.: Sustainable Production of Tropical Forest Reseruces for Establishment of Recyhcling-based Society (Indonesian Institute of Sciences, Indonesia)

Scholars from abroad

Bambang Subiyanto: Collaborative research work on "Sustainable Production of Tropical Forest Reseruces for Establishment of Recyhcling-based Society" September 16-September 22 (2006)

Endang Sukara: Collaborative research work on "Sustainable Production of Tropical Forest Reseruces for Establishment of Recyhcling-based Society" September 16-September 22 (2006)

Witjaksono: Collaborative research work on "Sustainable Production of Tropical Forest Reseruces for Establishment of Recyhcling-based Society" September 16-September 22 (2006)

Ermawar Riksfardini Annisa: Collaborative research work on "Sustainable Production of Tropical Forest Reseruces for Establishment of Recyhcling-based Society" November 21(2006)-January 24 (2007)

B. Educational Activities (2006.4-2007.3)

B-1. On-campus teaching

Undergraduate level: Introduction to mushroom 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)

B-2. Off-campus teaching

Part-time Lecturer

Graduate level: Special Lecture in Wood Science and Technology (Kyoto Prefectural University, Advanced Course) (Umezawa), Special Lecture in Tree Biochemistry (The University of Tokyo) (Umezawa)

Open seminar, etc.

Umezawa, T.: the 45th RISH Symposium the Current Status and Future Prospects of Chemical Characterization of Forest Biomass (Uji, June 30, 2006) (keynote, the chair of organizing committee), the 55th RISH Symposium the Future Prospects of Bioscience for Sustainable Production and Utilization of Forest Biomass (October 20, 2006, Nagoya, invited lecture), The 4th ISS Seminar (Uji, Nobember 20, 2006, Uji, invited lecture), The ISS Symposium Towards Reforestation (Uji, January 26, 2007, invited lecture), The 65th RISH Symposium Current Status and Future Prospects of RISH Exploratory and Mission Research Projects (March 15, 2007, Uji, Invited lecture), The 66th RISH Symposium

Current Status and Future Prospects of RISH Collaborative Research Programs (March 16, 2007, Uji, Invited lecture), The 70th RISH Symposium Roadmap for Sustainable Production and Utilization of Tropical Tree Biomass Resources (March 20, 2007, Uji, the Chair of organizing committee, keynote)

Hattori, T.: The 70th RISH Symposium Roadmap for Sustainable Production and Utilization of Tropical Tree Biomass Resources (March 20, 2007, Uji, 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.

Students and research fellows
Post-Doctral fellow : (4)
Doctor's program : (4)
Master's program : (7)

A. Research Activities (2006.4-2007.3)

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, functional oligosaccarides, feedstuff, physiologically active compounds and others by using microorganisms and their enzymes. The research subjects include pretreatments of wood by selective white rot fungi, 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 lignin-degrading enzymes from white rot fungi

Extracellular enzymes, such as peroxidases and laccase, 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.

c) Development of efficient biocatalysts for wood biomass conversion

Isolation of biocatalysts for efficient conversion of wood biomass is aimed by modifying microorganisms including lignin-degrading basidiomycetes, yeasts, and bacteria 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 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 biomass.

A-2. Publifications and presentations

a) Publifications

Books

Watanabe, T., Y. Ohashi, T. Tanabe, T. Watanabe, Y. Honda and K. Messner: Lignin biodegradation by selective white rot fungus and its potential use in wood biomass conversion. In ACS Symposium Series 954, Materials, Chemicals and Energy from Forest Biomass, American Chemical Society. pp 409-421, 2007

Watanabe, T., M. Samsuri, R. Amirta, N. Rahmawati, Syafwina, B. Prasetya, T. Tanabe, Y. Ohashi, T. Watanbae, Y. Honda, M. Kuwahara and K. Okano: Lignin-degrading fungi as biotechnological tool for biomass conversion, In Sustainable Development and Utilization of Tropical Forest Resources, RISH, Kyoto Univ., 167-173, 2006

Watanabe, T.: Biorecycle of waste rubber by white rot fungi and enzymatic radical reactions, Ecomaterial Handbook, Atsushi Suzuki et al. eds., Maruzen, Tokyo, 292-293, 2006

Suenaga, H., T. Watanabe, H. Fujihara, A. Nishi and K. Furukawa: Microbial degradation of polychlorinated biphenyls (PCB): genetic and biochemical bases, Kankyobaiodenanigadekirunoka (edited by Japan society for environmental biotechnology), Shoukadohshoten, Nakanishi Printing Co. Ltd., Kyoto, Japan, pp.19-30, 2006 (in Japanese)

Reviews

Watanabe, T.: Trends in biorefinery and pretreatments of lignocellulosics by white rot fungi, Mokuzai Gakkaishi 53; 1-13, 2007

Original papers

Ohashi, Y., Y. Kan, T. Watanabe, Y. Honda and T. Watanabe: Redox silencing of the Fenton reaction system by an alkylitaconic acid, ceriporic acid B produced by a selective lignin-degrading fungus, *Ceriporiopsis subvermispora*. Org Biomol Chem 5; 840-847, 2007

Tsukihara, T., Y. Honda, T. Watanabe and T. Watanabe: Molecular breeding of white rot fungus, *Pleurotus ostreatus*, by homologous expression of its versatile peroxidase MnP2. Appl Microbiol Biotechnol 71; 114-120, 2006

Tsukihara, T., Y. Honda, R. Sakai, T. Watanabe and T. Watanabe: Exclusive overproduction of recombinant versatile peroxidase MnP2 by genetically modified white rot fungus, *Pleurotus ostreatus*. J Biotechnol 126; 431-439, 2006

Okano, K., Y. Iida, M. Samsuri, B. Prasetya, T. Usagawa and T. Watanabe: Comparison of in vitro digestibility and chemical composition among sugarcane bagasses treated by four white-rot fungi, Animal Sci J 77; 308-313, 2006

b) Conference and seminar papers presented

Annual meeting of Japan Society of Bioscience, Biochemistry and Agrochemistry 2007: 3 presentaions

The 13th Annual meeting of The Japan Institute of Energy: 2 presentaions

Annual meeting of the Society for Bioscience and Bioengineering: 2 presentaions

The 56th Annual Meeting of Japan Wood Research Society: 5 presentaions

The 51th Lignin Symposium: 2 presentaions

The 10th Annual meeting of Japanese Society of Mushroom Science and Biotechnology: 1 presentaion

The 50th anniversary meeting of The Mycological Society of Japan: 2 presentaions

A-3. Off-campus activities

Membership in academic societies

Watanabe, T.: Japan Society of Bioscience, Biochemistry and Agrochemistry (council of Kansai branch), Japan Tappi (Committee member of Wood Sci.), The Society for Bioscience and Bioengineering (Member of Biorefinery Res. Div.), Japanese Society of Mushroom Science and Biotechnology (Council member)

Honda, Y.: Japan Wood Research Society (Secretary of the Institute., Editorial Boad member), Japanese Society of Mushroom Science and Biotechnology (Council member), The Mycological Society of Japan (Secretary)

Research grants

Grant-in-Aid for Exploratory Research, Development of wood preservatives by iron chelators which suppress active oxytgen species, hydroxyl radical (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, T.: KSI International Symposium on global sustainability（Invited lecture, Panelist）、The 3rd International Symposium on Emerging Technologies of Pulping and Papermaking (ISETPP), Guangzhou, (2 presentations), Renewable energy 2006 International Conference and Exhibition Joint Conference with The International Solar Energy Society ISES, Chiba, (2 presentations), The International Symposium on Mushroom Science, Akita (presentation)

Honda, Y.: The 3rd International Symposium on Emerging Technologies of Pulping and Papermaking (ISETPP), Guangzhou, (2 presentations), Renewable energy 2006 International Conference and Exhibition Joint Conference with The International Solar Energy Society ISES, Chiba, (2 presentations), The International Symposium on Mushroom Science, Akita (presentation)

Watanabe, T.: The 3rd International Symposium on Emerging Technologies of Pulping and Papermaking (ISETPP), Guangzhou, (2 presentations), Renewable energy 2006

International Conference and Exhibition Joint Conference with The International Solar Energy Society ISES, Chiba, (2 presentations), The International Symposium on Mushroom Science, Akita (presentation)

International joint researches, overseas research surveys

Watanabe, T.: Cooperative research between NRCT and Yamaguchi University under the Core University System of Japanese Society of Promotion of Science

Honda, Y.: Cooperative research between NRCT and Yamaguchi University under the Core University System of Japanese Society of Promotion of Science, Surveys of "biomass conversion using genetically modified basidiomycetes"

B. Educational Activities (2006.4-2007.3)

B-1. On-campus teaching

a) courses given

Undergraduate level: Science of Humanosphere –Conversion of Solar Energy- (Takahsi Watanabe, Honda), Mushroom Biology Seminar (Honda, Takahito Watanabe)

Graduate level: Chemistry of Wood Biomass Conversion (Advanced Course) (Takashi Watanabe, Honda), 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).

B-2. Off-campus teaching etc.

Part-time lecturer

Watanabe, T.i: Akita Prefectural Univ., Murata Manufacturing Co., Ltd. (Technical Adviser)

Open seminar, etc

Watanabe, T.: The 6th Conference of Fungal Molecular Biological Society of Japan, Symposium of The Society for Biotechnology, Japan "Biotechnology for Sustainable Society", Symposium on Chemical Processing of Wood, Humanosphere Symposium "Bioscience for Sustainable Utilization of Forest Resources", Special Seminar of Akita Prefectural Univ., The 1st Seminar of Institute of Sustainability Science, Symposium on Emachu Project, The Japan Wood Research Society, The 20th Memorial Annual Meeting of Research Society of Cellulase

Honda, Y.: The 3rd Energy Recycling Symposium - Biomass conversion and solar power statellite, The 66th Humanosphere Symposium –Domestic and International Inter-University Collaborative Programs–

Watanabe, T.: The 70th Humanosphere Symposium

Students and research fellows from abroad

Doctor course: 0

Cooperative research fellows: 3

B-3. Overseas teaching

Students and research fellows from abroad

JSPS PD fellow: Rudianto Amirta (Indonesia)