2.3 DIVISION OF APPLIED LIFE SCIENCES

Division of Applied Life Sciences was ofiginally 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 the subjects that involve microorganisms, animals, and plants, and offers educational and research programs in the following specialized fields; physical chemistry, organic chemistry, biochemistry, molecular biology. Original research projects are encouraged in both basic and applied aspects.

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

A. Research activities (2005.4-2006.3)

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 β-cell ATP-sensitive potassium (K_{ATP}) channels play an important role in the regulation of glucose-induced insulin secretion. The β-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 and Reviews

Ueda, K. et al.: ABC proteins(edited by Ueda et al.), Gakkai Shuppann Center, Tokyo, 2005

Matsuo, M., Y. Kimura and K. Ueda: KATP channel interaction with adenine nucleotides. J Mol Cell Cardiol 38; 907-916, 2005

Takahashi, K., Y. Kimura, K. Nagata, A. Yamamoto, M. Matsuo and K. Ueda: ABC proteins, key molecules for lipid homeostasis. Medical Molecular Morphology 38; 2-12, 2005

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

Original papers

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

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

Toda, Y., R. Aoki, Y. Ikeda, Y. Azuma, N. Kioka, M. Matsuo, M. Sakamoto, S. Mori, M. Fukumoto and K. Ueda: Detection of ABCA7-positive cells in salivary glands from patients with Sjogren's syndrome. Pathol. Int. 55; 639-643, 2005

Takahashi, H., M. Mitsushima, N. Okada, T. Ito, S. Aizawa, R. Akahane, T. Umemoto, K. Ueda and N. Kioka: Role of interaction with vinculin in recruitment of vinexins to focal adhesion. Biochem. Biophys. Res. Commun. 336; 239-246, 2005

Ban, N., M. Sasaki, H. Sakai, K. Ueda and N. Inagaki: Cloning of ABCA17, a novel, rodent sperm-specific ATP-binding cassette (ABC) transporter that regulates intracellular lipid metabolism. Biochem. J. 389; 577-585, 2005

Hayashi, M., S. Abe-Dohmae, M. Okazaki, K. Ueda and S. Yokoyama: Heterogeneity of High Density Lipoprotein Generated by ABCA1 and ABCA7. J. Lipid Res. 46; 1703-1711, 2005

Arakawa, R., N. Tamehiro, T. Nishimaki-Mogami, K. Ueda and S. Yokoyama: Fenofibric acid, an active form of fenofibrate, increases apolipoprotein A-I-mediated high-density lipoprotein biogenesis by enhancing transcription of ATP-binding cassette transporter A1 gene in a liver X receptor-dependent manner. Arterioscler Thromb Vasc Biol. 25; 1193-1197, 2005

Chen, H., D. M. Cohen, D. M., Choudhury, N. Kioka and S. W. Craig: Spatial distribution and functional significance of activated vinculin in living cells. J. Cell Biol. 169; 459-470, 2005

Paternotte, N., J. Zhang, I. Vandenbroere, K. Backers, D. Blero, N. Kioka, J. M. Vanderwinden, I. Pirson and C. Erneux: SHIP2 interaction with the cytoskeletal protein Vinexin. FEBS J. 272; 6052-6066, 2005

b) Conference and seminar paper presented

The 9th Annual Meeting of Japanese Association for Molecular Target Therapy of Cancer, 1 paper

The 58th Annual Meeting of JapaneseJapan Society for Cell Biology , 2 papers

The 78th Annual Meeting of Japanese Biochemistry Society, workshop, 4 papers

The 64th Annual Meeting of Japanese Cancer Association, symposium, 3 paper

The 28th Annual Meeting of Japan Society for Molecular Biology, 3 papers

The 2006 Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry, 11 papers

The 59th Annual Meeting of Japan Society of Nutrition and Food Science, symposium, 1 paper

The 82th Annual Meeting of Japan physiological Society, 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.) Encouragement of Young Scientists (B): Functional analysis of ABC proteins involved in cholesterol efflux (Matsuo, M.)

A-4. International cooperations and overseas activities

International meetings (roles)

Ueda, K.: 1st FEBS Special Meeting on ABC Proteins (Vice Organazer, presentation)
Gordon Reseach Conference, Molecular and Cellular Biology (presentation)
International Symposium on Biological Membrane Transport 2005 (invited lecture)

Kioka, N.: The 44th Symposium of Korean Society of Life Science (invited lecture)

Matsuo, M.: 1st FEBS Special Meeting on ABC Proteins (Invited lecture)
Gordon Reseach Conference, Molecular and Cellular Biology of Lipids (presentation)
International Symposium on Life of Proteins (presentation)

Membership in international academic societies

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

B. Educational Activities (2005.4-2006.3)

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 : Mima, Joji, Dr. Agric. Sci.
Students and research fellows
Doctor's program :(5)
Master's program :(9)
Undergraduate :(5)
Research fellow :(5)

A. Research Activities (2005.4-2006.3)

A-1. Main subjects

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

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

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

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

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

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

to create the concept of nano-biotechnology. Through these, we look forward to creating new bioactive proteins and cells which transcend the limitations of known genomic information.

A-2. Publications and presentations

a) Publications

Original papers

Shintani, Y., K. Hirako, M. Motokawa, T. Iwano, X. Zou, Y. Takano, M. Furuno, H. Minakuchi and M. Ueda: Development of miniaturized multichannel HPLC for high-throughput analysis. J Chromatog A 1073; 17-23, 2005

Mima, J., M. Hayashida, T. Fujii, Y. Narita, R. Hayashi, M. Ueda and Y. Hata: Structure of the carboxypeptidase Y inhibitor IC in complex with the cognate proteinase reveals a novel mode of the proteinase-protein inhibitor interaction. J Mol Biol 346(5); 1323-1334, 2005

Fukuda, T., S. Shiraga, M. Kato, Y. Morita, E. Tamiya, T. Hori, S. Suye and M. Ueda: Construction of novel single cell screening system using a yeast cell chip for nano-sized modified-protein-displaying libraries. Nano Biotechnology 1; 105-111, 2005

Shiraga, S., M. Kawakami, M. Ishiguro and M. Ueda: Enhanced reactivity of Rhizopus oryzae lipase displayed on yeast cell surface in organic solvents. Potential as a whole cell biocatalyst in organic solvents. Appl Environ Microbiol 71(8); 4335-4338, 2005

Shiraga, S., M. Ishiguro, H. Fukami, M. Nakno and M. Ueda: Creation of Rhizopus oryzae lipase having a unique oxyanion hole by combinatorial mutagenesis in the lid domain. Appl Microbiol Biotechnol 68; 779-785, 2005

Kato, M., H. Maeda, M. Kawakami, S. Shiraga and M. Ueda: Construction of a selective cleavage system for a protein displayed on the cell surface of yeast. Appl Microbiol Biotechnol 69(4); 423-427 2005

Nakamura, I., H. Yoneda, T. maeda, A. Makino, M. Ohmae, J. Sugiyama, M. Ueda, S. Kobayashi and S. Kimura; Enzymatic polymerization behavior using cellulose-binding domain deficient endoglucanase II. Macromol Biosci 5(7); 623-628, 2005

Khaw, T. S., Y. Katakura, J. Koh, A. Kondo, M. Ueda and S. Shioya: Evaluation of performance of different surface-engineered yeast strains for direct ethanol production from raw starch. Appl Microbiol Biotechnol 70(5); 573-579, 2005

b) Conference and seminar papers presented

Annual Meeting of the Society for Biotechnology, Japan 2005: 4 reports

The 78th Annual Meeting of the Japanese Biochemical Society: 2 reports

The 28th Annual Meeting of MBSJ: 1 reports

Annual Meeting of Japan Society for Bioscience, Biotechnology and Agrochemistry 2005: 6 reports

Enzyme Engineering XVIII: 2 reports

Pacifichem: 2 reports

The 2nd International Meeting on Microsensors and Microsystems: 1 report

A-4. International cooperations and overseas activities

International meetings (roles)

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

B. Educational Activities (2005.4-2006.3)

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), Experiments of Biomacromolecular Chemistry (Ueda, Kato and Mima)

Graduate level: Biomacromolecular Chemistry (Ueda), Experiments of Biomacromolecular Chemistry (Ueda, Kato and Mima)

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: (2)*
Master's program: (5) *Undergraduate : (4)*
Research student: (2)

A. Research Activities (2005.4-2006.3)

A-1. Main subjects

a) Chemistry of Secondary Metabolites in Plants

The transformation of rice with a transgene (*OASA1D*) that encodes anthranilate synthase alpha-subunit with decreased feedback sensitivity to tryptophan (Trp) results in a dramatic increase in the Trp content. Further transformation to overexpress the tryptophan decarboxylase gene of rice origin caused a decrease in the Trp level, but a marked increase in the level of tryptamine in rice calli, as expected. However, the increase was observed for many components in addition to tryptamine to produce rather complicated metabolic profile. One of the major increased components was identified as serotonin. The result indicates that TDC plays an important role in regulating the tryptophan-related secondary metabolism in rice.

b) Structure-Activity Relationships of Ecdysone Agonists

Scorpions are the terrestrial arthropods and belong to Arachinida of Chelicerata, a different taxon from that of common insects, Insecta of Mandibulata. Taxonomically, scorpions are closer to spider and mites than insects and crustaceans. To date, more than 1600 species have been found in all over the world. Scorpions grow via molting like insects. Whereas the molecular mechanisms concerning molting have been intensively studied in insects, those in other arthropods are not well understood. In this study, we succeeded in the cloning of the genes encoding ecdysone receptor (EcR) and ultraspiracle (USP), the heterodimeric receptor of molting hormone in insects, of Japanese scorpions *Liocheles australasiae*. Amino acid sequences of their ORF (open reading frame) were deduced to be 539 and 410, respectively. Both amino acid sequences had a structure typical for the nuclear receptor superfamily, and were homologous to those of a tick *Amblyomma*

americanum. The reporter gene assays for ecdysone agonists were also constructed against Sf-9 and CIRL-Lepd-SL1.

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. Peptides derived from bacterial flagellin are known to induce defense responses. In this study we synthesized a number of linear and cyclic flagellin-derived peptides which possess constrained conformation and measured their elicitor activity to investigate the structural features important for the activity. As a result, it is shown that a turn structure at the center part of the flagellin peptides is important for the activity. ii) The peptide isolated from the venom of the Japanese scorpion *Liocheles australasiae* was synthesized by Fmoc-solid phase synthesis to investigate the pattern of disulfide bridges. The peptide was digested by a protease and the molecular weight of the digested fragments was measured by LC/MS. Combinations among 4 Cys residues constructing two disulfide bridges were determined by MS.

d) Endocrine Disruptor

The phase-I metabolites of a chlorinated insecticide, methoxychlor, are known to show estrogen receptor binding activity. However, the phase-II metabolites such as glucuronate and sulfate conjugates were less active than the phase-I metabolites. In this study, the estrogen receptor binding activity of glucuronate and sulfate conjugates of the mono- and bis-OH methoxychlor was measured and their sturucture-activity relationship was carefully examined. All conjugates exhibited more than 20-fold lower activity than bis-OH methoxychlor.

e) Fungicidal mode of action of TF-991

Effects of a fungicidal agent TF-991 on the metabolic profile of a plant pathogenic fungus *Alternaria mali* was investigated. Analysis using GC-MS revealed an accumulation of fatty acids such as palmitate and stearate in the fungal mycelia after the treatment with TF-991. The levels of glycerides were also increased. These results suggested that TF-991 likely affected the lipid metabolism of the fungus.

A-2. Publications and presentations

a) Publications

Review papers

Nakagawa, Y.: Nonsteroidal ecdysone agonists. Vitam Horm 73; 131-173, 2005

Original papers

Matsuda, F., T. Yamada, H. Miyazawa, H. Miyagawa and K. Wakasa: Characterization of tryptophan-overproducing potato transgenic for a mutant rice anthranilate synthase alpha-subunit gene (OASA1D). Planta 222; 535-545, 2005

Matsuda, F., H. Miyazawa, K. Wakasa and H. Miyagawa: Quantification of indole-3-acetic acid and amino acid coniugates in rice by liquid chromatography-electrospray ionization-tandem mass spectrometry. Biosci Biotech Biochem 69; 778-783, 2005

Shimizu, B., H. Miyagawa, T. Ueno, K. Sakata, K. Watanabe and K. Ogawa: Morning glory systemically accumulates scopoletin and scopolin after interaction with *Fusarium oxysporum*. Z. Naturforsch C 60; 83-90, 2005

Miyashita, M., T. Shimada, S. Nakagami, N. Kurihara, H. Miyagawa and M Akamatsu: Evaluation of Estrogen Receptor Binding Affinity of DDT-Related Compounds and Their

Metabolites. In Environmental Fate and Safety Management of Agrochemicals (J. Marshall Clark, Hideo Ohkawa, Eds.), ACS Symp Ser, 899; American Chemical Society, Washington, D.C., pp. 159-166, 2005

Ogura, T., C. Minakuchi, Y. Nakagawa, G. Smagghe and H. Miyagawa: Molecular cloning, expression analysis and functional confirmation of ecdysone receptor and ultraspiracle from the Colorado potato beetle *Leptinotarsa decemlineata*. FEBS J 272; 4114-4128, 2005

Wheelock, C. E., Y. Nakagawa, T. Harada, N. Oikawa, M. Akamatsu, G. Smagghe, D. Stefanou, K. Itatrou 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

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

Proceedings and Reports

Miyashita, M., J. Otsuki, Y. Hanai, Y. Nakagawa and H. Miyagawa: Analysis of Peptide Components in the Venom of the Japanese Scorpion Liocheles Australasiae. In "Peptide Science 2005" (T. Wakamiya, Ed.); The Japanese Peptide Society, 185-186, 2006

b) Conferences and seminar papers presented

The 31th Annual Meeting of Pesticide Science Society of Japan: 4 reports

Annual Meeting of the Japan Society for Bioscience, Biotechnology, and Agrochemistry 2006: 7 reports

Japan Society for Bioscience, Biotechnology, and Agrochemistry (Kansai Branch Meeting): 2 reports

The 2nd COE International Meeting: 5 reports

The 21st Meeting of Pesticide Design: 1 report

The 42th Peptide Meeting: 1 report

The 47th Annual Meeting of Plant Pathology: 1 report

The 33rd Symposium of Structure-Activity Relationship: 1 report

PACIFICHEM 2005: 1 report

The 40th Meeting of Regulation of Plant Growth and Development: 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)

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).

International joint researches, overseas research surveys

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

B. Educational Activities (2005.4-2006.3)

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)

Graduate level: Bioregulation Chemistry Seminar (Miyagawa, Nakagawa, Miyashita), Experimental Course in Bioregulation Chemistry (Miyagawa, Nakagawa, Miyashita).

B-2. Off-campus teaching, etc.

H. Miyagawa: Graduate School of Life and Environmental Science, Shimane University (Special lecture on signal molecule technology)

Y. Nakagawa: Faculty of Agriculture, Kyoto Prefectural University (Industrial Organic Chemistry)

C. Other Remarks

H. Miyagawa: Chief Manager of Radio Isotope Experiments at College of Agriculture; Member of the Advisory Committee of Radio Isotope Center, Kyoto University.

Y, Nakagawa: 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.
Students and research fellows
Doctor's program : (2) *Master's program : (8)*
Undergraduate : (4)

A. Research Activities (2005.4-2006.3)

A-1. Main subjects

a) Sequestration of toxic substances from host plants in inseects

A number of insects sequester toxic substances from plants for defense. *Parastrachia japonensis* is a toxic stink bug with red warning coloration. The bugs were found to sequester toxic lipids selectively from drupe of its host, *Schoepfia jasminodora*. The stone seeds contained a large quantity of acetylenic triglycerides such as 9,11-octadecadiynoic and 9,11,13-octadecatriynoic acid found together with oleic and other normal fatty acids. Synthetic esters of the fatty acids exhibited lethal effects when orally administrated to mice, which cleally indicated the lipids to play a role as defensive substances in the bug. The fate of individual fatty acids in the body tissues suggests that these acetylenic fatty acids are utilized both as an energy source and defensive substances in the bug. The adaptive mechanisms of sequestration were examined in several toxic insects in association with plant secondary metabolites.

b) Fatty acid selectivity in biosynthesis of insect-derived elicitors

Volicitin [*N*-(17-hydroxylinolenoyl)-L-glutamine] and *N*-linolenoyl-L-glutamine, originally identified from the regurgitant of *Spodoptera exigu*a, induce damaged corn leaves to release volatiles which enable parasitic wasps to locate host caterpillars. In addition to the fatty acid amides, the linoleic acid analogs of these amides are also identified from the larval secretions. However, intensive chemical analyses of the composition of fatty acids in the oral secretions clearly showed that almost the same amount of palmitic acid was detected as those of linolenic and linoleic acids. When gut tissues of *S. litura* were incubated with unsaturated or saturated fatty acids sadium salt (linolenic, linoleic, oleic, palmitic and stearic acids) and L-[α-^{15}N] glutamine to investigate the specific incorporation of fatty acids, unsaturated fatty acids were incorporated into the amides more than three times as much as those of saturated fatty acids. These results suggested that volicitin related compounds are acitively synthesized by caterpillar tissues and might play important roles in unsaturated faty acids metabolism.

c) ß-Glycosylation of DIMBOA in *Mythimna separata* larvae

It is well-known that maize plants protect themselves against herbivory with a defense system with cyclic hydroxamic acids, especially DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one). This compound is stored as a glucoside in the plants. When the plant tissues are injured, the aglycone is released through the action of hydrolytic enzyme ß-glucosidase. DIMBOA shows many different biological activities, such as the reduced survival and reproductive rate of aphids, the feeding deterrent effect against European corn borer. However, we present evidence that the rice armyworm *Mythimna separata* overcome the toxicity of the maize plants by glucosylation of DIMBOA. When the fifth instar were fed on artificial diets

containing DIMBOA, three kinds of glucosides (DIMBOA-Glc, HMBOA-Glc, MBOA-Glc) were identified from frass extracts. Furthermore, the glucosides were obtained from the incubation of the midgut extracts of *M. separata* with UDP-glucose and DIMBOA. As far as we know, this is a first report on ß-glycosylation of DIMBOA by an insect herbivore.

A-2. Publications and presentations

a) Publications

Original papers

Tan, K. H. and R. Nishida: Synomone or kairomone? - *Bulbophyllum apertum* flower releases raspberry ketone to attract *Bactrocera* fruit flies. J. Chem. Ecol. 31; 509-519, 2005

Takada, W., T. Sakata, S. Shimano, Y. Enami, N. Mori, R. Nishida andY. Kuwahara: Scheloribatid mites as the source of pumiliotoxins in dendrobatid frogs. J. Chem. Ecol. 31; 2405-2417, 2005

Noge, K., M. Kato, T. Iguchi, N. Mori, R. Nishida and Y. Kuwahara: Biosynthesis of neral in *Carpoglyphus lactis* (Acari: Carpoglyphidae) and detection of its key enzyme, geraniol dehydrogenase, by electrophoresis. J. Acarol. Soc. Jpn. 14; 75-81, 2005

Yoshinaga, N., K. Kato, C. Kageyama, K. Fujisaki, R. Nishida and N. Mori: Ultraweak phton emission from herbivory-injured maize plants. Naturwissenschaften 93: 38-41, 2006

Ohta, N., N. 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 hirsute*. Phytochemistry 67: 584-588, 2006

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

Sawada, Y., N. Yoshinaga, K. Fujisaki, R. Nishida, Y. Kuwahara and N. Mori: Absolute configuration of volicitin from regurgitant of lepidopteran caterpillars and biological activity of volicitin-related compounds. Biosci. Biotech. Biochim. (accepted)

b) Conference and seminar papers presented

The 49th Annual Meeting of Japanese Society of Applied Entomology and Zoology: 2 papers

Annual Meeting of Japan Society of Bioscience, Biotechnology and Agrochemistry (2005): 6 papers

The 14th Annual Meeting of Acarological Society of Japan: 1 paper

The 21th Annual Meeting of the International Society of Chemical Ecology: 1 paper

The 5th Asia-pacifc Congress of Entomology: 7 papers

The 2nd International Symposium of Entomological Science COE: 8 papers

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). Research Grant, Integrated Research (C) (2), Biologically active compounds derived from arthropods (Mori: 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, Papua New Guinea, USA)

Mori, N. Biosynthesis of insect-derived elicitors (USA), DIMBOA biosynthesis induced by insect-derived elicitors (Germany)

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

B-2. Off-campus teaching

Nishida: Kobe University

Chair of Molecular and Cellular Sciences

2.3.5 Laboratory of Plant Nutrition

Staff *Professor* : *Sekiya, Jiro, Dr. Agric. Sci.*
Associate Professor : *Matoh, Toru, Dr. Agrjc. Sci.*
Assistant Professor : *Kobayashi, Masaru, Dr. Agric. Sci.*
Assistant Professor : *Koizumi, Yukio*
Research Associate : *Ochiai, Kumiko*

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

A. Research Activities (2005.4-2006.3)

A-1. Main subjects

a) Biosynthesis, catabolism and function of glutathione and its alalogs in higher plants.

Glutathione (GSH) is an important thiol compound in higher plants. In hiher plants, homoglutathione (hGSH) is involed in leguminous plants such as soybean and hydroxymethylglutathione (hmGSH) in Poaceae in addition to GSH. In this project, we focus our effort to elucidate biosynthesis, catabolism and function of GSH and related compounds.

Regarding with hGSH, we succeded to generate tobacco plants transformed with soybean hGSH synthetase. We analyzed features on hGSH synthesis and GSH *S*-conjugate formation of transformed tobacco plants. From rice leaves, we purified hmGSH synthetase which was a serine carboxypeptidase like enzyme. We are trying to produce recombinant hmGSH synthetase and to analyze properties of this enzyme.

In radish plants, GSH catabolism to yield cysteine is catalyzed by a sequential reaction of γ-glutamyltransferase (GGT) and dipeptidase (DPase). We have already purified 4 types of GGT from radish cotyledons and cyaracterized their major properties. We further demonstrated that most of the activity of soluble GGTs were localized to vacuoles and may play an important role in GSH *S*-conjugate degradation.

b) 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.

c) 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.

d) 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

Books and reviews

Matoh T: Agroecology. Cropping Systems(edited by S. Inamura,), pp66-78, Asakura Publishing, 2005

Original papers

Matoh, T. and K. Ochiai: Distribution and partitioning of newly taken-up boron in sunflower. Plant Soil. 278; 351-360, 2005

Kawachi, T., S. Aoyama, M. Yangyuoru, K. Unami, T. Matoh, D. Acquah and S. Quarshie: An irrigation tank for harvesting rainwater in semi-arid savannah areas –design and construction practices in Ghana/West Africa-. J. Rainwater Catchment Syst. 11; 17-24, 2005

b) Conference and seminar papers presented

Annual Meeting of the Japanese Society of Plant Physiologists, 2005: 2 reports

Annual Meeting of Japanese Society of Soil Science and Plant Nutrition, 2005: 4 reports

A-3. Off-campus activities

Membership in academic societies (roles)

Sekiya, Jiro: Japanese Society of Soil Science and Plant Nutrition (Board member), Japanese Association for Cellular and Molecular Biology (Board member, Award Committee), Japan Oil Chemists' Society (Journal of Oleo Science Executive Editor, Award Committee), Japanese Society of Plant Physiologists (Board member).

Matoh, Toru: Japanese Society of Soil Science and Plant Nutrition (Board member, Vice-chairperson of the 4th Committee, Editor)

Research grants

Matoh, Toru: General Scientific Research (B) (2) Environmental evaluation of the export-oriented farming in the tropical countries. General Scientific Research (A) (1) Studies of sustainable development in the mountain area of Laos (member). General Scientific Research (A) (1) Sustainable upland cultivation in Mainland Indochina (member). General Scientific Research (A) (1) Small-schale irrigation project in Ghana (member).

A-4. International cooperations and overseas activities

International meetings (roles)

Matoh, Toru: Organizing Committee for International Boron Symposium 2005

International joint researches, overseas research surveys

Matoh, Toru: 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 (2005.4-2006.3)

B-1. On-campus teaching

a) Course given

Undergraduate level: Introduction to Applied Life Science, Part II (Sekiya), Biochemistry II (Sekiya), Plant Biochemistry (Sekiya), Plant Nutrition (Matoh), Laboratory Course in Plant Biochemistry (Matoh, Kobayashi, Koizumi), Stress Physiology in Plants (Matoh)

Graduate level: Advanced Cource in Plant Biochemistry (Sekiya, Matoh), Experimental Course in Plant Nutrition (Sekiya, Matoh, Kobayashi, Koizumi), Plant Nutrition Seminar (Sekiya, Matoh, Kobayashi, Koizumi)

B-2. Off-campus teaching. etc.

Part-time lecturer

Sekiya, Jiro: Faculty of Environmental Science, Shiga Prefectural University (Plant Biochemistry)

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

C. Other Remarks

Sekiya, Jiro: The Japan Food Chemical Research Foundation (Board of Director), FFI Journal (Executive Editor), Society for Promotion of Food and Agri-Biotechnology in Kinki Area (Board member)

Matoh, Toru: Advisory member for Commitee for Promoting Ssutainable 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 (2005.4-2006.3)

A-1. Main subjects

a) X-ray structures of stereoselective carbonyl reductase

An NADPH-dependent carbonyl reductase isolated from *Sporobolomyces salmonicolor* produces (S)-alcohol from ethyl 4-chloro-3-oxobutanoate. The X-ray structures of the enzyme and its complex with a coenzyme, NADPH, have been determined. The enzyme has two domains, a

NADPH-binding domain and a substrate-binding domain, and belongs to the short-chain dehydrogenase/reductase (SDR) family. The structure of the NADPH-binding domain and the interaction between the enzymes and NADPH are very similar to those found in other structure-solved enzymes belonging to the SDR family, while the structure of the substrate-binding domain is unique. The X-ray structure of enzyme/NADPH complex and preliminary modeling show that the formation of the hydrophobic channel induced by the binding of NADPH is closely related to the stereoselective reduction.

b) Enzymatic properties and genetic organization of RrhJ1I restriction-modification system

RrhJ1I, a new NaeI isoschizomer, has been isolated from *Rhodococcus rhodochrous* J1 which is widely used for production of commodity chemicals such as acrylamide. RrhJ1I recognizes GCCGGC and cleaves between C and G to produce blunt end. A DNA fragment carrying the gene coding for RrhJ1I restriction-modification system was cloned, and the endonuclease and metyltransferase genes were in a head-to-head orientation. A third gene, which was assumed to encode HNH-endonuclease, was found in the intergenic region. Enzymatic activities of RrhJ1I endonuclease and methyltranferase were detected in recombinant *E. coli* cells carrying both genes.

c) Analysis of Gpx2, a homologue of glutathione peroxidase in *Saccharomyces cerevisiae*

We have previously reported that *S. cerevisiae* has three glutathione peroxidase homologues (*GPX1*, *GPX2*, and *GPX3*). Of these, *GPX2* gene product (Gpx2) shows the highest similarity to phospholipid hydroperoxide glutathione peroxidase. The redox status of Gpx2 was essentially in the reduced form even in mutants defective in glutathione reductase or glutaredoxin under oxidative stressed conditions. On the other hand, Gpx2 was partially oxidized in a mutant defective in cytosolic thioredoxin (*trx1*Δ*trx2*Δ) under non-stressed conditions, and completely oxidized in *tert*-butyl hydroperoxide (t-BHP)-treated cells of *trx1*Δ*trx2*Δ and thioreoxin reductase-deficient mutant cells. Alanine scanning of cysteine residues of Gpx2 revealed that intramolecular disulfide bond was formed between Cys^{37} and Cys^{83} *in vivo*. Gpx2 was purified to determine whether Gpx2 functions as a peroxidase that uses thioredoxin as an electron donor *in vitro*. As a result, Gpx2 reduced H_2O_2 and t-BHP in the presence of thioredoxin, thioredoxin reductase and NADPH; however, it showed remarkably lower activity toward these peroxides in the presence of glutathione, glutathione reductase and NADPH.

d) Cellular response to freeze-thaw stress in *Saccharomyces cerevisiae*.

Resistance to freeze-thaw stress of baker's yeast cells is clitical for the frowzen daugh technology in the bakery industry. In the process of freezing, yeast cells induce the adaptive responses to low-temperature through the activation of HOG-MAP kinase cascade and stress responsive transcription factors Msn2p/Msn4p. Additionally, it is well known that intracellular trehalose and glycerol function as cryoprotectant and confer the resistance to yeast cells. On the other hand, we have little information about the cellular events in the thawing process and its physiological roles in the resistance to freeze-thaw stress is still mysterious. We recently found that a certain transcription factor is activated and changes its cellular localization in the thawed cells, resulted in the inducuction of several genes and *HSP* genes. Such phenomena seem kind of stress responses in the thawed cells and we are investigating the importance of those responses in the acquirementof the resistance to freeze-thaw stress.

A-2. Publications and presentations

a) Publications

Book

Kita, K.: VIII.1&2 Industrial Microorganisms and Products, Applied Microbiology 2nd Ed. (edited by S. Shimizu and S. Horinouch), pp. 317-325, Bun-eido Shuppan, Tokyo, 2006 (in Japanese)

Original papers

Kamitori, S., A. Iguchi, A. Ohtaki, M. Yamada and K. Kita: X-ray structures of NADPH-dependent carbonyl reductase from *Sporobolomyces salmonicolor* provide insight into stereoselective reduction of carbonyl compounds. J Mol Biol 352(3); 551-558, 2005

Izawa, S., R. Takemura, Y. Miki and Y. Inoue: Characterization of the export of bulk poly $(A)^+$ mRNA in *Saccharomyces cerevisiae* during wine-making process. Appl Environ Microbiol 71(4); 2179-2182, 2005

Izawa, S., R. Takemura, K. Ikeda, Y. Fukuda, Y. Wakai and Y. Inoue: Characterization of Rat8-localization and mRNA export in *Saccharomyces cerevisiae* during Japanese sake brewing. Appl Microbiol Biotechnol 69(1); 86-91, 2005

Takatsume, Y., S. Izawa and Y. Inoue: Unique regulation of glyoxalase I activity during osmotic stress response in the fission yeast *Schizosaccharomyces pombe*: neither the mRNA nor protein level of glyoxalase I increases under conditions that enhance its activity. Arch Microbiol 183(3); 224-227, 2005

Maeta, K., K. Mori, Y. Takatsume, S. Izawa and Y. Inoue: Diagnosis of cell death induced by methylglyoxal, a metabolite derived from glycolysis, in *Saccharomyces cerevisiae*. FEMS Microbiol Lett. 243(1); 87-92, 2005

Takatsume, Y., K. Maeta, S. Izawa and Y. Inoue: Enrichment of yeast thioredoxin by green tea extract through activation of Yap1 transcription factor in *Saccharomyces cerevisiae.* J Agric Food Chem 53(2); 332-337, 2005

Maeta, K., S. Izawa and Y. Inoue: Methylglyoxal, a metabolite derived from glycolysis, functions as a signal initiator of the high osmolarity glycerol—mitogen-activated protein kinase cascade and calcineurin/Crz1-mediated pathway in *Saccharomyces cerevisiae*. J Biol Chem 280(1); 253-260, 2005

Zuin, A., A. P. Vivancos, M. Sansó, Y. Takatsume, J. Ayté, Y. Inoue and E. Hidalgo:. The glycolytic metabolite methylglyoxal actvates Pap1 and Sty1 stress responses in *Schizosaccharomyces pombe.* J Biol Chem 280(44); 36708-36713, 2005

Tanaka, T., S. Izawa and Y. Inoue: *GPX2*, encoding a phospholipid hydroperoxide glutathione peroxidase homologue, codes for an atypical 2-Cys peroxiredoxin in *Saccharomyces cerevisiae*. J Biol Chem 280(51); 42078-42087, 2005

Bi, W., Y. Inai, N. Shiraishi, K. Maeta, Y. Takatsume, Y. Inoue and M. Nishikimi: Cytosolic proteins as principal copper buffer in an early response to copper by yeast cells. J Clin Biochem Nutr. 36(1); 19-27, 2005

Review

Izawa, S., R. Takemura and Y. Inoue: Stress response in the process of mRNA export: unique response to ethanol of *Sake* yeast during sake brewing. Kagaku to Seibutsu 43(5); 278-280, 2005

Izawa, S.: Ethanol-stress response and tolerance in yeast. J Brew Soc Jpn 100 (5); 305-309, 2005

Patent

Kita, K., F. Yu and F. Watanabe: Enzymes and genes for RrhJ1I restriction-modification system. 2006.3.3 (patent-pending)

b) Conference and seminar papers presented

The 78th Japanese Biochemical Society Annual Meeting: 2 papers

The 27th Annual Meeting of the Molecular Biology Society of Japan: 4 papers

The 38th Meeting of Yeast Genetics and Molecular Biology, Japan: 3 papers

The Annual Meeting of the Society for Biotechnology 2005, Japan: 1 paper

Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry 2006: 6 paper

22th International Conference on Yeast Genetics and Molecular Biology: 3 papers

The 3rd Meeting of Internatinal Redox Network: 2 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)

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

International meetings (roles)

Kita, K.: The 13th German-Japan Workshop on Enzyme Technology, Germany (invited speaker)

B. Educational Activities (2005.4-2006.3)

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 Seminar (Kita, Inoue, Izawa), Experimental Course of Cellular Bioenergy Conversion (Kita, Inoue, Izawa)

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

Doctor's program : (6) *Postdoctral research fellow : (2)*
Master's program : (20) *Research fellow : (1)*
Undergraduate : (4) *Research student : (2)*

A. Research Activities (2005.4-2006.3)

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

Ogawa, J. and S. Shimizu: Screening for Unique Microbial Reactions Useful for Industrial Applications. Handbook of Industrial Biocatalysis (edited by Ching T. Hou), Taylor & Francis, Boca Raton, pp.2-1～2-21, 2005

Original papers

Abe, T., E. Sakuradani, T. Asano, H. Kanamaru, Y. Ioka and S. Shimizu: Identification of mutation sites on Δ6 desaturase genes from *Mortierella alpina 1S-4* mutants. Biosci. Biotechnol Biochem. 69 (5); 1021-1025, 2005

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. 17; 1-9, 2005

Abe, T., E. Sakuradani, T. Ueda and S. Shimizu: Identification of mutation sites on Δ5 desaturase genes from *Mortierella alpina* 1S-4 mutants. J. Biosci. Bioeng. 99 (3); 296-299, 2005

Honda, K., H. Tsuboi, T. Minetoki, H. Nose, K. Sakamoto, M. Kataoka and S. Shimizu: Expression of the *Fusarium oxysporum* lactonase gene in *Aspergillus oryzae* -molecular properties of the recombinant enzyme and its application-. Appl. Microbiol. Biotechnol. 66 (5); 520-526, 2005

Kumura, N., M. Izumi, S. Nakajima, S. Shimizu, H.-S. Kim, Y. Wataya and N. Baba: Synthesis and biological activity of fatty acid derivatives of quinine. Biosci. Biotechnol. Biochem. 69 (11); 2250-2253, 2005

Li, Q.S., J. Ogawa, R. .D. Schmid and S. Shimizu: Indole hydroxylation by bacterial cytochrome P450 BM-3 and modulation of activity by cumene hydroperoxide. Biosci Biotechnol Biochem. 69 (2); 293-300, 2005

Sakamoto, K., K. Honda, K. Wada, S. Kita, K. Tsuzaki, H. Nose, M. Kataoka and S. Shimizu: Practical resolution system for DL-pantoyl lactone using the lactonase from *Fusarium oxysporum*. J. Biotechnol. 118 (1); 99-106, 2005

Sakuradani, E., T. Abe, K. Iguchi and S. Shimizu: A novel fungal 3-desaturase with wide substrate specificity from arachidonic acid-producing *Mortierella alpina* 1S-4. Appl. Microbiol. Biotechnol. 66 (6); 648-54, 2005

Sakuradani, E., S. Takeno, T. Abe and S. Shimizu: Chapter 2. Arachidonic acid-producing *Mortierella alpina*: creaction of mutants ans molecular breeding. Single Cell Oils (edited by Z. Cohen, C. Ratledge), AOCS Press, Champaign, Illinois, pp.21-35, 2005

Sulistyaningdyah, W.T., J. Ogawa, Q.-S. Li, C. Maeda, Y. Yano, R.D. Schmid and S. Shimizu: Hydroxylation activity of P450 BM-3 mutant F87V towards aromatic compounds and its application to the synthesis of hydroquinone derivatives from phenolic compounds. Appl. Microbiol. Biotechnol. 67 (4); 556-562, 2005

Takeno, S., E. Sakuradani, A. Tomi, M. Inohara-Ochiai, H. Kawashima, T. Ashikari and S. Shimizu: Improvement of the fatty acid composition of an oil-producing filamentous fungus, *Mortierella alpina* 1S-4, through RNA Interference with 12-Desaturase Gene Expression. Appl Environ Microbiol. 71 (9); 5124-5128, 2005

Takeno, S., E. Sakuradani, S. Murata, M. Inohara-Ochiai, H. Kawashima, T. Ashikari, S. Shimizu: Molecular evidence that the rate-limiting step for the biosynthesis of arachidonic acid in *Mortierella alpina* is at the level of an elongase. Lipids 40 (1); 25-30, 2005

Takeno, S., E. Sakuradani, A. Tomi, M. I.-Ochiai, H. Kawashima and S. Shimizu: Transformation of oil-producing fungus, *Mortierella alpina* 1S-4, using Zeocin, and application to arachidonic acid production. J. Biosci. Bioeng. 100 (6); 617-622, 2005

Reviews

Ishige, T., K. Honda and S. Shimizu: Whole organism biocatalysis. Curr. Opin. Chem. Biol. 9 (2); 174-180, 2005

Ogawa, J., S. Kishino, A. Ando, S. Sugimoto, K. Mihara and S. Shimizu. Production of conjugated fatty acids by lactic acid bacteria. J. Biosci. Bioeng. 100 (4); 355-364, 2005

Ogawa, J., E. Sakuradani and S. Shimizu. Biotech-nics and -mix: Microbial production of functional lipids. Seibutsu Kogaku 83 (7); 339-341, 2005 (in Japanese)

Shimizu, S.: Sesamin. Vitamin 79 (1); 13-21, 2005 (in Japanese)

Takeno, S., E. Sakuradani and S. Shimizu: Screening and breeding of oleaginous microorganisms: Recent progress. Gakujutsu Geppo 58 (10); 29-33, 2005 (in Japanese)

b) Conference and seminar papers presented

Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry 2005: 26 reports

53rd Meeting of Japan Society of Enzyme Engineering: 1 report

400th Meeting of Vitamin B Research Comittie: 1 report

Meeting of Kansai Branch of Japan Society for Bioscience, Biotechnology, and Agrochemistry 2005: 1 report

54th Meeting of Japan Society of Enzyme Engineering: 2 reports

Annual Meeting of the Society for Fermentation and Bioengineering, Japan 2005: 9 reports

402nd Meeting of Vitamin B Research Comittie: 1 report

31st Meeting of Enzyme Application Association: 1 report

4th Lipid Reserch Seminar: 5 reports

9th Symposium on Biocatalysis Chemistry: 2 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)

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 (committieman of young scientist group)

Research grants

Monbukagakusho Research Grant: Scientific Research (S) Development of all-purpose asymmetric reductase module and their application to the production system of chiral alcohols (Shimizu, Kataoka, Ogawa), Scientific Research (B) (2) Stereoselective production of double chiral compounds by novel enzymatic reactions (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 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.: 96th American Oil Chemists' Society Annual Meeting and Expo, USA (invited speaker), BIOTRANS 2005 Symposium, The Netherlands (invited speaker), JP-EU Summit on Industrial and Environmental Biotechnology, Yokohama (invited speaker), The 13th German-Japanese Workshop on Enzyme Technology, Germany (organizer, invited speaker), Enzyme Engineering XVIII, Korea (organizer, invited speaker), International Symposium on Biocatalysis and Biotechnology, Taiwan (invited speaker), BioThailand 2005, Thailand (invited speaker)

Kataoka, M.: BIOTRANS 2005 Symposium, The Netherlands (speaker), Enzyme Engineering XVIII, Korea (speaker), International Interdisciplinary Conference on Vitamins, Coenzymes, and Biofactors 2005, Awaji (speaker), Pacific Rim Summit on Industrial Biotechnology and Bioenergy, USA (invited speaker)

Ogawa, J.: 96th American Oil Chemists' Society Annual Meeting and Expo, USA (invited speaker), BIOTRANS 2005 Symposium, The Netherlands (speaker), The 13th German-Japanese Workshop on Enzyme Technology, Germany (invited speaker), Enzyme Engineering XVIII, Korea (speaker), International Symposium on Biocatalysis and Biotechnology, Taiwan (invited speaker)

Sakuradani, E.: 96th American Oil Chemists' Society Annual Meeting and Expo, USA (speaker), 7th Northeastern Aisa Symposium on Biotechnology, Korea (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)

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)

B. Educational Activities (2005.4-2006.3)

B-1. On-campus teaching

a) Courses given

Undergraduate level: Applied microbiology II (Shimizu, Kataoka, Ogawa, Sakuradani), Applied microbiology IV (Shimizu, Kataoka), Laboratory course in applied microbiology (Kataoka, Ogawa, Sakuradani), Biotechnology (Shimizu)

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

b) Seminars

Campus tour on Open-Campus 2005 of Kyoto University (Kataoka, Ogawa, Sakuradani), Mini-lecture for students of Ichinomiya high school (Kataoka), Mini-lecture for students of Hokuryo high school (Kataoka)

B-2. Off-campus teaching, etc.

Part-time lecturer

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

Kataoka, M.: Shiga Prefectural University (Utilization of microorganism) , Mahidol University, Thailand (2nd International University of Biotechnology of UNESCO)

B-3. Overseas teaching

Students and research fellows from abroad

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

2.3.8 Laboratory of Microbial Biotechnology

Staff *Professor* *: Sakai, Yasuyoshi, Dr. Agric. Sci. (2005. 10. –)*
Assistant Professor : Yurimoto, Hiroya, Dr. (Agric. Sci.)
Students and research fellows
Doctor's program: (6)
Master's program: (11)
Undergraduate : (4)
Research fellow : (1)

A. Research Activities (2005.4-2006.3)

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

Sakai, Y.: Strategy for prevention from protein degradation in heterologous protein producion, Seibutsu-kougaku Handbook (edited by Seibutsu-kougakukai), pp.136-138, Korona, Tokyo, 2005 (in Japanese)

Original reports

Ano, Y., T. Hattori, M. Oku, H. Mukaiyama, M. Baba, Y. Ohsumi, N. Kato and Y. Sakai: A sorting nexin PpAtg24 regulates vacuolar membrane dynamics during pexophagy via binding to phosphatidylinositol-3-phosphate. Mol Biol Cell 16; 446-457, 2005

Orita, I., H. Yurimoto, R. Hirai, Y. Kawarabayasi, Y. Sakai and N. Kato: The archaeon *Pyrococcus horikoshii* possesses a bifunctional enzyme for formaldehyde fixation via the ribulose monophosphate pathway. J Bacteriol 187; 3636-3642, 2005

Yurimoto, H., R. Hirai, N. Matsuno, H. Yasueda, N. Kato and Y. Sakai: HxlR, a member of the DUF24 protein family, is a DNA-binding protein that acts as a positive regulator of the formaldehyde-inducible hxlAB operon in *Bacillus subtilis*. Mol Microbiol 57; 511-519, 2005

Limtong, S., N. Srisuk, W. Yongmanitchai, H. Yurimoto, T. Nakase and N. Kato: *Pichia thermomethanolica* sp. nov., a new thermotolerant methylotrophic yeast isolated in Thailand. Int J Syst Evol Microbiol 55; 2225-2229, 2005

Shinoda, Y., J. Akagi, Y. Uchihashi, A. Hiraishi, H. Yukawa, H. Yurimoto, Y. Sakai and N. Kato: Anaerobic degradation of aromatic compounds by *Magnetospirillum* strains: isolation and degradation genes. Biosci Biotechnol Biochem 69; 1483-1491, 2005

Ano, Y., T. Hattori, N. Kato and Y. Sakai: Intracellular ATP correlates with mode of pexophagy in *Pichia pastoris*. Biosci Biotechnol Biochem 69; 1527-1533, 2005

Goenrich, M., R. K. Thauer, H. Yurimoto and N. Kato: Formaldehyde activating enzyme (Fae) and hexulose-6-phosphate synthase (Hps) in *Methanosarcina barkeri*: a possible function in ribose-5-phosphate biosynthesis. Arch Microbiol 184; 41-48, 2005

Kajikawa, M., K. T. Yamato, H. Fukuzawa, Y. Sakai, H. Uchida and K. Ohyama: Cloning and characterization of a cDNA encoding beta-amyrin synthase from petroleum plant *Euphorbia tirucalli* L. Phytochemistry 66; 1759-1766, 2005

Kotani, T., H. Yurimoto, N. Kato and Y. Sakai: Purification and characterization of three NAD^+-dependent secondary alcohol dehydrogenases from propane-utilizing bacterium *Gordonia* sp. strain TY-5. Proc Appl Microbiol Res 3; 135-148, 2005

Reviews

Yurimoto, H., N. Kato and Y. Sakai: Assimilation, dissimilation, and detoxification of formaldehyde, a central metabolic intermediate of methylotrophic metabolism. Chem Rec 5; 367-375, 2005

Dunn Jr., W. A., J. M. Cregg, J. A. K. W. Kiel, I. J. van der Klei, M. Oku, Y. Sakai, A. A. Sibirny, O. A. Stasyk and M. Veenhuis: Pexophagy: The Selective Autophagy of Peroxisomes. Autophagy 1; 75-83, 2005

Sakai, Y: Understanding the C1-microorganisms towards efficient utilization of natural resources. Bioscience & Industry 63(10); 643-645, 2005 (in Japanese)

Yurimoto, H., N. Kato and Y. Sakai: Metabolism of C1 compounds in yeasts, bacteria and archaea. Bioscience & Industry 63(12); 773-776, 2005 (in Japanese)

Reports

Yurimoto, H.: Reports on "Annual Meeting of the Japan Society for Bioscience, Biotechnology, and Agrochemistry 2005". Bioscience & Industry 63(6); 398, 2005 (in Japanese)

b) Conference and seminar papers presented

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

reports

Annual meeting of The Society for Biotechnology, Japan, 2005: 2 reports

Annual Meeting of Japanese Society of Molecular Biology 2005: 2 reports

Annual Meeting of Japanese Society for Cell Biology 2005: 2 reports

Yeast Genetics and Molecular Biology News Japan No. 38: 4 reports

18th Annual meeting of Japan Society for Archaea: 1 report

General meeting of Kansai Branch of the Japan Society for Bioscience, Biotechnology, and Agrochemistry: 1 report

International Symposium on Life of Proteins: 2 reports

A-3. Off-campus activity

Membership in academic societies (roles)

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

Yurimoto, H: The Society for Biotechnology, Japan (Biomedia)

Research grants

Monbukagakusho Research Grant: Scientific research (S): Molecular cell biology and development of applied function of microorganisms which use future natural resources (Sakai), Scientific research on priority areas: Regulation of de novo membrane formation and physiological and Biochemichal function of phospho-lipid-binding domain (Sakai), Scientific research on priority areas: Peroxisomal proteins: molecular mechanism of their biogenesis and degradation (Sakai), Young Scientists (B): Transcriptional activator and signal transduction in methanol-inducible gene expression in yeasts (Yurimoto).

Other Research grant: Research Found by Noda Institute for Scientific Research: Molecular mechanism of microbial strategy for formaldehyde detoxification and its application (Yurimoto).

A-4. International cooperation and overseas activities

International meetings (roles)

Sakai, Y.: Gordon Resarch Conference on Autophagy in Stress, USA (invited speaker).

Yurimoto, H.: 7th Northeastern Asia Symposium on Biotechnology, Korea (invited speaker).

International joint researches

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 (2) (Ruhr Universit, Germany, Professor; Kasetsart University, Thailand, Associate Professor)

B. Educational Activities (2005.4-2006.3)

B-1. On-campus teaching

a) Courses given

Undergraduate level: Applied Microbiology I (Sakai), Applied Microbiology IV (Sakai), Laboratory Course in Applied Microbiology (Sakai, Yurimoto)

Graduate level: Microbial Biotechnology (Advanced Course) (Salkai), Microbial Biotechnology Seminar (Kato, Sakai), Experimental Course of Microbial Biotechnology (Kato, Sakai)

B-2. Off-campus teaching, etc.

Open Lecture

Sakai, Y.: Organization for Small & Medium Enterprises and Regional Innovation, JAPAN "Private Seminar on University-Industry Collaborative Research (lecture). Japan Science and Technology Agency "Seminar on Biological Production (lectiure).

B-3. Overseas teaching

Lecture in abroad

Sakai, Y., Korean Research Institute of Bioscience and Biotechnology (lecture)

C. Other Remarks

Sakai, Y.: Assistant chief of Radioisotope managing committee at Graduate School of Agriculture, Kyoto University. Associate Professor (Adjunct) of Division of Cell Proliferation, The National Institute for Basic Biology.

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: Kakutani, Tadaaki, Dr. Agric. Sci.
Assistant Professor : Tsujimura Seiya, Ms. Agric. Sci.

Students and research fellows

PD fellow	*: (1)*	*Doctor's program*	*: (2)*
Master's program:	*(8)*	*Undergraduate*	*: (4)*
Research fellow	*: (3)*	*Foreign reseach fellow*	*: (2)*

A. Research Activities (2005.4-2006.3)

A-1. Main subjects

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

Redox chemistry of quinonoid cofactors and quinoproteins (development of novel methods of cofactor synthesis, use as a mediator of bioelectrocatalytic reactions). Structure and function of

quinohemoprotein amine dehydrogenase (a new enzyme discovered by us), Molecular cloning and structural analysis of histamine dehydrogenase. Theory of bioelectrocatalytic reactions. Fundamental study of spectroelectrochemistry based on separator-less bulk electrolysis. Direct electron transfer of redox proteins at carbon electrodes with structurally regulated surface.

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. Functional analysis of the enzymes involved in the energy conversion system of *E. coli*. Bioelectrocatalytic oxidation of acetate catalyzed by whole cells of *E. coli*. Multiple oxidation process using enzymes of the TCA cycle. Modification of bioanodes based on quinoproteins. Metal complexes covalently modified on a carbon electrode as a mediator.

c) Construction of electrochemical biosensing systems.

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

d) Development of biophysical methods for analyzing transmembrane processes across biomembrane.

Development of supported planar lipid bilayers for functional reconstitution of membrane proteins. Development of a convenient method for detecting monolayer and bilayer supported on polymer substrate.

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. Biochemistry 70(3); 654-659, 2006

Fujieda, N., N. Tsuse, A. Satoh, T. Ikeda and K. Kano: Production of Completely Flavinylated Histamine Dehydrogenase, Unique Covalently Bound Flavin and Iron-sulfur Cluster Containing Enzyme, of Nocardioides simplex in Escherichia coli and its Properties. Biosci. Biotechnol. Biochem. 69 (12); 2459-2462, 2005

Ogata, H., S. Hirota, A. Nakahara, H. Komori, N. Shibata, T. Kato, K Kano and Y. Higuchi: Activation Process of [NiFe] Hydrogenase Elucidated by High Resolution X-ray Analyses: Conversion of the Ready to Unready State. Structure 13(11); 1635-1642, 2005

Nanjo, S., K. Ishii, T. Ueki, S. Imabayashi, M. Watanabe and K. Kano: Electron Transfer Reactions of Glucose Oxidase at Au(111) Electrodes Modified with Phenothiazine Derivatives. Anal. Chem. 77(13); 4142-4147, 2005

Tsujimura, S., A. Kuriyama, N. Fujieda, K. Kano and T. Ikeda: Mediated Spectroelectrochemical Titration of Proteins for Redox Potential Measurements by a Separator-less One-compartment Bulk Electrolysis Method. Anal. Biochem. 337 (2); 325-331, 2005

Tsujimura, S., K. Kano and T. Ikeda: Bilirubin Oxidase in Multiple Layer Catalyzes Four-electron Reduction of Dioxygen to Water Without Redox Mediators. J. Electroanal. Chem. 576(1); 113-125, 2005

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., K. Kano and T. Ikeda: Potentiality of Photosynthetic Respiration Cells. The Forefront of Development on Ecobioenergy (edited by S. Ueda and A. Kondo), Chapter 7 Future Eco Electrochemicall-Bio Cells, CMC press; 316-324, 2005 (in Japanese)

Tsujimura, S., K. Kano and T. Ikeda: Current Trends on Bioelectrochemical Cells. The Forefront of Development on Ecobioenergy (edited by S. Ueda and A. Kondo), Chapter 7 Future Eco Electrochemicall-Bio Cells, CMC press; 308-315, 2005 (in Japanese)

Tsujimura, S., K. Kano and T. Ikeda: Potentiality of Photosynthetic Respiration Cells. Eco Industry 10 (4); 12-18, 2005 (in Japanese)

Tsujimura, S., K. Kano and T. Ikeda: Current Trends on Bioelectrochemical Cells Eco Industry 10 (4); 5-11, 2005 (in Japanese)

Osakai, T. and K. Kano: Cyclic voltammetry(1) – Quasi-Reversible and Irreversible waves. Electrochemistry 73 (4); 310-313, 2005 (in Japanese)

Kano, K. and T. Osakai: Cyclic Voltammetry –Reversible waves. Electrochemistry 73 (3); 220-224, 2005 (in Japanese)

Tsujimura, S. and K. Kano: Electroanalytical Chemistry on Biomaterials. Bunseki 2005 (5), 269-270 (in Japanese)

Kano, K., A. Ichimura, I. Taniguchi, T. Sagara, F. Mizutani, K. Aoki, M. Yamamoto, K. Maeda: 50 th Symposium of the Polarographic Society of Japan. Rev. Polarogr., 51(1); 48-52, 2005 (in Japanese)

b) Conference and seminar papers presented.

The Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry in 2005: 6 reports

The 439th 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 2006: 5 reports

The 85th Annual Meeting of The Chemical Society of the Japan: 1 report

The 86th Annual Meeting of The Chemical Society of the Japan: 1 report

The 72th Electrochemical Society Meeting: 2 reports

The 73th Electrochemical Society Meeting: 2 reports

The 51th Annual Meeting on Polarography and Electroanalytical Chemistry: 3 reports

The Symposium on Organic Electrochemistry: 1 report

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), Journal of Biochemistry (an editoryal board member)

Research grants

Grants-in-Aid for Scientific Research From the Ministry of Education, Science, Sports and Culture of Japan: General Scientific Research (B) (2) (Ikeda, head; Kano, member). General Scientific Research (B) (2) (Kano). Young Scientific Research (B) (Tsujimura)

A-4. International cooperation and overseas activities

International meetings (roles)

Kano, K.: The 8th Asian Conference of Analytical Chemistry, Taipei, Taiwan, October (Invited speaker).

Kano, K.: The 2nd International Symposium of Environmental Biotechnologies on Bioremediation, National Cheng Kung University, Tainan, Taiwan, October (Invited speaker, Keynote speaker)

Sato, A.: International Conference on Flavin (one presentation)

Sato, A.: The 4 th International Conference on Vitamin, Awaji, Hyogo, Novenber (two presentations)

Fujieda, N.: The 4 th International Conference on Vitamin, Awaji, Hyogo, Novenber (one presentation)

Scholars from abroad

Collaborative researcher from Taiwan and China (2)

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 (Kano), Laboratory Course in Analytical Chemistry (Kano, Tsujimura), Laboratory Course in Biophysical Chemistry (Kano, Kakutani, Tsujimura), New Strategies in Agricultural Sciences (Kano and others)

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

B-2. Off-campus teaching, etc.

Part-time lecturer

Kano, K.: Kyoto Institute of Technology (Bioelectrochemistry), Shiga Medicinal University (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: (2) *Master's program* : *(4)*
Undergraduate : *(4)* *CREST Research Fellow: (1)*

A. Research Activities (2005.4-2006.3)

A-1. Main subjects

a) Isolation of odorant receptors in insects and plants.

In February 2004, we have successfully isolated the two sex pheromone receptor genes from the antennae of the female silkmoth, *Bombyx mori*. This is the first isolation of sex pheromone receptor genes in animals. We expressed the two receptors on *Xenopus* oocytes and confirmed that the one specifically responds to bombykol and the other to bombykal, which are the sex pheromones of the silkmoth. Each of the receptors strictly responded only to its sex pheromone but not to any of the other 43 odorants that male antennae of silkmoth electrophysiologically responded to. We are interested in the molecular mechanism of the evolution of sex pheromone receptors in *Lepidoptera* and that of the coevolution of sex pheromones and their receptors.

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

Book

Tomita, M. and T. Nishioka: Metabolomics. The Frontier of Systems Biology (edited by M. Tomita and T. Nishioka), pp. 256, Springer-Verlag, Tokyo, 2005

Original papers

Abe, M., M. Murai, N. Ichimaru, A. Kenmochi, T. Yoshida, A. Kubo, Y. Kimura, A. Moroda, H. Makabe, T. Nishioka and H. Miyoshi: Dynamic function of the alkyl spacer of acetogenins in their inhibitory action with mitochondrial complex I (NADH-ubiquinone oxidoreductase). Biochemistry 44; 14898-14906, 2005

Adachi, K., H. Endo, T. Watanabe, T. Nishioka and T. Hirata: Hemocyanin in the exoskeleton of crustaceans, enzymatic properties and immunolocalization. Pigment Cell Res. 18; 136-143, 2005

Adachi, K., K. Wakamatsu, S. Ito, M. Miyamoto, T. Kokubo, T. Nishioka and T. Hirata: An oxygen transporter hemocyanin can act on the late pathway of melanin synthesis. Pigment Cell Res. 19; 214-219, 2005

Fujita, D., N. Ichimaru, M. Abe, M. Murai, T. Hamada, T. Nishioka and H. Miyoshi: Synthesis of non-THF analogs of acetogenin toward simplified mimics. Tetrahedron Lett. 46; 5775-5779, 2005

Ichimaru, N., M. Murai, M. Abe, T. Hamada, Y. Yamada, S. Makino, T. Nishioka, H. Makabe, A. Makino, T. Kobayashi and H. Miyoshi: Synthesis and inhibition mechanism of delta.lac-acetogenins: a novel type of inhibitor of bovine heart mitochondrial complex I. Biochemistry 44; 816-825, 2005

Ishii, N., T. Soga, T. Nishioka and M. Tomita: Metabolome analysis and metabolic simulation. Metabolomics 1; 29-37, 2005

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 Physiol. 140; 704-715, 2006

Kao, M.-C., S. D. Bernardo, E. Nakamaru-Ogiso, H. Miyoshi, A. Matsuno-Yagi and T. Yagi: Characterization of the membrane domain subunit NuoJ (ND6) of the NADH-quinone oxidoreductase from *Escherichia coli* by chromosomal manipulation. Biochemistry 44; 3562-3571, 2005

Makabe, H., M. Higuchi, H. Konno, M. Murai and H. Miyoshi: Synthesis of (4R, 15R, 16R, 21S)- and (4R, 15S, 16S, 21S)-rollicosin. Tetrahedron Lett. 46; 4671-4675, 2005

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

Nomura T., A. Ishihara, R. C. Yanagita, T. R. Endo and H. Iwamura: Three gemones differentially contribute to the biosynthesis of benzoxazinones in hexaploid wheat. Proc. Natl. Acad. Sci. U. S. A. 102; 16490-16495, 2005

Shiomi, K., H. Ui, H. Suzuki, H. Hatano, T. Nagamitsu, D. Takano, H. Miyadera, T. Yamashita, K. Kita, H. Miyoshi, A. Harder, H. Tomoda and S Omura: A gamma-lactone form nafuredin, nafuredin-gamma, also inhibits helminth complex I. J. Antibiotics 58; 50-55, 2005

Reviews

Sakurai, T., T. Nakagawa, K. Touhara and T. Nishioka: Identification of the sex pheromone receptor genes of the sex pheromones of silkmoth, Saibo Kogaku 24; 150-151, 2005 (in Japanese)

Nakagawa, T., T. Sakurai, T. Nishioka and K. Touhara: High sensitive and high specific ligand reception in sex pheromone reception in insects, Jikken Igaku 23; 1210-1212, 2005 (in Japanese)

Nishioka, T., K. Matsuda and Y. Fujita: Combined analysis of metabolome and transcriptome: catabolism in Bacillus subtilis, Metabolomics. The Frontier of Systems Biology (edited by T. Tomita and T. Nishioka), pp. 127-140, Springer-Verlag, Tokyo, 2005

Nishioka, T.: Metabolomics and Medical Sciences. Metabolomics, The Frontier of Systems Biology (edited by T. Tomita and T. Nishioka), pp. 233-243, Springer-Verlag, Tokyo, 2005

Nakagawa, T., T. Sakurai, T. Nishioka and K. Touhara: Molecular mechanism of sex pheromone reception in insects. Tanpakushitsu Kakusan Kohso 50; 1563-1570, 2005 (in Japanese)

Nishioka, T. and T. Soga: Metabolomics by using CE-MS. Rinsho Kensa 49; 1015-1020, 2005 (in Japanese)

Nishioka, T., S. Terabe, S., Soga, K. Matsuda and Y. Fujita: Metabolome -A system for genome informatics interaction with environmental perturbations-, Tanpakushitsu Kakusan Kohso 50; 2198-2203, 2005 (in Japanese)

b) Conference and seminar papers presented

The 25th Annual Symposium of Capillary Electrophoresis (Invited lecture: 1)

The 28th Annual Meeting of the Molecular Biology Society of Japan: (Workshop Organizer: 1)

The 47th Annual Meeting of the Plant Physiology Society of Japan: 2 reports

The 50th Annual Meeting of the Japanese Society of Applied Entomology and Zoology: 1 report

The 78th Annual Meeting of the Japanese Society of Sericultural Science (Invited lecture: 1)

Annual Meeting of Japanese Society of Bioscience, Biotechnology, and Agrochemistry 2005: 7

reports (Symposium Organizer: 1)

The 77th Annual Meeting of the Japanese Biochemical Society: 1 report

The 30th Annual Meeting of Pesticide Science Society of Japan: 2 report

The 3rd Symposium "Function and Regulation of Plants", Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency (JST): 2 reports

The 4th Symposium "Function and Regulation of Plants", Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency (JST): 1 report

A-3. Off-campus activities

Membership in academic societies

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), 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); Scientific Research (S), Physiological function of respiratory enzymes in parasites mitochondria for adaptation to low oxygen circumstance (Miyoshi, member); Grant-in-Aids for Scientific Research (C) Metabolic fates of sencondary metabolites in plant defense (Ishihara, head).

Others: CREST from JST, Molecular mechanism of defenses induced in plants against insects (Nishioka, member); 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.: 27th Annual Meeting of the Association for Chemoreception Sciences, April 13 - 17, 2005, Florida, USA (1 report).

Nishioka, T.: 62nd Annual Meeting of the Korean Society of Biochemistry and Molecular Biology, May 19-20, 2005, Seoul, Korea, (Invited lecture).

Nishioka, T.: The First Annual Meeting of the Metabolomics Society, Institute for Advanced Biosciences, Keio University, June 20-23, Tsuruoka, Japan.

International joint researches

Miyoshi, H..: Dynamic structure of cytochrome bc1 complex revealed by 13C-labeled-stigmatellin and FT-IR spectroscopy. (Germany); Functional analysis of membrane domain subunits of mitochondrial complex-I through photoaffinity labeling study. (USA); Functional analysis of ubiquinone binding site of cytochrome bo enzyme through 13C-labeled ubiquinone and EPR spectroscopy. (Germany)

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

B. Educational Activities (2005.4-2006.3)

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) Head of the undergraduate course of 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 *Associate Professor: Aibara, Shigeo, D. Agric. Sci.*
Assistant Professor : Takahashi, Nobuyuki, D. Agric. Sci.
Mizutani, Kimihiko, D. Agric. Sci.

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

A. Research Activities (2005.4-2006.3)

A-1. Main subjects

a) Studies of the acquisition of serpin activity by ovalbumin

Ovalbumin, a major egg white protein, is a member of serpin (serine proteinase inhibitors) superfamily. It has, however, no inhibitory activity. We have made attempts to confer the serpin activity on ovalbumin by the site-directed mutagenesis approach. A hinge mutant in which the P14 arginine residue is replaced by threonine (R339T) was found by X-ray crystallographic analysis to undergo the large conformational transition upon the P1-P1' cleavage by the loop-insertion mechanism that is an essential structural mechanism for the exertion of the serpin inhibitory activity. The crystallographic data along with successful productions for alternative mutants with an increased loop-insertion rate strongly suggested that the acquisition of the serpin inhibitory activity is possible for ovalbumin by further site-directed mutagenesis.

b) Iron release mechanism of egg white transferrin as revealed by X ray crystallographic analyses.

Transferrin is the transporter protein that bind tightly iron in blood before transporting the metal ion to target cells. On the target cells, transferrin-iron complex binds with a specific

receptor, internalized into the cell, and then release iron in presence of an anion. To find the anion-dependent iron release mechanism, the anion-binding site on transferrin was studied by X ray crystallographic analysis. We found that the anion occupies some of iron binding ligand. To examine the feasibility of iron release mechanism by the competitive binding of anion and iron, the kinetic analysis of iron release was done. The time course of iron release displayed a biphasic progress curve. The obtained kinetic parameter supported the competitive binding mechanism obtained structural analysis.

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 the space-grown crystals prepared in space. Protein single crystals of good diffraction quality were obtained in space although 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 rate of protein molecules was controlled to the diffusion transport. I explained that it was a factor in growing single crystals of good diffraction quality.

A-2. Publications and presentations

a) Publications

Original papers

Nomura, K., N. Takahashi, M. Hirose, S. Nakamura and F. Yagi: Overall carbohydrate-binding properties of *Castanea crenata* agglutinin (CCA). Carbohydrate Research 340(12); 2004-2009, 2005

Takahashi, N., M. Onda, K. Hayashi, M. Yamasaki, T. Mita and M. Hirose: Thermostability of refolded ovalbumin and S-ovalbumin. Bioscience, Biotechnology, and Biochemistry 69(5); 922-931, 2005.

Takahashi, N., K. Terakado, G. Nakamura, C. Soekmadji, T. Masuoka, M. Yamasaki and M. Hirose: Dynamic mechanism for the serpin loop insertion. Journal of Molecular Biology 348(2); 409-418, 2005.

Mizutani, K., B. Mikami, S. Aibara and M. Hirose: Structure of aluminium-bound ovotransferrin at 2.15 Angstroms resolution. Acta Crystallogr. D Biol. Crystallogr. 61; 1636-1642, 2005.

b) Conference and seminar papers presented

The 2005 Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry : 1 paper

The 78th Annual Meeting of The Japanese Biochemical Society: 2 papers

The 22th Space Utilization Symposium : 3 papers

A-3. Off-campus activities

Membership in academic societies

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

Research grants

Takahashi, N.: Grant-in-Aid for Scientific Research (C) (2). Investigation for a transition state of loop insertion in serpin: An approach for prevention of amyroidosis (Principal investigator).

A-4. International cooperations and overseas activities

International joint researchers, overseas research surveys

Aibara, S.: Space Experiment of Crystal Growth of Proteins (Russia)

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

B. Educational Activities (2005.4-2006.3)

B-1. On-campus teaching

a) Courses given

Undergraduate level: Laboratory Course in Biological Chemistry (Aibara, Takahashi, Mizutani)

Graduate level: Applied Structural Biology Seminar (Aibara, Takahashi, Mizutani), Experimental Course of Applied Structural Biology (Aibara, Takahashi, Mizutani)

B-2. Off-campus teaching, etc.

Part-time lecturer

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

Doctor's program : (7) *Master's program : (10)*
Research student: (2) *Research fellow : (2)*

A. Research Activities (2005.4-2006.3)

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 2.0 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 is in progress.

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. A series of *p*-substituted monophenyl phosphonates were synthesized as mechanism-based inhibitors of *E. coli* and human GGTs. The effect of the electronic nature of the leaving group on the rate of enzyme inactivation (a Broensted plot) revealed that the transition state for the reaction of the enzyme with the monophosphonate inhibitors was totally different between the *E. coli* and human GGTs. A series of novel phosphonate diester-type mechanism-based inhibitors of GGT were developed and used successfully for probing the active-site geometry 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 inbibitors 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 lieaving 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.

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 parameters for the amide hydrolysis of this mutant is measured to understand the rationale for improved amide hydrolysis.

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 ofbrassinosteroids (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.: Enzyme Inhibitors as Chemical Tools to Study Enzyme Catalysis: Rational Design, Synthesis, and Applications. The Chemical Record 5; 209-228, 2005

Mizutani, M.: Diversity of cytochromes P450 and chemical evolution in plants. Regulation of Plant Growth and Development 40; 67-82, 2005

Mizutani, M. and Saito S: Inhibitors of cytochromes P450 enhance drought-stress tolerance in plants. - GA biosynthesis inhibitors also act as ABA catabolism inhibitors?!- . Kagaku to

Seibutu 43; 628-630, 2005

Mizutani, M. and K. Sakata: Diglycosidase deeply concerned with the tea aroma formation and its application for food industries. Bioscience and Industry 64; 145-150, 2006

Kinoshita T. and K. Sakata: Mysterious Aroma of Oriental Beauty. Koryo 229; 113-120, 2006 (in Japanese).

Original papers

Ueno, K., H. Yoneyama, S. Saito, M. Mizutani, K. Sakata, N. Hirai and S. Todoroki: A Lead Compound for the Development of ABA 8'-hydroxylase Inhibitors, Bioorg. Med. Chem. Lett. 15; 5226-5229, 2005

Kinoshita, T., J. –Y. Cho, M. Mizutani, B. Shimizu, H. –T. Tsai, Y. -L. Chen and K. Sakata: Gene Expression Profiling during the Fermentation Process of "Oriental Beauty", Proceedings of 2005 International Symposium on Innovation in Tea Science and Sustainable Development in Tea Industry, Nov. 11-15, p. 541-545, Hangzhou China, 2005

Sakata, K., M. Mizutani, Y. –O. Ahn and B. Shimizu: Floral Aroma of Oolong Tea are Results of Stress-Responded Reactions in Tea Leaves during the Tea Proecessing, Proceedings of 2005 International Symposium on Innovation in Tea Science and Sustainable Development in Tea Industry, Nov. 11-15, p. 607-617, Hangzhou China, 2005

Cho, J. -Y., M. Mizutani M., B. Shimizu, T. Kinoshita, M. Ogura, K. Tokoro, M. –L. Lin and K. Sakata: Chemical Profiling of Aroma and Charbohydrates during the Fermentaion Process of Formosa Oolong Tea "Oriental Beauty". Proceedings of 2005 International Symposium on Innovation in Tea Science and Sustainable Development in Tea Industry, Nov. 11-15, Hangzhou China, p. 928-932, 2005

Nakanishi, T., T. Nakatsu, M. Matusoka, K. Sakata and H. Kato: Crystal Structures of pyruvate phosphate dikinase from maize revbaled an alternative conformation in the swiveling-domain mortion, *Biochemistry* 44; 1136-1144, 2005

Tsuruhami, K., S. Mori, K. Sakata, S. Amarume, S. Saruwatari, T. Murata and T. Usui: Efficient synethsis of beta-primeverosidase as aroma precursors by transglycosylation of beta-diglycosidase *Penicillium multicolor*. J. Carbohydr. Chem. 24; 849-863, 2005

Sawai, Y., J. –H. Moon, K. Sakata and N. Watanabe: Effects of structure on radical-scavenging abilities and antioxidative activities of tea polyphenols. NMR Analytical approach using 1,1-diphenyl-2-picrylhydorazyl radicals. J. Agric. Food Chem. 53(9); 3598-3604, 2005

Kato, M., Y. Uno, J. Hiratake and K. Sakata: β-Glucopyranoimidazolines as intermediate analogue inhibitors of family 20 β-N-acetylglucosaminidases. Bioorg. Med. Chem. 13; 1563-1571, 2005

Ueno, K., Y. Araki, N. Hirai, S. Saito, M. Mizutani, K. Sakata and Y. Todoroki: Differences between the structural requirements for ABA 80-hydroxylase inhibition and for ABA activity. Bioorg. Med. Cehm. 13(10); 3359-3370, 2005

Shimizu, B., F. Saito, H. Miyagawa, K. Watanabe, T. Ueno, K. Sakata and K. Ogawa: Phytotoxic components produced by pathogenic *Fusarium* against morning glory. Z. Naturforsch. 60c; 862-866, 2005

Kitahata, N., S. Saito, Y. Miyazawa, T. Umezawa, Y. Shimada, Y. K. Min, M. Mizutani, N. Hirai, K. Shinozaki, S. Yoshida and T. Asami: Chemical regulation of abscisic acid catabolism in plants by cytochrome P450 inhibitors. Bioorg Med Chem. 13; 4491-8, 2005

Fujita, K., F. Oura, N. Nagamine, T. Katayama, J. Hiratake, K. Sakata, H. Kumagai and K.

Yamamoto: Identification and molecular cloning of a novel glycoside hydrolase family of core 1 type *O*-glycan-specific endo-alfa-N-acetylgalactosaminidase from Bifidobacterium longum. J. Biol. Chem. 280; 37415-37422, 2005

Tsuruhami, K., S. Mori, S. Amarume, S. Saruwatari, T. Murata, J. Hirakake, K. Sakata and T. Usui: Isolation and Characterization of a beta-Primeverosidase-Like Enzyme from *Penicillium multicolor*. Biosci. Biotechnol. Biochem. 70; 691-698, 2006

Kai, K., B. Shimizu, M. Mizutani, K. Watanabe and K. Sakata: Accumulation of coumarins in *Arabidopsis thaliana.* Phytochemistry 67; 379-386, 2006

Sakamoto, T., Y. Morinaka, T. Ohnishi, H. Sunohara, S. Fujioka, M. Ueguchi-Tanaka, M. Mizutani, S. Takatsuto, S. Yoshida, H. Tanaka, H. Kitano and M. Matsuoka: Erect leaf caused by brassinosteroid deficiency increases biomass production and grain yield in rice. Nat Biotechnol. 24; 105-109, 2006

Fujita, S., T. Ohnishi, B. Watanabe, T. Yokota, S. Takatsuto, S. Fujioka, S. Yoshida, K. Sakata and M. Mizutani: *Arabidopsis* CYP90B1 catalyzes the early C-22 hydroxylation of C_{27}, C_{28}, and C_{29} sterols. Plant J. 45; 765-74, 2006

Nakatsu, T., S. Ichiyama, J. Hiratake, A. Saldanha, N. Kobayashi, K. Sakata and H. Kato: Structural basis for spectral difference in luciferase bioluminescence. Nature 440; 372-376, 2006

b) Conference and seminar papers presented

RIKEN Plant Science Center Seminar (Yokohama, 2005. 5. 16): 1 paper

Anglo-German-Japanese Biochemistry Meeting (Kyoto, 2005. 9. 5): 1 paper

The 2002 Annual Meeting of the Japanese Society for Applied Glycoscience (Tsu, 2005.9.28-30): 2 papers

The 2005 Joint Meeting of Kansai, Chushikoku, and Nishinihon Branch of Japan Society for Bioscience, Biotechnology, and Agrochemistry (Osaka, 2005.9.30-10.1): 2 papers

Enzyme Engineering XVIII (Gyongju, Korea,2005.10,9-14): 1 paper

The 40th Annual Meetng of Regulation of Plant Growth & Development (Tokyo, 2005.10.31-11.2): 4 papers

2005 International Symposium on Innovation in Tea Science and Sustainable Development in Tea Industry (Hangzhou, China, 2005.11.11-15): 3 papers

34th Tocklai Conference -- Strategies for Quality – (Assam, India, 2005.11.28-30): 1 paper

The 28th Annual Meeting of the Molecular Biology Society of Japan (Kobe, 2005.12.7-10): 1 paper

2005 International Chemical Congress of Pacific Basin Societies (Honolulu, Hawaii, USA, 2005.12.15-20): 2 papers

21COE Kyoto University Alliance for Chemistry, Chemical Biology Mini Sympojium (Kyoto, 2006. 3. 16): 1 paper

The 2003 Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry (Kyoto, , 2003.3.26-28): 14 papers

The 47th Annual Meeting of the Japanese Society for Plant Physiologists (Tsukuba, 2005.3.19-21): 7 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): A new disaccharide-glycoside specific glycosidase (diglycosidase) family in plant kingdom (K. Sakata)(J. Hiratake, M. Mizutani, and B. Shimizu, collaborators); Grant-in-Aid for Exploratory Research: Investigation of substrate recognition mechanism of 6-modified β-glucoside specific-glycosidases, β-primeverosidase and furcatin hydrolase (K. Sakata) (J. Hiratake and B. Shimizu, collaborators); Grant-in-Aid for Scientific Research (B) (2): Bioorganic chemical studies on glycosidases by using substrate : transition-state analogue inhibitors of glycosidases as research tools (J. Hiratake) (M. Mizutani and B. Shimizu, collaborators); Grant-in-Aid for Exploratory Research: A chemical knock-out of biosynthesis and degradation of indole-3-acetic acid (IAA) conjugates (J. Hiratake) (M. Mizutani, collaborator); Grant-in-Aid for JSPS Fellows: Molecular basis of the elevated aroma formation in Oriental Beauty prepared from tea leaves infested by the tea green leafhopper (K. Sakata)(J-Y. Cho, collaborator); Grant-in-Aid for JSPS Fellows: Functional analysis of cytochrome P450 monooxygenases involved in brassinosteroids catabolism (T. Ohnishi)

A-4. International cooperation and overseas activities

International meetings (roles)

Sakata, K.: Member of American Chemical Society (Division of Agricultural Food Chemistry); 34th Tocklai Conference -- Strategies for Quality --, Assam (India), (2005.11.28-30) (Invited lecture)

Others

Sakata, K.: The 16th Korea-Japan Joint Committee for Basic Scientific Research (2005.5.23-24)

Sakata, K.: Foreign Research Professor of Sunchon National University (Korea) (2004.2.1-2009.3.1)

B. Educational Activities (2005.4-2006.3)

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

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)

Hiratake, J.: Kyoto University of Education, Faculty of Education (A primary course of organic 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 (2005. 6. 4 and 7.16)

B-3. Overseas teaching

Students and research fellows from abroad

PhD student: 1 (China)

Research Fellow (JSPS): 1 (Korea)

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 : (6) *Master's Program : (15)*

Research student: (1)

A. Research Activities (2005.4-2006.3)

A-1. Main subjects

a) Construction of a protein expression system by using a psychrotrophic bacterium, *Shewanella livingstonensis* Ac10, as the host

Protein expression system that works at low temperatures would be useful for the production of thermolabile proteins and proteins that are toxic to the host cells due to their enzyme activities at moderate temperatures. We have constructed a novel protein expression system by using a psychrotrophic bacterium *Shewanella livingstonensis* Ac10 isolated from Antarctic seawater as the host. We found that the upstream region of the gene coding for the homolog of alkyl hydroperoxide reductase AhpC exhibited strong promoter activity in *S. livingstonensis* Ac10. By using this promoter, thermolabile proteins from a psychrophilic bacterium, *Desulfotalea psychrophila*, were successfully overproduced.

b) The Cellular Function of Selenocysteine Lyase in Selenoprotein Synthesis

Selenocysteine lyase (SCL) is a pyridoxal 5'-phosphate (PLP)-dependent enzyme that specifically acts on L-selenocysteine to yield L-alanine and selenium. We found that decreasing the level of SCL by RNAi in HeLa cells results in significant reduction in protein levels of cytosolic glutathione peroxidase (cGPx) and activities of cGPx and thioredoxin reductase. We also found that RNAi-mediated reduction of SCL induces cell growth inhibition even in the presence of selenomethionine, selenocysteine, selenite, or FBS in a serum-free medium. These results provide new insights into possible pathways for selenoprotein biosynthesis.

A-2. Publications and presentations

a) Publications

Original Papers

Abe, K., I. Ebata, T. Kazuoka, H. MIhara, T. Kurihara and N. Esaki: Protein Interaction between Selenophosphate Synthetase and IscS. Biomed. Res. Trace Elem. 16; 325-327, 2005

Goto, M., H. Muramatsu, H. Mihara, T. Kurihara, N. Esaki, R. Omi, I. Miyahara and K. Hirotsu: Crystal Structures of Δ^1-Piperideine-2-carboxylate/Δ^1-Pyrroline-2-carboxylate Reductase Belonging to a New Family of NAD(P)H-dependent Oxidoreductases. J. Biol. Chem. 280; 40875-40884, 2005

Kurata, A., T. Kurihara, H. Kamachi and N. Esaki: 2-Haloacrylate reductase: A novel enzyme of the medium-chain dehydrogenase/reductase superfamily that catalyzes the reduction of a carbon-carbon double bond of unsaturated organohalogen compounds. J. Biol. Chem. 280; 20286-20291, 2005

Kurokawa, S., H. Mihara, T. Kurihara and N. Esaki: Protein Interaction between Selenophosphate Syntetase and IscS. Biomed. Res. Trace Elem. 16; 325-327, 2005

Mihara, H., H. Muramatsu, R. Kakutani, M. Yasuda, M. Ueda, T. Kurihara and N. Esaki: *N*-Methyl-L-amino acid dehydrogenase from *Pseudomonas putida*, a novel member of unusual NAD(P)-dependent oxidoreductase superfamily., FEBS Journal 272; 1117-1123, 2005

Muramatsu, H., H. Mihara, M. Goto, I. Miyahara, K. Hirotsu, T. Kurihara and N. Esaki: A New Family of NAD(P)H-Dependent Oxidoreductases Distinct from Conventional Rossmann-Fold Proteins. J. Biosci. Bioeng. 99; 541-547, 2005

Yamamoto, H., K. Mitsuhashi, N. Kimoto, Y. Kobayashi and N. Esaki: Robust NADH-regenerator: improved α-haloketone-resistant formate dehydrogenase. Appl. Microbiol. Biotechnol. 67; 33-39, 2005

Yoshimune, K., Andrey Galkin, Ljudmila Kulakova, T. Yoshimura and N. Esaki: Cold-active DnaK of an Antarctic psychrotroph *Shewanella* sp.Ac10 supporing the growth of *dnaK*-null mutant of *Escherichia coli* at cold temperatures. Extremophiles 9; 145-150, 2005

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

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

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), Grant-in-Aid for Creative Scientific Research; Collaboratory on electron correlations- Toward a new research network between physics and chemistry – (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), Grant-in-Aid for Exploratory Research; Development of protein expression systems operating at low temperatures to suppress the formation of inclusion bodies (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 research surveys (invited speaker),

International meetings (roles)

Esaki, N.: International Symposium on Extremophiles and Their Applications (speaker)

Esaki, N.: Pacifichem2005 (speaker)

Esaki, N.: International Conference on Alpine and Polar Microbiology (speaker)

Kurihara, T.: Pacifichem2005 (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 (2005.4-2006.3)

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.: Physical Science Chemistry, Osaka Prefecture University (Biological Science), Department of Sciences, Kyoto Univiersity of Education (Applied Biochemistry)

B-3. Overseas teaching

Students and research fellows from abroad

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

Chair of Wood Biosciences (Research Institute For Sustainable Humanosphere)

2.3.14 Laboratory of 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 (1)

( ) number of students comissioned for research from Grad. Sch. Biostdies

A. Research Activities (2005.4-2006.3)

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 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 cDNAs involved in polyketide biosyntheses and in water-ion transports, from 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.: ABC proteins in plants, ABC proteins (edited by K. Ueda), p. 45-78, Gakkaishuppan Center, 2005

Sudarmonowati, E. and T. Hayashi: Tree biotechnology in Indonesia, *In* Sustainable Development and utilization of tropical forest resources (edited by Y. Imamura, T. Umezawa and T. Hata), pp.113-122, Universe Printing, Kyoto, Japan, 2006

Hayashi, T.: Tissue culture and molecular breeding of tropical trees, *In* Sustainable Development and utilization of tropical forest resources (edited by Y. Imamura, T. Umezawa and T. Hata), pp. 189-190, Universe Printing, Kyoto, Japan, 2006

Hayashi, T., T. Konishi, Y. Ohmiya and T. Nakai: Is cellulose synthesis enhanced by expression of sucrose synthase in poplar?, *In* Abiotic stress Tolerance in Plants (edited by A.K. Rai and T. Takabe), pp. 187-193, Springer, Tokyo, Japan, 2006

Original papers

Otani, M., N. Shitan, K. Sakai, E. Martinoia, F. Sato and K. Yazaki: Characterization of vacuolar transport of the endogenous akaloid berberine in *Coptis japonica*. Plant Physiol. 138 (4); 1939-1946, 2005

Sasaki, K., K. Ohara and K. Yazaki: Gene expression and characterization of isoprene synthase from *Populus alba*. FEBS Lett. 579 (11); 2514-2518, 2005

Shitan, N., F. Kiuchi, F. Sato, K. Yazaki and K. Yoshimatsu: Establishment of *Rhizobium*-mediated transformation of *Coptis japonica* and molecular analyses of transgenic plants. Plant Biotech. 22 (2); 113-118, 2005

Terasaka, K., J. J. Blakeslee, B. Titapiwatanakun, W. A. Peer, A. Bandyopadhyay, S. N. Makam, O. R., E. L. Lee, A. S. Murphy, F. Sato and K. Yazaki: PGP4, an ATP-binding cassette P-glycoprotein, catalyzes auxin transport in *Arabidopsis thaliana* roots. Plant Cell 17 (11); 2922-2939, 2005

Hayashi, T.: Callose Syndromes, http://www.glycoforum.gr.jp/science/word/glycobiology/PS-A03E.html, 2005

Sudarmonowati, E., S. Hartati, R. Hartati, Y. W. Park and T. Hayashi: Expression of cellulase gene in *Paraserianthes falcataria*, Towards Ecology and Economy Harmonization of Tropical Forest Resources, ed. W. Dwianto, pp. 388-394, Bali, Indonesia, 2005

Hartati, S., Y. W. Park, E. Sudarmonowati and T. Hayashi: *Agrobacterium*-mediated genetic transformation of *Acacia mangium* bearing xyloglucanase gene, Towards Ecology and Economy Harmonization of Tropical Forest Resources, ed. W. Dwianto, pp. 395-399, Bali, Indonesia, 2005

Patents

Horiuchi, K. and K. Yazaki: Cleaning of cadmium from environment by use of plants, and the plants transformed with modified Ycf1 genes encoding yeast cadmium factor-1. January 28th, 2005.

Horiuchi, K. and K. Yazaki: Takashi Hirooka: Clearn up method of organic pollutants from environment. March 23rd, 2005.

Yano, S. and K. Yazaki: Monoterpene synthase gene from eucalyptus. August 10th, 2005.

Horiuchi, K. and K. Yazaki: Establishment of heavy metal tolerant plants and plant cells. September 27th, 2005.

Reviews

Yazaki, K.: Plant morphology and ABC proteins. Kagaku-to-Seibutsu 43 (5); 288-295, 2005.

Yazaki, K.: Transporters of secondary metabolites. Curr. Opin. Plant Biol. 8 (3); 301-307, 2005

Hayashi, T. and K. Baba: Functional analysis of cellulose and xyloglucan, Plant Growth regulation 40; 167-174, 2005

Hayashi, T., K. Yoshida, Y. W. Park, T. Konishi and K. Baba: Cellulose metabolism in plants, International Review of Cytology 247; 1-34, 2005

b) Conference and seminar papers presented

Annual Meeting of Japanese Society for Plant Physiologyists 2005 : 11 reports

Annual Meeting of Japanese Society for Bioscience, Biotechnology and Agrochemistry 2005 : 5 report

The 23rd Annual Meeting of Japanese Society for Plant Cell and Molecular Biology : 9 reports

The 28th Annual Meeting of The Molecular Biology Society of Japan: 1 reports

Annual Meeting of Japanese Society for Plant Science 2005 : 2 reports

Annual Meeting of Japan Cellulose 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), The Japanese Bioindustry Association (Editorial Board), Association of Bio Quinone (Executive Board).

Hayashi, T.: Japan Society for Carbohydrate (Councilor, Editor), National Institute of Science and Technology Policy (Researcher)

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); Nitta Corp, Phytoremediation (Yazaki); Research grant for Sustainable Humanosphere for Mission 1, Impact on atmospheric chemistry by plant-derived volatile organic compounds and its physiological roles (Yazaki), Research grant for Sustainable Humanosphere for Hoga Mission, Molecular analysis of gene expression influenced by microwave irradiation to plants (Yazaki).

Others: Nitta Corp, Phytoremediation (Yazaki); Research grant for Sustainable Humanosphere for Mission 1, Impact on atmospheric chemistry by plant-derived volatile organic compounds and its physiological roles (Yazaki), Research grant for Sustainable Humanosphere for Hoga Mission, Molecular analysis of gene expression influenced by microwave irradiation to plants (Yazaki), Biodesign project in Japan (Hayashi, Head), Joint research grant with RITE (Hayashi, Head), Japan International Research Center for Agricultural Sciences (Hayashi, Head), Research Grant for Sustainable Humanosphere (Hayashi, Head), Research Grant from the Forestry and Forest Products Institute (Kuroda)

A-4. International cooperations and overseas activities

International meetings (roles)

Yazaki, K.: 3rd Japanese-German Joint Symposium –New Deployment of Post Genome Research in Plant- Kanazawa (Organizer), 17 th International Symposium on Sustainable Humanosphere, Uji, (Organizer). Pacifichem 2005 (December, 2005, Honolulu, USA, Invited speaker), 6th International Wood Science Symposium (August, 2005, Bali,

Indonesia), 3rd Japanese-German Joint Symposium (September, 2005, Kanazawa, Symposist), 17 th International Symposium on Sustainable Humanosphere (September, 2005, Uji, Symposist), ABC 2006 FEBS advanced course (March, 2006, Innsbruck, Austria, Speaker).

Hayashi, T.: Expression of xyloglucanase and cellulase in mangium (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)

International Joint Researches, overseas research sruveys

Yazaki, K.: ABC proteins involved in auxin polar transport in plants (Purdue University, USA), Physiological roles of ABC proteins in plants (Syngenta, Switzerland), 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)

Hayashi, T.: Cellulose (Editoral board)

Editorial work for international journals (roles)

Yazaki, K.: Plant & Cell Physiology (Editorial Board), Plant Biotechnology (Associate Editor), J. Wood Sci. (Editorial Board)

Scholars from abroad

Ph D scientists (2) (Korea and Brasil)

Ph D student (1) (Spain)

B. Educational Activities (2005.4-2006.3)

B-1. On-campus teaching

a) Courses given

Undergraduate level: Wood Biomass (Hayashi, Hata, Kawai)

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.: Toyama Medical and Pharmaceutical University, Applied Natural Product Course (April, 2005, Graduate level), Kobe Pharmaceutical University, Pharmaceutical Chemistry Course (September 2005, Graduate level), Natural Product Course (December 2005, Undergraduate level).

Hayashi, T.: Nagoya University, Agricultural Life Sciences for graduate course (December, 2005), Kyushu Univesity, Special lecture course for graduate course (June, 2005)

Open seminar, etc.

Yazaki, K.: The Pharmaceutical Society of Japan Hokuriku Branch Lecture (April 26th, 2005, Toyama, Invited lecturer), Nagoya University Bioscience and Biotechnology Center, Special Seminar (August 2005, Nagoya), Kobe Pharmaceutical University Special Seminar, (September, 2005, Kobe), Takara Bio Co. Ltd, Biotechnology Research

Laboratories, Invited lecture (October, 2005, Otsu), 15th Symposium on the Development and Application of Naturally Occurring Drug Materials, Speaker (November, 2005, Tokyo), Hoga Mission Symposium, Symposist (March, 2006, Uji).

Hayashi, T.: The 2 nd RISH Open Seminor (Uji, Octorber, 2005), Symposium of Plant Scientist Network (Tokyo, October, 2005)

B-3. Overseas teaching

Lectures and seminars

Yazaki, K.: Special Lecture, VTT Biotechnology (May 2005, Espoo, Finland), Special Seminar, VTT Biotechnology, Cancer Research Group (June 2005, Turku, Finland), Special Seminar, Helsinki University (June 2005, Helsinki, Finland), Special Seminar, Ghent University (June 2005, Ghent, Belgium), Special Seminar, Leipniz Institute of Plant Biochemistry (March 2006, Halle, Germany), Special Seminar, Cadarache Institute (March 2006, Cadarache, France).

Hayashi, T.: Special Lecture, Hebrew University (2006, 3, Transgenic poplar) (Rehbot, Iarael)

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

2.3.15 Laboratory of Biochemical Control

Staff *Professor* *: Umezawa, Toshiaki, Dr. Agric. Sci.*

Assistant Professor : Hattori, Takefumi, Dr. Agric. Sci.

Students and research fellows

Doctor's Program: (2)

Master's Program: (6)

A. Research Activities (2005.4-2006.3)

A-1. Main subjects

a) 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 in relation to heartwood formation.

b) 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 established for the first time that all the carbon atoms of a norlignan, *cis*-hinokiresinol, is derived from cinnamate.

c) Biosynthetic mechanisms for lignins

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 energy production. To obtain basic knowledge for metabolic engineering of lignin biosynthesis, we are working on the molecular mechanisms of lignin biosynthesis in various plant species by comprehensive metabolite analysis.

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 based on knowledges of biosynthetic mechansisms for wood components.

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

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

A-2. Publications and presentations

a) Publications

Books and reviews

Umezawa, T.: Lignan is a key compound to elucidate tree phylogeny, Ki no bikkuribanashi 100, Kodansha; 64-65, 2005 (in Japanese)

Imamura, Y. and T. Umezawa: Sustainable Development and Utilization of Tropical Forest Resources –Outline of JSPS-LIPI Core University Program in the Field of Wood Science-, Sustainable Development and Utilization of Tropical Forest Resources (edited by Y. Imamura, T. Umezawa and T. Hata), pp. 1-9, RISH, Kyoto University, Japan, 2006

Umezawa, T.: Biotechnology of Tropical Fast-growing Trees, Sustainable Development and Utilization of Tropical Forest Resources (edited by Y. Imamura, T. Umezawa and T. Hata), pp. 108-112, RISH, Kyoto University, Japan, 2006

Munir, E., T. Hattori and M. Shimada: Role of oxalate biosynthesis in growth of copper tolerant wood-rotting and pathogenic fungi, Sustainable Development and Utilization of Tropical Forest Resources (edited by Y. Imamura, T. Umezawa and T. Hata), pp. 108-112, RISH, Kyoto University, Japan, 2006

Original papers

Tokunaga, N., N. Sakakibara, T. Umezawa, Y. Ito, H. Fukuda and Y. Sato: Involvement of extracellular dilignols in lignification during tracheary element differentiation of isolated Zinnia mesophyll cells. Plant Cell Physiology 46; 224-232, 2005

Li, L., X. Cheng, S. Lu, T. Nakatsubo, T. Umezawa and V.L. Chiang: Clarification of cinnamoyl Co-enzyme A reductase catalysis in monolignol biosynthesis of aspen. Plant Cell Physiology 46; 1073-1082, 2005

Tokimatsu, T., N. Sakurai, H. Suzuki, H. Ohta, K. Nishitani, T. Koyama, T. Umezawa, N. Misawa,

K. Saito and D. Shibata: Kappa-View: A web-based analysis tool for integration of transcript and metabolite data on plant metabolic pathway maps. Plant Physiology 138; 1289-1300, 2005

Watanabe, T., T. Hattori, S. Tengku and M. Shimada: Purification and characterization of NAD-dependent formate dehydrogenase from the white-rot fungus Ceriporiopsis subvermispora and a possible role of the enzyme in oxalate metabolism. Enzyme and Microbial Technology 37; 68-75, 2005

Kawasaki, T., H. Koita, T. Nakatsubo, K. Hasegawa, K. Wakabayashi, H. Takahashi, K. Umemura, T. Umezawa and K. Shimamoto: Cinnamoyl-CoA reductase, a key enzyme in lignin biosynthesis, is a novel effector of small GTPase Rac in defense signaling in rice. Proc. Acad. Natl. Sci. USA 103; 230-235, 2006

b) Conference and seminar papers presented

55th Annual Meeting of Japan Wood Res. Soc. (Kyoto): 5 papers

The 23rd Annual Meeting of the Japanese Society of Plant Cell and Molecular Biology (Kyoto): 3 papers

50th Lignin Symposium, (Nagoya): 1 paper

The mycological society of America & the mycological society of Japan joint meeting 2005: 1 paper

IUFRO tree Biotechnology 2005 (South Africa): 3 papers

International Symposium on Wood Science and Technology 2005 (Yokohama): 4 papers

8th Annual Meeting of Indonesian Wood Research Society (Indonesia): 1 paper

6th International Wood Science Symposium (Indonesia): 5 papers

International Chemical Congress of Pacific Basin Societies 2005 (Hawai): 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)

Hattori, T.: Mycological Society of Japan (Treasurer for The mycological society of America & the mycological society of Japan joint meeting 2005)

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 Scientific Research (B) (2): Gene expression mechanisms in bamboo. (Joint 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., The General Environmental Technos Co., Ltd., A grant-in-aid from Hokuto Foundation for Bioscience

A-4. International cooperations and overseas activities

International meetings (roles)

Umezawa, T.: Establishment of International Cooperative Research on Science of Sustainable

Humanosphere in South-East Asia-Development of sustainable production and utilization systems oftropical plantation forest resources and their contribution to global environment- (organizer, key note), 6th International Wood Science Symposium (organizer)

Oral presentation

Umezawa, T.: Keynote lecture in 8th Annual Meeting of Indonesian Wood Research Society, Tenggarong, Indonesia, Invited lecture in International Symposium on Wood Science and Technology 2005, Yokohama, Japan, Lecture in Wood Science School in Cibinong 2006, Cibinong, Indonesia

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 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)

Hattori, T.: Cooperative work on Screeing of forest microorganisms based on their phosphate solubilizing ability for reforestation (The Univ. of North Sumatra, Indonesia)

Hattori, T.: Field study of root-rot fungi for Acacia mangium (PT Musi Hutan Persada, Indonesia)

Scholars from abroad

Erman Munir: Collaborative research work on "Elucidation of the mechanisms for carbon metabolism of ectomycorrhizal fungi" October 1-October 7 (2005)

Myrtha Karina: Collaborative research work on "Sustainable production and utilization of tropical forest resources for establishment of recycling-based society" November 25-November 30 (2005)

Faizatul Falah: Collaborative research work on "Sustainable production and utilization of tropical forest resources for establishment of recycling-based society" November 26-December 1 (2005)

B. Educational Activities (2005.4-2006.3)

B-1. On-campus teaching

Undergraduate level: Introduction to mushroom science

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)

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
Doctor's program : (5) *Master's program: (11)*
Post-Doctral fellow: (2)

A. Research Activities (2005.4-2006.3)

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 engineered technique, structure-function relationship 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.: Pretreatments of wood biomass for saccharification and fermentation by selective white rot fungi, in Frontiesrs of Eco-Bioenergy –Construction of Sustainable Society

Systems Orineted Zero Emission (edited by M.Ueda and A. Kondo), CMC Publication, Tokyo, 68-78, 2005

Original papers

Amirta R., T. Tanabe, T. Watanabe, Y. Honda, M. Kuwahara and T. Watanabe: Methane fermentation of Japanese cedar wood pretreated with a white rot fungus, *Ceriporiopsis subvermispora*. J. Biotechnol.; in press, 2005

Rahmawati, N., Y. Ohashi, T. Watanabe, Y. Honda and T. Watanabe: Ceriporic acid B, an extracellular metabolite of *Ceriporiopsis subvermispora* suppresses the depolymerization of cellulose by the Fenton reaction. Biomacromolecules 6; 2851-2856, 2005

Rahmawati, N., Y. Ohashi, Y. Honda, M. Kuwahara, K. Fackler, K. Messner and T. Watanabe: Pulp bleaching by hydrogen peroxide activated with copper 2,2'-dipyridylamine and 4-aminopyridine complexes. Chem. Eng. J. 112; 167-171, 2005

Punnapayak H., M. Kuhirun and T. Watanabe: Liquid-state prebleaching of paper pulp with white rot fungi, J. Natl. Res. Council Thailand; in press, 2005

Kohzu, A., T. Miyajima, T. Tateishi, T. Watanabe, M. Takahashi and E. Wada: Dynamics of 13C natural abundance in wood decomposing fungi and their ecophysiological implications. Soil Biol. and Biochem. 37; 1598-1607, 2005

Reviews

Watanabe, T.: Biorefinery of wood biomass using biological functions of white rot fungi, Onkochishin, 41; 1-6, 2004

b) Conference and seminar papers presented

The 12 th Annual meeting of The Japan Institute of Energy: 2 presentaion

Annual meeting of the Society for Bioscience and Bioengineering: 3 presentaion

The 9 th Annual meeting of Japanese Society of Mushroom Science and Biotechnology: 1 presentaion

Annual meeting of Japan Society of Bioscience, Biochemistry and Agrochemistry 2006: 9 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 Scientific Research (B)(2), Conversion of wood to ethanol by the system incorporated overexpression of laccase (Takashi Watanabe), 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)(2), Molecular breeding of white rot fungi suitable for highly effective sccahrification of wood biomass (Honda), Grant in Aid for Young Scientists (B),

Characterization of the fatty acid synthesis-related genes in selective lignin degradation (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.: International Chemical Congress of Pacific Basin Societies (Pacifichem 2005), Honolulu (Invited lecture), NEDO White Bio Workshop, Kawasaki (Invited lecture), The 6th International Symposium, The 21st Century COE program, Nagaoka Univ. of Technol., "Global Renaissance by Green Energy Revolution", Nagaoka (Invited lecture), 6th International Wood Science Symposium (IWSS), Bali, (Presentation), International Symposium on Wood Science and Technology (IAWPS2005), Yokohama, (Presentation)

Honda, Y.: The Mycological Society of America/The Mycological Society of Japan Joint Meeting 2005, Hilo, (Presentation)

Watanabe, T.: 6th International Wood Science Symposium (IWSS), Bali (Presentation), Internatinal Symposium on Wood Science and Technology (IAWPS2005), Yokohama (Presentation)

International joint researches, overseas research surveys

Watanabe, T.: Cooperative research between RISH, Kyoto University and R & D Center for Applied Physics, LIPI, Indonesia, under the Core University System of Japanese Society of Promotion of Science, Cooperative research between NRCT and Yamaguchi University under the Core University System of Japanese Society of Promotion of Science

Honda, Y.: Cooperative research between RISH, Kyoto University and R & D Center for Applied Physics, LIPI, Indonesia, under the Core University System of Japanese Society of Promotion of Science, Cooperative research between NRCT and Yamaguchi University under the Core University System of Japanese Society of Promotion of Science, Surveys of "biomass comversion using genetically modified basidiomycetes"

Watanabe, T.: Cooperative research between RISH, Kyoto University and R & D Center for Applied Physics, LIPI, Indonesia, under the Core University System of Japanese Society of Promotion of Science,

B. Educational Activities (2005.4-2006.3)

B-1. On-campus teaching

a) courses given

Undergraduate level: Mushroom Biology Seminar (Honda, Takahito Watnabe)

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

B-2. Off-campus teaching etc.

Open seminar, etc

Watanabe, T.: Kyoto University Open Seminar for Forest Science, The 2nd Energy Recycling Symposium - Biomass conversion and solar power statellite, ST/GSC Roadmap Workshop, 11th Bio-industry Seminar

Students and research fellows from abroad

Doctor course: 1

Cooperative research fellows: 4

B-3. Overseas teaching

Watanabe, T.: Special lecture in Lembang University

Students and research fellows from abroad

Watanabe, T.: Special lecture in Novozymes (Davis)