2.3 DIVISION OF APPLIED LIFE SCIENCES

Division of Applied Life Sciences was established in 1997 by combining 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), and, in 2001, was divided into Divisions of Applied Life Sciences and Food Science and Biotechnology.

The new Division of Applied Life Sciences focuses on the subjects of that involve microorganisms, animals, and plants, and carries a wide area of research and education 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
Research fellow : (6)
Doctor's program: (7)
Master's program: (11)
Undergraduate : (5)

A. Research activities (2004.4-2005.3)

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

Ueda, K. et al.: New strategy for Bioscience, Maruzen, Tokyo, 2004

Ueda, K. et al.: ABC proteins, Gakkai Shuppann Center, Tokyo, 2005

Original papers

Abe-Dohmae, S., Y. Ikeda, M. Matsuo, M. Hayashi, K. Okuhira, K. Ueda and S. Yokoyama: Human ABCA7 supports apolipoprotein-mediated release of cellular cholesterol and phospholipid to generate high density lipoprotein. J Biol Chem 279; 604-611, 2004

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

Fujise, H., S. Sasawatari, T. Annoura, T. Ikeda and K. Ueda: 3,3',4,4',5 -Pentachlorobiphenyl Inhibits Drug Efflux Through P-Glycoprotein in KB-3 Cells Expressing Mutant Human

P-Glycoprotein. J Biomed Biotechnol 2004; 137-142, 2004

Kimura, Y., M. Matsuo, K. Takahashi, T. Saeki, N. Kioka, T. Amachi and K. Ueda: ATP hydrolysis-dependent multidrug efflux transporter: MDR1/P-glycoprotein. Curr Drug Metab 5; 1-10, 2004

Mitsushima, M., A. Suwa, T. Amachi, K. Ueda and N. Kioka: Extracellular Signal-regulated Kinase Activated by Epidermal Growth Factor and Cell Adhesion Interacts with and Phosphorylates Vinexin. J Biol Chem 279; 34570-34577, 2004

Munehira, Y., T. Ohnishi, S. Kawamoto, A. Furuya, K. Shitara, M. Imamura, T. Yokota, S. Takeda, T. Amachi, M. Matsuo, et al.: Alpha1-syntrophin modulates turnover of ABCA1. J Biol Chem 279; 15091-15095, 2004

Nagata, K., A. Yamamoto, N. Ban, A. R. Tanaka, M. Matsuo, N. Kioka, N. Inagaki and K. Ueda: Human ABCA3, a product of a responsible gene for abca3 for fatal surfactant deficiency in newborns, exhibits unique ATP hydrolysis activity and generates intracellular multilamellar vesicles. Biochem Biophys Res Commun 324; 262-268, 2004

Suzuki, S., T. Nishimaki-Mogami, N. Tamehiro, K. Inoue, R. Arakawa, S. Abe-Dohmae, A. R. Tanaka, K. Ueda and S. Yokoyama: Verapamil increases the apolipoprotein-mediated release of cellular cholesterol by induction of ABCA1 expression via Liver X receptor-independent mechanism. Arterioscler Thromb Vasc Biol 24; 519-525, 2004

b) Conference and seminar paper presented

The 8th Annual Meeting of Japanese Association for Molecular Target Therapy of Cancer, 1 paper

The 57th Annual Meeting of Japan Society for Cell Biology, 2 papers

The 24th Sapporo Cancer Seminar, invited speaker

The 77th Annual Meeting of Japanese Biochemistry Society, workshop, 3 papers

The 63th Annual Meeting of Japanese Cancer Association, symposium, 1 paper

The 27th Annual Meeting of Japan Society for Molecular Biology, 3 papers

The 2005 Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry, 12 papers

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 (2): Regulation of anchorage-dependent cell proliferation and tumor metastasis by membrane cytoskeletal protein. (Kioka, N.) Developmental 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.) Priority Area (2): Quality control of ABCG protein complex. (Matsuo, M.)

Research grant from The Naito Foundation: Functional analysis of membrane cytoskeletal proteins in the convergence and cooperation between cell adhesion and signaling. (Kioka, N.)

A-4. International cooperations and overseas activities

International meetings (roles)

Ueda, K.: The 2004 HDL workshop, Crete, Greece (presentation)

Kioka, N.: Gordon Research Conferences, "Signaling by adhesion receptor" USA (presentation)

Matsuo, M.: Seminer in Inje University School of Medicine (Invited lecture)

Symposium "Current topics on trends in molecular medicine" Keimyung University School of Medicine (Invited lecture)

Membership in international academic societies

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

B. Educational activities (2004.4-2005.3)

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 :(3)
Master's program :(8)
Undergraduate :(4)
Research fellow :(1)
Research student :(1)

A. Research Activities (2004.4-2005.3)

A-1. Main subjects

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

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

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

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

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

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

known as combinatorial bioengineering and through fusion with nanotechnology and other fields to create the concept of nano-biotechnology. Through these, we look forward to creating new bioactive proteins and cells which transcend the limitations of known genomic information.

A-2. Publications and presentations

a) Publications

Books

Ueda, M. and A. Kondo: Combinatorial Bioengineering Development of Molecular Evolution. J Mol Catalys 28; 4-6, 2004

Original papers

Fujita, Y., J. Ito, M. Ueda, H. Fukuda and A. Kondo: Synergistic saccharification and direct fermentation to ethanol of amorphous cellulose by use of an engineered yeast strain codisplaying three types of cellulolytic enzyme. Appl Environ Microbiol 70(2); 207-1212, 2004

Ito, J., Y. Fujita, M. Ueda, H. Fukuda and A. Kondo: Improvement of cellulose-degrading ability of a yeast strain displaying *Trichoderma reesei* endoglucanase II by recombination of cellulise-binding domains. Biotechnol Prog 20(3); 688-691, 2004

Kimura, Y., S. Shibasaki, K. Morisato, N. Ishizuka, H. Minakuchi, K. Nakanishi, M. Matsuo, T. Amachi, M. Ueda and K. Ueda: Microanalysis for MDR1 ATPase by HPLC with a titanium dioxide column. Anal Biochem 326(2); 262-266, 2004

Kimura, H., T. Tanigawa, H. Morisaka, T. Ikegami, K. Hosoya, N. Ishizuka, H. Minakuchi, K. Nakanishi, M. Ueda, K. Cabrera and N. Tanaka: Simple 2D-HPLC using a monolithic silica column for peptide separation. J Separation Sci 27(10-11); 897-904, 2004

Lin, Y., S. Shiraga, T. Tsumuraya, T. Matsumoto, A. Kondo, I. Fujii and M. Ueda: Comparison of two forms of catalytic antibody displayed on yeast-cell surface. J Mol Catalys 28(4-6); 241-246, 2004

Lin, Y., S. Shiraga, T. Tsumuraya, T. Matsumoto, A. Kondo, I. Fujii and M. Ueda: Isolation of novel catalytic antibody clones from combinatorial library displayed on yeast-cell surface. J Mol Catalys 28(4-6); 247-252, 2004

Mima, J., M. Hayashida, T. Fujii, Y. Hata, R. Hayashi and M. Ueda: Crystallization and preliminary crystallographic analysis of carboxypeptidase Y inhibitor I^C complexed with the cognate proteinase. Acta Crystallog 60(9); 1622-1624, 2004

Nagao, K., M. Yamashita and M. Ueda: Production of auto-proteolytically subunit-assembled 7-beta-(4-carboxybutanamido)cephalosporanic acid (GL-7ACA) acylase from *Pseudonomas* sp. C427 using a chitin-binding domain. Appl Microbiol Biotechnol 65; 407-413, 2004

Shigechi, H., Y. Fujita, J. Koh, M. Ueda, H. Fukuda and A. Kondo: Energy-saving direct ethanol production from low-temperature-cooked corn starch using a cell-surface engineered yeast strain co-displaying glucoamylase and alpha-amylase. Biochem Eng J 18; 149-153, 2004

Shigechi, H., J. Koh, Y. Fujita, T. Matsumoto, Y. Bito, M. Ueda, E. Satoh, H. Fukuda and A. Kondo: Direct ethanol fermentation from non-cooked corn starch using a novel surface-engineered yeast strain co-displaying glucoamylase and α-amylase. Appl Environ Microbiol 70(8); 5037-5040, 2004

Shiraga, S., M. Kawakami and M. Ueda: Construction of combinatorial library of the

starch-binding domain of *Rhizopus oryzae* glucoamylase and screening of clones with enhanced activity by yeast display method. J Mol Catalys 28(4-6); 229-234, 2004

Sugiyama, K., T. Niki, K. Inokuchi, Y. Teranishi, A. Tanaka and M. Ueda: Heterologous expression of metabotropic glutamate receptor subtype1 in *Saccharomyces cerevisiae*. Appl Microbiol Biotechnol 64(4); 531-536, 2004

Ueda, M.: Combinatorial bioengineering - Development of molecular evolution. J Mol Catalys 28(4-6); 137, 2004

Ueda, M.: Future direction of molecular display by yeast-cell surface engineering. J Mol Catalys 28(4-6); 139-144, 2004

Reviews

Fujita, Y., M. Ueda, H. Fukuda and A. Kondo: Construction of a cellulose-degrading yeast strain by co-displaying endoglucanase, cellobiobiohydrolase and ß-glucosidase. Proc Mie Bioforum 764-772, 2004

Katahira. S., Y. Fujita, M. Ueda, H. Fukuda and A. Kondo: Construction of yeast strain for xylan utilization through co-display of two types of xylanolytic enzyme on cell surface of *Saccharomyces cerevisiae*. Proc Mie Bioforum 773-777, 2004

Kondo, A. and M. Ueda: Yeast cell surface display - Application of molecular display. Appl Microbiol Biotechnol 64(1); 28-40, 2004

Kondo, A., Y. Fujita, M. Ueda and H. Fukuda: Direct and efficient production of ethanol from cellulosic materials with a yeast strain displaying cellulolytic enzymes. Proc Mie Bioforum 755-763, 2004

b) Conference and seminar papers presented

Annual Meeting of the Society for Biotechnology, Japan 2004: 6 reports

The 77th Annual Meeting of the Japanese Biochemical Society: 5 reports

The 27th Annual Meeting of MBSJ: 5 reports

Annual Meeting of Japan Society for Bioscience, Biotechnology and Agrochemistry 2004: 17 reports

A-4. International cooperations and overseas activities

International meetings (roles)

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

Scholars from abroad

Invited scholar :1 (from Thailand)

B. Educational Activities (2004.4-2005.3)

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: (7) *Undergraduate : (4)*

A. Research Activities (2004.4-2005.3)

A-1. Main subjects

a) Chemistry of Secondary Metabolites in Plants

The transformation of plants with a transgene (*OASA1D*) that encodes anthranilate synthase alpha-subunit with decreased feedback sensitivity to tryptophan (Trp) has been shown to result in a dramatic increase in the level of Trp in plant tissues. In order to examine the effect of Trp biosynthesis activation on the level of indole-3-acetic acid (IAA), a plant hormone (auxin) derived from Trp, the amount of IAA in the *OASA1D*-transformed rice was quantitated, using LC-MS/MS. The result demonstrated that the increase in the Trp level also resulted in the increase in the IAA level, although the increase apparently had no effects on morphology or growth of the trasformant. This suggests that the increased IAA is somehow isolated in the plant tissue from its site of action. The quantitation of some IAA metabolites showed that alanine conjugate of IAA was increased in its level, while the levels of glutamate and aspartate conjugates were relatively unchanged. Therefore, only the specific conjugation pathway(s) is likely activated to deal with the elevation of IAA level in the transformant. .

b) Structure-Activity Relationships of Ecdysone Agonists

An insect molting hormone, 20-hydroxyecdysone, binds to the heterodimer of ecdysone receptor (EcR) and ultraspiracle (USP), and regulate the hormonal action. We performed the cloning of EcR and USP genes of Colorado potato beetles *Leptinotarsa decemlineata*, and prepared their proteins using rabbit reticulocyte lysate *in vitro*. The binding affinity of various ecdysone agonists was measured against in vitro translated EcR/USP heterodimers. The binding affinity of nonsteroidal ecdysone agonists was lower than that against Lepidoptera rice stem borers *Chilo suppressalis*, although the binding of steroidal compounds was similar among various EcR/USP heterodimers.

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 cyclic flagellin-like peptides and measured their elicitor activity to investigate the structural features important for the activity. A cyclic analog in which N- and C-terminal residues were linked exhibited the almost same activity with the linear peptide. This indicates that the N-terminal part of flagellin peptides is spatially located in proximity to the C-terminal part when the peptide interacts with its receptor. ii) The rearing procedure of Japanese scorpions *Liocheles australasiae* was established. The venom was

collected from the telsons of scorpions and a number of peptides were separated by HPLC. The molecular mass of a bioactive peptide was determined by mass spectrometer and its primary sequence was determined by Edman sequencing analysis.

d) Endocrine Disruptor

Metabolites of a chlorinated insecticide, methoxychlor, have been known to show estrogen receptor binding activity. These metabolites (phase-I metabolites) are further metabolized to glucuronate and sulfate conjugates by phase-II metabolic reactions. However, the estrogenic activity of phase-II metabolites has never been evaluated. In this study, we synthesized glucuronate and sulfate conjugates of mono- and bis-demethylated methoxychlor and measured their estrogen receptor binding activity. All conjugates exhibited much lower activity than mono- and bis-demethylated methoxychlor which are phase-I metabolites. This suggests that the estrogenic activity of phase-I metabolites of methoxychlor were decreased following conjugation by glucuronate or sulfate in vivo

A-2. Publications and presentations

a) Publications

Original papers

Matsuda, F., K. Morino, R. Ano, M. Kuzawa, K. Wakasa and H. Miyagawa: Metabolic flux analysis of the phenylpropanoid pathway in elicitor-treated potato tuber tissue. Plant Cell Physiol 46; 454-446, 2005

Matsuda, F., K. Morino, H. Miyazawa, M. Miyashita and H. Miyagawa: Determination of Potato Glycoalkaloids using High-pressure Liquid Chromatography-Electrospray Ionizaion/Mass Spectrometry. Phytochem Anal 15; 121-124, 2004

Minakuchi, C., Y. Nakagawa, Y. Soya and H. Miyagawa: Preparation of functional ecdysteroid receptor proteins (EcR and USP) using a wheat germ cell-free protein synthesis system. J Pestic Sci 29; 189-194, 2004

Minakuchi, C., Y. Nakagawa, M. Kamimura and H. Miyagawa: Measurement of receptor-binding activity of non-steroidal ecdysone agonists using in vitro expressed receptor proteins (EcR/USP complex) of Chilo suppressalis and Drosophila melanogaster In "New Discoveries in Agrochemicals." (J. Marshall Clark, Hideo Ohkawa, Eds.). ACS Symp Ser, 892; American Chemical Society, Washington, D.C., pp. 191-200, 2005

Miyashita, M.; T. Shimada, H. Miyagawa, and M. Akamatsu: Surface plasmon resonance-based immunoassay for 17beta-estradiol and its application to the measurement of estrogen receptor-binding activity. Anal Bioanal Chem 381; 667-73, 2005

Miyashita, M.; T. Shimada, S. Nakagami, N. Kurihara; H. Miyagawa and Akamatsu, M: Enantioselective recognition of mono-demethylated methoxychlor metabolites by the estrogen receptor. Chemosphere 54; 1273-1276, 2004

Morino, K., F. Matsuda, H. Miyazawa, A. Sukegawa, H. Miyagawa and K. Wakasa: Metabolic profiling of tryptophan-overproducing rice calli that express a feedback-insensitive a subunit of anthranilate synthase. Plant Cell Physiol 46; 514-521, 2005

Nakagawa, Y., K. Takahashi, H. Kishikawa, T. Ogura, C. Minakuchi and H. Miyagawa: Classical and three-dimensional QSAR for the inhibition of [3H]ponasterone A binding by diacylhydrazine-type ecdysone agonists to insect Sf-9 cells. Bioorg Med Chem 13; 1333-1340, 2005

Nishiwaki, H., K. Sato, Y. Nakagawa, M. Miyashita and H. Miyagawa: Metabolism of imidacloprid in houseflies. J Pestic Sci 29; 110-116, 2004

Ogura, T., Y. Nakagawa, C. Minakuchi and H. Miyagawa: QSAR for binding affinity of substituted dibenzoylhydrazines to intact Sf-9 cells. J Pestic Sci 30; 1-6, 2005

Okazaki, Y., T. Isobe, Y. Iwata, T. Matsukawa, F. Matsuda, H. Miyagawa, A. Ishihara, T. Nishioka and H. Iwamura: Metabolism of avenanthramide phytoalexins in oats. Plant J 39; 560-572, 2004

Swevers, L., L. Kravariti, S. Ciolfi, M. Xenou-Kokoletsi, N. Ragoussis, G. Smagghe, Y. Nakagawa, B. Mazomenos and I. Kostas: A cell-based high-throughput screening system for detecting ecdysteroid agonists and antagonists in plant extracts and libraries of synthetic compounds. FASEB J 18; 134-136, 2004

Watanabe, B., S. Yamamoto, K. Sasaki, Y. Nakagawa, and H. Miyagawa: A simple synthesis of 6-deoxoteasterone and its 20-epimer. Tetrahedron Lett 45; 2767-2769, 2004

Watanabe, B., Y. Nakagawa, T. Ogura and H. Miyagawa: Stereoselective synthesis of (22R)- and (22S)-castasterone/ponasterone A hybrid compounds and evaluation of their molting hormone activity. Steroids 69; 483-493, 2004

Uesusuki, S., B. Watanabe, S. Yamamoto, J. Otsuki, Y. Nakagawa and H. Miyagawa: Synthesis of Brassinosteroids of Varying Acyl Side Chains and Evaluation of Their Brassinolide-like Activity. Biosci Biotech Biochem 68; 1097-1105, 2004

b) Conferences and seminar papers presented

The 30th Annual Meeting of Pesticide Science Society of Japan: 4 reports

Annual Meeting of the Japan Society for Bioscience, Biotechnology, and Agrochemistry 2005: 10 reports

70th Anniversary Kansai Branch Meeting of the Japan Society for Bioscience, Biotechnology, and Agrochemistry: 1 report

80th Anniversary Symposium on the Japan Society for Bioscience, Biotechnology, and Agrochemistry (Kansai Branch Meeting): 1 report

Workshop for Insects: 2 reports

The 1st COE International Meeting: 4 reports

The 20th Meeting of Pesticide Design: 1 report

The 41th Peptide Meeting: 1 report

The 46th Annual Meeting of Plant Pathology: 2 reports

The 19th Workshop of Pesticide Design Research: 1 report

The 55th Annual Meeting of the Japan Wood Research Society: 1 report

A-3. Off-campus activities

Membership in academic societies (roles)

Miyagawa, H.: Japan Society for Pesticide Science (editorial board member, councilor), Japan Society for Bioscience, Biotechnology, and Agrochemistry (councilor of Kansai branch), Japanese Society for Lichenology (councilor)

Nakagawa, Y.: The Division of Structure-Activity Studies, The Pharmaceutical Society of Japan (board member), 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 (2004.4-2005.3)

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 (Advanced Course) (Miyagawa, Nakagawa), Bioregulation Chemistry Seminar (Miyagawa, Nakagawa, Miyashita), Experimental Course in Bioregulation Chemistry (Miyagawa, Nakagawa, Miyashita).

B-2. Off-campus teaching, etc.

H. Miyagawa: Faculty of Agriculture, Tottori University (Biological and Environmental Chemistry II)

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

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.

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 : (3) *Master's program : (7)*
Undergraduate : (4)

A. Research Activities (2004.4-2005.3)

A-1. Main subjects

a) Chemical factors controlling feeding behavior of aphids

Aphids feed on plants by sucking from phloem tissues and often seriously damage agricultural crops. The feeding behavior is composed of two distinct phases: insertion of the proboscis into the plant tissues (probing) and sucking the phloem sap (ingestion). Plant secondary compounds specific to hosts appeared to a play significant role in their host recognition during probing. We examined profiles of flavonoid glycosides that potentially act as probing stimulants in the host assessments of aphids in the tribe Macrosiphini. A bean aphid, *Megoura crassicauda*, feeds selectively on the plant genus *Vicia* (Fabaceae), but it never infests a hairy vetch, *V. hirsuta*. A specific probing deterrent was isolated from a *V. hirsuta* extract by monitoring the inhibitory effect, and characterized as (*E*)-2-methyl-2-butene-1,4-diol 4-O-β-D-glucopyranoside.

b) Electrophysiological analyses of taste chemosensilla in Lepidoptera.

Larvae of most lepidopterous insects feed on a limited number of plants often belonging to a single family, by sensing specific allelochemicals commonly contained in their hosts. We examined feeding stimulants and deterrents in the hosts and non-host plants and tested these compounds to the gustatory chemosensory organs located on their mouthparts using a tip-recording method. Typical electrophysiological responses were obtained from the lateral and medial styloconic chemosensilla of two swallowtail butterfly species.

c) Ultraweak photon emission from herbivory injured maize plants

Against herbivory or infection, plants make various defense responses. In this present study, we found ultraweak photon emission from maize leaves damaged by *Helicoverpa armigera* (Noctuidae). Interestingly, mechanically damaged maize leaves treated with the caterpillar regurgitants emitted the same intensity and pattern of photon emission as those from maize leaves damaged by caterpillars. Furthermore, two-dimensional imaging of the leaf section treated with the oral secretions clearly showed that photon emission was observed specifically at the lip of wound exposed to the secretions. These results suggest that the direct interaction between maize leaf cells and chemicals contained in caterpillar regurgitants triggers the photon emission.

d) Scheloribatid mites as the source of pumiliotoxins in dendrobatid frogs

The strawberry poison frog *Dendrobates pumilio* (Anura: Dendrobatidae) and related poison frogs contain a variety of dendrobatid alkaloids that are considered to be sequestered through the consumption of alkaloid-containing arthropods microsympatrically distributed in the habitat. In addition to ants, beetles, and millipedes, we found that adults of two species of oribatid mites belonging to the cohort Brachypylina, trophically a lower level of animal than ants and beetles, contain dendrobatid alkaloids. GC/MS of hexane extracts of adult *Scheloribates azumaensis*

(Oribatida: Acari) revealed the presence of not only pumiliotoxin **251D** (8-hydroxy-8-methyl-6-(2'-methylhexylidene)-1-azabicyclo[4.3.0]nonane), but also precoccinelline 193C and another coccinelline-type alkaloid. From the corresponding extracts of an unidentified *Scheloribates* sp., pumiliotoxin **237A**

A-2. Publications and presentations

a) Publications

Original papers

Mizoguchi, A., K. Murakami, N. Shimizu, N. Mori, R. Nishida and Y. Kuwahara: S-isorobinal as the female sex pheromone from an alarm pheromone-emitting mite, *Rhizoglyphus setosus*. Experimental and Applied Acarology 36; 107-117, 2005

Morita, A., N. Mori, R. Nishida, N. Hirai and Y. Kuwahara: Neral (the alarm pheromone) biosynthesis *via* the mevalonate pathway, evidenced by D-glucose-1-^{13}C feeding in *Carpoglyphus lactis* ^{13}C incorporation into other opisthonotal gland exudates. J Pestic Sci 29; 27-32, 2004

Nishimura, K., N. Mori, K. Okabe and Y. Kuwahara: Chemical ecology of astigmatid mites LXXVI identification of α-acaridial as the female sex pheromone of Schwiebea similis (Acari: Acaridae). Jpn J Environ Entomol Zool 15(2); 107-117, 2004

Noge, K., N. Mori, C. Tanaka, R. Nishida, M. Tsuda and Y. Kuwahara: Identification of astigmatid mites using the second internal transcribed spacer (ITS2) region and its application for phylogenetic study. Experimental and Applied Acarology 35; 29-46, 2005

Oikawa, A., A. Ishihara, C. Tanaka, N. Mori, M. Tsuda and H. Iwamura: Accumulation of HDMBOA-Glc is induced by biotic stresses prior to the release of MBOA in maize leaves. Phytochemistry, 65; 2995-3001 2004

Ono, H., Y. Kuwahara and R. Nishida: Hydroxybenzoic acid derivatives in a non-host rutaceous plant, *Orixa japonica*, deter both oviposition and larval feeding in a Rutaceae-feeding swallowtail butterfly, *Papilio xuthus* L. J Chem Ecol 30; 287-301, 2004

Shelly, T. E., and R. Nishida: Larval and adult feeding on methyl eugenol and the mating success of male oriental fruit flies, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). Entomol Exp Appl 112; 155-158, 2004

Shimizu, N., K. Noge N. Mori. R. Nishida and Y. Kuwahara: Chemical ecology of astigmatid mites LXXIII. Neral as an alarm pheromone of the acarid mite, *Oulenzia* sp. (Astigmata: Winterschmidtiidae). J Acarol Soc Jpn 13; 57-64, 2004

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

Yoshinaga, N., N. Morigaki, F. Matsuda, R. Nishida and N. Mori: In vitro biosynthesis of volicitin in Spodoptera litura. Insect Biochem Molec Biol 35; 175-184, 2005

Review papers

Nishida, R.: Chemosensory basis of host recognition in butterflies – multi-component system of oviposition stimulants and deterrents. In T. Yamamoto, T. Fushiki, H. Kaba and Y. Ninomiya eds. Chemical Senses Supplement 30. Oxford University Press, Oxford, UK. i293-i294, 2005

b) Conference and seminar papers presented

The 48th Annual Meeting of Japanese Society of Applied Entomology and Zoology: 4 papers

Annual Meeting of Japan Society of Bioscience, Biotechnology and Agrochemistry (2004): 5 papers
The 10th Annual Meeting of Acarological Society of Japan: 3 papers
The 14th Annual Meeting of Environmental Entomology and Zoology of Japan: 1 paper
The 14th International Symposium on Olfaction and Taste: 1 paper
International Symposium on Chemical Sense and Insect Behavior: 1 paper
The 1st International Symposium of Entomological Science COE "Development and Metamorphosis": 7 paper

A-3. Off-campus activities

Membership in academic societies (roles)

Ritsuo Nishida: Japanese Society of Applied Entomology and Zoology (councilor, editorial board)
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 (secretary). 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, Laos, USA)

B. Educational Activities (2004.4-2005.3)

B-1. On-campus teaching

a) Course given

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, R.: Mie University, Kobe University, Kyoto University of Education, Osaka Prefectural University, Research Institute for Subtropics

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

A. Research Activities (2004.4-2005.3)

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: Apoplast and Plant Nutrition 3. Function of Ca in apoplast. Jpn J Soil Sci Plant Nutr 75; 381-386, 2004

Kobayashi M and T Matoh. Boron nutrition of cultured tobacco BY-2 cells. In Tobacco BY-2 Cells, Biotechnology in Agriculture and Forestry vol., 53, Eds. T Nagata, S Hasegawa and D Inze, Springer 2004

Original papers

Kobayashi, M., T Mutoh and T Matoh: Boron nutrition of cultured tobacco BY-2 cells. IV. Genes induced under low boron supply. J Exp Bot 55, 1441-1443, 2004

Ochiai, K. and T Matoh. Alleviation of salinity damage to rice plants by the use of polyethylene glycols (PEGs) through reduction of Na^+ tranprot to shoots. Soil Sci Plant Nutr, 50; 129-133, 2004

Ochiai, K., Y. Iwata and T. Matoh: Relationship between nitrate-nitrogen and total nitrogen concentrations in spinach and Komatsuna sold at local markets. Jpn J Soil Sci Plant Nutr 75, 693-695

Nakano, Y., S. Okawa, T. Yamauchi, Y. Koizumi and J. Sekiya: Occurrence of two forms of γ-glutamyltransferases in radish plant. Plant Biotechnol 21; 243-246, 2004

Patcharaporn, P., C. Chitbuntanorm, N. Chinoim, P. Kangyawongha and T. Matoh: Phosphorus accmulation in soils and nitrate contamination in underground water under export-oriented asparagus farming in Nong Ngu Lauem village, Nakhon Pathom province, Thailand. Soil Sci Plant Nutr 50, 385-393, 2004

Phimsirikul, P. and T. Matoh: Effect of polyolefin-coated EDTA (ethylenediamine tetraaceteic acid)-K_2SO_4 on the growth of Italian ryegrass and red clover in sandy soil. Soil Sci Plant Nutr 50; 135-140, 200

b) Conference and seminar papers presented

Annual Meeting of the Japanese Society of Plant Physiologists, 2004: 2 reports

Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry, 2004: 3 reports

Annual Meeting of Japanese Society for Plant Cell and Molecular Biology, 2004: 2 reports

Annual Meeting of Japanese Society of Soil Science and Plant Nutrition, 2004: 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) Function of inorganic elements in higher plant cell walls. 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). Small-schale irrigation project in Ghana (member)

A-4. International cooperations and overseas activities

International meetings (roles)

Sekiya, Jiro: 6th International Workshop on Plant Sulfur Metabolism, 2005 (Organizing Committee)

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 (2004.4-2005.3)

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 Textile Science, Kyoto Institute for Textile and Technology (Plant Nutrition), Faculty of Agriculture, Kyoto Prefectural University (Plant Nutrition Ⅰ, Ⅱ), Graduate School of Agriculture, Shimane University (Plant Nutrition)

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: (5)
Undergraduate : (2)

A. Research Activities (2004.4-2005.3)

A-1. Main subjects

a) Structure-function analysis of restriction-modification enzymes

Crystal structures of EcoO109I restriction endonuclease and its complexes with DNA were solved by multiple wavelength anomalous dispersion and SIRAS, respectively. A comparison of the two structures shows that the catalytic domain moves drastically to capture the DNA. One metal ion and two water molecules are observed near the active site of the DNA complex. One water molecule, activated by Lys-126, attack the phosphorus atom in an S_N2 mechanism. EcoO109I is similar to EcoRI family enzymes in terms of its DNA cleavage pattern and folding topology of the common motif in the catalytic domain, but it differs in the manner of DNA recognition. These results propose a novel classification of the type II restriction endonucleases and lead to the suggestion that EcoO109I represents a new subclass of the EcoRI family.

b) Inducible production of thioredoxin in yeasts focusing on the development of thioredoxin-containing functional foods

Thioredoxin (TRX) is an important antioxidant present in all types of organism. Besides its role as an antioxidant, TRX protects the gastric mucosa due to its anti-inflammatory effect. In addition, TRX decreases allergenicity; therefore, the oral administration of TRX is of considerable interest with respect to its clinical use as well as the development of functional foods containing TRX. We have attempted to enrich the cellular TRX content in *Saccharomyces cerevisiae*, and found that green tea extract, which is rich in catechins (polyphenols), activates the Yap1 transcription factor, leading to the induction of *TRX2*, a target of Yap1. Production of yeast TRX was monitored by both *TRX2-lacZ* reporter expression assay and Western blotting using anti-yeast TRX antibody. Maximal production of TRX was achieved in a medium containing 0.1% green tea extract at pH 7.6.

c) Stress response in the mRNA export in *Saccharomyces cerevisiae*.

In eukaryotic cells including yeast, mRNA is synthesized and processed in the nucleus and exported to the cytoplasm through nuclear pore complexes. It is well known that mRNA export is a target in the regulation of stress. Yeast cells alter not only their transcriptional patterns but also the types of mRNA to be exported from the nucleus in order to adapt rapidly to stress. Under conditions of heat shock, stress-induced transcripts such as *SSA4* encoding Hsp70 are efficiently exported, whereas bulk poly $(A)^+$ mRNA accumulates in the nucleus. The mechanisms of such a selective mRNA export caused by the stress seem to be quite complex and remain controversial. Additionally, it still remains to be clarified whether ethanol and heat shock affect mRNA export in

the same way. We have been investigating about the mechanisms of the selective export of mRNA under stressed conditions, including the making process of sake and wine.

A-2. Publications and presentations

a) Publications

Original papers

Hashimoto, H., T. Shimizu, T. Imasaki, M. Kato, N. Shichijo, K. Kita, and M. Sato: Crystal structures of type II restriction endonuclease EcoO109I and its complex with cognate DNA. J Biol Chem 280(7); 5605-5610, 2005

Imasaki, T., H. Hashimoto, T. Shimizu, M. Kato, J. Tsuda, K. Kita, and M. Sato: Crystallization and preliminary X-ray crystallographic analyses of EcoO109I and its complex with DNA. Acta Cryst D60(6); 1165-1166, 2004

Izawa, S., N. Kuroki and Y. Inoue: Nuclear thioredxoin peroxidase Dot5 in *Saccharomyces cerevisiae*: roles in oxidative stress response and disruption of telomeric silencing. Appl Microbiol Biotechnol 64(1); 120-124, 2004

Izawa, S. and Y. Inoue: A screening system for antioxidants using thioredoxin-deficient yeast: discovery of thermostable antioxidant activity from *Agaricus blazei* Murill. Appl Microbiol Biotechnol 64(4); 537-542, 2004

Izawa, S., M. Sato, K. Yokoigawa and Y. Inoue: Intracellular glycerol influences resistance to freeze stress in *Saccharomyces cerevisiae*: analysis of a quadruple mutant in glycerol dehydrogenase genes and glycerol-enriched cells. *Appl Microbiol Biotechnol* 66(1); 108-114, 2004

Izawa, S., K. Ikeda, K. Maeta and Y. Inoue: Deficiency in the glycerol channel Fps1p confers increased freeze tolerance to yeast cells: application of the *fps1*Δ mutant to frozen dough technology. Appl Microbiol Biotechnol 66(3); 303-305, 2004

Izawa, S., R. Takemura and Y. Inoue: Gle2p is essential to induce adaptation of the export of bulk poly $(A)^+$ mRNA to heat shock in *Saccharomyces cerevisiae.* J Biol Chem 279(34); 35469-35478, 2004

Kataoka, M., A.-R.G. Delacruz-Hidalgo, M.A. Akond, E. Sakuradani, K. Kita and S. Shimizu: Gene cloning and overexpression of two conjugated polyketone reductases, novel aldo-keto reductase family enzymes, of *Candida parapsilosis*. Appl Microbiol Biotechnol 64(3); 359-366, 2004

Maeta, K., S. Izawa, S. Okazaki, S. Kuge and Y. Inoue: Activity of the Yap1 transcription factor in *Saccharomyces cerevisiae* is modulated by methylglyoxal, a metabolite derived from glycolysis. Mol Cell Biol 24(19); 8753-8764, 2004

Takatsume, Y., S. Izawa and Y. Inoue: Identification of thermostable glyoxalase I from *Schizosaccharomyces pombe.* Arch Microbiol, 181(5); 371-377, 2004

Tsuzi, D., K. Maeta, Y. Takatsume, S. Izawa and Y. Inoue: Regulation of the yeast phospholipid hydroperoxide glutathione peroxidase *GPX2* by oxidative stress is mediated by Yap1 and Skn7. FEBS Lett 565(1-3); 148-154, 2004

Tsuzi, D., K. Maeta, Y. Takatsume, S. Izawa and Y. Inoue: Distinct regulatory mechanism of yeast *GPX2* encoding phospholipid hydroperoxide glutathione peroxidase by oxidative stress and a calcineurin/Crz1-mediated Ca^{2+} signaling pathway. FEBS Lett 569(1-3); 301-306, 2004

Takemura, R., Y. Inoue and S. Izawa: Stress response in the yeast mRNA export factor: reversible change in the localization of Rat8p is caused by ethanol stress but not heat shock. J Cell Sci 117(18); 4189-4197, 2004

b) Conference and seminar papers presented

The 77th Japanese Biochemical Society Annual Meeting: 1 paper

The 26th Annual Meeting of the Molecular Biology Society of Japan: 4 papers

The 37th Meeting of Yeast Genetics and Molecular Biology, Japan: 2 papers

The Annual Meeting of the Society for Biotechnology 2004, Japan: 2 papers

Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry 2005: 4 papers

A-3. Off-campus activities

Membership in academic societies

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

Inoue, Y.: Yeast Society (Committee Member)

Membership in Science Council of Japan, etc.

Kita, K.: Council of Fermentation and Bioconversion (Member)

Research grants

Research Grants from Ministry of Education, Culture, Sports, Science, and Technology: Grant-in-Aid for Scientific Research (B)(2) Suctural analysis of recognition mechanism of endonucleases and its application to alteration of specificity. (Kita, K.) Grant-in-Aid for Young Scientist (B)(2) Function of thioredoxin in alcoholic fermentation and its transport. (Izawa, S.)

B. Educational Activities (2004.4-2005.3)

B-1. On-campus teaching

a) Courses given

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

Graduate level: Cellular Bioenergy Conversion Seminar (Advanced course) (Kita), Cellular Bioenergy Conversion Seminar (Kita, Inoue), Experimental Course of Cellular Bioenergy Conversion (Kita, Inoue)

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 : (9)	*Reserch fellow : (1)*
Master's program : (20)	*COE research fellow : (1)*
Undergraduate : (5)	*Foreign research fellow : (1)*

A. Research Activities (2004.4-2005.3)

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: Enzymes. Bioprocesses and Biotechnology for Functional Foods and Nutraceuticals (edited by J.-R. Neese, J. Bruce German). p.197-205, Marcel Dekker, New York-Basel, 2004

Shimizu, S.: Part VI Approach to the microbial factory; chapter 11 Microbial factory -useful microorganisms-. New strategy of Bioscience (edited by Faculty of Agriculture, Kyoto University). p.146-160, Maruzen, 2004 (in Japanese)

Original papers

Ando, A., J. Ogawa, S. Kishino and S. Shimizu: Conjugated linoleic acid production from castor oil by *Lactobacillus plantarum* JCM 1551. Enzyme Microb Technol 35 (1); 40-45, 2004

Kataoka, M., A.-R. Delacruz-Hidalgo, M.A. Akond, E. Sakuradani, K. Kita and S. Shimizu: Gene cloning and overexpression of two conjugated polyketone reductases, novel aldo-keto reductase family enzymes, of *Candida parapsilosis*. Appl Microbiol Biotechnol 64 (3); 359-366, 2004

Kataoka, M., A. Kotaka, R. Thiwthong, M. Wada, S. Nakamori and S. Shimizu: Cloning and overexpression of the old yellow enzyme gene of *Candida macedoniensis*, and its application to the production of a chiral compound. J Biotechnol 114 (1-2); 1-9, 2004

Kajikawa, M., K.T. Yamato, Y. Kohzu, M. Nojiri, E. Sakuradani, S. Shimizu, Y. Sakai, H. Fukuzawa and K. Ohyama: Isolation and characterization of Δ6-desaturase, an ELO-like enzyme and Δ5-desaturase from the liverwort *Marchantia polymorpha* and production of arachidonic and eicosapentaenoic acids in the methylotrophic yeast *Pichia pastoris*. Plant Mol Biol 54 (3); 335-352, 2004

Ogawa, J., W.T. Sulistyaningdyah, Q.-S. Li, H. Tanaka, S.-X. Xie, K. Kano, T. Ikeda and S. Shimizu: Two extracellular proteins with alkaline peroxidase activity, a novel cytochrome *c* and a catalase-peroxidase, from *Bacillus* sp. No.13. Biochim Biophys Acta 1699 (1-2); 65-75, 2004

Sakuradani, E., Y. Hirano, N. Kamada, M. Nojiri, J. Ogawa and S. Shimizu: Improvement of

arachidonic acid production by mutants with lower n-3 desaturation activity derived from *Mortierella alpina* 1S-4. Appl Microbiol Biotechnol 66 (3); 243-248, 2004

Sulistyaningdyah, W.T., J. Ogawa, H. Tanaka, C. Maeda and S. Shimizu: Characterization of alkaliphilic laccase activity in the culture supernatant of *Myrothecium verrucaria* 24G-4 in comparison with bilirubin oxidase. FEMS Microbiol Lett 230 (2); 209-214, 2004

Sulistyaningdyah, W.T., J. Ogawa, Q.S. Li, R. Shinkyo, T. Sakaki, K. Inouye, R.D. Schmid and S. Shimizu: Metabolism of polychlorinated dibenzo-*p*-dioxins by cytochrome P450 BM-3 and its mutant. Biotechnol Lett 26 (24); 1857-1860, 2004

Takeno, S., E. Sakuradani, S. Murata, M. Inohara-Ochiai, H. Kawashima, T. Ashikari and S. Shimizu: Cloning and sequencing of the *ura3* and *ura5* genes, and isolation and characterization of uracil auxotrophs of the fungus *Mortierella alpina* 1S-4. Biosci Biotechnol Biochem 68 (2); 277-285, 2004

Takeno, S., E. Sakuradani, S. Murata, M. Inohara-Ochiai, H. Kawashima, T. Ashikari and S. Shimizu: Establishment of an overall transformation system for an oil-producing filamentous fungus, *Mortierella alpina* 1S-4. Appl Microbiol Biotechnol 65 (4); 419-425, 2004

Reviews

Delacruz-Hidalgo, A.-R. G., M. Kataoka and S. Shimizu: Biochemical and applied studies of microbial oxidoreductases. Ouyou biseibutsugaku kenkyu 2 (1); 39-46, 2004

Ogawa, J.: Screening and development of novel microbial reactions involved in nucleic acid and lipid metabolism. Nogei Kagaku Kaishi 78 (9); 830-835, 2004 (in Japanese)

Ogawa, J., S. Kishino, A. Ando, S. Sugimoto and S. Shimizu: Microbioal production of conjugated fatty acids. Seibutsu Kogaku Kaishi 82 (7); 285-287, 2004 (in Japanese)

Ogawa, J. and S. Shimizu: Use of enzymes as functional foods and nutritional supplements, and fermentative microorganisms. Nihon Jozo Kyokaishi 99 (12); 832-849, 2004 (in Japanese)

Ogawa, J., N. Horinouchi and S. Shimizu: Microbial production of 2'-deoxyribonucleosides. Bioscience & Industry 62 (6); 389-390, 2004 (in Japanese)

Shimizu, S.: Basic studies on development of microbial processes for useful compound production. Nogei Kagaku Kaishi 78 (1); 4-13, 2004 (in Japanese)

Shimizu, S.: Screening, development and breeding of oleaginous microoganisms, and their application to the functional lipid production. Bioscience & Industry 62 (1); 11-16, 2004 (in Japanese)

Takeno, S., E. Sakuradani and S. Shimizu: Creation and application of host-vector system in oleaginous fungus *Mortierella alpina* 1S-4. Ouyou Biseibutsugaku Kenkyu 2 (2); 82-87, 2004 (in Japanse)

Reports

Ogawa, J.: Enzyme for foods. Seibutsu Kogaku Kaishi 82 (2); 70, 2004 (in Japanese)

Sakuradani, E.: Versatile biosynthetic pathway of polyunsaturated fatty acids. Seibutsu Kogaku Kaishi 82 (6); 264, 2004 (in Japanese)

b) Conference and seminar papers presented

Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry 2004: 27 reports

Annual Meeting of the Vitamin Society of Japan 2004: 1 report

4th Collaboration Meeting on Agri-Bio Technolgy of Kyoto University: 1 report

Annual Meeting of the Society for Fermentation and Bioengineering, Japan 2004: 12 reports

Meeting of Kansai Branch of Japan Society for Bioscience, Biotechnology, and Agrochemistry 2004: 3 reports

6th CLA Seminar: 1 report

52nd Meeting of Japan Society of Enzyme Engineering: 6 reports

Challenge to the Bio-process of 21st Century: 1 report

30th Meeting of Enzyme Application Association: 1 report

437th Meeting of Kansai Branch of Japan Society for Bioscience, Biotechnology, and Agrochemistry: 1 reports

3rd Lipid Reserch Seminar: 4 reports

SFBJ Technology Seminar 2005, Biotechnics: 1 report

438th Meeting of Kansai Branch of Japan Society for Bioscience, Biotechnology, and Agrochemistry: 1 report

A-3. Off-campus activities

Membership in academic societies (roles)

Shimizu: Japan Society for Bioscience, Biotechnology, and Agrochemistry (director); The Society for Fermentation and Bioengineering, Japan (councilor); The Japanese Biochemical Society (councilor, chairman of Kinki branch); The Vitamin Society of Japan (councilor); The Society of Enzyme Engineering (committieman); Japan Bioindustry Association (councilor, editor), Japan Applied Microbiology Society (director)

Kataoka: The Society of Enzyme Engineering (secretary); The Japanese Biochemical Society (general affairs of Kinki branch); The Vitamin Society of Japan (topics editor)

Ogawa: The Society for Fermentation and Bioengineering, Japan (secretary of lipid technology group)

Sakuradani: 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 Analysis and application of membrane component molecules of lipid ball secreted by filamentous fungi (Shimizu, Kataoka, Ogawa), Exploratory Research Analysis of bio-functional regulation by Ce (Ogawa, Shimizu, Kataoka) 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)

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: 95th American Oil Chemists' Society Annual Meeting and Expo, USA (invited speaker), 2nd International Congress on Biocatalysis, biocat2004, Germany (invited speaker), 9th Swiss-Japanese Meeting on Biotechnolgy and Bioprocess Development, Switzerland (organizer, invited speaker), Japan-Finland Joint Seminar on New Aspects in Microbial Biotechnology, Kyoto (invited speaker), The 80th Anniversary JSBBA international Symposium, Tokyo (invited speaker)

Kataoka: 2nd International Congress on Biocatalysis, biocat2004, Germany (speaker), The 8th China-Japan-Korea Joint Symposium on Enzyme Engineering, China (speaker), The 4th JSPS-NRCT Joint Seminar on Development of Thermotolerant Microbial Resources and Their Applications, Yamaguchi (speaker)

Ogawa: 95th American Oil Chemists' Society Annual Meeting and Expo, USA (invited speaker), 9th Swiss-Japanese Meeting on Biotechnolgy and Bioprocess Development, Switzerland (participant), Metabolic Engineering V, USA (speaker)

Sakuradani: 95th American Oil Chemists' Society Annual Meeting and Expo, USA (speaker)

Membership in international academic societies

Shimizu: 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: Applied Miciobiology and Biotechnology (editor)

International joint researchers, overseas research surveys

Shimizu: Development of thermotolerant microbial resources and their applications in Thailand and Japan (Thailand)

Kataoka: Development of thermotolerant microbial resources and their applications in Thailand and Japan (Thailand)

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

Scholars from abroad

Post-Doctoral fellow of JSPS (1) (Germany)

B. Educational Activities (2004.4-2005.3)

B-1. On-campus teaching

a) Courses given

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

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

b) Seminars

Mini-lecture on Open-Campus 2004 of Kyoto University (Kataoka), Mini-lecture for students of Nishinomiya high school

B-2. Off-campus teaching, etc.

Part-time lecturer

Shimizu: Shiga Prefectural University (Utilization of microorganism), Osaka University (32nd International University of Microbiology of UNESCO)

Kataoka: Shiga Prefectural University (Utilization of microorganism)

B-3. Overseas teaching

Students and research fellows from abroad

Foreign students: Doctor's program (3) (China, Thailand, Indonesia), undergraduate (1) (China)

C. Other Remarks

Kataoka: "Award for young scientists 2004" by Japanese Society of Enzyme Engineering

Ogawa: "Award for young scientists 2004" by the Japan Society for Bioscience and Biotechnology, and Agrochemistry

2.3.8 Laboratory of Microbial Biotechnology

Staff *Professor : Kato, Nobuo, Dr. Agric. Sci.*
Associate Professor: Sakai, Yasuyoshi, Dr. Agric. Sci.
Assistant Professor : Yurimoto, Hiroya, Dr. (Agric. Sci.)

Students and research fellows
Research fellow : (3)
Doctor's program: (5)
Master's program: (12)
Undergraduate : (4)

A. Research Activities (2004.4-2005.3)

A-1. Main subjects

a) Development of new raw materials for microbial production

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) Microbial degradation of pollutants

Bacteria that assimilate long-chain hydrocarbons (oil wax) were found in natural sources and characterized. Microorganisms that degrade aliphatic compounds under anaerobic condition were found in natural sources and analyzed enzymologically. These organisms are promising to bioremediation of oil-spilled soil.

A-2. Publications and presentations

a) Publications

Books

Sakai, Y.: Genome analysis of a methylotrophic yeast *Pichia pastoris* (in Japanese). In: T. Imanaka. (Ed.), Genomics and proteomics. NTS, Tokyo, pp.215-220, 2004

Original reports

Kajikawa, M., K. T. Yamato, Y. Kohzu, M. Nojiri, E. Sakuradani, S. Shimizu, Y. Sakai, H. Fukuzawa and K. Ohyama: Isolation and characterization of delta(6)-desaturase, an ELO-like enzyme and delta(5)-desaturase from the liverwort Marchantia polymorpha and production of arachidonic and eicosapentaenoic acids in the methylotrophic yeast Pichia pastoris. Plant Mol Biol 54: 335-352, 2004

Komeda, T., Y. Tamai, K. Kondo, H. Yurimoto, N. Kato and Y. Sakai: Mechanism of formate-inducible gene expression and development of heterologous protein production system in the methylotrophic yeast (in Japanese). Proc Appl Microbiol Res 2(2): 90-96, 2004

Limtong, S., N. Srisuk, W. Yongmanitchai, H. Kawasaki, H. Yurimoto, T. Nakase and N. Kato: Three new thermotolerant methylotrophic yeasts, Candida krabiensis sp. nov., Candida sithepensis sp. nov., and Pichia siamensis sp. nov., isolated in Thailand. J Gen Appl Microbiol 50: 119-127, 2004

Mukaiyama, H., M. Baba, M. Osumi, S. Aoyagi, N. Kato, Y. Ohsumi and Y. Sakai: Modification of a ubiquitine-like protein Paz2 conducted micropexophagy through formation of a novel membrane structure. Mol Biol Cell 15: 58-70, 2004

Nakagawa, T., T. Ito, S. Fujimura, M. Chikui, T. Mizumura, T. Miyaji, H. Yurimoto, N. Kato, Y. Sakai and N. Tomizuka: Molecular characterization of glutathione-dependent formaldehyde dehydrogenase gene FLD1 from methylotrophic yeast Pichia methanolica. Yeast 21: 445-453, 2004

Sakai, Y., H. Takahashi, Y, Wakasa, T. Kotani, H. Yurimoto, N. Miyachi, P. P. Van Veldhoven and N. Kato: Role of a-methylacyl coenzyme A racemase in the degradation of methyl-branched alkanes by Mycobacterium sp. strain P101. J Bacteriol 186: 7214-7220, 2004

Shinoda, Y., Y. Sakai, H. Uenishi, Y. Uchihashi, A. Hiraishi, H. Yukawa, H. Yurimoto and N. Kato: Aerobic and anaerobic toluene degradation by a newly isolated denitrifying bacterium, Thauera sp. strain DNT-1. Appl Environ Microbiol 70: 1385-1392, 2004

Yurimoto, H., B. Lee, F. Yasuda, Y. Sakai and N. Kato: Alcohol dehydrogenases that catalyse methyl formate synthesis participate in formaldehyde detoxification in the

methylotrophic yeast Candida boidinii. Yeast 21: 341-350, 2004

Yurimoto, H., M. Yamane, Y. Kikuchi, H. Matsui, N. Kato and Y. Sakai: The pro-peptide of Streptomyces mobaraensis transglutaminase functions in cis and in trans to mediate efficient secretion of active enzyme from methylotrophic yeasts. Biosci Biotechnol Biochem 68: 2058-2069, 2004

Reviews

Kato, N., T. Ishige, and Y. Sakai: Wax ester production from *n*-parafin (in Japanese). Kagaku To Kogyo 78(6): 302-309, 2004

Kato, N.: Novel metabolic functions of microorganisms and their application (in Japanese). Nippon Nougeikagaku Kaishi 78(6): 548-555, 2004

Sakai, Y: Optimization of production of useful enzymes using gene expression system in methylotrophic yeasts (in Japanese). Seibutsukougaku Kaishi 82(11): 540-547, 2004

Shinoda, Y., Y. Sakai, and N. Kato: Anaerobic degradation of aromatic compounds by denitrifying bacteria: new frontier of microbial metabolism. Yosui To Haisui 47(3): 182-189, 2004

b) Conference and seminar papers presented

Annual meeting of the Japan Society for Bioscience, Biotechnology, and Agrochemistry 2005: 11 reports

Annual meeting of The Society for Biotechnology, Japan, 2004: 7 reports

Annual meeting of the Japanese Biochemical Society 2004: 2 reports

Annual Meeting of Japanese Society of Molecular Biology 2004: 1 report

Yeast Genetics and Molecular Biology News Japan No. 37: 5 reports

17th Annual meeting of Japan Society for Archaea: 1 report

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

3rd Mini Symposium of 21st Century COE: 1 report

7th Symposium of Research Center for Water Environment Technology: 1 report

Open Symposium of Protein Degradation: 1 report

Amano Enzyme 5th Practical Symposium: 1 report

16th Joint Symposium on Yeasts: 1 report

A-3. Off-campus activity

Membership in academic societies (roles)

Kato, N.: Japan Society for Bioscience, Biotechnology, and Agrochemistry (Councilor). The Society for Fermentation and Bioengineering, Japan (Inspector).

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

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 (B)(2): Molecular basis and its application of formaldehyde-fixing reactions in bacteria and archaea (Kato), Exploratory research: Metabolic engineering

for bacterial wax ester production (Kato), Scientific research on priority areas: Regulation of de novo membrane formation and physiological and Biochemichal function of phospho-lipid-binding domain (Sakai), Young Scientists (B): Transcriptional activator and signal transduction in methanol-inducible gene expression in yeasts (Yurimoto).

Other Research grant: Research grant from RITE: Screening of novel microorganisms (enzymes) that can uptake CO_2 (Kato), Research grant from RITE: Studies on degradation mechanism of aromatic ring" (Kato), Research grant from Toyobo Co. Ltd.: Development and its application of functional protein probes by using *in vivo* manipulation technology within cells as a verification system" (Sakai), Fermentation and Metabolism Resarch Foundation: Gene expression of C1 metabolism in microorganisms and its application (Yurimoto).

A-4. International cooperation and overseas activities

International meetings (roles)

Kato, N.: Gordon Resarch Conference on Molecular Basis of Microbial One-Carbon Metabolism, USA (invited speaker). The 95th AOCS Annual Meeting & EXPO, USA (invited speaker). The 4th JSPS-NRCT Joint Seminar on Development of Thermotolerant Microbial Resources and Their Applications, Fukuoka (invited speaker). The 9th Swiss-Japanese Joint Meeting on Biotechnology and Bioprocess Development, Switzerland (invited speaker).

Sakai, Y.: Gordon Resarch Conference on Molecular Basis of Microbial One-Carbon Metabolism, USA (invited speaker). The American Society for Cell Biology 44th Annual Meeting, USA (invited speaker), 2004 International Meeting of the Microbiological Society of Korea, Korea (invited speaker).

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

Membership in international academic societies

Kato, N.: Biodegradation (editor)

International joint researches

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

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 (8) (Max-Planck Institute, Germany, Professor; University of Freiburg, Germany, Associate Professor; University of Marburg, Germany, Associate Professor; Oregon State University, USA, Professor; Robert Wood Johonson Medical School, USA, Professor; University of Washington, USA, Professor; Korea University, Korea, Professor; Chinease Academy of Sciences, China, Associate Professor)

B. Educational Activities (2004.4-2005.3)

B-1. On-campus teaching

a) Courses given

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

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

B-2. Off-campus teaching, etc.

Open Lecture

Sakai, Y.: Kyoto University Internationla Innovation Center "Free Discussion on University-Industry Collaborative Research (lecture. Carl Zeiss Microscopic Imaging Seminar (lectiure).

B-3. Overseas teaching

Lecture in abroad

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

C. Other Remarks

Yurimoto, H., Kato, N., Sakai, Y.: The Japan Bioscience, Biotechnology and Agrochemistry Society Award for Excellence to Authors Publishing in Bioscience, Biotechnology, and Biochemistry in 2004.

Sakai, Y.: Aword for Amano Enzyme 5th Practical Symposium. 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* *: Ikeda, Tokuji, Dr. Agric. Sci.*
Associate Professor: Kakutani, Tadaaki, Dr. Agric. Sci.
Associate Professor: Kano, Kenji, 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 (2004.4-2005.3)

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), and molecular cloning and structural analysis of histamine dehydrogenase. Theory of bioelectrocatalytic reactions. Fundamental study of spectroelectrochemistry based on sepalator-less bulk electrolysis. Direct electron transfer of redox proteins at surface structure-regulated electrodes.

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

Bilirubin oxidase as a very efficient catalyst for electrocatalytic reduction of dioxygen to water at pH 7.0 (excellent mediator properties of cyano-metal complexes, preparation of mediator-enzyme-modified electrodes, and direct bioelectrocatalysis). Functional analysis of the enzymes involved in the energy conversion system of *E. coli*. Bioelectrocatalytic oxidation of alcohol catalyzed by whole cells of acetic acid bacteria, and the utilization in an electrochemical H_2 production. Diffusion-controlled electrochemical oxidation of NADH at a diaphorase/naphthoquinone derivative-modified electrode. Modification of bioanodes based on quinoproteins.

c) Construction of electrochemical biosensing systems.

Fundamental study for the development of a micro H_2O_2 sensor and the development of a cholesterol sensor based on the micro H_2O_2 sensor. Development of a method of total-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

Fujieda, N., A. Satoh, N. Tsuse, K. Kano and T. Ikeda: 6-S-Cysteinyl Flavin Mononucleotide-containing Histamine Dehydrogenase from Nocardioides simplex: Molecular Cloning, Sequencing, Over-expression and Characterization of Redox Centers of Enzyme. Biochemistry 43 (33); 10800–10808, 2004

Hayashi, Y., T. Shimasaki, H. Takasu, K. Kano and T. Ikeda: Cholesterol Sensors Composed of Multienzyme Systems and Its Application to Flow-type Sensor. Chem. Sens. (Supplement B); 66–67, 2004

Kojima, S., S. Tsujimura, K. Kano, T. Ikeda, M. Sato, H. Sanada, H. Omura and S. Shimizu: Fundamental Study for an Oxygen-insensitive Amperometric Glucose Sensor Using a Novel Glucose Dehydrogenase. Chem Sens (Supplement B); 768–769, 2004

Kuriyama, A., M. Arasaki, N. Fujieda, S. Tsujimura, K. Kano and T. Ikeda: Separator-less One-compartment Bulk Electrolysis with a Small Auxiliary Electrode and Its Application to Spectroelectrochemistry. Electrochemistry 72 (7); 484–486, 2004

Kondo, K., E. Kondo, H. Maki, K. Yasumoto, K. Takagi, K. Kano and T. Ikeda: Purification and Characterization of Aromatic Amine Dehydrogenase from Alcaligenes xylosoxidans. Biosci Biotech Biochem 68 (9); 1921–1928, 2004

Ogawa, J., W. T. Sulistyaningdyah, Q,-A. Li, H. Tanaka, S.-X. Xie, K. Kano, T. Ikeda and S. Shimizu: Two Extracellular Proteins with Alkaline Peroxidase Activity, A Novel Cytochrome c and a Catalase-peroxidase, from Bacillus sp. No. 13. Biochim Biophys Acta 1699 (1/2); 65–75, 2004

Sakaguchi, S., K. Kano and T. Ikeda: Effect of pH on the Hydrogenase Activity of Desulfovibrio vulgaris. Electroanalysis 16 (13/14); 1166–1171, 2004

Tsujimura, S. K. Kano and T. Ikeda: Kinetic Study of Direct Electron Transfer between Enzyme and Carbon Electrodes. Chem Sens (Supplement B); 770–771, 2004

Tsujimura, S., T. Nakagawa, K. Kano and T. Ikeda: Kinetic Study of Direct Bioelectrocatalysis of Dioxygen Reduction with Bilirubin Oxidase at Carbon Electrodes. Electrochemistry 72 (6); 437–439, 2004

Reviews and others

Tsujimura, S., K. Kano and T. Ikeda: Biofuel Cells Basic Principle and Applications. Bioscience and Industry 62(3); 175-178, 2004

Tsujimura, S., K. Kano and T. Ikeda: New Posibility Driven by Apactical Application ofBiofuel Cells. Material Stage 3(12); 60-65, 2004

Tsujimura, S., K. Kano and T. Ikeda: Structrue and Principle of Biofuel Cells. ChemicalEngineering 49 (9); 717–722, 2004

Ikeda, T.: A Novel Electrochemical Approach to the Characterization of Oxidoreductase Reactions. The Chemical Record 4, 192-203, 2004

b) Conference and seminar papers presented.

The Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry in 2004: 6 reports

The 65th Meeting of the Japan Society for Analytical Chemistry: 1 reports

The 54th Annual Meeting of the Japan Society for Analytical Chemistry: 4 reports

Kansai Branch Annual Meeting of Japan Society for Bioscience, Biotechnology, and

Agrochemistry: 2 reports

The 77th Annual Meeting of The Japanese Biochemical Society: 3 reports

The 50th Annual Meeting on Polarography and Electroanalytical Chemistry: 9 reports

The 433th Kansai Branch Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry: 1 reports

The 73th Electrochemical Society Meeting: 3 reports

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

The 27 th Annual Meeting of the Membrane Society of Japan: 1 report

A-3. Off-campus activities

Membership in academic societies (roles)

Ikeda T.: The Japan Society for Bioscience, Biotechnology, and Agrochemistry (a council member); The Polarographic Society of Japan (an Auditor), Kinki Branch of The Japan Society for Analytical Chemistry (an auditor and chairman of the 50th anniversaly)

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)

Ikeda, T.: XVIIth International Symposium on Bioelectrochemistry and Bioenergetics, Florence, June. (one presentation).

Kano, K.: XVIIth International Symposium on Bioelectrochemistry and Bioenergetics, Florence June. (one presentation).

Ikeda, T.: 204th Meeting of The Electrochemical Society, Orlando, October (Organizer and a key note speaker of the session: Biological Fuel Cells).

Tsujimura, S. Kano, K.and Ikeda, T.: 204th Meeting of The Electrochemical Society, Orlando, October ; (one presentation).

Ikeda, T.: The 12th Japanese-Germany Workshop on Enzyme Technology, Ohtsu, November (invited speaker).

Ikeda, T.: International Seminar on Sensor and Biosenor, Bogor, March, 2004 (principal guest and plenarylecturer).

Kano, K.: Japan-U.S. Joint Scientific Project (member)

Ikeda, T.: The 8th Word Congress on Biosensors, Granada, Spain, March (invited lecture), International Society of Electrochemistry – 55th Annual Meeting, Thessaloniki, Greece, September (one presentation)

Kano, K.: The 8th Word Congress on Biosensors, Granada, Spain, March (one presentation), Post

Symposium of International Conference for Analytical Surface Chemistry, ,Tsukuba, July (invited lecture), International Society of Electrochemistry – 55th Annual Meeting, Thessaloniki, Greece, September (one presentation)

Analytical Biochemistry (an editorial board member)

Tsujimura, S. The 10th International Meeting on Chemical Sensors,Tsukuba, July (one presentation), 206th Meeting of The Electrochemical Society, Hawai, U.S.A. December (one presentation)

Scholars from abroad

Collaborative researcher from Taiwan and chaina (2)

B. Educational Activities (2004.4-2005.3)

B-1. On-campus teaching

a) Courses given

Undergraduate level: Biophysical Chemistry I (Ikeda), Biophysical Chemistry II (Ikeda), Introduction to Applied Life Science I (Ikeda), Analytical Chemistry (Kano), Laboratory Course in Analytical Chemistry (Kano, Tsujimura), Laboratory Course in Biophysical Chemistry (Kakutani, Kano, Tsujimura), New Strategies in Agricultural Sciences (Ikeda and others)

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

B-2. Off-campus teaching, etc.

Part-time lecturer

Kano, K.: Kyoto Institute of Technology (Bioelectrochemistry), Shiga Medicinal University (Chemistry)

C. Other remarks

Kano, K., The Electrochemical Society, Scientific Award in 2004.

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 : (9)*
Undergraduate : (4) *CREST Research Fellow: (1)*

A. Research Activities (2004.4-2005.3)

A-1. Main subjects

a) Isolation of odorant receptors in insects and plants.

It is generally accepted that in mammals, each odorant receptor detects not a specific odor substance but a group of odor substances. So it is difficult to find the relationships between the structure of odorant receptors and that of odorants. On the other, such relationship is obvious between the sex pheromones, bombykol and bombykal, released by female silkworm moths and their receptors. Their receptors have not been isolated. We searched odorant receptor genes and found 20 genes in the silkworm moth genome. Among them, two are expressed only in the male moth antennae. By *in situ* hybridization, one of the two is expressed in odorant neurons surrounded by the cells that the pheromone binding protein is expressed. We suppose that these two are the receptors of bombykol and bombykal. To confirm our identification, we are now constructing the male silk moths in which the bombykol receptor gene is suppressed by RNAi.

b) Development of a comprehensive and high-throughput chemical analysis for metabolites.

Most small molecular metabolites are ionic and difficult to measure because no analytical method was available for them. Recently we have successfully developed a method for metabolomics by using capillary electrophoresis coupled to mass spectrometry (CE/MS) and applied it to *Escherichia coli*, *Bacillus subtilis*, and *Oryza sativa*. Profile of metabolites varied with the environmental perturbations and mutations in a highly sensitive and specific manner. With a combination of DNA microarray and proteomics, metabolomics are a powerful tool for functional genomics, systems biology, and metabolic engineering.

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 Arabidopsis mutants.

A-2. Publications and presentations

a) Publications

Original papers

Abe, M., A. Kenmochi, N. Ichimaru, T. Hamada, T. Nishioka and H. Miyoshi: Essential structural features of acetogenins: role of hydroxy groups adjacent to the bis-THF rings. Bioorg Med Chem Lett 14; 779-782, 2004

Elias, M. D., S. Nakamura, C. T. Migita, H. Miyoshi, H. Toyama, K. Matsushita, O. Adachi and M. Yamada: Occurrence of a bound ubiquinone and its function in *Escherichia coli* membrane-bound quinoprotein glucose dehydrogenase. J Biol Chem 279; 3078-3083, 2004

Hamada, T., N. Ichimaru, M. Abe, D. Fujita, A. Kenmochi, T. Nishioka, K. Zwicker, U. Brandt and H. Miyoshi: Synthesis and inhibitory action of novel acetogenin mimics with bovine heart mitochondrial complex I. Biochemistry 43; 3651-3658, 2004

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 Δlac-acetogenins: a novel type of inhibitor of bovine heart mitochondrial complex I. Biochemistry 44, 816-825, 2005

Itoh, A., Y. Ohashi, Y. Soga, H. Mori, T. Nishioka and M. Tomita: Application of capillary electrophoresis-mass spectrometry to synthetic *in vitro* glycolysis studies. Electrophoresis 25, 1996-2002 2004

Jang, S.-M., A. Ishihara and K. Back: Production of coumaroylserotonin and feruloylserotonin in transgenic rice expressing pepper hydroxycinnamoyl-CoA: serotonin *N*-(hydroxycinnamoyl)transferase. Plant Physiol 135, 346-356, 2004

Jia, L., B.-F. Liu, S. Terabe and T. Nishioka: Two-dimensional separation method for analysis of Bacillus subtilis metabolites via hyphenation of micro-liquid chromatography and

capillary electrophoresis. Anal Chem 76, 1419-1428 2004

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

Konno, H., N. Hiura, H. Makabe, M. Abe and H. Miyoshi: Synthesis and mitochondrial complex I inhibition of dihydroxy-cohibin A, non-THF annonaceous acetogenin analogue. Bioorg Med Chem Lett 14; 629-632, 2004

Makabe, H., Y. Hattori, Y. Kimura, H. Konno, M. Abe, H. Miyoshi, A. Tanaka and T. Oritani: Total synthesis of cis-solamin and its inhibitory action with bovine heart mitochondrial complex I. Tetrahedron 60; 10651-10657, 2004

Makabe, H., A. Miyawaki, R. Takahashi, Y. Hattori, H. Konno, M. Abe and H. Miyoshi: Synthesis of two possible diastereomers of reticulatain-1. Tetrahedron Lett 45; 973-977, 2004

Oikawa, A., A. Ishihara, C. Tanaka, N. Mori, M. Tsuda and H. Iwamura: Accumulation of the activated benzoxazinone glucoside (HDMBOA-Glc) is induced by biotic stresses prior to the release of a benzoxazolinon (MBOA) in maize leaves. Phytochemistry 65, 2995-3001, 2004

Okazaki, Y., A. Ishihara, T. Nishioka and H. Iwamura: Identification of a dehydrodimer of avenanthramide B in elicited oat leaves. Tetrahedron 60, 4765-477, 2004

Okazaki, Y., T. Isobe, Y. Iwata, T. Matsukawa, F. Matsuda, H. Miyagawa, A. Ishihara, T. Nishioka and H. Iwamura: Metabolism of avenanthramide phytoalexins in oats. Plant J 39, 560-572, 2004

Ozawa, R., K. Shijiri, M. W. Sabelis, G. Arimura, T. Nishioka and J. Takabayashi: Corn plants treated with jasmonic acid attract more specialist parasitoids, thereby increasing parasitization of the common armyworm. J Chem Ecol 30, 1797-1808 2004

Ritter, M., H. Palsdottir, M. Abe, W. Mäntele, C. Hunte, H. Miyoshi and P. Hellwig: Direct evidence for the interaction of stigmatellin with a protonated acidic group in the bc_1 complex from S. cerevisiae as monitored by FTIR difference spectroscopy and ^{13}C specific labeling. Biochemistry 43, 8439-8446, 2004

Sakurai, T., T. Nakagawa, H. Mitsuno, H. Mori, Y. Endo, S. Tanoue, Y. Yasukochi, K. Touhara and T. Nishioka: Identification and functional characterization of a sex pheromone receptor in the silkmoth *Bombyx mori*. Proc Nat Acad Sci USA 101, 16653-16658 2004

Sato, S., T. Soga, T. Nishioka and M. Tomita: Simultaneous determination of the main metabolites in rice leaves using capillary electrophoresis mass spectrometry and capillary electrophoresis diode array detection. Plant J 40, 151-163 2004

Soga, T., Y. Kakazu, M. Robert, M. Tomita and T. Nishioka: Qualitative and quantitative analysis of amino acids by capillary electrophoresis electrospray ionization tandem mass spectrometry. Electrophoresis 25, 1964-1972 2004

Yamashita, T., T. Ino, H. Miyoshi, K. Sakamoto, A. Osanai, E. Nakamaru-Ogiso and K. Kita: Rhodoquinone reaction site of mitochondrial complex I in parasitic helminth, *Ascaris suum.* Biochim Biophys Acta (Bioenergetics), 1608; 97-103, 2004

Yang, Q., H. X. Trinh, S. Imai, A. Ishihara, L. Zhang, H. Nakayashiki, Y. Tosa and S. Mayama: Analysis of the involvement of hydroxyanthranilate hydroxycinnamoyltransferase and caffeoyl-CoA 3-*O*-methyltransferase in phytoalexin biosynthesis in oat. Mol

Plant-Microbe Interact 44, 81-89 2004

Reviews

Nishioka, T.: Reconstruction of a living cell from the genome, In *New Bioscience* edited by Graduate School of Agriculture, Kyoto University. Maruzen, Tokyo. pp.161-178, 2004

Nishioka, T.: Metabolome analysis; from the development to application. Bioindustry 62, 229-233, 2004

b) Conference and seminar papers presented

Annual Meeting of Japanese Society of Bioscience, Biotechnology, and Agrochemistry 2005: 9 reports

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

The 15th Joint Symposium of Tokai and Hokuriku Branches of Japanese Society of Biomedical Mass Spectrometry: 1 report (invited speaker)

The 27th Annual Meeting of the Molecular Biology Society of Japan: 2 reports (Workshop Organizer: 1)

The 30th Annual Meeting of Pesticide Science Society of Japan: 1 report

The 2nd Symposium "Function and Regulation of Plants", Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency (JST): 4 reports

Division of Technology and Informatics, Japan Bioindustry Association (JBA) "Significance and Application of Metabolome Analysis in Bioscience": 1 report

The 12nd Meeting of the Genome based Drug Discovery Forum. Pharmacoinfomatics: 1 report (invited speaker)

Riken Symposium "The 5th Frontier Symposium on the Analytical Technology and Chemistry": 1 report

The Workshop on the Chemical Acceptance of Insects: 1 report

The 6th Workshop on the System Biology: 1 report

The symposium of Annual Meeting of Japanese Society of Bioscience, Biotechnology, and Agrochemistry 2005: 1 report

A-3. Off-campus activities

Membership in academic societies

Nishioka, T.: Japanese Society of Bioinformatics (councilor)

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

Research grants

Monbu-Kagakusho Research Grant: Grants-in-Aids for Priority Areas Research, Development of the comprehensive chemical analysis for the measurement of the cellular concentrations of metabolites (Nishioka, head); Grants-in-Aids for Scientific Research (A), Molecular basis of chemical communications among plants (Nishioka, member); Grants-in-Aids for Scientific Research (B), Synthetic studies of the molecular probes for elucidation of respiratory enzymes in C. elegans (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 Young Scientists (B) Involvement of hydroxycinnamic acid amides in the defense mechanism in gramineous plants (Ishihara,

head).

Others: Grants for the Rice Genome Research from the Ministry of Agriculture, Forestry, and Fishery, Japan (Nishioka, head); 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.: 2nd International E.Coli Alliance Conference on Systems Biology Project Gemini, Banff, Alberta, Canada, June 18-22, 2004 (1 report)

Nishioka, T.: ISOT/JASTS 2004, Kyoto, July 5-9, 2004 (2 report)

Nishioka, T.: 5th International Conference on Systems Biology. Convention Center, Heidelberg. Germany, October 9-13, 2004 (2 report)

Nishioka, T.: 5th Asia-Pacific International Symposium on Microscale Separations and Analysis, Hotel President, Seoul, South Korea, December 5-8, 2004 (invited speaker)

Nishioka, T.: Genome Informatics Workshop 2004, Pacifico Convention Plaza Yokohama, Japan, December 13-15, 2004 (1 report)

Nishioka, T.: The Pacific Symposium on Biocomputing (PSB) PSB2005, The Fairmont Orchid, Hawaii, U.S.A., January 4-8, 2005 (1 report)

Miyoshi, H.: 13th European Bioenergetics Conference (1 report)

International joint researches

Miyoshi: 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: Characterization of rice plants expressing THT gene form pepper (Korea)

B. Educational Activities (2004.4-2005.3)

B-1. On-campus teaching

a) Courses given

Undergraduate level: Bioorganic chemistry II (Nishioka, Miyoshi), Structure analysis of organic compounds (Nishioka), Biotechnology – Strategy of agricultural sciences- (Nishioka), Introduction of Applied Life Sciences IV (Miyoshi), Laboratory Course in Bioorganic Chemistry (Miyoshi, Ishihara)

Graduate level: Chemistry of biologically active compounds (Nishioka), Biofunction chemistry seminar (Nishioka, Miyoshi), Experimental course of biofunctional chemistry (Nishioka, Miyoshi).

B-2. Off-campus teaching, etc.

Part-time lecturer

Nishioka, T.: Professor, Graduate School of Media and Governance, Keio University.

2.3.11 Laboratory of Applied Structural Biology

Staff *Professor* *: Hirose, Masaaki, D. Agric. Sci.*
Associate Professor: Aibara, Shigeo, D. Agric. Sci.
Assistant Professor : Takahashi, Nobuyuki, D. Agric. Sci.
Mizutani, Kimihiko, D. Agric. Sci.

Students and research fellows
Doctor's program: (1) *Master's program: (3)*
Undergraduate : (2)

A. Research Activities (2004.4-2005.3)

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

Books

Aibara, S. et al.: Crystallization of Proteins, Kyoto University Press, Kyoto, 2005

Original papers

Aibara, S., N. Ogawa, and M. Hirose: Microstructures of bread dough and the effects of shortening on frozen dough. Biosci. Biotech. Biochem., 69; 397-402, 2005

Okamoto I., K. Mizutani, M. Hirose: Iron-binding process in the amino- and carboxyl-terminal lobes of ovotransferrin: quantitative studies utilizing single Fe^{3+}-binding mutants. *Biochemistry* 43; 11118-11125, 2004

b) Conference and seminar papers presented

The 2003 Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry : 2 papers

The meeting of Kansai Branch of the Japan Society for Bioscience, Biotechnology, and Agrochemistry: 1 paper

The 2003 Annual Meeting of the Crystallographic Society of Japan: 1 paper

The 77th Annual Meeting of The Japanese Biochemical Society: 2 papers

The 10th International Conference on the Crystallization of Biological Macromolecules: 1 paper

A-3. Off-campus activities

Membership in academic societies

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

Research grants

Monbu-Kagakusho Research Grant: 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) (Takahashi, N.) Grant-in-Aid for Young Scientists (B). Structural mechanism of alminium ion binding by transferrin (Principal investigator) (Mizutani, K.).

A-4. International cooperations and overseas activities

International joint researchers, overseas research surveys

Aibara: Space Experiment of Crystal Growth of Proteins (Russia)

B. Educational Activities (2004.4-2005.3)

B-1. On-campus teaching

a) Courses given

Undergraduate level: Introduction into Applied Life Sciences III (Hirose), Chemistry of Bio-catalist (Hirose), Laboratory Course in Biological Chemistry (Aibara, Takahashi, Mizutani)

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

B-2. Off-campus teaching, etc.

Part-time lecturer

Hirose, Masaaki: Kyoto Prefectural University, Graduate Course (Protein engineering)
Aibara, Shigeo: Mukogawa Women's University, Junior College Division (Biochemistry)

B-3. Overseas teaching

Students and research fellows from abroad

Foreign Student: 1 (China)

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 (2004.4-2005.3)

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 which many β-glucosidases from various plants are also present. In addition to β-primeverosidase, various kinds of disaccharide-specific diglycosidases such as β-acuminosidase, β-vicianosidase, and β-rutinosidase are present in a wide range of plant kingdom, and we isolated the diglycosidase cDNAs encoding furcatin hydrolase and vicianin hydrolase. 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. After the deglycosidation with mannosidase, we have succeeded in crystallization of β-primeverosidase.

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

The glycosylamidines were elaborated for selective inhibitiors of glycosidases and were applied for glycosidase studies as research tools. A series of glycosylamidine derivatives with

B-2. Off-campus teaching, etc.

Part-time lecturer

Hirose, Masaaki: Kyoto Prefectural University, Graduate Course (Protein engineering)
Aibara, Shigeo: Mukogawa Women's University, Junior College Division (Biochemistry)

B-3. Overseas teaching

Students and research fellows from abroad

Foreign Student: 1 (China)

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 (2004.4-2005.3)

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 which many β-glucosidases from various plants are also present. In addition to β-primeverosidase, various kinds of disaccharide-specific diglycosidases such as β-acuminosidase, β-vicianosidase, and β-rutinosidase are present in a wide range of plant kingdom, and we isolated the diglycosidase cDNAs encoding furcatin hydrolase and vicianin hydrolase. 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. After the deglycosidation with mannosidase, we have succeeded in crystallization of β-primeverosidase.

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

The glycosylamidines were elaborated for 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 assayed for inhibition of family 20 *N*-acetylglucosaminidase. The cyclic glucosylamidines were found to serve as extremely potent and selective inhibitors of this class of enzymes, indicating that the glycosylamidines serve as "tailor-made" inhibitors according to the reaction mechanisms, as well as to the substrate specificities of enzymes. These properties of glyccosylamidines have been used successfully as a ligand for affinity chromatography of glycosidases for purification according to the enzyme's glycon substrate specificity. The diglycoside-specific glycosidases have also been purified successfully by β-primeverosylamidine as an affinity ligand. For assay of *in vivo* activities, the glycosylamidnes were applied to seedlings of *Arabidopsis thaliana* to observe an interesting morphological change that was dependent on the glycon moiety of glycosylamidines.

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 and were assayed for irreversible inhibition of *E. coli* and human GGTs. For both the enzymes, the monophosphonates served as a potent affinity-labelling agent that modified the catalytic Thr to cause time-dependent inhibition of the enzymes. However, the effect of the electronic nature of the leaving group (*p*-substituted phenols) on the rate of enzyme inactivation (a Broensted plot) has 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. The monophosphonates were thus found to serve as useful tools to unravel the structure and mechanisms of GGTs. Novel phosphonate-based inhibitors have been synthesized as potent inhibitors of human GGT.

d) Directed evolution of *Pseudomonas* lipase:

Pseudomonas lipase is a synthetically useful and chiral acyl-transfer catalyst with extremely broad substrate specificity. Directed evolution of this lipase was explored for improved amide-hydrolyzing activities. The gene encoding the lipase was randomized by error-prone PCR, and the mutant lipases expressed in *Ps. aeruginosa* were screened for improved amide-hydrolyzing activity. A sensitive colony assay identified six mutant lipases with improved amidase activity. The amino acid sequences of the mutant lipases was determined to find that the mutations are located far from the active site, but near the Ca^{2+} binding site on the surface of the protein. A highly sensitive active-site titration of lipase using a novel fluorescent phosphonate affinity-labelling agent was used to determine the moles of the active mutant enzymes. By this method, the molecular activities for ester- and amide-hydrolyzing activities have been calculated for mutant lipases to find that the absolute activities for the ester and the amide vary independently according to the mutational site.

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 and catabolism of phytohormones such as abscisic acid (ABA) and brassinosteroid (BR). ABA is

involved in normal growth and development as well as in adaptive responses to environmental stresses. The hydroxylation at the 8'-position of ABA is known as the key step of ABA catabolism, and we previously identified a P450 CYP707A as ABA 8'-hydroxylase. We have found that uniconazole-P, known as a gibberellin biosynthesis inhibitor, strongly inhibits CYP707A activity. The uniconazole-P treated plants accumulated ABA and showed remarkable drought tolerance, indicating that the P450 is a noble target enzyme for plant growth regulator. In addition, we have studied on biochemistry of P450 enzymes in BR biosynthesis and catabolism and found new reactions and pathways in the activation/inactivation process of BR.

f) Coumarin biosynthesis in plants:

The coumarin derivatives are often found in the plant kingdom, although physiological and biological functions of the coumarin derivatives are not obvious. Investigation into the coumarin contents in wild type and the mutants of Arabidopsis was performed, resulting that the roots of Arabidopsis accumulates 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. Investigation into the glucosylation step of scopoletin is also performed.

g) Clarification of molecular basis of 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-dimethyl-3.7-octadiene-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.

A-2. Publications and presentations

a) Publications

Books

Mizutani, M. and K. Sakata: C. Enzymes work on glycosides and oligosaccharides, ß-primeverosidase (EC 3.2.2.149), "Hirokawa Protein Chemistry, Vol. 4, Enzymes 4.3 Hydrolases [II]", Ed. by Irie M, Tsuru D, *etc.* Hirokawa Shoten, Tokyo, pp. 232-240, 2004 (in Japanese)

Guo W, Lu Y, Luo S. and K. Sakata: "All about Chinese Dark Tea produced *via* microbial fermentation process", Saiwai Shobo, Tokyo, 2004 (in Japanese)

Reviews

Guo W, Lu Y, Luo S, Sakata, K: Dark tea --- Tea produced *via* microbial fermentation process,

Nippon Shokuhin Kagaku Kogaku Kaishi, **51**(7); 323-331, 2004

Mizutani M, Y-O. Ahn and K. Sakata: Plant original disaccharide-specific glycosidases --- Coevolution of the enzymes with substrates is a way of self-defense of the plants?, Kagakuto Seibutsu **42**(12); 774-776, 2004

Sakata K: Attracted to the floral aroma of oolong tea --- Clarification of biological roles of the enzymes involved in the aroma formation ---, Chemistry, 59(8); 18-23, 2004

Original papers

Ahn Y-O, M. Mizutani, H. Saino and K. Sakata: Furcatin Hydrolase from *Viburnum furcatum* Blume Is a Novel Disaccharide-specific Acuminosidase in Glycosyl Hydrolase Family 1. J Biol Chem 279(22); 23405-23414 (2004).

Amarume, S, T. Murata, T. Tsuruhami, S. Mori, J. Hiratake, K. Sakata and T. Usui: Characterization and utilization of a β-primeverosidase-like endo-β-glycosidase from *Penicillium multicolor*. Glycobiology 14; 1082-1082 (2004)

Cho J-Y., B. Shimizu, T. Kinoshita, M. Mizutani, K-R. Chen, C-L. Chen and K. Sakata: Chemical Profiling in the Manufacturing Process of "Oriental Beauty", 2004 International Conference on O-Cha (tea) Culture and Science, (Shizuoka, Nov. 4-6) p. 260-262 (2005).

Fujii, R., Y. Nakagawa, J. Hiratake, A. Sogabe and K. Sakata: Directed evolution of *Pseudomonas aeruginosa* lipase for improved amide-hydrolyzing activity. Protein Engineering, Design and Selection 18; 93-101 (2005).

Hayashi, S., K. Yagi, T. Ishikawa, M. Kawasaki, T. Asai, J. Picone, C. Turnbull, J. Hiratake, K. Sakata, M. Takada, K. Ogawa and N. Watanabe: Emission of 2-Phenylethanol from its β-D-Glucopyranoside and the Biogenesis of these Compounds from [$^2H_8$] L-Phenylalanine in Rose Flowers, Tetrahedron 60; 7005-7013 (2004)

Hibi T., H. Nii, T. Nakatsu, A. Kimura, H. Kato, J. Hiratake and J. Oda: Crystal structure of gamma-glutamylcysteine synthetase: Insights into the mechanism of catalysis by a key enzyme for glutathione homeostasis. Proc Natl Acad Sci USA 101; 15052-15057 (2004).

Katayama, T, A. Sakuma, T. Kimura, Y. Makimura, J. Hiratake, K. Sakata, T. Yamanoi, H. Kumagai, and K. Yamamoto: Molecular Cloning and Characterization of *Bifidobacterium bifidum*1,2-α-L-Fucosidase (AfcA), a Novel Inverting Glycosidase (Glycoside Hydrolase Family 95). J Bacteriol 186(15); 4885-4893 (2004).

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

Kinoshita T, J-Y. Cho, M. Mizutani, B. Shimizu, H-T. Tsai, Y-L. Chen, M-L.Lin and K. Sakata: Gene Expression Profiling in the Manufacturing Process of "Oriental Beauty", Proceedings of 2004 International Conference on O-Cha (tea) Culture and Science, p. 161-164 (2005).

Nakanishi T, Y. Ohki, J. Oda, M. Matsuoka, K. Sakata and H. Kato: Purification, Crystallization and Preliminary X-Ray Diffraction Studies on Pyruvate Phosphate Dikinase from Maize. Acta Cryst D60; 193-194 (2004)

Ohta, D. and M. Mizutani: Redundancy or flexibility: Molecular diversity of the electron transfer components for P450 monooxygenases in higher plants. Frontiers in Bioscience 9; 1587-1597 (2004)

Ogura, M, I. Terada, F. Shirai, K. Tokoro, K-R. Chen, C-L. Chen, M-L. Lin, B. Shimnizu, T.

Kinoshita and K. Sakata: Tracing Aroma Characteristics Changes during Porecessing of the Famous Formosa Oolong Tea "Oriental Beauty", Proceedings of 2004 International Conference on O-Cha (tea) Culture and Science, p. 240-242 (2005).

Saito, S, N. Hirai, C. Matsumoto, H. Ohigashi, D. Ohta, K. Sakata and M. Mizutani: Arabidopsis *CYP707A*s Encode (+)-Abscisic Acid 8'-Hydroxylase, a Key Enzyme in the Oxidative Catabolism of Abscisic Acid. *Plant Physiology* 134, 1439-1449 (2004).

Sakata, K., M. Mizutani and Guo W. Ma S-J: Improvement of Flavour Quality of CTC Black Tea by Glycosidases in Tea Leaves. Internat. J Tea Sci 3 (3&4); 167-173 (2004).

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. Naturforschung 60 c, 83-90 (2005)

Sawai, Y., Y. Yamaguchi, Y. Mizukami, K. Sakata and N. Watanabe: Comparison of Radical-Scavenging Abilities and Influence of Conjugated Double Bond on Antioxidative Activities of Tea Polyphenols, 2004 International Conference on O-Cha (tea) Culture and Science, (Shizuoka, Nov. 4-6) p. 256-257 (2005).

b) Conference and seminar papers presented

The 7th International Symposium on Cytochrome P450 Biodiversity and Biotechnology (Awaji, 2004.8.1-5): 3 papers

The 18th International Conference on Plant Growth Substances (Canberra, 2004.9.20-24): 4 papers

The 27th Annual Meeting of the Molecular Biology Society of Japan (Kobe, 2004.12.8-11): 1 paper

The 2004 International Conference on O-Cha (tea) Culture and Science (Shizuoka, 2004.11.4-6): 4 papers

The 46th Symposium on the Chemistry of Natural Products (Hiroshima, 2004.10.6-8): 1 paper

The 70th Anniversary Memorial Meeting and Symposium of Kansai Branch of Japan Society for Bioscience, Biotechnology, and Agrochemistry (Hikone, 2004.10.2-3): 4 papers

The 2002 Annual Meeting of the Japanese Society for Applied Glycoscience (Kagoshima, 2004.9.15-16): 2 papers

German-Japan Seminar on Molecular Regulation of Plant Secondary Metabolism (Kisaradzu, 2004.9.20-23): 1 paper

The 30th Meeting of Applied Glycoscientists (Kyoto, 2004.11.19): 1 paper

The 1st International Forum of Pu-erh Tea (Kunming, 2004.11.24-26): 1 paper

Enzyme Molecular Design Forum (Tokyo, 2004.11.26): 1 paper

1st Education Development Seminar (Kanazawa, 2004. 11.28): 1 paper

104th Annual Research Seminar at Institute for Chemical Research, Kyoto University (Kyoto, 2004.12.3): 3 papers

Protein Crosstalk Salon '05: Informatics, Folding and Directed evolution (Tsukuba, 2005.3.4): 1 paper

The 2003 Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry (Sapporo, 2005.3.28-30): 13 papers

The 46th Annual Meeting of the Japanese Society for Plant Physiologists (Niigata, 2005.3.24-26): 8 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, 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); Research Grant: Grant-in-Aid for Scientific Research (B)(2): An exploratory study to clarify the molecular basis of the manufacturing process of the famous Formosa Oolong Tea (Oriental Beauty) prepared from tea shoots infested by the green leaf-hopper (K. Sakata) (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 from the Ministry of Education, Culture, Sports, Science and Technology (21 COE on Kyoto University Alliance for Chemistry), Mini-project (J. Hiratake) (J. Hiratake and B. Shimizu, collaborators); Grant-in-Aid for Young Scientists (B): Investigation of gene clusters involved in terpenoid biosynthesis in Arabidopsis genome (M. Mizutani); 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 (J-Y. Cho)(K. Sakata, collaborator); Grant-in-Aid for JSPS Fellows: Functional analysis of cytochrome P450 monooxygenases involved in brassinosteroids catabolism (T. Ohnishi)

Norinsuisannsho Research Grant: Technical Development Program for Making Agribusiness in the Form of Utilizing the Concerned Know-how from Private Sector (Amano Enzyme Co. Ltd.) (K. Sakata, M. Mizutani and B. Shimizu, collaborator)

A-4. International cooperation and overseas activities

International meetings (roles)

K. Sakata: Member of American Chemical Society (Division of Agricultural Food Chemistry); The 1st International Forum of Pu-erh Tea, Kunming (China), (2004.11.24-26) (Invited lecture)

J. Hiratake: Member of American Chemical Society

M. Mizutani: Member of American Society of Plant Biologists

B. Shimizu: Member of American Chemical Society

Others

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

B. Educational Activities (2004.4-2005.3)

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: Chemistry of Molecular Biocatalysts (Sakata and Hiratake), Seminar in Molecular Biocatalysts (Hiratake and Sakata), Laboratory Course in Molecular Biocatalysts (Hiratake and Sakata)

B-2. Off-campus teaching

Part-time lecturer

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

Hiratake, J.: Kyoto University of Education, Faculty of Education (A primary course of organic chemistry)

An extension lecture etc.

Sakata, K.: "Being attracted by the floral aroma of oolong tea," a lecture at O-CHA Ryori Kenkyukai, Ohtsuma Women's University (2004.7.10)

Sakata, K.: New developments of glycosidase studies triggered by the studies on tea aroma formation mechanism, the 40th meeting of Sirasagi Seminar, Osaka Prefectural University (2004.1.20)

Hiratake, J.: The 2004 Science Partnership Program (SPP) by the Ministry of Education, Culture, Sports, Science and Technology: Special lectures at Momoyama high school, Kyoto (2004.7.11, 16)

B-3. Overseas teaching

Sakata, K : Molecular basis of aroma formation during tea manufacturing, Nanjing Agricultural University, Nanjing, China, 2004.11.10

Mizutani, M: "Characterization of Cytochromes P450 involved In ABA Catabolism," Florigene Ltd, Melbourne, Australia, 2004. 9. 27

Mizutani, M: "Molecular basis of floral aroma formation in Oolong tea," Florigene Ltd, Melbourne, Australia, 2004. 9. 27

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 (2004.4-2005.3)

A-1. Main subjects

a) Reaction mechanism of fluoroacetate dehalogenase

Fluoroacetate dehalogenase catalyzes the hydrolytic defluorination of fluoroacetate. The enzyme is unique in that it catalyzes the cleavage of the strong carbon-fluorine bond of aliphatic organofluorine compounds. The activity of the enzyme from *Burkholderia* sp. FA1 toward fluoroacetate and chloroacetate was completely lost by substituting Ala for each of the catalytic residues (Asp104 and His271) and the residues in the vicinity of the active site (Arg105, His149, Arg108, Tyr147, Phe34, Tyr212, Phe272, and His103). In contrast, when Trp150 was replaced by Ala, 71% activity toward chloroacetate remained, whereas the activity toward fluoroacetate was completely lost. Thus Trp150 plays an essential role in the defluorination reaction.

b) Reaction mechanism of base modification in tRNA

5-Methylaminomethyl-2-thiouridine, which exists in the first base at the anticodon of tRNA, is important for aminoacylation and translation on ribosome. Cysteine desulfurase plays a crucial role in biosynthesis of various sulfur-containing molecules such as iron-sulfur cluster and thiamine. We purified and characterized MnmA, which is proposed to collaborate with cysteine desulfurase to produce 2-thiouridine in the first base at the anticodon of $tRNA^{Lys}$. We found that the reaction catalyzed by MnmA proceeds through ATP hydrolysis, and two cysteine residues of MnmA are essential for the thiolation reaction.

A-2. Publications and presentations

a) Publications

Original Papers

Ashida, H., A. Galkin, L. Kulakova, Y. Sawa, N. Nakajima and N. Esaki: Conversion of cofactor specificities of alanine dehydrogenases by site-directed mutagenesis. J Mol Catal B: Enzym 30; 173-176, 2004

Igarashi, M., N. Maruoka, S. Kato, H. Mihara, T. Kurihara and N. Esaki: Characterization of Slr0077 of *Synechocystis* sp. PCC6803, a homolog of chloroplastic cysteine desulfurase of higher plants. Trace Nutrients Research 21; 51-58, 2004

Ishihara, K., H. Yamaguchi, T. Omori, T. Uemura, N. Nakajima and N. Esaki: A Novel Zinc-containing alpha-Keto Ester Reductase from Actinomycete: An Approach Based on Protein Chemistry and Bioinformatics. Biosci Biotechnol Biochem 68; 2120-2127, 2004

Kurata, A., T. Kurihara, H. Kamachi and N. Esaki: Asymmetric reduction of 2-chloroacrylic acid to (*S*)-2-chloropropionic acid by a novel reductase from *Burkholderia* sp. WS. Tetrahedron: Asymmetry 15; 2837-2839, 2004

Kurokawa, S., H. Mihara, T. Kurihara and N. Esaki: Expression analysis of mammalian selenocysteine lyase. Biomed. Res. Trace Elem. 15; 278-280, 2004

Muramatsu, H., H. Mihara, R. Kakutani, M. Yasuda, M. Ueda, T. Kurihara and N. Esaki: Enzymatic synthesis of *N*-methyl-L-phenylalanine by a novel enzyme, *N*-methyl-L-amino acid dehydrogenase, from *Pseudomonas putida*. Tetrahedron: Asymmetry 15; 2841-2843, 2004

Muramatsu, H., H. Mihara, R. Kakutani, M. Yasuda, M. Ueda, T. Kurihara and N. Esaki: The Putative Malate/Lactate Dehydrogenase from *Pseudomonas putida* is a NADPH-dependent D^1-Piperideine-2-carboxylate/D^1-Pyrroline-2-carboxylate Reductase Involved in the Catabolism of D-Lysine and D-Proline. J Biol Chem 280; 5329-5335, 2004

Yamamoto, H., K. Mitsuhashi, N. Kimoto, A. Matsuyama, N. Esaki and Y. Kobayashi: A Novel NADH-Dependent Carbonyl Reductase from *Kluyveromyces aestuarii* and Comparison of NADH-Regeneration System for the Synthesis of Ethyl (*S*)-4-Chloro-3-hydroxybutanoate. Biosci. Biotechnol. Biochem 68; 2004

Yow, G.Y., T. Uo, T. Yoshimura and N. Esaki: D-Amino acid-*N*-acetyltransferase of *Saccharomyces cerevisiae*: a close homologue of histone acetyltransferase Hpa2p acting exclusively on free D-amino acids. Arch Microbiol 182; 396 - 403, 2004

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

Yoshimune, K., N. Esaki and M. Moriguchi: Site-directed mutagenesis alters DnaK-dependent folding process. Biochem Biophys Res Commun 326; 74-78, 2005

Yoshimune, K., A. Galkin, L. Kulakova, T. Yoshimura and N. Esaki: DnaK from *Vibrio proteolyticus*: Complementation of a *dnaK*-null mutant of *Escherichia coil* and the role of its ATPase domain. J BIosci Bioeng 99; 136-142, 2005

A-3. Off-campus activities

Membership in academic societies

Esaki, Nobuyoshi: The Japanese Biochemical Society (councilor), The Japan Trace Nutrients Research Society (director), The Japan Society for Bioscience, Biotechnology and Agrochemistry (councilor), The Society of Bioscience and Bioengineering, Japan (councilor), The Vitamin Society of Japan (councilor), Japan Society for Biomedical Research on Trace Element (councilor)

Kurihara, Tatsuo: The Society for Biotechnology, Japan (editorial board)

Research grants

Research Grants from Japan Society for the Promotion of Science: Grant-in-Aid for Scientific Research (B)(2); Mechanisms of activation of essential trace element selenium and co-translational insertion of selenium into polypeptides (N. Esaki), Grant-in-Aid for Scientific Research (B)(2); Isolation of novel cold-adapted microorganisms and their gene resources development (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 Young Scientists A; Bioconversion of fluorinated organic compounds (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.: Japan-Finland Joint Seminar on New Aspects in Microbial Biotechnology (speaker)

Esaki, N.: 4th European-Japanese Bioorganic Conference (speaker)

Membership in international academic societies

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

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

B. Educational Activities (2004.4-2005.3)

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)

B-3. Overseas teaching

Students and research fellows from abroad

Foreign students: Research student (China), Guest Research Associate (Korea)

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. (2004, 8-)*

Students and research fellows
Doctor's program: 1 (1) *Research fellow* *: 1*
Master's program: 4 (2) *Post doc research fellow: 6*

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

A. Research Activities (2004.4-2005.3)

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.: Chapter 43 Natural Products and Metabolites. The Handbook on Plant Biotechnology (Edited by Christou, P. and Klee, H). p. 811-857, John Wiley & Sons, London, 2004

Original papers

Hayashi, T., Y.W. Park, T. Takeda, S. Nakamura and Y. Ohmiya: Involvement of cellulose in cellulose biosynthesis. *In* Biotechnology of Lignocellulose Degradation and Biomass Utilization, ed. K. Ohmiya, pp. 869-873, Uni Publishers, Tokyo, 2004

Konishi, T., Y. Ohmiya, and T. Hayashi: Evidence that sucrose loaded into the phloem of a poplar leaf is used directly by sucrose synthase associated with various β-glucan synthases in the stem, Plant Physiology **134**, 1146-1152 (2004).

Kuroda, H., K. Yazaki and M. Shiotani: Molecular diagnosis of forest trees, the case studies on pine trees. *In* Sustainable Production and Effective Utilization of Tropical Forest Resources, eds. T Umezawa et al., p. 390, JSPS, Kyoto, 2004

Ohara, K., Y. Kokado, H. Yamamoto, F. Sato and K. Yazaki: Engineering of ubiquinone biosynthesis using the yeast *coq2* gene confers oxidative stress tolerance in transgenic tobacco, Plant J **40** (5), 734-743 (2004).

Okada, K., K. Ohara, K. Yazaki, K.,Nozaki, N.,Uchida, M.,Kawamukai, H. Nojiri and H. Yamane:

The *AtPPT1* gene encoding 4-hydroxybenzoate polyprenyl diphosphate transferase in ubiquinone biosynthesis is required for embryo development in *Arabidopsis thaliana*, Plant Mol Biol **55** (4), 567-577 (2004).

Park, Y.W., K. Baba, Y. Furuta, I. Iida, K. Sameshima, M. Arai, and T. Hayashi: Enhancement of growth and cellulose accumulation by overexpression of xyloglucanase in poplar, FEBS Lett **564**, 183-187 (2004).

Sasaki, K., K. Ohara and K. Yazaki: Molecular cloning and characterization of isoprene synthase from *Populus alba. In* Sustainable Production and Effective Utilization of Tropical Forest Resources, eds. T Umezawa et al., p. 390, JSPS, Kyoto.

Sudarmonowati, E., S. Hartati, R. Hartati, Y.W. Park and T. Hayashi: Production of transgenic *Acacia mangium* expressing cellulase gene to enhance growth. *In* Sustainable Production and Effective Utilization of Tropical Forest Resources, eds. T Umezawa et al., pp. 270-275, JSPS, Kyoto, 2004

Patents

T. Nomura and T. Hayashi: Method for producing palm wood material, FPO4-001-MY (Patent in Malaysia) (2004).

Reviews

Ohara, K., K. Yazaki: Aromatic substrate prenyltrnsferases and their involvement in the production valuable compounds. Bioscience & Industry **62** (7), 441-444 (2004).

Shitan, N., K. Yazaki: The diversity of plant ABC protein superfamily. (review in Japanese), Seikagaku **76**, 1221-1224 (2004).

Shitan, N., F. Sato, K. Yazaki: Accumulation mechanism of antibacterial alkaloid in plant cell. Bioscience & Industry 62 (4), 34-35 (2004)

Yazaki, K.: Metabolic engineering of isoprenoids in plants. APAST **14** (5), 100-105 (2004).

Reports

b) Conference and seminar papers presented

Annual Meeting of Japanese Society for Plant Physiologyists 2004 : 7 reports

Annual Meeting of Japanese Society for Bioscience, Biotechnology and Agrochemistry 2004 : 3 report

The 22nd Annual Meeting of Japanese Society for Plant Cell and Molecular Biology : 3 reports

The 27th Annual Meeting of The Molecular Biology Society of Japan: 2 reports

The 50th Annual Meeting of Japanese Society for Pharmacognosy : 1 report

The 53nd Meeting of Japan Wood Research Society : 7 report

Annual Meeting of Japan Cellulase Society: 2 reports

Annual Meeting of Japanese Society for Cellulose : 1 report

Memorial Symposium of Japanese Carbohydrate Sciences: 1 report

The 116th Annual Meeting of Japanese Society for Forestry : 1 report

A-3. Off-campus activities

Membership in academic societies (roles)

Yazaki, K. : The Japanese Society for Plant Cell and Molecular Biology (Executive Board, Editorial Board), The Japanese Society for Plant Physiologist (Board member, Editorial Board),Japan Society for Bioscience, Biotechnology, and Agrochemistry (Boad member), The Japan Wood Research Society (Editorial Boad), The Japanese Bioindustry

Association (Editorial Boad)

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

Research grants

Monbusho Research Grant: Priority Areas (2) Molecular mechanism of auxin polar transport by MDR-type ABC transporter in plants (Yazaki, Head), Scientific Research (B) Structural and functional analyses of prenyltransferase accepting aromatic substrates (Yazaki, Head), Phytoremediation technology by transport engineering with ABC transporter (Yazaki, Head). Research for the Future Program, Regulation of secondary metabolism and intracellular transport (Yazaki, Head); Sumitomo Foundation, Anti-green house gas trees by isoprene metabolic engineering (Yazaki, Head); Uehara Life Science Foundation, Molecular dissection of ABC protein, Cjmdr1, an alkaloid transporter (Yazaki, Head); Nitta Corp, Phytoremediation; Research grant for Sustainable Humanosphere, Studies on molecular diagnostics of woody plants. Hoga: Origin of cellulases (Hayashi, Head), RITE research grant (Hayashi, Head), Biodesign project in Japan (Hayashi), JICA research grant (Hayashi, Head), Joint research grant with RITE (Hayashi), Japan International Research Center for Agricultural Sciences (Hayashi)

Others: Research grant from Forestry and Forest Products Institute (Kuroda), Research Grant for Sustainable Humanosphere (Kuroda, Head)

A-4. International cooperations and overseas activities

International meetings (roles)

Yazaki, K.: German-Japan Seminar on Molecular Regulation of Plant Secondary Metabolism (Kazusa, September, invited speaker), Plant Membrane Biology (Montpellier July, Poster), The 5th International Wood Science Symposium (Kyoto, September, Poster)

Hayashi, T.: X Cell Wall Meeting (Sorrento, Italy, August to September, speaker), Vth International Wood Science Symposium (Kyoto, Japan, September, speaker), ACS meeting (San Diego, USA, March, speaker)

Kuroda, H.: The 5th International Wood Science Symposium (Kyoto, September, research presentation)

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.: 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)

Editorial work for international journals (roles)

Yazaki, K.: Plant & Cell Physiology (editorial board), Plant Biotechnology (editorial board), J. Wood Sci. (editorial board)

Hayashi, T.: Cellulose (Editoral board)

Scholars from abroad

Ph D student (2) (Korea and Brasil)

B. Educational Activities (2004.4-2005.3)

B-1. On-campus teaching

a) Courses given

Undergraduate level: Wood Biomass (Yazaki, Sugiyama, Yoshimura), Short semonor for wood science and technology (Kuroda)

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

B-2. Off-campus teaching, etc.

Part-time Lecturer

Yazaki, K.: Kobe Pharmaceutical University, Pharmacognosy course for undergraduate (January, 2005)

Kuroda, H.: Kyushu Univesity, Special lecture course for graduate course (October, 2004)

Open seminar, etc.

Yazaki, K.: The 1st RISH Open Seminor (Uji, Octorber, 2004), Open lecture of Kyoto University "World of woods and trees" (Kyoto, Octorber, 2004), Special Seminar in Riken (Yokohama, November, 2004), The 41st Phytochenistry Symposium (Kyoto, November, 2004), The 57th Open Seminar of Wood Material Science (Kyoto, January, 2005), Mission Symposium toward Sustainable Humanosphere (Uji, March, 2005)

Kuroda, H: The 1st RISH Open Seminor (Uji, Octorber, 2004)

B-3. Overseas teaching

Lectures and seminars

Yazaki, K.: Special Lecture (University of Ulm, Ulm, Germany, July, 2004)

Hayashi, T.: Universita di Roma, Italy (2004, 9, Two cellulases in poplar), INRA, France (2005, 2, Expression of xyloglucanase in poplar), Université Claude Bernard Lyon, France (2005, 2, Expression of xyloglucanase in poplar), UPM, Malaysia (2005, 2, Expression of xyloglucanase in poplar)

2.3.15 Laboratory of Biochemical Control

Staff *Professor* *: Shimada, Mikio, Dr. Agric. Sci.*
Associate Professor: Umezawa, Toshiaki, Dr. Agric. Sci.
Assistant Professor : Hattori, Takefumi, Dr. Agric. Sci.
Students and research fellows
Doctor's Program: (5)
Master's Program: (4) *Research Fellow : (1)*

A. Research Activities (2004.4-2005.3)

A-1. Main subjects

a) Enzymatic and genetic biochemistry of the glyoxylate cycle related to fruit body formation in wood-destroying basidiomycetes

Wood-destroying basidiomycetes including edible mushrooms produce oxalic acid, with which they degrade cell walls of host plants. In order to protect woods from fungal attack, it is important to elucidate the physiological role and biochemical mechanisms for biosynthesis of oxalic acid. We first extracted two different oxalate-producing enzymes, oxaloacetase and glyoxylate oxidase, from both white-rot and brown-rot fungi. We have discovered that one of them is a novel oxalate-producing enzyme, cytochrome *c* dependent glyoxylate dehydrogenase which has never been recorded in the list of enzyme nomenclatures. Recently we demonstrated the close relationships between oxalate production by TCA and Glyoxylate cycles and the fruit body formation. The key enzymes of Glyoxylate cycle, isocitratelyase and malate synthase have been purified for the first time from basidiomycetes. Enzymatic and genetic studies including cDNA cloning of the glyoxylate dehydrogenase are in progress.

b) Biochemical mechanisms for production of symbiotic basidiomycetes which support the growth of forest trees

Symbiotic mycorrhizal fungi may serve as "phosphate supplying biofertilizers" helping trees grow well in forests, but their biochemical interactions remain to be elucidated. Focusing on possible role of dicarboxylic acids produced by symbiotic mushrooms, effects of organic acids on growth of forest plants are being investigated.

c) 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.

d) 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.

e) 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.

A-2. Publications and presentations

a) Publications

Books and reviews

Hattori, T., K. Iwase and M. Shimada: Proposed role of organic acids in an application of forest microorganisms for mycorrhizal remediation. Bioscience and Industry 63; 27-30, 2005

Umezawa, T.: Perspectives of future studies of wood extractives. Mokuzai Gakkaishi 51; 48-49 2005

Original papers

Muta, Y. S. Ohyama, T. Umezawa, M. Shimada and K. Inouye : Inhibitory effects of lignans on the activity of human matrix metalloproteinase (Matrilysin). J Agri Food Chemistry 52; 5888-5894, 2004

Okunishi, T., N. Sakakibara, S. Suzuki, T. Umezawa and M. Shimada: Stereochemistry of matairesinol formation by of *Daphne* secoisolariciresinol dehydrogenase. J Wood Sci 50; 77-81, 2004

Okunishi, T., T. Umezawa and M. Shimada: Semi-micro chiral HPLC analysis of lignans. J Wood Sci 50; 93-96, 2004

Suzuki, S., M. Yamamura, M. Shimada and T. Umezawa: A heartwood norlignan, (*E*)-hinokiresinol, is formed from 4-coumaryl 4-coumarate by a *Cryptomeria japonica* enzyme preparation. Chem Commun 2838-2839, 2004

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

b) Conference and seminar papers presented

54th Annual Meeting of Japan Wood Res. Soc. (Sapporo): 6 papers

49th Lignin Symposium, (Tsukuba): 1 paper

The Fifth International Wood Science Symposium, (Kyoto): 6 papers

9th International Conference on Biotechnology in the Pulp and Paper Industry, (South Africa): 1 paper

4th Regional Coordination of Wood Science (RCCWS) International Symposium, (Russia): 1 paper

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

A-3. Off-campus activities

Membership in academic societies (roles)

Shimada, M.: Japan Society for Bioscience, Biotechnology, and Agrochemistry (Council of Kansai subdivision), The Japanese Forestry Society, The Mycological society of Japan (Director), International Academy of Wood Science (Fellow)

Umezawa, T.: International Academy of Wood Science (Fellow)

Hattori, T.: Mycological Society of Japan (Treasurer)

Research grants

Monbukagakusho Research Grants: Grant-in-Aid for Scientific Research (C)(2): Elucidation of a possible role of lipid metabolism in germination of ectomycorrhizal (ECM) fungi and in an early stage of symbiosis between ECM fungi and the host plant (Head Investigator: Hattori, T., Joint Investigator: Shimada, M.). Grant-in-Aid for Scientific Research (C)(2): cDNA Cloming of enzymes involved in oxalic acid biosynthesis in copper tolerant brown-rot fungus *Fomitopsis palustris* (Head Investigator: Shimada, M., Joint Investigator: Hattori, T.). 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-4. International cooperations and overseas activities

International meetings (roles)

Oral presentation

Shimada, M.: IAWPS 2003 (International Conference on Forest Proudcts) (Daejon, Korea)The 34th International Research Group on Wood Preservation (Brisbane, Australia)

Umezawa, T.: Keynote lecture in The Fifth International Wood Science Symposium, Kyoto, Japan, Invited lecture in Lignan and norlignan biosynthesis Germany-Japan Seminar on Molecular Regulation of Plant Secondary Metabolism, Kisarazu, Japan.

International Joint Researches, overseas research surveys

Umezawa, T.: Cooperative work on molecular biology of phenylpropanoid biosynthesis (North Carolina State University, USA), Cooperative work on biosynthesis of antitumor lignans (Düsserdorf University, Germany), Cooperative work on Sustainable production and utilization of tropical forest resources for establishment of recycling-based society (Research and Development Unit for Biomaterials, LIPI, Indonesia)

Scholars from abroad

Bambang Subiyanto: Collaborative research work on “Sustainable production and utilization of tropical forest resources for establishment of recycling-based society” September 10-29 (2004)

Ee Ding Wong: Collaborative research work on “Sustainable production and utilization of tropical forest resources for establishment of recycling-based society” September 14-20 (2004)

Mohannad Muslich: Collaborative research work on “Sustainable production and utilization of tropical forest resources for establishment of recycling-based society” September 15-21 (2004)

Syamsul Falah: Collaborative research work on “Sustainable production and utilization of tropical forest resources for establishment of recycling-based society” September 15-21 (2004)

Jeon-Jun Yoon: Collaborative research work on “Elucidation of the mechanisms for carbon

metabolism of wood-rotting fungi" March 18-March 20 (2005)

Geon-Sik Seo: Collaborative research work on "Elucidation of the mechanisms for carbon metabolism of ectomycorrhizal fungi" March 18-March 20 (2005)

Erman Munir: Collaborative research work on "Elucidation of the mechanisms for carbon metabolism of ectomycorrhizal fungi" March 15-March 20 (2005)

B. Educational Activities (2004.4-2005.3)

B-1. On-campus teaching

Undergraduate level:

Graduate level: Control Biochemistry of Wood (Advanced course) (Shimada and Umezawa) , Laboratory course in Control Biochemistry of Wood (Shimada and Umezawa).

B-2. Off-campus teaching, etc.

Part-time lecturer

Shimada, M.: Wood Biochemistry for the graduate course students at the Graduate school of Gifu University; Wood Biochemistry for the graduate course students at the Graduate school of Kagawa University

Umezawa, T.: Heirich-Heine Universität Düsserdorf (Special lecture), Phillips- Universität Marburg (Special lecture), Universiti Putra Malaysia (Special lecture), North Carolina State University (Special lecture), Kazusa DNA Research Institute (Special lecture), Special lecture of wood science and technology for the graduate course students at the Graduate School of Shiduoka University, Special lecture of wood biochemistry the graduate course students at the Graduate School of Tokyo University, Special lecture of wood biomass science for the graduate course students at the Graduate School of Kyoto Prefectural University, Plantech Research Institute (Special seminar).

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 : (6) *Master's program: (10)*
Post-Doctral fellow: (1)

A. Research Activities (2004.4-2005.3)

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., Y. Ohashi, N. Rahmawati, R. Amirta, T. Watanabe, Y. Honda and M. Kuwahara: Control of the generation of active oxygen species for selective lignin biodegradation by *Ceriporiopsis subvermispora*. in "Biotechnology of lignocellulose degradation and biomass utilization", ed. by K. Ohmiya et al., Uni Publishers, Tokyo, 697-704, 2004

Watanabe, T.: Molecular conversion technology of plact cell walls for refinery, in Advanced Technologies for woody organic resources, ed. By M.Funaoka, CMC Publication, Tokyo, 68-79, 2005

Honda, Y: Biotechnology in Mushroom. in "Introduction to Mushroom Science", ed. by Ohga, S., Kaiseisha Press, Ohtsu, 133-147, 2004

Original papers

Kamitsuji, H., T. Watanabe, Y. Honda and M. Kuwahara: Direct oxidation of polymeric substrates by multifunctional manganese peroxidase isozyme from *Pleurotus ostreatus* without redox mediators. Biochem J 386; 387-393, 2005

Kamitsuji, H., Y. Honda, T. Watanabe, and M. Kuwahara: Mn^{2+} is indispensable for the production of active MnP2 by *Pleurotus ostreatus*. Biochem Biophys Res Commun 327; 871-876, 2005

Okano, K., M. Kitagawa, Y. Sasaki, and T. Watanabe: Conversion of Japanese red cedar (*Cryptomeria japonica*) into feed for ruminants by white-rot basidiomycetes. Animal Feed

Sci and Technol 120; 235-243, 2005

Kamitsuji, H., Y. Honda, T. Watanabe and M. Kuwahara: Production and induction of manganese peroxidase isozymes in a white-rot fungus *Pleurotus ostreatus*. Appl. Microbiol. Biotechnol 65; 287-294, 2004

Widyorini, R., J. Xu, T. Watanabe and S. Kawai: Chemical changes of steam-pressed kenaf core binderless particleboards. J Wood Sci 51; 26-32, 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 54th Annual meeting of the Japan Wood Science Society: 3 presentaions

The 55th Annual meeting of the Japan Wood Science Society: 4 presentaions

The 49 th Lignin Symposium: 3 presentaions

The 12 th Annual meeting of The Japan Institute of Energy: 1 presentaion

Annual meeting of Analytical Polymer Science: 1 presentaion

Annual meeting of Cellulase Research: 2 presentaion

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

Annual meeting of the Society of Polymer Science, Japan: 1 presentaion

Annual meeting of the Japanese Society of Mushroom Science and Biotechnology: 1 presentaion

Annual meeting of Japan Society of Bioscience, Biochemistry and Agrochemistry 2005: 3 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.)

Honda, Y.: Japan Wood Research Society (Secretary of the Institute., Editorial Boad member), Japanese society of mushroom science and biotechnology (Council member)

Research grants

Grant-in-Aid for Scientific Research (B)(1), Conversion of wood to ethanol by the system incorporated overexpression of laccase (Watanabe), Grant-in-Aid for Exploratory Research, Suppression of cellulose degradation by metabolites of basidiomycetes –New strategy for pulp bleaching (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 (Watanabe)

Others: Grant: RITE Research grant for advanced research, Aanaysis and molecular breeding of selective white rot fungi for the production of ethanol (Watanabe)

A-4. International cooperations and overseas activities

International meetings (roles)

Watanabe, T.: 229th American Chemical Society National Meeting, San Diego, USA (Invited lecture), RITE International Symposium on Biorefinery (Invited lecture), The IV

Asia-Pacific Mycological Congress, Chiang Mai, Thailand (Invited lecture), 5th International Wood Science Symposium, Kyoto (Committee member, Moderator), 9th International Conference on Biotechnology in the Pulp and Paper Industry (Durban, South Africa (Presentation), 7th Biennial Symposium on International Society of Environmental Biotechnology, Cicago, USA (Presentation), The 4th JSPS-NRCT Joint Seminar on Development of Thermotorelant Microbial Resourtces and Their Applications, Fukuoka (Presentation), The 2nd International Symposium on Sustainable Energy System, Kyoto (Presentation)

Honda, Y.: 5th International Wood Science Symposium (Organising committee member, Moderator)

International joint researches, overseas research surveys

Watanabe, T.: Cooperative research between Wood Research Institute, 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 Wood Research Institute, 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"

B. Educational Activities (2004.4-2005.3)

B-1. On-campus teaching

a) courses given

Graduate level: Chemistry of Wood Biomass Conversion (Watanabe, Honda)

B-2. Off-campus teaching etc.

Open seminar, etc

Watanabe, T.: Seminar of energy recycling symposium - Solar power stations and biomass conversion

B-3. Overseas teaching

Watanabe, T.: Special lecture in Lembang University

Students and research fellows from abroad

Doctor course: 6

Cooperative research fellows: 3