Chair Applied Microbiology

2.3.7 Laboratory : Fermentation Physiology and Applied Microbiology

Member :	Professor	Ogawa, Jun, Dr. Agric. Sci.
	Assistant Professor	Sakuradani, Eiji, Dr. Agric. Sci.
	Professor	Shima, Jun, Ph.D.
	Assistant Professor	Ando, Akinori, Dr. Agric. Sci.
	Doctor's program	1
	Master's Program	20
	Undergraduate	5
	Post-Doctoral fellow	2
	Researcher	4

A. Research Activities (2010.4-2011.3)

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-g-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. 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. The same enzymatic reaction was applied to nicotinamide production from 3-cyanopyridine and industrialized from 1998.

e) Collaboration with the Research Division of Microbial Sciences

The purposes are to discover and develop the latent possibilities of microorganisms and to tune these possibilities into useful applications such as bioprocess design, bio-fuel production, biomass utilization, bioremediation, bio-control, probiotics development, functional food production, pharmaceutical synthesis, etc. For the purposes, the research division encourages broad collections of various kinds of microorganisms and fundamental understand-ings of microbial metabolisms. The examples of the research topics are: 1) analysis on microbial amino acid metabolism for production of hydroxyl amino acids useful as functional food materials, 2) analysis on microbial reductive fatty acid metabolism for the production of conjugated fatty acids useful for prevention of lifestyle-related diseases. Furthermore, researches on nucleic acid metabolism in lactic acid bacteria for the devel-opment of probiotics for hyperuricemia prevention, microbial laccase as a module of bio-battery, and extracellular production of lipids by filamentous fungi are also on going.

A-2.Publications and presentations

a) Publications

Books

- Kishino, S., J. Ogawa. Chapter 7: Bio-products and novel platform -Synthesis of fatty acid derivatives-. Earth environment series. Eco-bio-refinery. Ueda, M., Tamaru. H. eds. CMC press. 213-219 (2010) (in Japanese)

- Ogawa, J., E. Sakuradani, S. Kishino, A. Ando, S. Shimizu. Volume 5. Chapter 1. Section 5., 3. Production of useful fatty acid. Enzyme application technology, Komiyama M. ed., NTS press. 430-433 (2010) (in Japanese)

- Sakuradani, E., J. Ogawa, S. Kishino, A. Ando, K. Yokozeki, S. Shimizu. Oils, Microbial Production. Encyclopedia of Industrial Biotechnology: Bioprocess, Bioseparation, and Cell Technology (ed. by M.C. Flickinger) John Wiley & Sons, 6, 3693-3706 (2010).

- Sakuradani, E. A. Ando, J. Ogawa S. Shimizu. Part2-2 Arachidonic Acid-Producing Mortierella alpina : Creation of Mutants, Isolation of the Related Enzyme Genes, and Molecular Breeding. Single Cell Oils -Microbial and Algal Oils 2nd Edition-, ed. Zvi Cohen, Colin Ratledge, AOCS Press, USA, 29-49 (2010).

- Shimizu, S., M. Kataoka. Aldehyde reductase. Encyclopedia of Industrial Biotechnology: Bioprocess, Bioseparation, and Cell Technology (ed. by M.C. Flickinger) John Wiley & Sons, 1, 177-184 (2010).

- Shimizu, S., M. Kataoka. Lactonohydrolase. Encyclopedia of Industrial Biotechnology: Bioprocess, Bioseparation, and Cell Technology (ed. by M.C. Flickinger) John Wiley & Sons, 5, 3116-3123 (2010).

- Shimizu, S., M. Kataoka. Pantothenic Acid and Related Compounds. Encyclopedia of Industrial Biotechnology: Bioprocess, Bioseparation, and Cell Technology (ed. by M.C. Flickinger) John Wiley & Sons, 6, 3767-3774 (2010).

Original Papers(including book-reviews)

- Kishino, S., J. Ogawa, A. Ando, K. Yokozeki, S. Shimizu. Microbial production of conjugated γ-linolenic acid from γ-linolenic acid by Lactobacillus plantarum AKU 1009a. J. Appl. Microbiol., 108 (6), 2012-2018 (2010).

- Murai, M., T. Yamashita, M. Senoh, Y. Mashimo, M. Kataoka, H. Kosaka, A. Matsuno-Yagi, T. Yagi, H. Miyoshi. Characterization of the ubiquinone binding site in alternative NADH-quinone oxidoreductase of Saccharomyces cerevisiae by photoaffinity labeling. Biochemistry., 49 (13), 2973-2980 (2010).

- Nakatani, M., M. Hibi, M. Minoda, J. Ogawa, K. Yokozeki, S. Shimizu. Two laccase isoenzymes and a peroxidase of a commercial laccase-producing basidiomycete, Trametes sp. Ha1. N Biotechnol., 27 (4), 317-323 (2010).

- Ohto, C., M. Muramatsu, S. Obata, E. Sakuradani, S. Shimizu. Production of geranylgeraniol on overexpression of a prenyl diphosphate synthase fusion gene in Saccharomyces cerevisiae. Appl. Microbiol. Biotechnol., 87 (4), 1327-1334 (2010).

- Sakuradani, E. Advances in the production of various polyunsaturated fatty acids through oleaginous fungus mortierella alpina breeding. Biosci. Biotechnol. Biochem., 74 (5), 908-917 (2010).

- Sasaki, Y., M. Kataoka, N. Urano, J. Ogawa, A. Iwasaki, J. Hasegawa, K. Isobe, S. Shimizu. Cloning, sequencing and expression analysis of a gene encoding alcohol oxidase in Paenibacillus sp. AIU 311. J. Biosci. Bioeng., 110 (2), 145-171 (2010).

- Smirnov, S.V., T. Kodera, N.N. Samsonova, V.A. Kotlyarova, N.Y. Rushkevich, A.D. Kivero, P. M. Sokolov, M. Hibi, J. Ogawa, S. Shimizu. Metabolic engineering of Escherichia coli to produce (2S, 3R, 4S)-4-hydroxyisoleucine. Appl. Microbiol. Biotechnol., 88 (3), 719-726 (2010).

Reviews

- Kishino, S., J. Ogawa. Special issue: The front line of useful chemical production from bio-mass - Synthesis of fatty acid derivatives-. BIO INDUSTRY, 27 (11), 32-37 (2010) (in Japanese)

Patents

- Kawashima, H., E. Sakuradani., J. Ogawa, S. Shimizu. Production of triglyceride with three same fatty acids. (Open patent: 2010-042037)

b) Conference and seminar papers presented

- 101st AOCS Annual Meeting & Expo：4 reports

- 19th International Symposium on Plant Lipids:１report

- The Eleventh China-Japan-Korea Joint Symposium on Enzyme Engineering:１report

- The Eleventh China-Japan-Korea Joint Symposium on Enzyme Engineering:１report

- 6th International Symposium on Biocatalysis and Biotechnology:１report

- Gordon Research Conferences Plant Lipids: Structure, Metabolism and Function: 1report

- JBA Bio-conversion new resource conference symposium "2020 bioindustry": １report

- The 71th Yeast Research Society of Japan：１report

- Fermentation Technology ― Core Competence of JapanBio” in BioJapan 2010:１report

- The 21th microorganism workshop "The development of manufacturing by microorganisms"

- The 10th Conference on Fungal Genetics and Molecular Biology in 2010
"Our good partners: The inspiring fungi in industrial field"：１report

- The 49th Annual Meeting of the Japan Oil Chemists’ Society”
Hakodate, Japan:3 reports

- Bio Japan 2010　:1 report

- 466th Meeting of Kansai Branch of Japan Society for Bioscience, Biotechnology, and Agrochemistry：
３ reports

- The 12th Japan-Swiss biotechnology・bioprocess conferences: 2 reports

- The 10th Conference on Fungal Genetics and Molecular Biology in 2010：１report

- The 64th Japanese Society of Enzyme Engineering : 2report

- "Th 62th Society for Biotechnology, Japan / Annual Meeting 2011 : 9 reports

- "The Young Scientist Symposium of Brewing Sciety of Japan: 2reports

- Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry 2011: 18 reports

A-3.Off-campus activities 1

Membership in academic societies

- gawa, Jun, D.Agric.Sci : Japanese Society for Bioscience, Biotechnolgy, and Agrochemistry (member of Industry-Government-Academy young person interchange society, Secretory of Kansai branch), The Society for Fermentaton and Bioengineering, Japan (chairman of lipid biotechnology group), Japan Bioindustry Asociation (secretary of the soceity of Fermentation and Metabolism), Japanese Society of Brewing (secretary of young person's society), Industry Research Association, Osaka (secretary of new frontier material group)

A-3.Off-campus activities 2

Research grants

1. Grants-in-aid for Scientific Research(KAKENHI)

- Scientific Research (B) : Ogawa, Jun : Screening and development of microbial fatty acid and organic acid reducing activity

- Challenging Exploratory Research : Ogawa, Jun : The production of novel branced amino acids with function of metabolic control for useful as prevention of lifestyle-related diseases.

- Scientific Research (B) : Sakuradani, Eiji : Screening of novel lipid bioconversion reaction and the development of functional lipid producing process

2.Other Research Grants

- Target Protein Research program : Ogawa, Jun : Structural analysis and modification of enzymes useful for chiral compound production

- Bio-oriented Technology Research Advancement Institution : Ogawa, Jun : Development of omega 3 fatty acid resourses for creation of lipid nutritional balance products

- Research project funded by New Energy and Industrial Technology Development Organization : Ogawa, Jun : The Project for Development of a Technological Infrastructure for Industrial Bioprocesses

- Research project funded by New Energy and Industrial Technology Development Organization : Ogawa, Jun : Platform technology development for dicarboxylic acid production from non-food plant biomass

- Research project funded by JST : Ogawa, Jun : Oil-producing microorganisms breeding for polymer substrate production

- Research project funded by New Energy and Industrial Technology Development Organization : Kishino, Shigenobu : Selective production of functional lipids by microorganisms

A-4.International cooperation and overseas activities 1

Membership in academic societies

- gawa, Jun: Applied Microbiology and Biotechnology (editor), International Society of Biocatalysis and Biotechnology (editor)

A-4.International cooperation and overseas activities 2

Visiting Research Scholars

- Researcher 1（Korea）

- Entrusted researcher 1（Korea）

B.Educational Activities(2010.4-2011.3)

B-1.On-campus teaching

a) Courses given

- Undergraduate level: Primer course of applied life sciences II (Ogawa), Biochemistry I (Ogawa), Applied microbiology I (Ogawa), Applied microbiology II (Ogawa), Applied microbiology III (Ogawa), Applied microbiology IV (Ogawa), Laboratory course in applied microbiology (Sakuradani, Ando)

- Graduate level: Fermentation physiology and applied microbiology special course (Ogawa, Shima), Fermentation physiology and applied microbiology seminar (Ogawa, Sakuradani, Shima, Ando), Experimental course of fermentation physiology and applied microbiology (Ogawa, Sakuradani, Shima, Ando)

B-3.Overseas teaching 1

International students

- International students : Doctral 1 (China)