

Chair Industrial Microbiology (Endowed Chair)

2.10.1 Laboratory : Industrial Microbiology

Member:	Professor	Kenzo Yokozeki D. Agric.Sci
	Associate Professor	Tairo Hagishita, Ph.D.
	Assistant Professor	Mak
	Program-Specific Researcher	1
	Researcher	1

A. Research Activities (2010.4-2011.3)

A-1. Main Subjects

a) Development of novel process of N-acetylneuraminic acid

N-acetylneuraminic acid is used as remedy of flu. We aim for process development of N-acetylneuraminic acid from cheap materials like N-acetylglucosamine and pyruvic acid. Epimerase which catalyzes conversion of N-acetylglucosamine to N-acetylmannosamine, and aldolase which catalyzes condensation of N-acetylmannosamine and pyruvic acid are obtained by microbial screening. By various combinations of these epimerases and aldolases, efficiency of N-acetylneuraminic acid production is examined in one pot reaction.

b) Screening for novel enzymes useful for the production of hydroxyl amino acids

Hydroxy amino acids, including 4-hydroxyisoleucine possessing anti-diabetes effect, are known to have various physiological activities, and their effective production methods are desired. By selection of α -ketoglutarate-dependent dioxygenases from genome database, enzyme catalysts useful for hydroxylation of various amino acids are obtained. Also, process of optically-active hydroxy amino acids are developed, by using NAD(P)⁺-dependent reductases which reduce amino acid having prochiral carbonyl group.

c) Selective formation of functional fatty acids by microorganism

We are developing the conjugated fatty acids having various kinds of useful physiology. We are making research in microorganisms for the production of functional fatty acids, and found that lactic acid bacteria produce conjugated linoleic acids. Studies on purification, characterization and expression system of the enzymes involved in each reaction are also carried out. Further development of conjugated fatty acids production by lactic acid bacteria is going on.

d) Novel microbial oxidizing enzyme and its application

A variety of microbial oxidases like peroxidases and laccases, which are industrially used for pulp bleaching and waste treatment, are screened and its applicabilities are evaluated. Also, this study is aimed at the development of laccase-mediator reaction systems, which could catalyze the oxidative degradation of persistent chemical substances when there was a certain low molecular compound. We are exploring the mediators from natural.

d) The microbial conversion useful for the production of steroids

Attention has been focused on the microbial conversion of cholesterol as the supply method of a steroid hormone. We are exploring various kinds of microorganisms producing novel useful enzymes responsible for the modification of the side chain in the sterol backbone. The microbial production of steroid hormones having an acetylated residue at the 17th carbon position on cholesterol has recently become a subject of considerable interest.

A-2.Publications and presentations

a) Publications

Books

- Kishino S., J. Ogawa. Synthesis of Fatty Acid Derivatives. BIO INDUSTRY, 27 (11), 32-37 (2010)

Original Papers(including book-reviews)

- Kishino S., J. Ogawa, K. Yokozeki, S. Shimizu. Linoleic acid isomerase in *Lactobacillus plantarum* AKU1009a proved to be a multi-component enzyme system requiring oxidoreduction cofactors. Biosci. Biotechnol. Biochem., 75(2), 318-322 (2011)

- Ogawa, J., T. Kadera, S.V. Smirnov, M. Hibi, N.N. Samsonova, R. Koyama, H. Yamanaka, J. Mano, T. Kawashima, K. Yokozeki, S. Shimizu. A novel l-isoleucine metabolism in *Bacillus thuringiensis* generating (2S,3R,4S)-4-hydroxyisoleucine, a potential insulinotropic and anti-obesity amino acid. Appl. Microbiol. Biotechnol., 89 (6), 1929-1938 (2011)

- Smirnov, S.V., T. Kadera, 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)

- 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)

- 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)

Reviews

- Kishino S., J. Ogawa. *Biorefinery Strategy for Change to Sustainable Bioproduction from Petroleum-dependent Production*, CMC Publishing Co.,Ltd., 213-219 (2010)

- Ogawa J., E. Sakuradani, S. Kishino, A. Ando, S. Shimizu. *Applications of enzyme technology*, NTS Inc., 430-433 (2010)

Patents

- Smirnov, S.V., Samsonova, N.N., Kotliarova, V.A., Rushkevich, N.Y., Fedorina, E.A., Sokolov, P.M., Kolokolova, A.V., Ogawa, J., Hibi, M., Shimizu, S., Imabayashi, Y., Suzuki, S., Sugiyama, M.
Method for producing hydroxylated amino acids
WO/2011/021717 (2011)

- Hibi M, Kodera T, Kotliarova VA, Ogawa J, Rushkevich NY, Samsonova NN, Shimizu S, Smirnov SV, Kozlov YI.
Manufacturing 4-hydroxy isoleucine or its salt for promoting insulin secretion, by contacting 2-amino-3-methyl 4-keto pentanoic-acid reductase derived from microorganisms e.g. Nocardia with 2-amino-3-methyl 4-keto pentanoic WO2009060963-A1; JP2009131254-A (2010)

- Fat degrading microorganisms, microbial immobilization, treatment of wastewater, and wastewater treatment systems
2010-26324

- Fat degrading microorganisms, microbial immobilization, treatment of wastewater, and wastewater treatment systems
2010-26325

b) Conference and seminar papers presented

- The 12th Japanese-Swiss Meeting on Biotechnology and Bioprocess Development: 1 Presentations

- The Society for Biotechnology, Japan / Annual Meeting 2010: 3 Presentations

- Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry 2011: 12 Presentations

- 101th AOCS Annual Meeting & Expo: 2 Presentations

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

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

- 64th Japanese Society of Enzyme Engineering: 1 Presentations

- 1st Interdisciplinary Lipid Creation Forum: 3 Presentations

A-3.Off-campus activities 1

Membership in academic societies

- Yokozeki kenzo, D.Agric.Sci : The Society for Fermentation and Bioengineering, Japan (councilor),
The Society of Enzyme Engineering (honorary member)

A-3.Off-campus activities 2

Research grants

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

- Grant-in-Aid for Young Scientists (B) : Makoto Hibi, Ph. D. : Screening of novel microbial enzymes for the production of on-demand hydroxylated amino acids

- Scientific Research (B) : Yokozeki kenzo, D.Agric.Sci : Screening and development of novel peptide synthesizing enzymes using unprotected amino acids

2.Other Research Grants

- Research project funded by New Energy and Industrial Technology Development Organization (NEDO) : Kishino Shigenobu, Ph.D. : Selective formation of functional fatty acids by microorganism

B.Educational Activities(2010.4-2011.3)

B-1.On-campus teaching

a) Courses given

- Undergraduate level: Industrial Microbiology (Yokozeki), Laboratory course in applied microbiology (Hagishita, Hibi and Kishino)

- Graduate level: Industrial Microbiology (Yokozeki), Fermentation Physiology and Applied Microbiology Seminar (Yokozeki, Hagishita, Hibi and Kishino), Experimental Course of Fermentation Physiology and Applied Microbiology (Yokozeki, Hagishita, Hibi and Kishino)

B-2.Off-campus teaching etc.

Part-time lecturer

- Yokozeki kenzo, D.Agric.Sci: University of Tokyo

- Yokozeki kenzo, D.Agric.Sci: Kyusyu University

- Yokozeki kenzo, D.Agric.Sci: Kyoto Gakuen University

- Yokozeki kenzo, D.Agric.Sci: Soka University