2.10.1 Laboratory : Industrial Microbiology

Member :	Professor	Kenzo Yokozeki D. Agric.Sci
	Associate Professor	Tairo Hagishita, Ph.D.
	Assistant Professor	Makoto Hibi, Ph.D.
	Assistant Professor	Kishino Shigenobu, Ph.D.
	Post-Doctoral fellow	1
	Researcher	2

A. Research Activities (2009.4-2010.3)

A-1. Main Subjects

a) outline

Our laboratory aims at creating industrial innovation by the application of novel functions being involved in microorganisms. It is important to find out something new while closely looking at nature without prejudice. Approach for the finding of novel and useful potential being involved in microorganisms is a key component.

b) Industrial application of novel microbial aldolase

From a standpoint of manufacturing technology, the condensation reaction for the carbon-carbon bond formation that is extremely difficult by organic synthesis is very attractive reaction. Under this background we are exploring novel aldolase that can catalyze the condensing reaction of carbon-carbon coupling to develop the production process of N-acetylneuraminic acid.

c) Screening for novel enzymes synthesizing hydroxylated amino acids

The hydroxyisoleucine that is slightly extracted from a plant seed is receiving particular attention as an anti-obesity drug. There are eight kinds of isomers to the hydroxyisoleucine. If novel enzymes catalyzing asymmetrical reaction were found, an epoch-making method would be built. We are exploring various kinds of enzymes participating in hydroxyisoleucine production widely.

d) 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.

e) Novel microbial oxidizing enzyme and its application

A variety of microbial oxidases, such as peroxidases and laccases, are screened and its applicabilities are evaluated. This study was aimed at the development of laccase-mediator reaction systems, which could catalyze the oxidation of various compounds when there was a certain low molecular compound. We are exploring the mediator from natural.

f) 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.

g) Efficient microbial production of deoxyribonucleoside useful for genetic engineering

There will be a need for 2'-deoxyribonucleoside (dNS) in order to be increasing demand in new medical and biotechnology field in near future. The current dNS sources hydrolyzed herring and salmon sperm DNA, which are not suitable sources for sudden high demand. Microbial production of dNS from cheap sugar materials by reverse reactions of nucleoside degradation is investigated.

A-2.Publications and presentations

a) Publications

Books

- Ogawa, J., N. Horinouchi and S. Shimizu: Retrosynthetic production of 2'-deoxyribonucleoside from glucose, acetaldehyde, and nucleobase through multistep enzyme reactions. Biocatalysis and Agricultural Biotechnology (ed. by C.T. Hou, J.-F. Shaw) CRC Press, p.p. 269-278 2009
- Yatagai, M., T. Hamada, H. Nozaki, S. Kuroda, K. Yokozeki and K. Izawa: Synthesis of Optically Active α-Methyl Amino Acids using Biotransformation as a Key Step. ACS Symposium Series, 1009 (Asymmetric Synthesis and Application of α-Amino Acids), American Chemical Society, p.p. 394-406, 2009

Original Papers

- Kishino, S., J. Ogawa, K.. Yokozeki and S. Shimizu: Microbial production of conjugated fatty acids. Lipid Technol., 21; 177-181, 2009

- Kodera, T., T.S. Smirnov, N. Samsonova, Y.I. Kozlov, R. Koyama, M. Hibi, J. Ogawa, K. Yokozeki, S. Shimizu: Novel L-isoleucine hydrolating enzyme, L-isoleucine dioxygenase from Bacillus thuringiensis, produces (2S,3R,4S)-4-hydroxyisoleucine. Biochem. Biophy. Res. Commun. 390; 506-510, 2009
- Kira, I., Y. Asano and K. Yokozeki: Screening, purification, and identification of the enzyme producing
N-(L-α-L-aspartyl)-L-phenylalanine methyl ester from L-isoasparagine and L-phenylalanine methyl ester. J. Biosci. Bioeng. 108; 190–193, 2009
- Ogawa, J., J. Mano, T. Hagishita and S. Shimizu: Enantioselective ester hydrolase from Sphingobacterium sp. 238C5 useful for chiral resolution of β-phenylalanine and for its β-peptide synthesis. J. Mol. Catal., B Enzym. 60; 138-144, 2009
- Koyanagi, T., T. Katayama, H. Suzuki, A. Onishi, K. Yokozeki and H. Kumagai: Hyperproduction of 3,4-dihydroxyphenyl-L-alanine (L-Dopa) using Erwinia herbicola cells carrying a mutant transcriptional regulator TyrR.Biosci Biotechnol Biochem. 73; 1221-1223, 2009
- Ando, A., J. Ogawa, S. Sugimoto, S. Kishino, E. Sakuradani, K. Yokozeki and S. Shimizu: Selective production of cis-9,trans-11 isomer of conjugated linoleic acid from trans-vaccenic acid methyl ester by Delacroixia coronata.
J. Appl. Microbiol. 106; 1697-1704, 2009
- Horinouchi, N., T. Kawano, T. Sakai, S. Matsumoto, M. Sasaki, Y. Mikami, J .Ogawa and S. Shimizu: Screening and characterization of a phosphopentomutase useful for enzymatic production of 2'-deoxyribonucleoside. N. Biotechnol. 26; 75-82, 2009
- Kishino, S., J. Ogawa, K. Yokozeki and S. Shimizu: Metabolic diversity in biohydrogenation of polyunsaturated fatty acids by lactic acid bacteria involving conjugated fatty acid production. Appl. Microbiol. Biotechnol. 84; 87-97, 2009

b) Conference and seminar papers presented

- 5th German-Japanese Workshop on Enzyme Technology: 1 report
- Engineering Conferences International Enzyme Engineering XX: 1 report
- Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry 2010: 18 reports
- 100th AOCS Annual Meeting & Expo： 1 report
- The 5th Takeda Science Foundation Symposium on PharmaSciences： 1 report
- The International Health Food Symposium： 1 report
- Italy-Japan Symposium： 1 report

- 11th Meeting of Conjugated Linoleic Acid: 2 reports
- 8th Lipid Research Seminar: 2 reports

A-3.Off-campus activities

Membership in academic societies

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

Research grants

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

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

2.Other Research Grants

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

B.Educational Activities(2009.4-2010.3)

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: Kyoto Gakuen University, Faculty of Bioenvironmental Science (Special Lecture Series B), Soka University, Faculty of Engineering (Indtroduction to Industrial Biotechnology), Iwate University, Faculty of Agriculture (Industrial

Microbiology)