Chair Food Production Technology

2.7.8 Laboratory : Basic and Applied Molecular Biotechnology

Member :	Professor	Murata, Kousaku, Dr. Agric. Sci.
	Associate Professor	Hashimoto, Wataru, Dr. Agric. Sci.
	Assistant Professor	Kawai, Shigeyuki, Dr. Agric. Sci.
	Doctor's program	1
	Master's Program	6
	Undergraduate	2
	Post-Doctoral fellow	3
	Researcher	7

A. Research Activities (2010.4-2011.3)

A-1. Main Subjects

a) Bacterial cell-surface mechanism for macromolecule recognition

A Gram-negative Sphingomonas sp. A1 forms a huge mouth-like pit on the cell surface and incorporates alginate into the cytoplasm through the pit. The strain A1 flagellin-like protein functions as a cell-surface receptor for alginate. In this study, localization mechanism of the cell-surface receptor was analyzed. Another cell-surface protein SPH2681 was found to interact with the receptor. The protein contains a metallopeptidase-dependent HXXEH motif essential for Zn2+ binding and catalytic activity. Two genes coding for SPH2681 and SPH2682 were found to assemble into a single operon in the bacterial genome. Both proteins were purified from recombinant E. coli cells and characterized. SPH2681 associated with SPH2682 and formed a heterosubunit enzyme with peptidase activity. These results suggest that a monomeric SPH2681 functions as an anchor for the cell-surface receptor while a heterodimeric form of SPH2681 and SPH2682 show a metal-ion dependent peptidase.

b) Structural biology of polysaccharide lyase

Almost all alginate lyases act endolytically on substrate, thereby yielding unsaturated oligouronic acids having 4-deoxy-L-erythro-hex-4-enopyranosyluronic acid at the nonreducing end. In contrast, Agrobacterium tumefaciens alginate lyase Atu3025, a member of polysaccharide lyase family 15, acts on alginate polysaccharides and oligosaccharides exolytically and releases unsaturated monosaccharides from the substrate terminal. The crystal structures of Atu3025 and its inactive mutant in complex with alginate trisaccharide (H531A/ΔGGG) were determined at 2.10 and 2.99 Å resolutions with final R-factors of 18.3% and 19.9%, respectively, by X-ray crystallography. Two structural determinants, i.e., a short α-helix in the central α/α-barrel domain and a conformational change at the interface between the central and C-terminal domains, were found to be essential for the exolytic mode of action.

c) Biopolymer production from glycerol and atmospheric nitrogen by a nitrogen-fixing bacterium

Glycerol is generated as a major by-product during biodiesel production, and its efficient utilization is now sought in various areas. Glycerol metabolic pathway in A. vinelandii was determined through metabolite analysis, enzyme assay, and RT-PCR. Glycerol-3-phosphate was accumulated in A. vinelandii cells grown on glycerol to the exponential phase, and its level drastically decreased in the cells grown to the stationary growth phase. The bacterial cells grown on glycerol inducibly produced glycerol kinase GlpK and glycerol-3-phosphate dehydrogenase GlpD, but not glycerol dehydrogenase. The inducible expression of GlpD was regulated at the transcriptional stage.The PHB production level reached 33% per dry cell weight in nitrogen-free glycerol medium. This bioprocess using atmospheric nitrogen by the nitrogen-fixing bacterium is expected to produce valuable biopolymers from glycerol.

d) The structure of the NADP(H)-biosynthetic enzyme, NAD(H) kinase, of eukaryotic cell

NAD kinase is the NADP-biosynthetic enzyme catalyzing the phosphorylation of NAD. Pos5 is located in mitochondria of budding yeast Saccharomyces cerevisiae and is NADH kinase, which shows strong NADH kinase activity. Detailed analysis, including a comparison of the tertiary structure of Pos5 with the structures of human and bacterial NAD kinases revealed that Arg-293 of Pos5, corresponding to His-351 of human NAD kinase, confers positive charge on the surface of NADH-binding site, whereas the corresponding His-residue does not. Furthre analyes including site-deirected mutagenesis revealed that Arg-293 of Pos5 is a major determinant of NADH selectivity. Moreover, we found function-unknown C5orf33 gene, that is homologous with the plant NADK3 (NADH kinase) gene, in the human chromosome 5. Detailed study of C5orf33 protein suggested that this is a novel NAD kinase that functions in low NAD+ level.

e) The molecular biology of NAD-biosynthesis in Saccharomyces cerevisiae

Quinolinic acid (QA) is an intermediate of the kynurenine pathway. We have discovered that the budding yeast Saccharomyces cerevisiae secretes a significant amount of QA into the medium and also physiologically utilizes extracellular QA as a new vitamin (NAD+ precursor). Evidences are provided showing that extracellular QA enters the cell via Tna1, a high-affinity nicotinic acid permease. Physiological relevance of reutilization of extracellular QA is also evidenced. Moreover, possibility was pointed out that QA functions as a universal vitamin (NAD+ precursor) for fungi, even for those lacking the kynurenine pathway such as Schizosaccharomyces pombe. Thus, our data shed light on the significance of the kynurenine pathway, which is well known for NAD+ biosynthesis.

f) The oxygen-biology on Saccharomyces cerevisiae

An oxygen, especially a high-concentration-oxygen, is frequently used to promote healthy or to treat patients for medical purpose. However, a mechanism underlying how eucaryotic cells respond to a high-concentration-oxygen is elusive. To elucidate the mechanism, S. cerevisiae cells, which were cultured to log-phase aerobically, were exposed to 100 % oxygen for 5 and 15 min, and then a microarray analysis was conducted. As a result, several genes were up-regulated by this exposure. In particular, SRX1, of which product probably participates in the repair of proteins containing cysteine-sulfinic acid modifications, was up-regulated by about 30-fold. High expression of Srx1 against 100 % oxygen were demonstrated through western blotting and fluorescent microscopic observation of Srx1-GFP fusion protein. Phenotypic analysis of srx1 and tsa1 suggested the significance of both Srx1/Tsa1 and functional mitochondrial respiratory chain for protection to 100 % oxygen.

g) Biofuel production from marine biomass

Bioethanol production from algae is a promising approach that resolves problems associated with biofuel production from land biomass, such as bioethanol–food conflicts and the indirect land use change. In this study, an integrated bacterial system for converting alginate to ethanol was established using a metabolically modified, alginate-assimilating, pit-forming bacterium, Sphingomonas sp. A1 (strain A1). Overexpression of Zymomonas mobilis pdc and adhB was achieved using a strong constitutive expression promoter newly identified in strain A1 and by inserting multiple gene copies. Metabolome analysis revealed by-product accumulation, and its synthesis pathway was blocked by gene disruption. The ethanologenic recombinant strain A1 accumulated 13.0 g·L−1 ethanol in 3 days using alginate as the sole carbon source. We also searched yeast strains utilizing mannitol, an another promising marine biomass, for growth out of our 47 yeast laboratory strains and found 14 strains utilized it for growth. Among the 14 strains, 6 strains produced ethanol from mannitol and displayed tolerance to at least 5% extracellular ethanol.

h) Degradation of extracellular matrices by pathogenic bacteria

Streptococcal unsaturated glucronyl hydrolases degrade unsaturated heparin disaccharides as well as unsaturated hyaluronate and chondroitin disaccharides. This result indicates that each glycosaminoglycan in mammalian extracellular matrices is first depolymerized to unsaturated disaccharides by a specific polysaccharide lyase, and the resultant unsaturated disaccharides are degraded to the constituent monosaccharides by a single enzyme, UGL. In streptococcal genomes, the UGL gene is located near the heparin lyase III (HepC) gene, suggesting that the two proteins are cooperatively involved in degrading glycosaminoglycans. This gene organization is also observed in the genome of various pathogenic bacteria, suggesting that the UGL gene cluster is probably involved in the interaction of pathogenic bacteria with mammalian host cells.

i) Molecular biology of endophytic nitrogen fixing bacterium

Gluconacetobacter diazotorophicus (Gdi) is a endophytic, non-rhizobial acetic acid bacterium that is originally isolated from sugarcane. In order to utilize this bacterium for promotion of plant growth, it is important to deeply understand the growth characteristics and the conditions for expression of nif genes. In rhis study, it was revealed that, in the presence of nitrogen source (1.0 mM ammonium sulfate), high concentration of phosphate in the medium supresses the growth of Gdi, while low phosphate facilitates it. Conversely, in the absence of this nitogen source, high concentration of phosphate in the medium facilitated the growth of Gdi, while low phosphate suppressed it. These growth phenotypes solely depended on phosphate concentration, but not of pH. These data suggest that not onlythe concentration of nitorogen-compound but also that of phoshate in host plant is critical determinant to utilize Gdi for promotion of nonlegume.

A-2.Publications and presentations

a) Publications

Original Papers(including book-reviews)

- Maruyama, Y., A. Ochiai, B. Mikami, W. Hashimoto and K. Murata:
Crystal structure of bacterial cell-surface alginate-binding protein with an M_75 peptidase motif. Biochemical and Biophysical Research Communications 405; 411-416, 2011

- Nakamichi, Y., Y. Maruyama, B. Mikami, W. Hashimoto and K. Murata:
Structural determinants in streptococcal unsaturated glucuronyl hydrolase for recognition of glycosaminoglycan sulfate groups. Journal of Biological Chemistry 286; 6262-6271, 2011

- Maruyama, Y., A. Chuma, B. Mikami, W. Hashimoto and K. Murata:
Heterosubunit composition and crystal structures of a novel bacterial M16B metallopeptidase. Journal of Molecular Biology 407; 180-192, 2011

- Ochiai, A., M. Yamasaki, B. Mikami, W. Hashimoto and K. Murata:
Crystal structure of exotype alginate lyase Atu3025 from Agrobacterium tumefaciens. Journal of Biological Chemistry 285; 24519-24528, 2010

- Takase, R., A. Ochiai, B. Mikami, W. Hashimoto and K. Murata:
Molecular identification of unsaturated uronate reductase prerequisite for alginate metabolism in Sphingomonas sp. A1. Biochimica et Biophysica Acta 1804; 1925-1936, 2010

- Maruyama, Y., A. Ochiai, T. Itoh, B. Mikami, W. Hashimoto and K. Murata:
Mutational studies of the peptidoglycan hydrolase FlgJ of Sphingomonas sp. strain A1. Journal of Basic Microbiology 50; 311-317, 2010

- Pham, T. A., S. Kawai, E. Kono and K. Murata:
The role of cell wall revealed by the visualization of Saccharomyces cerevisiae transformation. Current Microbiology 62; 956-961, 2011

Reviews

- Ochiai, A., R. Takase, W. Hashimoto and K. Murata:
Structure and function of alginate-metabolizing enzymes. Vitamins 84; 525-531, 2010

- Kawai, S., W. Hashimoto and K. Murata:
Transformation of Saccharomyces cerevisiae and other fungi: Methods and possible underlying mechanism. Bioengineered Bugs 1; 395-403, 2010

Reports,others

- Nakamichi, Y., Y. Maruyama, R. Takase, Y. Nishitani, B. Mikami, W. Hashimoto and K. Murata:
Loop movement over the active cleft in streptococcal unsaturated glucuronyl hydrolase. SPring-8 User Experiment Report, 2010A1279

- Nakamichi, Y., Y. Maruyama, R. Takase, Y. Nishitani, B. Mikami, W. Hashimoto and K. Murata:
The flexible catalytic residues prerequisite for activity expression in bacterial glycosaminoglycan-degrading enzyme. SPring-8 User Experiment Report, 2010B1149

b) Conference and seminar papers presented

- The Annual Meeting (2010) of Japan Society for Bioscience, Biotechnology, and Agrochemistry (9)

- The Annual Meeting (2010) of Japan Society for Bioscience, Biotechnology, and Agrochemistry (Kansai Branch) (3)

- The 11 th Kansai Glycoscience Forum (1)

- The Annual Meeting (2010) of the Japanese Biochemical Society (1)

- The Annual Meeting (2010) of the Society for Biotechnology, Japan (1)

- The Annual Meeting (2010) of the Vitamin Society of Japan (1)

A-3.Off-campus activities 1

Membership in academic societies

- Murata, Kousaku, Dr. Agric. Sci. : Japan Society for Bioscience, Biotechnology, and Agrochemistry (Special Committee of Ethics, Member), Japan Society for Bioscience, Biotechnology, and Agrochemistry (Councilor of Nation-Wide), Japan Society for Bioscience, Biotechnology, and Agrochemistry (Selection Committee of Directors, Member), Japan Society for Japan Society for Bioscience, Biotechnology, and Agrochemistry (Editor-in-Chief of "Chemistry and Biology (Japanese)"), Japan Society for Bioscience, Biotechnology, and Agrochemistry (Councilor of Kansai Branch), Japan Society for Bioscience, Biotechnology, and Agrochemistry (Councilor of the Agricultural Chemical Research Foundation), Japan Society for Bioscience, Biotechnology, and Agrochemistry (The 100th Anniversary Memorial Implementation Committee of Vitamin B1 Discovery by Dr. Umetaro Suzuki, Member), The Society for Biotechnology, Japan (Councilor), The Society for Biochemistry, Japan (Councilor), The Vitamin Society of Japan (Councilor), The Japanese Society for Food Science and Technology (Councilor of Kansai Branch)

- Hashimoto, Wataru, Dr. Agric. Sci. : Japan Society for Bioscience, Biotechnology, and Agrochemistry (Representative), The Society for Biotechnology, Japan (Representative), Yeast Research Society of Japan (Operator)

- Shigeyuki Kawai, Dr. Agric. Sci. : Japan Society for Bioscience, Biotechnology, and Agrochemistry (Member of the Program organization) of the Annual meeting (2011)

A-3.Off-campus activities 2

Research grants

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

- Grant-in-Aid for Scientific Research (B) : Murata, Kousaku : Structure/function relationship and cell surface localization of bacterial flagellar flagellin

- Challenging Exploratory Research : Murata, Kousaku : Analysis of bacterial regulation for nitrogen-fixing reaction through identification of nitrogen import proteins

- Grant-in-Aid for Scientific Research (C) : Hashimoto, Wataru : Structure/function relationship of streptococcal system for heparin degradation and transport and its involvement in the bacterial infection disease

- Grant-in-Aid for Young Scientists (B) : Kawai, Shigeyuki : The mechanism underlying the regulation of biosynthesis and degradation of NADP(H) in Saccharomyces cerevisiae

2.Other Research Grants

- Bio-oriented Technology Research Advancement Institution：Murata, Kousaku：Ethanol production basis from marine biomass alginate

- Monbukagakusho Targeted Proteins Research Program：Hashimoto, Wataru：Structural biology of bacterial super-biosystem for import and degradation of polysaccharides

- Funding Program for Next Generation World-Leading Researchers：Kawai, Shigeyuki：Establishment of the practical ethanol-production system from marine biomass by utilizing the bacterium with regulated oxidation –reduction system

- Institute for Fermentation, Osaka, General Research Grant-in-Aid in 2010：Kawai, Shigeyuki：NAD-biosynthetic pathway focusing on the utilization and synthesis of quinolinate

A-4.International cooperation and overseas activities 1

Membership in academic societies

- Murata, Kousaku, Dr. Agric. Sci.: Bioengineered Bugs（International Editor）, American Society for Microbiology (Member), American Society for Biochemistry and Molecular Biology (Member)

- Hashimoto, Wataru, Dr. Agric. Sci.: Applied Microbiology and Biotechnology (Editor), American Society for Microbiology (Member)

- Kawai, Shigeyuki, Dr. Agric. Sci.:

B.Educational Activities(2010.4-2011.3)

B-1.On-campus teaching

a) Courses given

- Undergraduate level : Food Microbiology (Murata); Basic and Applied Molecular Biotechnology (Murata, Hashimoto); Introduction and Practice in Department of Food Science and Biotechnology (Murata, Hashimoto); Introduction to Foreign Literature II in Food Science and Biotechnology (Hashimoto, allotment); Laboratory Course in Microbiology (Hashimoto, Kawai, allotment)

- Graduate level : Food Production and Engineering (Advanced Course) (Murata, allotment); Molecular Biotechnology (Advanced Course) (Murata, Hashimoto); Molecular Biotechnology Seminar (Murata, Hashimoto); Experimental Course in Molecular Biotechnology (Murata, Hashimoto)

C.Other Remarks

- Murata, Kousaku, Dr. Agric. Sci. : Applied Microbial Research Foundation (Director), Special Investigator of Trend Research Center of Science and Technology, National Institute of Science and Technology Policy, the Ministry of Education, Culture, Sports, Science & Technology in Japan, Member of Selection Committee for Proposals to the Funding Program for Next Generation World-Leading Researchers, the Japan Society for the Promotion of Science, Member of Preliminary Selection Committee for Proposals to the Program for Practical Applications of Industrial Technologies, New Energy and Industrial Technology Development Organization, Member of Documentary Selection Committee for Proposals to the Promotion of Basic Research Activities for Innovative Biosciences, Bio-oriented Technology Research Advancement Institution, National Agriculture and Food Research Organization , Member of Selection Committee for Proposals to the Program of Asahi Breweries Foundation, World Innovation Foundation (Fellow), Deputy Director General of the International Biographical Center (Director), Chairman of Certification Institute of Safety and Sanitary Controls for Miyako and Foods, Public Health and Welfare Bureau, Kyoto City

Special notes :

The following reaerch presentation in the 2011 Annual Meeting was awarded as a Topics Prize by Japan Society for Bioscience,
Title: Production of ethanol from marine biomass alginate by molecular breeding of pit-forming bacterium.
After presentation, the research project was reported by newspapers with a national circulation (Asahi, Yomiuri, and Mainichi) and industrial newspapers. The national broadcast NHK is now preparing to