

## 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	2
	Master's Program	4
	Undergraduate	3
	Post-Doctoral fellow	5
	Researcher	5

### A. Research Activities (2009.4-2010.3)

#### A-1. Main Subjects

##### a) Macromolecule-sensing mechanism on bacterial cell surface

Flagellin homologues (p5 and p6) on the cell surface of alginate-assimilating bacterium, *Sphingomonas* sp. A1, function as alginate receptors. To clarify the structure of alginate-binding regions in the receptor p5, The three-dimensional structure of the fragmented receptor  $\Delta$ N20C20 (mutant with a lack of N and C-terminal 20 residues) with an alginate-binding ability was determined by X-ray crystallography. Finally, this structural model lacks N-terminal 43 and C-terminal 20 residues ( $\Delta$ N43C20, residue no. 44-364). Based on the crystal structure, C-terminal region responsible for alginate binding was found to constitute  $\alpha$ -helix and show a high average B-factor. On the other hand, due to its flexibility, the structure of N-terminal alginate-binding region could not be determined. Thus, alginate-binding regions in N and C-terminal domains were demonstrated to show a flexibility.

##### b) Structural proteomics of polysaccharide lyases

The *Chlorella* virus enzyme vAL-1 (38 kDa), a member of polysaccharide lyase family 14, consists of an N-terminal cell wall-attaching domain (11 kDa) and a C-terminal catalytic module (27 kDa). The catalytic module, truncated form vAL-1(S), exhibited alginate lyase activity, and released di- to hexasaccharides from alginate at pH 7.0, while disaccharides were preferentially generated at pH 10.0. This indicates that vAL-1(S) shows two pH-dependent modes of action—endo- and exotypes. The structure of GlcA-bound vAL-1(S) at pH 7.0 and

10.0 was determined by X-ray crystallography: GlcA was bound outside and inside the cleft at pH 7.0 and 10.0, respectively. This suggests that the electric charges at the active site greatly influence the binding mode of substrates and regulate endo/exo activity. This is the first study in which the structure of a family 14 polysaccharide lyase with two different modes of action has been determined.

c) Gas biology in a nitrogen-fixing bacterium in response to low nitrogen

A nitrogen-fixing *Azotobacter vinelandii* secretes iron-chelating siderophores for efficient iron uptake under iron-limited conditions and produces iron-bound nitrogenases for nitrogen fixation. In this study, an *A. vinelandii* gene cluster inducibly expressed in the low nitrogen gas concentration was identified to be responsible for the production of a fluorescent siderophore azotobactin. The culture supernatants of six gene disruptants in the cluster showed no azotobactin-derived absorbance at 380 nm, while the wild-type strain produced azotobactin. The absorbance at 310 nm appeared in the culture supernatants of the gene disruptants, but not in the wild-type strain. *A. vinelandii* produces another catechol-containing siderophore with a maximal absorption at 310 nm, suggesting that azotobactin-deficient mutants induced the production of the catechol siderophore. This is the first finding on the gene cluster for azotobactin production and synthetic regulation of two types of siderophores in *A. vinelandii*.

d) The structure of the NADP(H)-biosynthetic enzyme, NAD(H) kinase, of eukaryotic cell NAD kinase (NADK) is the NADP-biosynthetic enzyme catalyzing the phosphorylation of NAD. The tertiary structure as well as the detailed mechanism underlying the regulation of its activity are not known. Pos5 is located in mitochondria of budding yeast *Saccharomyces cerevisiae* and is NADH kinase, which shows strong NADH kinase activity. In this study, we successfully established an optimum condition to express human NADK (HsNADK) in *Escherichia coli*. We achieved an over-expression by about 20-fold compared with that of the previous report. NADK activity of the purified enzyme was inhibited by NADH and NADPH, but not by NADP. The enzyme exhibits the sigmoidal kinetics to ATP. These data suggest that intracellular states of redox and energy regulate NADK activity in human cell. Furthermore, tertiary structure of the Pos5/NADH complex was solved for the first time. We revealed the structural component, which possibly confers strong NADH kinase activity to Pos5.

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

During the construction of *Saccharomyces cerevisiae* npt1bna4 strain, we fortuitously discovered that *S. cerevisiae* can secrete a quinolinic acid (QA), an intermediate in NAD-biosynthetic pathway, into a medium. Based on this finding, npt1bna4nrk1 strain that exhibits a strict QA-auxotrophy was constructed. The method to determine the concentration of QA easily was established. Deeper understanding of the molecular mechanism underlying

the synthesis and secretion of QA, which is seriously concerns with the pathogenesis of Huntington's disease and Alzheimer's disease in human, was expected by using *S. cerevisiae* as a model system.

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.

g) Biofuel production from marine biomass

Microbial system for bioethanol production from marine biomass alginate was established by use of alginate-assimilating bacterium *Sphingomonas* sp. A1. In order to improve redox balance, *Saccharomyces cerevisiae* regeneration system of coenzymes was introduced to the recombinant bacterial strain having *Zymomonas mobilis* genes for pyruvate decarboxylase and alcohol dehydrogenase. The resultant strain produced over 1% ethanol from alginate within 5 days.

h) Degradation of glycosaminoglycans by streptococci

Pathogenic streptococci such as *S. agalactiae*, *S. pneumoniae*, and *S. pyogenes* invade mammalian host cells through degradation of extracellular matrices, glycosaminoglycans, by polysaccharide lyases and unsaturated glucuronyl hydrolase (UGL). Streptococcal UGLs preferred sulfated substrates. The crystal structure of *S. agalactiae* UGL mutant in complex with the sulfated substrate ( $\Delta 6S$ , sulfation at C6 position of GalNAc) was determined by X-ray crystallography. Ser368 and Lys370 were found to be important for binding to sulfate group of the substrate. These are also supported by site-directed mutagenesis study. This substrate specificity of streptococcal UGLs is suggested to be feasible for bacterial infection through degradation of mammalian extracellular matrices with sulfate groups.

## A-2.Publications and presentations

a) Publications

Books

- Hashimoto, W., Y. Maruyama, T. Itoh, B. Mikami, and K. Murata:

Bacterial system for alginate uptake and degradation. In "Alginates: Biology &

Applications" Microbiology Monographs 13: (Ed. Bernd Rehm), Springer, pp73-94, 2009

#### Original Papers

- Ogura, K., M. Yamasaki, T. Yamada, B. Mikami, W. Hashimoto and K. Murata:  
Crystal structure of family 14 polysaccharide lyase with pH-dependent modes of action. J. Biol. Chem., 284; 35572-35579, 2009
- Maruyama Y., Y. Nakamichi, T. Itoh, B. Mikami, W. Hashimoto and K. Murata:  
Substrate specificity of streptococcal unsaturated glucuronyl hydrolases for sulfated glycosaminoglycan. J. Biol. Chem., 284; 18059-18069, 2009
- Hashimoto, W., A. Ochiai, J. He, T. Itoh, B. Mikami and K. Murata:  
Crystallization and preliminary crystallographic analysis of cell-surface alginate-binding protein Algp7 from *Sphingomonas* sp. A1. Acta Crystallograph. Sect. F Struct. Biol. Cryst. Commun., 65; 515-517, 2009

#### Reviews

- Hashimoto W, S. Kawai and K. Murata:  
Bacterial supersystem for alginate import/metabolism and its environmental and bioenergy applications. Bioengineered Bugs, 1; 1-13, 2010

#### Reports

- Takase, R., A. Ochiai, T. Itoh, K. Ogura, B. Mikami, W. Hashimoto and K. Murata:  
Crystal structure of NADP-bound  $\alpha$ -keto acid reductase prerequisite for alginate metabolism in *Sphingomonas* sp. A1. SPring-8 User Experiment Report, 2009B1177, 2009
- Maruyama, Y., A. Chuma, T. Itoh, A. Ochiai, K. Ogura, B. Mikami, W. Hashimoto and K. Murata:  
Crystal structure of the metallopeptidase family M16 enzyme from *Sphingomonas* sp. strain A1. SPring-8 User Experiment Report, 2009B1192, 2009
- Nakamichi, Y., Y. Maruyama, T. Itoh, A. Ochiai, K. Ogura, B. Mikami, W. Hashimoto and K. Murata:  
Crystal structure of streptococcal unsaturated glucuronyl hydrolase in complex with sulfated substrate. SPring-8 User Experiment Report, 2009B1201, 2009
- Maruyama, Y., A. Chuma, A. Ochiai, K. Ogura, Y. Nakamichi, B. Mikami, W. Hashimoto and K. Murata:  
X-ray structural analysis of the metallopeptidase family M16 enzyme. SPring-8 User Experiment Report, 2009A1179, 2009

- Takase, R., A. Ochiai, Y. Nakamichi, T. Itoh, K. Ogura, B. Mikami, W. Hashimoto and K. Murata:

X-ray crystal structure of Sphingomonas sp. A1  $\alpha$ -keto acid reductase for alginate metabolism. SPring-8 User Experiment Report, 2009A1329, 2009

#### Patents

- Patent pending/applied for Patent application no. 2009-198972 “Etanol-production from marine biomass”, inventors: Murata K, Hashimoto W, Kawai S, Orita K, Ihara K, Mikami B, Takeda H, Yoneyama N, Otchiai A, applicant: Kyoto university, Maruha Nichiro Holdings, Inc., application date: Aug. 28, 2009

#### b) Conference and seminar papers presented

- The Annual Meeting (2009) of Japan Society for Bioscience, Biotechnology, and Agrochemistry (7)
- The 62th Annual Meeting of the Vitamin Society of Japan (2)
- The 82th Annual Meeting of the Japanese Biochemical Society (7)
- The 2nd Meeting for Phospho-compounds (the 29th Meeting for C-P Compounds)(2)
- The Annual Meeting (2009) of The Society for Biotechnology, Japan (1)

### **A-3.Off-campus activities**

#### Membership in academic societies

- Murata, Kousaku, Dr. Agric. Sci. : Japan Society for Bioscience, Biotechnology, and Agrochemistry (Councilor of Nation-Wide, Editor-in-Chief of “Chemistry and Biology (Japanese)”, The Society for Biotechnology, Japan (Councilor), The Society for Biochemistry, Japan (Councilor. Member of Organizing Committee of the 82nd Annual Meeting of the Society for Biochemistry, Japan), The Japan Society for Nutrition and Food (Director), The Vitamin Society of Japan (Councilor of Nation-Wide)
- 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)
- Kawai, Shigeyuki, Dr. Agric. Sci. : Japan Society for Bioscience, Biotechnology, and Agrochemistry (Representative)

#### Research grants

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

- Scientific Research (B) : Murata, Kousaku : Structure/function relationship and cell surface

localization of bacterial flagellar flagellin

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

- 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

## A-4. International cooperation and overseas activities

### Membership in academic societies

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

## B. Educational Activities(2009.4-2010.3)

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