Division of Environmental Chemistry
– Molecular Microbial Science –

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Scope of Research

Microorganisms are found almost everywhere on Earth. They have a great diversity of capacities to adapt to various environments, including chemically and physically unusual environments. Our main subject is to clarify the molecular basis of environmental adaptations of microorganisms and their application. Specific functions of proteins and lipids with essential roles in environmental adaptation of extremophilic microorganisms are of our particular interest. We also undertake mechanistic analysis of microbial enzymes, in particular, those involved in unique metabolic pathways, and their application.

KEYWORDS

Extremophiles
Phospholipid Acyltransferase
Bacterial Cold-adaptaion Mechanism
Membrane Vesicle
Polyunsaturated Fatty Acid

Selected Publications

Ogawa, T.; Tanaka, A.; Kawamoto, J.; Kurihara, T., Purification and Characterization of 1-Acyl-*sn*-glycerol-3-phosphate Acyltransferase with a Substrate Preference for Polyunsaturated Fatty Acyl Donors from the Eicosapentaenoic Acid-producing Bacterium *Shewanella livingstonensis* Ac10, *J. Biochem.*, **164,** 33-39 (2018).

Toyotake, Y.; Cho, H. N.; Kawamoto, J.; Kurihara, T., A Novel 1-Acyl-*sn*-glycerol-3-phosphate *O*-Acyltransferase Homolog for the Synthesis of Membrane Phospholipids with a Branched-Chain Fatty Acyl Group in *Shewanella livingstonensis* Ac10, *Biochem. Biophys. Res. Commun.*, **500,** 704-709 (2018).

Tokunaga, T.; Watanabe, B.; Sato, S.; Kawamoto, J.; Kurihara, T., Synthesis and Functional Assessment of a Novel Fatty Acid Probe, ω-Ethynyl Eicosapentaenoic Acid Analog, to Analyze the in Vivo Behavior of Eicosapentaenoic Acid, *Bioconjugate Chem.*, **28,** 2077-2085 (2017).

Yokoyama, F.; Kawamoto, J.; Imai, T.; Kurihara, T., Characterization of Extracellular Membrane Vesicles of an Antarctic Bacterium, *Shewanella livingstonensis* Ac10, and Their Enhanced Production by Alteration of Phospholipid Composition, *Extremophiles*, **21,** 723-731 (2017).

Sato, S.; Kawamoto, J.; Sato, S. B.; Watanabe, B.; Hiratake, J.; Esaki, N.; Kurihara, T., Occurrence of a Bacterial Membrane Microdomain at the Cell Division Site Enriched in Phospholipids with Polyunsaturated Hydrocarbon Chains, *J. Biol. Chem.*, **287,** 24113-24121 (2012).

Elucidation of a Selective Protein-secretion Mechanism via Extracellular Membrane Vesicle of a Psychotropic Bacterium, *Shewanella* sp. HM13

Shewanella sp. HM13, a cold-adapted bacterium isolated from fish intestine, can produce larger amounts of extracellular membrane vesicles (EMVs) than the related strains, and the EMVs harbor a functionally unknown protein, P49, as a major single cargo. To elucidate the molecular mechanism of the P49-selective cargo loading to the EMVs, whole genome sequence of this strain was determined, and we found that a gene coding for P49 is located in a gene cluster composed of genes coding for homologs of subunits of type II secretion machinery (T2SS) of Gram-negative bacterium, proteins involved in bacterial surface lipoglycan, and functionally unknown proteins. When the genes coding for homologs of T2SS-like translocon were disrupted, P49 was accumulated in the cell and the post-vesicle fraction (PVF) of the culture supernatant. On the other hand, P49 localized to the EMVs disappeared in these mutants. Deletion mutants of each of lipoglycan-synthesis related genes in the gene cluster showed accumulation of P49 at the PVF and marked decrease in the amount of P49 both in the EMVs and cell fraction, suggesting that defects of these genes affect the cell surface structure and enhance secretion of P49 to the PVF without the cargo loading to EMVs. These results also suggest that T2SS-like machinery coded by P49-containing gene cluster translocates P49 to the outer membrane, and P49 interacts with the EMVs or the precursors on the outer membrane surface.

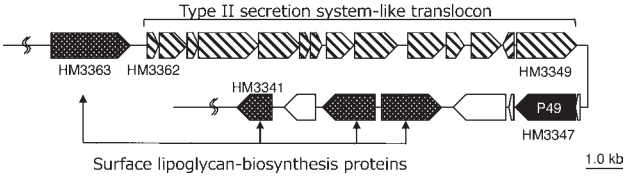

Figure 1. Genetic map of P49-containing gene cluster of *Shewanella* sp. HM13.

In vivo Characterization of a Novel Lysophosphatidic Acid Acyltransferase Homolog of *Escherichia coli*

Lysophosphatidic acid acyltransferase (LPAAT) introduces fatty acyl groups into the *sn*-2 position of membrane phospholipids. Multiple LPAAT homologs occur in some bacteria, and they are proposed to generate the membrane diversity by introducing different fatty acyl groups. *Shewanella livingstonensis* Ac10 isolated from Antarctic seawater has five LPAAT homologs (SlPlsC1 to SlPlsC5). SlPlsC1 and SlPlsC4 play an essential role in the synthesis of phospholipids containing an eicosapentaenoyl group and branched-chain fatty acyl groups, respectively. On the other hand, it has long been believed that *Escherichia coli* has one essential LPAAT homolog named PlsC. However, we demonstrated that *E. coli* possesses a physiologically functional SlPlsC4 ortholog named YihG, showing 39.1% sequence identity to SlPlsC4. YihG is also conserved in some γ-proteobacteria such as *Salmonella typhimurium* and *Vibrio cholerae*. Overexpression of YihG in *E. coli* JC201 carrying a temperature-sensitive mutation in *plsC* allowed its growth at non-permissive temperature. ESI-MS/MS and GC-MS analyses revealed that YihG introduces a *cis*-vaccenoyl group ($18{:}1^{\Delta 11}$) at the *sn*-2 position of phospholipids. A soft agar assay and microscopic observation showed enhanced swimming motility of the *yihG*-deleted mutant cells compared with the wild-type cells. These results suggested that *E. coli* YihG modulates the swimming motility by introducing the specific fatty acyl group into membrane phospholipids.

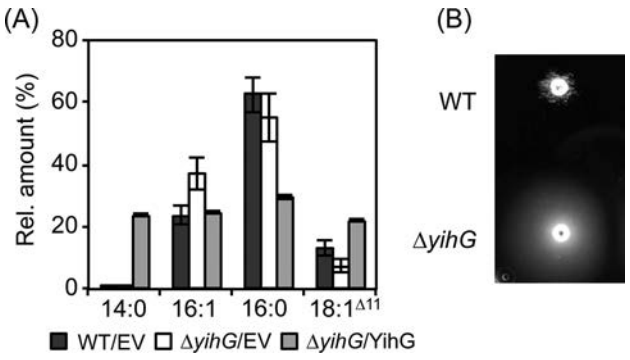

Figure 2. (A) Composition of fatty acyl groups at the *sn*-2 position of phospholipids from the wild-type strain harboring the empty vector (dark gray) and the *yihG*-deleted mutant harboring the empty vector (white) or YihG expression vector (light gray). Phospholipids were extracted and hydrolyzed by phospholipase A2, and resulting fatty acids were extracted and analyzed by GC-MS. (B) Motilities of the wild-type and *yihG*-deleted mutant cells on a 0.2% soft-agar plate. The cells were incubated at 37°C for 12 h.