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

Recent Selected Publications

Yokoyama, F.; Imai, T.; Aoki, W.; Ueda, M.; Kawamoto, J.; Kurihara, T., Identification of a Putative Sensor Protein Involved in Regulation of Vesicle Production by a Hypervesiculating Bacterium, *Shewanella vesiculosa* HM13, *Frontiers in Microbiology*, **12,** 629023 (2021).

Chen, C.; Kawamoto, J.; Kawai, S.; Tame, A.; Kato, C.; Imai, T.; Kurihara, T., Isolation of a Novel Bacterial Strain Capable of Producing Abundant Extracellular Membrane Vesicles Carrying a Single Major Cargo Protein and Analysis of Its Transport Mechanism, *Frontiers in Microbiology*, **10,** 3001 (2020).

Toyotake, Y.; Nishiyama, M.; Yokoyama, F.; Ogawa, T.; Kawamoto, J.; Kurihara, T., A Novel Lysophosphatidic Acid Acyltransferase of *Escherichia coli* Produces Membrane Phospholipids with a *cis*-vaccenoyl Group and Is Related to Flagellar Formation, *Biomolecules*, **10,** 745 (2020).

Ogawa, T.; Hirose, K.; Yusuf, Y.; Kawamoto, J.; Kurihara, T., Bioconversion from Docosahexaenoic Acid to Eicosapentaenoic Acid in the Marine Bacterium *Shewanella livingstonensis* Ac10, *Frontiers in Microbiology*, **11,** 1104 (2020).

Study on the Roles of Multiple Lysophosphatidic Acid Acyltransferases in Bacteria

Phospholipids (PLs), which are major constituents of biological membranes, comprise fatty acyl groups that vary in their chemical structures (*e.g.*, carbon chain length and unsaturation level) and correspondingly form PL membranes with various physical properties (*e.g.*, fluidity and thickness). Bacteria control the fatty acid compositions of the PL membranes in response to environmental changes to maintain membrane integrity. Lysophosphatidic acid acyltransferase (LPAAT), which is an enzyme that introduces an *sn*-2 fatty acid of PLs during their biosynthesis, is a regulator for the membrane fatty acid compositions. Many bacteria have multiple LPAAT paralogs, suggesting that these enzymes differ in functions, localizations, and timing of expression, and their coordinated actions enable the accurate regulation of membrane fatty acid compositions. We have been studying the LPAAT multiplicity using the marine bacterium *Shewanella livingstonensis* Ac10, which has five LPAATs (designated as PlsC1−PlsC5). To understand their division of roles, we studied the enzymatic and physiological functions of the two closely homologous LPAATs, PlsC4 and PlsC5. Mutagenesis analysis revealed that PlsC4 selectively utilizes *iso*-tridecanoic acid (medium-chain length and methyl-branched) for PL production, while PlsC5 prefers hexadecenoic acid (long-chain length and monounsaturated), thus generating PLs with different physicochemical properties. Consistent with a beneficial effect of branched-chain fatty acids in bacterial cold-adaptation, the *plsC4*-deficient cell grew slower than the *plsC5*-deficient and parent cells at 4 °C, whereas their growth was comparable at 18 °C. In addition, bioinformatic analysis indicated that the two LPAAT paralogs, which are distant from principal LPAATs, are widely distributed among γ-proteobacteria such as *Escherichia*, *Pseudomonas*, and *Vibrio*. Therefore, regulation of the membrane fatty acid compositions by multiple LPAATs might be more common than previously considered.

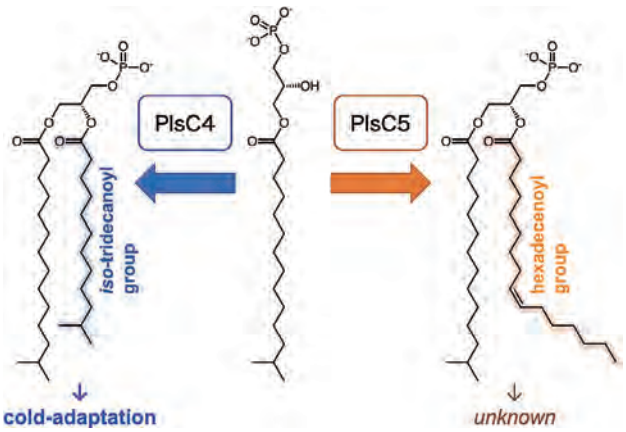

Figure 1. PlsC4 and PlsC5 are selective to different fatty acyl donor substrates and produce distinct PLs.

Construction of a Hypervesiculation Strain of *Shewanella vesiculosa* HM13 to Develop a Secretory Protein Production System at Low Temperatures

Bacteria secrete spherical nanoparticles enclosed by lipid membranes called extracellular membrane vesicles (EMVs), which selectively transport various biomolecules, including nucleic acids, lipids, lipopolysaccharides, and proteins, to their extracellular environments. EMVs serve a number of important roles in microbial interactions and survival in hostile environments. Besides, EMVs have attracted the attention of biotechnological industries for their potential use as a platform of vaccine, drug-delivery systems, and recombinant protein production systems.

Shewanella vesiculosa HM13, a psychrotrophic Gram-negative bacterium isolated from the intestinal contents of horse mackerel, abundantly produces EMVs carrying a single major cargo protein, P49, of unknown function and thus has a great potential for the secretory production of heterologous proteins. In Gram-negative bacteria, the outer membrane is connected to the peptidoglycan predominantly through Braun's lipoprotein (Lpp), and the formation of this linkage is catalyzed by L,D-transpeptidase (Ldt). The importance of this linkage for cell membrane stability implies that its disruption may lead to increased EMV production. In this study, to enhance the EMV productivity of *S. vesiculosa* HM13, we identified and disrupted genes coding for proteins involved in the outer membrane integrity, Lpp and Ldt. According to the bioinformatics analysis using amino acid sequences of *E. coli* Lpp and Ldt, we found genes coding for these protein homologs in the genome of *S. vesiculosa* HM13. These proteins have sequence similarities of 34.8% and 59.8% to those of *E. coli*, respectively. *lpp*- and *ldt*-gene disrupted mutants, ΔLpp and ΔLdt, generated by single crossover recombination demonstrated normal growth characteristics compared to the wild type. Next, we analyzed their EMV productivity, size distribution, and morphology. As a result, about 2.5-fold increase in EMV production was observed for ΔLpp and ΔLdt, while the morphology of EMVs of these mutants remained identical to those of the parent strain. In accordance with the increase in EMV production, the mutants secreted 2.3–2.5 mg/OD_{600}·L of P49 into the culture broth through EMVs as the cargo (Figure 2), which is around two times of the parent strain. These findings will contribute to the development of the EMV-based protein production system by using *S. vesiculosa* HM13 as the host.

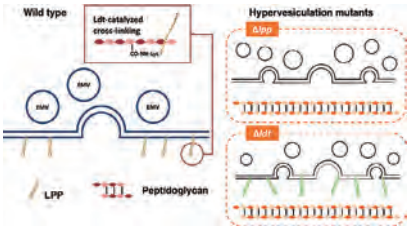

Figure 2. Schematic model of hypervesiculation strain of *Shewanella vesiculosa* HM13.