Division of Environmental Chemistry
– Molecular Microbial Science –

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Scope of Research

Microorganisms are found almost everywhere on Earth. They have 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
Membrane Vesicle

Selected Publications

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).
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, *Bioconjug. Chem.*, **28,** 2077-2085 (2017).
Kurihara, T.; Kawamoto, J.; Ogawa, T., Biosynthesis and Physiological Functions of ω-3 Long Chain Polyunsaturated Fatty Acids in Bacteria, *Vitamins*, **91,** 555-562 (2017).
Kurihara, T., Diversity of Bacterial Membrane Phospholipids: Their Biosynthesis and Functions, *Membrane*, **42,** 175-180 (2017).
Kawamoto, J.; Kurihara, T.; Esaki, N., Proteomic Insights of Psychrophiles, *Psychrophiles: From Biodiversity to Biotechnology*, 423-435 (2017).

Isolation and Characterization of a Membrane-Vesicle-Producing Bacterium, *Shewanella* sp. HM13

Protein expression at low temperatures is expected to alleviate the heat denaturation of proteins and would be suitable for the production of thermolabile proteins. To develop an efficient protein-production system, we isolated a cold-adapted Gram-negative bacterium, *Shewanella* sp. HM13, from fish intestine. This strain can produce about 5 mg/L culture of a secretory protein (P49), which was co-purified with the extracellular membrane vesicles (EMV) at the temperature range of 4~18°C. To determine the localization of P49, we prepared the EMV-containing fraction by ultracentrifugation and analyzed proteins co-purified with the EMVs, demonstrating that P49 is a major cargo protein of the EMVs, and more than 80% of P49 was recovered with the EMVs. These results suggested that specific cargo selection mechanism is operating for EMV proteins. Whole genome sequencing of *Shewanella* sp. HM13 demonstrated that the gene coding for P49 was located in a gene cluster containing *pulD* encoding a component of a general secretory pathway. The secretion of P49 within the EMVs was decreased in the *pulD*-disrupted mutant, suggesting that PulD is involved in the association of P49 with EMVs.

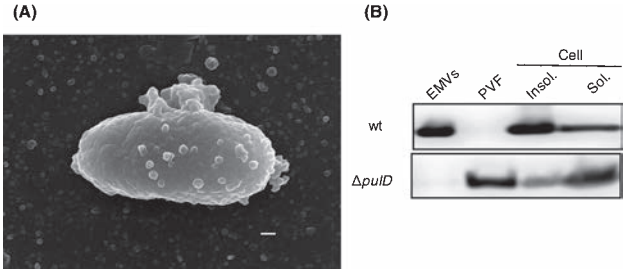

Figure 1. EMV-producing bacterium, *Shewanella* sp. HM13.
(A) SEM image of *Shewanella* sp. HM13. This strain can secrete EMVs from its cell surfaces to the extracellular milieu. Bar indicates 100 nm. (B) Localization of a cargo protein, P49. In the wild type strain, P49 was observed from the EMVs and the insoluble fraction of the cells. Gene deletion of *pulD* alters the localization of P49 from the EMVs to the post-vesicle fraction (PVF) and the soluble fraction of the cells, indicating that PulD is involved in the membrane association of P49.

Analysis of Protein-Protein Interaction between Polyunsaturated Fatty Acid-producing Enzymes

Some marine bacteria produce ω-3 long-chain polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid. They exist mainly as an acyl chain of membrane phospholipids and are inferred to play important physiological roles in extreme environments. Bacterial PUFA biosynthesis requires five enzymes, *i.e.* Orf2, Orf5, Orf6, Orf7, and Orf8, that catalyze a cycle of acyl chain elongation and C=C bond formation. Because each of the enzymes has different catalytic domains, it is proposed that they work cooperatively to accomplish PUFA biosynthesis. However, it has been uncertain whether and how the five enzymes interact with each other. To reveal it, we developed monoclonal antibodies and performed pull-down assays for the enzymes from an Antarctic EPA-producing bacterium *Shewanella livingstonensis* Ac10. As a result, we found that Orf5, Orf6, Orf7, and Orf8 tightly interact with each other, whereas Orf2 transiently. We also found their interaction with a lysophosphatidic acid acyltransferase, namely PlsC, from *S. livingstonensis* Ac10, an enzyme that incorporates EPA into membrane phospholipids *in vivo*. These results suggested that the protein-protein interactions enable the efficient production of EPA and EPA-containing phospholipids, which may contribute to the survival in hostile environments.

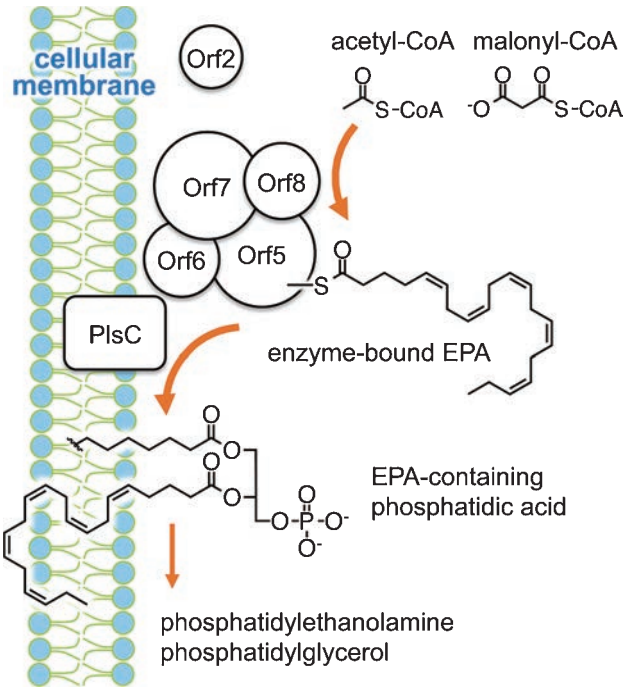

Figure 2. Schematic view of EPA and EPA-containing phospholipid biosynthesis.