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

Selected Publications

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, *Front. Microbiol.*, **10,** 3001 (2020).

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

Kamasaka, K.; Kawamoto, J.; Chen, C.; Yokoyama, F.; Imai, T.; Ogawa, T.; Kurihara, T., Genetic Characterization and Functional Implications of the Gene Cluster for Selective Protein Transport to Extracellular Membrane Vesicles of *Shewanella vesiculosa* HM13, *Biochem. Biophys. Res. Commun.*, **526,** 525-531 (2020).

Yustina, Y.; Ogawa, T.; Kawamoto, J.; Kurihara, T., Role of Acyl-CoA Dehydrogenases from *Shewanella livingstonensis* Ac10 in Docosahexaenoic Acid Conversion, *Biochem. Biophys. Res. Commun.*, **528,** 453-458 (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).

Genetic Characterization of the Gene Cluster for Selective Cargo Transportation to Extracellular Membrane Vesicles of a Hyper-vesiculating Bacterium, *Shewanella vesiculosa* HM13

Bacteria release spherical nanoparticles enclosed by lipid membranes called extracellular membrane vesicles (EMVs), which selectively deliver various cellular components, such as DNA, lipids, lipopolysaccharides, and proteins, to their extracellular milieu. EMVs are involved in microbial interactions and survival in the hostile environment. Besides, EMVs have attracted the attention of biotechnological industries for their potential use as a vaccine, a carrier of drugs, and a platform for recombinant protein production.

Shewanella vesiculosa HM13, a Gram-negative bacterium isolated from fish intestine, produces a large amount of EMVs carrying an S-layer-like protein, P49, as a single major cargo of the EMVs. Secretion of P49 with high purity could be useful for secretory production of foreign protein. Whole genome sequencing of this strain has revealed that the gene coding for P49 is present in a gene cluster including genes encoding homologs of cell-surface glycolipid-synthesis proteins and components of the type II protein secretion system (T2SS). To identify the genes involved in P49-selective protein transportation, we disrupted the genes in the cluster and analyzed the transportation of P49 to EMVs of the mutants. P49 of the mutants of the genes encoding components of the T2SS was localized mainly at the cellular fraction, not in the EMV fraction and the post-vesicle fraction removed EMVs from the culture supernatant. This result reveals that these homologs are components of a protein secretion machinery that plays an essential role in the transport of P49 to EMVs. Because P49 has an N-terminal signal sequence, the nascent protein synthesized in the cytoplasm is translocated across the inner membrane. Thus, it is assumed that the possible protein secretion machinery delivers P49 from the periplasm to the cell surface or extracellular space (Figure 1). Disruption of the genes encoding cell-surface polysaccharide-synthesis protein (Wzx2 and LptA), lipid-modification protein (GdpD), and nitroreductase (NfnB) changed the localization of P49. P49 of these mutants disappeared from EMVs and was found mainly from the PVF. On the other hand, it was also found that these genes are not essential for the EMV synthesis, suggesting that proteins encoded by these genes are required for the association of P49 with EMVs after its translocation to the cell surface or extracellular space. Wzx2 is a homolog of Wzx flippase family protein, which contributes to the biosynthesis of the O-antigen of lipopolysaccharides. Thus, it is hypothesized that Wzx2 plays a role in the biosynthesis of a surface glycolipid of EMVs required for tethering of P49 to EMVs (Figure 1).

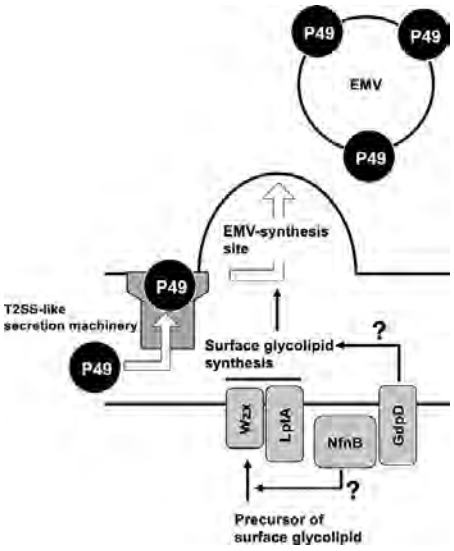

Figure 1. Possible model of P49-transportation pathway to EMVs of *Shewanella vesiculosa* HM13.

Detailed Analysis of Bacterial Bioconversion of DHA into EPA

Some marine microorganisms produce ω-3 polyunsaturated fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are beneficial to human health. To develop the microbial production system for EPA/DHA, their *de novo* biosynthesis has been intensively investigated. However, a comprehensive understanding of EPA/DHA metabolism including degradation and bioconversion is lacking. The EPA-producing bacterium *Shewanella livingstonensis* Ac10 is capable of not only biosynthesizing EPA-containing phospholipids but also converting exogenous DHA into EPA. We previously found that the unique conversion is mediated by an auxiliary β-oxidation enzyme, *i.e.* 2,4-dienoyl-CoA reductase. To further reveal the conversion mechanism, we examined the involvement of two homologs of acyl-CoA dehydrogenase (FadE1 and FadE2), which presumably catalyze the first step of β-oxidation reactions. Mutagenesis experiments demonstrated that the disruption of *fadE1*, but not of *fadE2*, led to the decreased DHA conversion. The expression of *fadE1* was enhanced when a cell was supplemented with DHA, while that of *fadE2* remained unchanged. Consistent with these *in vivo* results, we found that the recombinant FadE1 catalyzed the dehydrogenation of DHA-CoA *in vitro*. Altogether, these data suggest that FadE1 is a β-oxidation enzyme that contributes to the conversion from DHA to EPA.