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

Kawano, K.; Kamasaka, K.; Yokoyama, F.; Kawamoto, J.; Ogawa, T.; Kurihara, T.; Matsuzaki K., Structural Factors Governing Binding of Curvature-Sensing Peptides to Bacterial Extracellular Vesicles Covered with Hydrophilic Polysaccharide Chains, *Biophys. Chem.*, **299,** 107039 (2023).

Kamasaka, K.; Kawamoto, J.; Tsudzuki, T.; Liu, Y.; Imai, T.; Ogawa, T.; Kurihara, T., Capsular Polysaccharide-Mediated Protein Loading onto Extracellular Membrane Vesicles of a Fish Intestinal Bacterium, *Shewanella vesiculosa* HM13, *bioRxiv*, 04.25.538355 (2023).

Mullane, K. K.; Nishiyama, M.; Kurihara, T.; Bartlett, D. H., Compounding Deep Sea Physical Impacts on Marine Microbial Motility, *Front. Mar. Sci.*, **10** (2023).

Ogawa, T.; Kuboshima, M.; Suwanawat, N.; Kawamoto, J.; Kurihara, T., Division of the Role and Physiological Impact of Multiple Lysophosphatidic Acid Acyltransferase Paralogs, *BMC Microbiol.*, **1,** 241 (2022).

Conversion of Docosahexaenoic Acid to Eicosapentaenoic Acid by β-Oxidation Enzymes in *Shewanella livingstonensis* Ac10

Shewanella livingstonensis Ac10, a cold-adapted Gram-negative bacterium isolated from Antarctic seawater, produces eicosapentaenoic acid (EPA) at low temperatures. An EPA-less mutant strain (ΔEPA) showed delayed growth and cold-sensitive phenotypes. ΔEPA cultured in the medium supplemented with docosahexaenoic acid (DHA)-containing phospholipids grew normally at low temperatures. Interestingly, the mutant contained not only DHA-containing phospholipids but also EPA-containing phospholipids even though it cannot produce EPA *de novo*. These results suggested *S. livingstonensis* Ac10 requires EPA or DHA to adapt to low temperatures, and it has an unknown conversion pathway of DHA to EPA to generate EPA selectively. Previous studies demonstrated that first two β-oxidation enzymes, acyl-CoA dehydrogenase (FadE) and 2,4-dienoyl-CoA reductase (FadH), are involved in this conversion of DHA to EPA. Therefore, the conversion pathway is likely identical or similar to the β-oxidation pathway. On the other hand, it is unclear how EPA is produced from DHA by β-oxidation, in which a series of oxidation reactions repeatedly occur to degrade fatty acids into acetyl-CoA in general, and whether the last two β-oxidation enzymes, FadB and FadA, or FadJ and FadI, are involved in this conversion. Gene-deletion analysis of these genes demonstrated that, in the single *fad* mutant strains, EPA converted from DHA was accumulated, and EPA-containing phospholipids were produced. ΔEPA/ΔFadJ had DHA more than the others, and unknown fatty acids predicted as intermediates were observed in the mutant. However, every fad mutant, including ΔEPA/ΔFadJ, still has the conversion ability, indicating the redundant role of FadB/FadJ and FadA/FadI in the conversion. Therefore, double knockout strains, ΔEPA/ΔFadBJ and ΔEPA/ΔFadAI, were constructed. In ΔEPA/ΔFadBJ strain, the DHA-to-EPA conversion rate was decreased by 32% compared with ΔEPA. These results revealed the ß-oxidation enzymes play a role in the DHA-EPA conversion of this bacterium.

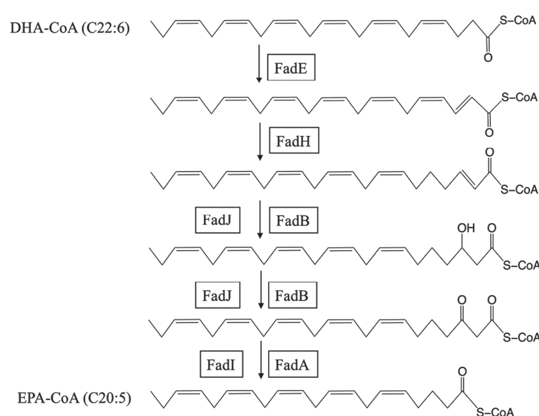

Figure 1. Putative conversion pathway of DHA to EPA.

Screening and Identification of Genes Involved in Extracellular Membrane Vesicle Production of *Shewanella vesiculosa* HM13

Extracellular membrane vesicles (EMVs) are lipid nanoparticles secreted by almost all bacteria, and their physiology and biotechnological applications have been attracting significant attention. However, the molecular basis of EMV biogenesis has yet to be fully elucidated. To facilitate the elucidation of bacterial EMV production, a curvature-sensing peptide, nFAAV5-NBD, was developed by using a hyper-vesiculating bacterium, *Shewanella vesiculosa* HM13, as a model organism. nFAAV5-NBD can bind to EMVs of this bacterium but not to the cells. In this study, we applied nFAAV5-NBD to screen a hyper-vesiculation and hypo-vesiculation mutant from the mutant library generated by transposon random mutagenesis (Fig. 2). As a result, we identified 16 or six genes whose transposon insertions caused hyper- or hypo-vesiculation, respectively. Identification of the transposon-insertion site indicated that genes involved in various cell processes, including protein quality control, cell wall synthesis, and signal transduction, are involved in the EMV production of *S. vesiculosa* HM13. A target gene disruption analysis of the identified genes also demonstrated the changes in EMV production of the mutants. These strains obtained in this study would contribute to the elucidation of bacterial vesicle formation mechanisms. Additionally, the hyper-vesiculating mutants obtained from this study would provide a clue for the application of this bacterium, for example, the production of valuable substances as a cargo of EMVs and the development of surface-engineered vesicles.

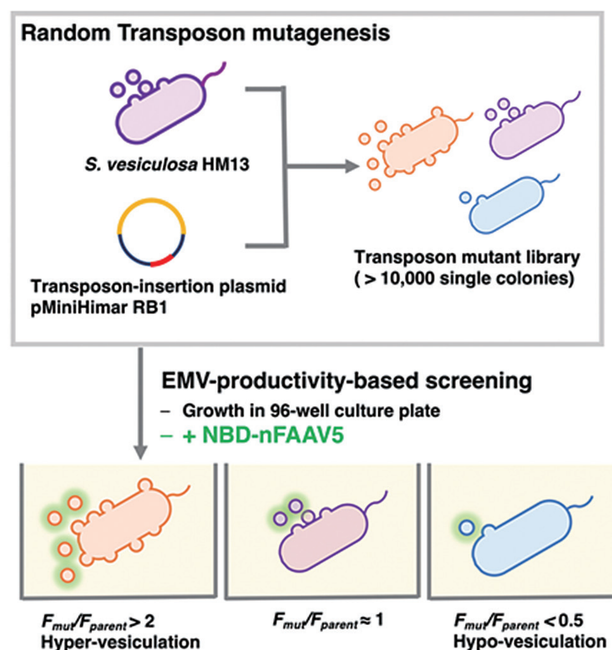

Figure 2. Schematic illustration of the high-throughput screening of the mutants with changes in EMV production of *S. vesiculosa* HM13.