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

Kawamoto, J.; Kurihara, T., Membrane Vesicles Produced by *Shewanella vesiculosa* HM13 as a Prospective Platform for Secretory Production of Heterologous Proteins at Low Temperatures, *Methods Mol. Biol.*, **2414**, 191-205 (2022).

Casillo, A.; Di Guida, R.; Cavasso, D.; Stellavato, A.; Rai, D.; Yokoyama, F.; Kamasaka, K.; Kawamoto, J.; Kurihara, T.; Schiraldi, C.; Kulkarni, S.; Paduano, L.; Corsaro, M. M., Polysaccharide Corona: The Acetyl-Rich Envelope Wraps the Extracellular Membrane Vesicles and the Cells of *Shewanella vesiculosa* Providing Adhesiveness, *Carbohydr. Polym.*, **297,** 120036 (2022).

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.*, **22**, 241 (2022).

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).

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).

Capsular Polysaccharide-Mediated Cargo Loading to Extracellular Membrane Vesicles of *Shewanella vesiculosa* HM13

Shewanella vesiculosa HM13, a Gram-negative bacterium isolated from fish intestine, secretes a large amount of extracellular membrane vesicles (EMVs) compared to the related strains and *Escherichia coli*, and the EMVs carry a functionally unknown protein, P49, as a single major cargo. This strain has been expected to be a clue to understanding the molecular mechanism of bacterial vesiculation and the EMV-targeted cargo transfer. A whole genome analysis of this strain demonstrated that the P49-coding gene is included in a gene cluster consisting of genes coding for protein secretion machinery and polysaccharide synthesis. An O-antigen-related flippase, Wzx, found in the gene cluster is a key protein for the cargo loading of P49 to EMVs and the synthesis of capsular polysaccharide (CPS) of the EMV surface. To verify whether CPS synthesized by the Wzx-dependent pathway interacts with P49, *in vitro* binding assay using purified P49 and P49-free EMVs was conducted. When EMVs harboring CPS were used, P49 bound to the EMVs. In contrast, P49 did not interact with EMVs collected from the *wzx*-less mutant. In the absence of CPS, no changes in the secondary structure of P49 were observed. These results revealed that CPS mediates the superficial interaction between P49 and EMVs. This system can be applied to the surface engineering of EMVs without modifying membrane components such as phospholipids.

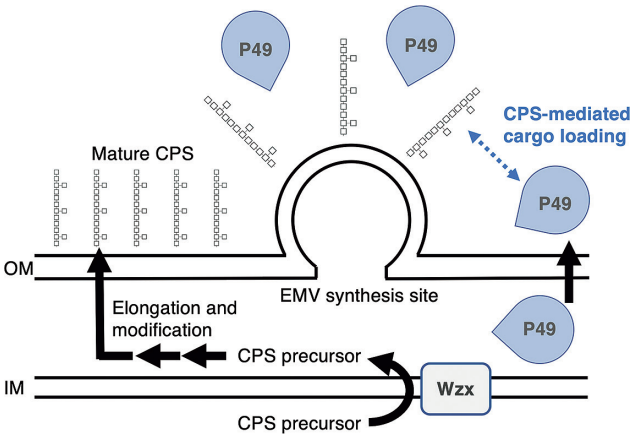

Figure 1. Schematic illustration of the capsular polysaccharide-mediated cargo loading.
An O-antigen flippase, Wzx, translocates a precursor of CPS from the inner leaflet to the outer leaflet of the inner membrane. After the elongation and maturation, CPS localizes at the surface of the cells and EMVs. P49 secreted to the surface of the cells by the function of a bacterial Type II-like secretion machinery interacts with CPS and is loaded onto the EMV surface.

A Novel and Second Lysophosphatidic Acid Acyltransferase YihG in *Escherichia coli*

A biological membrane largely comprises of glycerophospholipids, of which a fatty acyl group at the *sn*-2 position is introduced by a lysophosphatidic acid acyltransferase (LPAAT). For more than 30 years, PlsC had been believed to be a sole LPAAT in *Escherichia coli*, which is a well-studied, model Gram-negative bacterium. However, we demonstrated that an uncharacterized protein YihG is a second LPAAT in *E. coli*. We found some differences of the two LPAATs; PlsC is an essential LPAAT, whereas YihG is dispensable; YihG produces phospholipids that are distinct from ones generated by PlsC; and YihG, but not PlsC, controls the motility of *E. coli* cells. To gain further insights into the functions of YihG and how YihG controls cell physiology, we comparably analyzed the membrane proteome and transcriptome of wild-type and *yihG*-deficient cells. As a result, many genes required for cell motility (*e.g.* flagella formation and chemotaxis) were 2.2−40 times up-regulated in the mutant cells compared with the wild-type cells. This is in line with the enhanced motility of *yihG*-deficient cells. On the other hand, genes responsible for Glu-dependent acid resistance and biofilm formation were down-regulated in the mutant (up to 28-folds). As these genes and motility-related ones are known to be inversely controlled by regulatory factors such as a histone-like nucleoid structuring protein (H-NS) and a cAMP receptor protein (CRP), it is supposed that YihG affects the action of some transcriptional factors to modulate acid resistance and biofilm formation, in addition to cell motility.

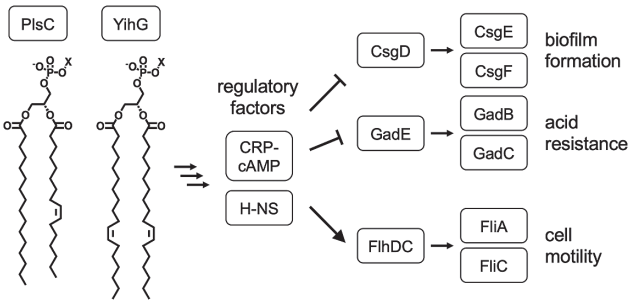

Figure 2. Proposed mechanism of how YihG controls cell physiology.