Division of Environmental Chemistry
– Molecular Microbial Science –

http://www.scl.kyoto-u.ac.jp/~mmsicr/mmstojp/Top_en.html

Prof
KURIHARA, Tatsuo
(D Eng)

Assist Prof
KAWAMOTO, Jun
(D Agr)

Assist Prof
OGAWA, Takuya
(D Agr)

PD
CHO, Hyun-Num
(D Agr)

Assist Res Staff

KITAYAMA, Kaori

Students

SUGIURA, Miwa (D3)
KAWAI, Soichiro (D2)
OHKE, Yoshie (D2)
CHEN, Chen (D1)
TOKUNAGA, Tomohisa (D1)
TOYOTAKE, Yosuke (M2)
NINOMIYA, Takemasa (M2)
OTA, Masaki (M1)
KUMAGAI, Fumihito (M1)
SUGIMOTO, Saki (M1)
CHEN, Tsai-min (M1)
FURUKAWA, Takahiro (M1)
YOKOYAMA, Fumiaki (M1)

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-adaptation Mechanism
Polyunsaturated Fatty Acid
Membrane Proteins
Acyltransferase

Selected Publications

Goto, S.; Kawamoto, J.; Sato, S. B.; Iki, T.; Watanabe, I.; Kudo, K.; Esaki, N.; Kurihara, T., Alkyl Hydroperoxide Reductase Enhances the Growth of *Leuconostoc mesenteroides* Lactic Acid Bacteria at Low Temperatures, *AMB Express*, **5,** 11 (2015).
Kawamoto, J.; Kurihara, T., Cold-adaptation Mechanism of Psychrophiles, *Seibutsu-Kogaku Kaishi*, **93,** 477-480 (2015).
Park, J.; Yamaura, T.; Kawamoto, J.; Kurihara, T.; Sato, S. B., Reciprocal Modulation of Surface Expression of Annexin A2 in a Human Umbilical Vein Endothelial Cell-derived Cell Line by Eicosapentaenoic Acid and Docosahexaenoic Acid, *PLoS One*, **9,** (1):e85045 (2014).
Kawamoto, J.; Kurihara, T., Proteins and Lipids of Cold-Adapted Microorganisms, *CSJ Current Review*, **17,** 55-61 (2014).
Kurihara, T.; Kawamoto, J., Chemical Approach to Analyze the Physiological Function of Phospholipids with Polyunsaturated Fatty Acyl Chain, *Yakugaku Zasshi*, **134,** 507-513 (2014).

Synthesis of Omega-ethynyl Eicosapentaenoic Acid and Its Application

Eicosapentaenoic acid (EPA) is an ω-3 polyunsaturated fatty acid (ω-3 PUFA) with a 20-carbon chain and five *cis* double bonds; the first double bond is located at the third carbon from the omega end. EPA acts as a precursor for a group of physiologically active lipid compounds and has many physiological functions, such as anti-inflammatory and anti-tumor. Although EPA commonly exists in membrane phospholipids as an acyl chain, how EPA behaves *in vivo* has not been elucidated in detail. Here, we developed an efficient synthesis method for ω-ethynyl EPA with an ethynyl group at omega position of EPA. This synthetic EPA analog is available for direct visualization by Raman microscopy and allows *in situ* chemical modification of EPA via click chemistry. To evaluate the usability of synthesized ω-ethynyl EPA, we used an Antarctic bacterium, *Shewanella livingstonensis* Ac10, as a model organism. This strain inducibly produces EPA at low temperatures, and the EPA-less mutant (ΔEPA) shows growth retardation and abnormal morphology at low temperatures. When EPA or ω-ethynyl EPA was exogenously supplemented to ΔEPA, both growth retardation and abnormal morphology were suppressed at 4 °C. Under the same conditions, membrane phospholipids of ΔEPA contained ω-ethynyl EPA as an acyl chain, and the amount of ω-ethynyl EPA was about 7% of total fatty acids in the membrane. These results reveal that ω-ethynyl EPA, similar to the natural form, is incorporated into *S. livingstonensis* Ac10 and performs the required physiological functions, suggesting that ω-ethynyl EPA can be used for *in situ* functional studies of EPA.

Physiological Function of a Novel Acyltransferase of an Antarctic Bacterium, *Shewanella livingstonensis* Ac10

In bacterial cell membrane biogenesis, phospholipid synthesis is catalyzed by two acyltransferases, PlsB and PlsC. The second acyltransferase PlsC catalyzes the acylation of the *sn*-2 position of the glycerol backbone of 1-acylglycerol phosphate. These two enzymes contribute to an asymmetric distribution of fatty acids between the *sn*-1 and 2 positions of the glycerol phosphate backbone. In *Shewanella livingstonensis* Ac10, a cold-adapted microorganism isolated from Antarctic seawater, eicosapentaenoic acid (EPA)-containing phospholipids play important roles in cell division at low temperatures. *S. livingstonensis* Ac10 has five putative PlsC proteins, PlsC1–5, in which PlsC1 is a key enzyme for EPA-containing phospholipids at low temperatures. On the other hand, the physiological functions of other PlsCs are still unclear. We generated gene-deletion mutants of each *plsC* gene and analyzed their phospholipid compositions. In the *plsC4*-deletion mutant (Δ*plsC4*), the amount of phospholipids containing the saturated fatty acid, 11-methyllauric acid (isoC13:0), was significantly decreased. The *plsC4*-expression vector restored the production of isoC13:0-containing phospholipids. An *in vitro* acyltransferase assay using 18:1-containing lysophosphatidic acid and various acyl donors indicated that PlsC4 has PlsC activity for relatively short-chain saturated acyl groups, 12:0, 13:0, and 14:0, but not for relatively long-chain saturated and unsaturated acyl groups, demonstrating that PlsC4 has a different substrate specificity from that of PlsC1 and is essential for the synthesis of isoC13:0-containing phospholipids.

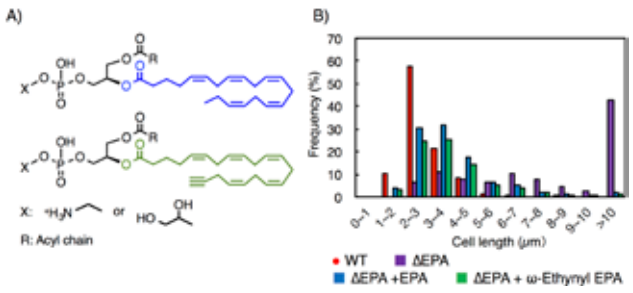

Figure 1. Structure of EPA-/ω-ethynyl EPA–containing phospholipids (A) and cell-size distribution of *S. livingstonensis* Ac10 and the EPA-less mutant grown with EPA/ω-ethynyl EPA (B). The EPA-less mutant forms filamentous cells with an average cell length of ΔEPA > 10 μm. In the presence of EPA/ω-ethynyl EPA, the average cell length of ΔEPA was 2-4 μm, similar to that of the parent strain (wild type).

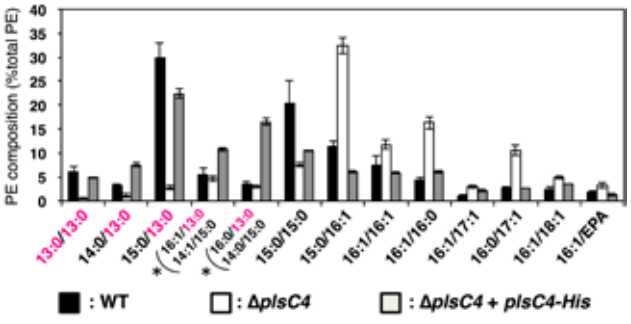

Figure 2. Composition of phosphatidylethanolamine of *S. livingstonensis* Ac10 (wild type), the *plsC4*-deleted mutant (Δ*plsC4*), and the *plsC4*-exoression strain (Δ*plsC4* + *plsC4-His* expression vector). The cells were harvested at 4 °C, and the membrane phospholipids were extracted. Phospholipid extracts were analyzed by ESI-MS. Red characters indicate the phospholipids containing 11-methyllauric acid (13:0). Asterisks indicate the isomers.