

“A critical role of cell surface structure and non-conventional cation-coupled flagellar motors from the alkaliphilic *Bacillus* spp.”

Alkaliphilic bacteria are well known for the utilization of their enzyme products for the food and chemical industry. In the early days of the study of alkaliphiles, extracellular enzymes produced by these extremophiles were added to detergents to improve the removal of dirt from laundry, and were used for the mass production of cyclodextrin with added value from cheap starch raw material.

For the extremely alkaliphilic *Bacillus*, a Na^+ -cycle across the cytoplasmic membrane is essential for growing at high pH. They display robust Na^+/H^+ antiport-dependent pH homeostasis and also contain NaChBac-type voltage-gated sodium channels that participate in Na^+ circulation, which supports pH homeostasis and motility.

Some Gram-positive bacteria possess a surface layer (S-layer) consisting of SLH (S-Layer Homology) domain-containing surface layer proteins, as the outermost component of the cell envelope. In recent years, surface layer proteins have attracted attention in the field of nanotechnology.

Bacillus pseudofirmus OF4 is a genetically accessible and non-pathogenic alkaliphile, and its genome contains 17 hypothetical genes which encode SLH domain containing surface layer proteins. The SLH domain-containing surface layer proteins assemble by binding to the cell wall in a manner that requires the putative *cell wall-modifying enzyme* CsaB. SlpA is a dominant S-layer protein of OF4. To observe the cell envelope of OF4, an *slpA* mutant strain, and a *csaB* mutant strain, ultra-thin section transmission electron microscopy was used. In the wild-type strain, the cytoplasmic membrane, peptidoglycan layer, and S-layer were observed. A *csaB* mutant strain lacked an S-layer part, and its peptidoglycan layer was disturbed. An *slpA* disrupted mutant strain also lacked an S-layer part, however, its peptidoglycan layer was not disturbed. The putative SLH domain-containing peptidoglycan hydrolases encoding genes have been found in the genome of *B. pseudofirmus* OF4. This suggested that the peptidoglycan hydrolases involved in cell separation and/or peptidoglycan biosynthesis are delocalized from the cell wall, which causes chained morphology, disturbance of peptidoglycan synthesis and alkaline sensitivity in the *csaB* mutant strain.

As another topic, I will present the flagellar motor of bacteria is a sophisticated nanomachine, which is embedded in the cell envelope. Prior to 2008, all previously studied conventional bacterial flagellar motors appeared to utilize either H^+ or Na^+ as coupling ions. Membrane-embedded stator complexes support conversion of energy using transmembrane electrochemical ion gradients. The main H^+ -coupled stators, known as MotAB, differ from Na^+ -coupled stators, PomAB of marine bacteria, and MotPS of alkaliphilic *Bacillus*. However, in 2008, a MotAB-type flagellar motor of alkaliphilic *Bacillus clausii* KSM-K16 was revealed as an exception with the first dual-function motor. This bacterium was identified as the first bacterium with a single stator-rotor that can utilize both H^+ and Na^+ for ion coupling at different pH ranges. Subsequently, another exception, a MotPS-type flagellar motor of alkaliphilic *Bacillus alcalophilus* AV1934, was reported to utilize Na^+ plus K^+ and Rb^+ as coupling ions for flagellar rotation. In addition, the alkaline-tolerant bacterium *Paenibacillus* sp. TCA20, which can utilize divalent cations such as Ca^{2+} , Mg^{2+} , and Sr^{2+} , was recently isolated from a hot spring in Japan, which contains a high Ca^{2+} concentration. These findings show that bacterial flagellar motors isolated from unique environments utilize unexpected coupling ions. This suggests that bacteria that grow in different extreme environments adapt to local conditions and evolve their motility machinery.

Faculty of Life Sciences, Toyo University **Dr. Masahiro Ito**

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1F Natural Science and Technology Main Hall

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【Contact】

Faculty of Natural System
Azuma Taoka 076-264-6234