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Basic Research for Development of Alternative Compounds to Preserve Low-Sodium Food
－Identification of Universal Factors Sensitive to Na^+－

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Summary

Sodium (Na^+) is important for food preservation. Na^+ is essential for bacterial proliferation and survival, but excess amount of Na^+ causes growth retardation or inhibition. However, mechanism of growth inhibition by Na^+ is unknown. So, we tried to investigate transcriptomic analyses under high intracellular Na^+ condition, and to identify proteins which is responsible for Na^+ toxicity.

Since intracellular Na^+ concentration is essentially maintained constant by some transporters, such as Na^+/H^+ antiporter(s) and respiratory Na^+ pump (NADH-quinone oxidoreductase), it is difficult to change the concentration to desired level for living cell. Previously, we constructed Na^+-sensitive *Vibrio parajaemolyticus* (MMabd), which all of *nhaA*, *nhaB*, *nhaD*, and *nqrABCDEF* were deleted. We have already shown that intracellular Na^+ concentration in MMabd was significantly higher than that in wild-type strain AQ3334. It means we can increase intracellular Na^+ concentration in MMabd. So, mRNA of MMabd exposed to higher Na^+ concentration medium was prepared, and we performed whole transcriptomic analysis with next-generation sequencer.

Genes of enzymes and transporters for biosynthetic pathway of compatible solutes against osmotic stress such as glycine, betaine, choline, diaminobutylate, proline, and ectoine were highly expressed at higher Na^+ condition. However, these genes didn't seem to be Na^+ specific since these genes were expressed higher also in wild-type strain. DUF2383 domain-containing protein (VPUCM_0083), RND-type drug efflux pump (VPUCM_2037 -2039), putative multidrug efflux pump (VPUCM_0909) were significantly higher expressed in MMabd, but not in AQ3334.

When expression level of our target proteins were not changed between low and high Na^+ condition, it would be difficult to identify them with transcriptomic analysis. We so tried to isolate Na^+-resistant mutants from MMabd. We obtained five mutants, and they showed higher minimum inhibitory concentrations (MIC) for NaCl and LiCl, but not KCl. In these mutants, it was expected that novel Na^+ efflux transporters are expressed, and/or loss of Na^+-sensitivity of target proteins are occurred.

We also tried to construct Mnh operon deleted strain in *Staphylococcus aureus*, which is a Na^+/H^+ antiporter. Now, we are investigating these mutants in detail.