

**Expression of salt-tolerance induced by osmotic stress in non-halophilic bacteria,  
*Escherichia coli* and *Bacillus subtilis***

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**Summary**

Most bacteria are known to accumulate  $K^+$  and/or organic solutes such as glycine betaine, glutamate, and trehalose in their cells when cells are grown in the medium with hyper-salinity. The accumulation of these compatible solutes in their cells is suggested to be very important for the salt-tolerance against hyper-salinity. However, little is known of the mechanism for the expression of salt-tolerance in non-halophilic bacteria. In the course of our study, we found that the salt-tolerance of *E. coli* and *B. subtilis* against hyper-salinity could be induced easily by prior exposure to moderate osmotic stress using NaCl or sorbitol containing 1% yeast extract for a short time (30-60 min). Therefore, we attempted to clarify the accumulation mode of compatible solutes in related to the salt-tolerance expression induced by the stress treatment. NMR and ion meter analysis revealed the accumulation of glycine betaine, glutamate, trehalose and  $K^+$  in *E. coli* cells during the stress treatment, although the content of intracellular free  $Na^+$  was not significantly changed by such stress treatment. The experiments using the defined stress medium suggested that not all of these solutes accumulated was required for inducing the expression of the salt-tolerance expression, and that among the solutes the combination of glutamate and  $K^+$  was found to be very effective for the induction. Similar results were obtained from the experiments in *B. subtilis*. Ultrastructural observation revealed pronounced condensation of the nuclear area after treatment with osmotic stress. From these results, we discussed the role of compatible solutes on the induction of salt-tolerance expression in non-halophilic bacteria, *E. coli* and *B. subtilis*.