

No. 08S2-09S2

## Functional Analysis of Genes in a Halotolerant Cyanobacterium Isolated from Dead Sea and Its Application

Teruhiro Takabe <sup>1</sup>, Takashi Hibino <sup>2</sup>, Yoshito Tanaka <sup>2</sup>, Tetsuko Takabe <sup>3</sup>, Incharoensakdi Aran <sup>4</sup>,  
Kirdmanee Chalermopol <sup>5</sup>

<sup>1</sup> Research Institute, Meijo University, <sup>2</sup> Graduate School of Environmental and Human Sciences, Meijo University, <sup>3</sup> Graduate School of Agricultural Science, Nagoya University, <sup>4</sup> Faculty of Science, Chulalongkorn University, <sup>5</sup> National Center for Genetic Engineering and Biotechnology

### Summary

*Aphanothece halophytica* is a halotolerant (halophilic) cyanobacterium which can grow in a wide range of salinity conditions (0.25 to 3.0 M NaCl) and accumulate betaine concomitantly. This cyanobacterium has unique systems for salt tolerance. Previous studies have shown that a NhaP-type Na<sup>+</sup>/H<sup>+</sup> antiporter (cation: proton antiporter-1, CPA1) of *A. halophytica*, Ap-NhaP1, has a novel ion specificity and its Na<sup>+</sup>/H<sup>+</sup> exchange activity was high over wide range of pH. In addition, *A. halophytica* synthesizes glycine betaine from glycine via three-step methylations although most biosynthetic pathways of betaine are oxidations of choline.

In this project, we found that *A. halophytica* has unique 12 Na<sup>+</sup>/H<sup>+</sup> antiporter genes. Among them, five transporters belong to NhaP-type Na<sup>+</sup>/H<sup>+</sup> antiporters, five are NapA-type antiporters, and one is unknown. It has also a multi resistance and pH adaptation Na<sup>+</sup>/H<sup>+</sup> antiporter (CPA3). Functional analysis of Na<sup>+</sup>/H<sup>+</sup> antiporters has been carried out. In addition to Ca<sup>2+</sup>/H<sup>+</sup> antiporter, one more Ca<sup>2+</sup>/cation antiporter has been found. The functional studies of these transporters on the regulation of Ca<sup>2+</sup> has been carried out. It was also suggested that *A. halophytica* has unique Na<sup>+</sup>-ATPase. On the osmoprotectant glycine betaine, it was found that in addition to a single component betaine transporter, *A. halophytica* has an ABC-type betaine transporter. Its functional properties have been studied. Moreover, aminoacid transporters have been studied. A molecular chaperon DnaK of *A. halophytica* has very high folding activity even at high salinity. Rice and poplar plants expressing *A. halophytica* DnaK showed enhanced seed yields as well as enhanced tolerances for salt and heat stresses. The plants exhibited the enhanced activities for Calvin-cycle enzymes, and showed faster growth and higher seed yield compared with the wild-type under normal growth conditions. Thus, it becomes clear that *A. halophytica* has unique genes which could be used for the construction of salt and drought stress tolerant crop plants.