Characterization and Utilization of Na^+-Efflux Genes from a Salt-Tolerant Yeast

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Summary

The salt-tolerant yeast *Zygosaccharomyces rouxii* is utilized industrially in making of miso-paste and soy-sauce. Its salt-tolerance seems to be achieved by the regulations of osmotic-pressure and intracellular ionic concentrations. Na^+-ATPase and Na^+/H^+-antiporter are supposed as factors that function in Na^+-efflux mechanisms. The Na^+/H^+-antiporter gene (*ZSOD2*) cloned from *Z. rouxii* has been demonstrated to participate in the salt-tolerance by its gene-disruption analysis. To elucidate the role of Na^+-ATPase gene (*ZENA1*), we constructed the *ZENA1*-disrupted mutant strain and examined the changes of resistant level to NaCl and plasma membrane ATPase activity of its disruptant. Moreover, we describe that the salt-tolerance could be elevated by functional expression of *ZSOD2* in a salt-sensitive yeast.

A *ZENA1::LEU2* mutant gene was constructed by the insertion of *LEU2* into *ZENA1* DNA and used to transform the *Z. rouxii leu2* strain. The level of salt-tolerance of an obtained *ZENA1* disruptant was almost same to that of the wild type strains by the estimation of growth on the agar plates containing various concentrations of NaCl. The level of plasma membrane ATPase activity of a *ZENA1* disruptant was not decreased by the gene disruption. From these results, it is thought that the functions of *ZENA1* product participate scarcely in the salt-tolerance of *Z. rouxii*. We found the presence of a second gene (*ZENA2*) homologous to *ZENA1*. The function of *ZENA2* remains to be clear.

We constructed a plasmid vector to express functionally the Na^+/H^+-antiporter gene (*ZSOD2*). It was under the regulations of a *GAL1* promoter and a *CYC1* terminator. When a salt-sensitive yeast was transformed by its plasmid, the obtained transformant could grow in the medium containing 5mM LiCl. This proposes that the elevation of resistant to salt could be achieved by functional expression of Na^+/H^+-antiporter gene.