NISR Research GRANT

2005 Young Investigator Research Grant

Molecular Evolutionary Analysis of High Sugar Tolerance of Wine Yeasts and Its Application for Breeding Industrial Yeasts

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Research aims

Technologies for enhancing the high sugar tolerance of *Saccharomyces cerevisiae* have not been established in spite of the importance of such tolerance in the fermentation industry. We have found that wine yeast strains, which have been selected and have evolved over many years to adapt to fermenting grape musts containing a high sugar concentration, are tolerant to high sugar stress, that the overexpression of Sed1p, a cell wall mannoprotein, confers high sugar tolerance to *S. cerevisiae*, and that the length of the *SED1* coding regions differs among strains working under different industrial conditions. The research aims of this study are to elucidate the mechanism of the high sugar tolerance mediated by *SED1*, and to develop a technology for enhancing the high sugar tolerance of industrial yeasts using the high-sugar-tolerance mechanisms of wine yeasts.

Methods

The high sugar tolerance of *S. cerevisiae* cells was assayed by spotting cells on synthetic dextrose (SD) agar plates containing 50% (w/v) glucose, unless otherwise indicated. Intracellular glycerol concentration was determined by enzymatic assay (Glycerol UV-method, Boehringer Mannheim). The assay for Zymolyase resistance of *S. cerevisiae* cells was performed by periodically measuring the decrease in the optical density at 660 nm (OD_{660}) of cell suspensions containing 40 μg/ml of Zymolyase 20T (Seikagakukogyou).

Results

A high sugar concentration causes high osmotic stress by altering water activity. The HOG pathway is the most prominent signalling pathway for responding to high osmotic stress in *S. cerevisiae*. To investigate whether high sugar tolerance induced by *SED1* overexpression requires other factors regulated by the HOG pathway, we constructed a *Δhog1* strain in which *HOG1* encoding the mitogen-activated protein kinase of the HOG pathway is deleted, and analyzed the effect of *SED1* overexpression in the *Δhog1* strain. The result showed that *SED1* overexpression in the *Δhog1* strain did not improve the high sugar tolerance, indicating that Hog1p is required for the high sugar tolerance induced by *SED1* overexpression, suggesting that other factors regulated by the HOG pathway are essential for such tolerance.

S. cerevisiae cells induce the intracellular accumulation of glycerol as a compatible solute in response to high osmotic stress. To investigate whether the high sugar tolerance induced by *SED1* overexpression is due to the change in intracellular glycerol concentration, we measured the intracellular glycerol concentration of the cells overexpressing *SED1* and control cells. There was no significant difference in intracellular glycerol concentration between the cells overexpressing *SED1* and the control cells. This indicates that the high sugar tolerance induced by *SED1* overexpression is not due to a change in intracellular glycerol concentration.

To elucidate the role of *SED1* in the high sugar tolerance, we constructed the *Δsed1* strain and analyzed the effect of *SED1* disruption on such tolerance. There was little difference in the growth on the SD medium containing 50% (w/v) glucose between the wild-type and *Δsed1* cells from the exponential-phase culture. In contrast, the growth of the wild-type cells from the stationary-phase culture was significantly better than that of the *Δsed1* cells from the stationary-phase culture on the SD medium containing 50% (w/v) glucose. These results indicate that Sed1p is required for the high sugar tolerance of stationary-phase *S. cerevisiae* cells.

Sed1p has recently been shown to function in ribosomes and mitochondria, as well as in the cell wall. To examine the association between the high sugar tolerance induced by *SED1* overexpression and the cell wall structure, the wild-type, *SED1*-overexpressing, and *Δsed1* cells were treated with Zymolyase, a mixture of cell wall-digesting enzymes. The resistance to Zymolyase was enhanced by *SED1* overexpression and reduced by *SED1* disruption, corresponding to the high sugar tolerance. Furthermore, the observation of the cells by scanning electron microscopy revealed that the *SED1*-overexpressing cells have a rough surface in contrast to the control cells with a smooth surface. These results suggest that a change in cell wall structure is involved in the high sugar tolerance induced by Sed1p.

To clarify whether the high sugar tolerance induced by Sed1p depends on its function in the cell wall, we constructed a disruptant of *KRE6*, which is required for the incorporation of Sed1p into the cell wall, and analyzed the high sugar tolerance of *Δkre6* cells overexpressing *SED1*. The result showed that *Δkre6* cells overexpressing *SED1* exhibit no high sugar tolerance, indicating that Sed1p incorporated into the cell wall is required for such tolerance.

SED1 is characterized by many length and sequence polymorphisms within both the species *S. cerevisiae* and

the genus *Saccharomyces*. However, no evidence indicating that these polymorphisms have physiological significance was obtained. We hypothesized that *SED1* polymorphisms are involved in the high sugar tolerance mentioned above. Therefore, we sequenced the *SED1* coding regions of the wine yeast strain OC-2 and the laboratory yeast strain W303 and compared the primary structure of Sed1p between both strains. The result showed that the Sed1p of W303 had two 51-amino-acid repeat units containing three cysteine residues. On the other hand, there were three repeat units in the Sed1p of OC-2. This difference in the number of cysteine residues in Sed1p might affect the high sugar tolerance through disulfide bond formation.

Conclusion

The high sugar tolerance induced by *SED1* overexpression is dependent on Hog1p, but is not due to a change in intracellular glycerol concentration. Sed1p is required for the high sugar tolerance of stationary-phase *S. cerevisiae* cells. *SED1* overexpression modifies the cell wall structure. Incorporation of Sed1p into the cell wall is required for the high sugar tolerance induced by *SED1* overexpression. There are two and three 51-amino-acid repeat units containing three cysteine residues in the Sed1p proteins of the laboratory yeast strain W303 and the wine yeast strain OC-2, respectively.