

Cloning of cDNAs by yeast expression system in the halophyte sea aster.

Noboru Inagaki, Michio Kanechi, Yuichi UNO and Migiwa Takeda*

Faculty of Agriculture, Kobe University, 1-1, Kobe, 657-8501, Japan

*The Graduate School of Science & Technology, Kobe University

Summary

The yeast expression vector responsive to multistress was constructed for the isolating genes concerned with salt tolerance in the halophyte sea aster (*Aster tripolium* L.). InvSc1, a strain of *Saccharomyces cerevisiae* was used for salt tolerance test; the survival and growth in the medium containing various concentration of NaCl was tested. The growth inhibition of yeast transformant with pYES2 vector was observed at more than 700mM NaCl when cultured in SD-Ura agar plate at 30C for 48 h. A 51-bp promoter fragment of the *DDR2* gene of *Saccharomyces cerevisiae*, oligo 31/32 (Kobayashi and McEntee, Proc.Natl.Acad.Sci.USA 87, p.6550-p.6554, 1990) was previously identified that conferred multistress-responsive inducibility on heterologous *CYC1-lacZ* reporter gene. The yeast expression vector was constructed with oligo 31/32 fused to *CYC1* minimal promoter based on pYES2 vector (pYES2-oligo31/32). The responsibility to salt stress of the expression system was evaluated by the β -galactosidase assay with *S. cerevisiae* cells transformed with the reporter vector containing *lacZ* reporter gene (pYES2-Oligo 31/ 32-*lacZ*). The β -galactosidase activity was increased with elevated concentration of NaCl in the medium. These results indicated that the dose-dependent expression is induced by oligo 31/32 -*CYC1* promoter in the yeast. An expression library with *Aster tripolium* L. cDNA under the control of the oligo 31/32 was transformed into the yeast strain. The transformants were screened with media containing 700 mM NaCl and some positive clones are obtained.