CHAPTER VII
CONCLUSION

In this study, we investigated the effect of ethanol on the growth of S. cerevisiae wild-type strain and its isogenic deletion mutants lacking genes encoding components of the MAPK pathways. We found that the components of the cell wall integrity pathway, i.e. BCK1, MPK1, SWI4, and SWI6, are required for ethanol tolerance. These finding suggested that the cell wall integrity pathway may play an important role in response to ethanol-induced cell wall damage. In addition, we found that STE3 and AKR1, encoding genes involved in pheromone signaling pathway, are also required for ethanol tolerance. Moreover, the *Δ*ste3 and *Δ*akr1 mutants were also sensitive to Calcofluor white, suggesting the role of these proteins in transducing cell wall stress signals.

To investigate whether cell wall remodeling occurs in response to ethanol stress, Zymolyase sensitivity test was used to monitor the cell wall alterations after exposure to either ethanol or Calcofluor white in the wild-type strain. We found that the sensitivity to Zymolyase was decreased with increasing concentrations of ethanol and Calcofluor white. Our results indicated that ethanol and Calcofluor white induce cell wall remodeling in dose- and time-dependent manner. The Zymolyase sensitivity test was next examined in the *Δ*bck1, *Δ*mpk1, *Δ*swi4, *Δ*swi6, Δste3, and Δakr1 mutants after exposure to ethanol and Calcofluor white. As expected, after growth in YPD media for 12 hours, the *Δ*bck1 and *Δ*mpk1 mutants were more sensitive to Zymolyase than the wild-type strain, supporting the idea that the cell wall remodeling is regulated by the cell wall integrity pathway. Surprisingly, we found that, after exposure to ethanol, the *Δ*bck1 and *Δ*mpk1 mutants were less sensitive to Zymolyase, even though the levels were lower than that of the wild-type strain. Our results suggested that ethanol may induce the cell wall remodeling through the alternative signaling pathways when lacking the signaling moleucles of the cell wall integrity pathway.

In addition, we also found that Akr1p, but not Ste3p, is involved in cell wall remodeling in response to cell wall stress. To test the possibility that the cell wall integrity pathway is activated in response to ethanol stress, we examined the expression of the cell wall-related genes, i.e. FKS2, CHS3, CRH1, and SED1 that are the target genes of this pathway. We found that these cell wall-related genes were significant upregulated after exposure to ethanol and Calcofluor white, suggesting the induction of cell wall-related genes in response to cell wall stress caused by ethanol to modify cell wall structure. The expression levels of FKS2, CHS3, and CRH1 are rapidly increased after exposure to ethanol for 30 minutes. However, in the presence of Calcofluor white, the expression levels of these genes were induced after prolonged incubation for 4 hours. We further investigated the expression levels of the cell wall-related genes in the *Δ*bck1, *Δ*mpk1, *Δ*swi4, *Δ*swi6, Δste3, and Δakr1 mutants, and found that, in the *Δ*bck1 and *Δ*mpk1 mutants, the expression levels of cell wall-related genes, i.e. FKS2 and CRH1, were slightly increased after exposure to ethanol. These results suggested the role of alternative signaling pathways in response to ethanol when the cell wall integrity pathway is inactivated. On the other hand, the induction of expression of these cell wall-related genes does not depend on the Swi4p/Swi6p transcription factor complex. In addition, we also found that Ste3p and Akr1p may not be involved direcly in the regulation of the cell wall-related gene expression in response to cell wall stress caused by ethanol and Calcofluor white.

We examined the effect of sorbitol on growth of the yeast wild-type strain and its isogenic deletion mutants during ethanol and Calcofluor white and found that sorbitol suppressed the ethanol-sensitivity phenotype of the Δbck1, Δmpk1, Δswi4, Δswi6 Δste3, and Δakr1 mutants. On the other hand, the addition of sorbitol mitigated the Calcofluor white-induced growth inhibition of the Δbck1, Δmpk1, and Δswi4 mutants, but not the Δste3, Δakr1, and Δswi6 mutants. We next examined the role of increased osmolarity in cell wall remodeling process during cell wall stresses caused by ethanol and Calcofluor white, and found that sorbitol has no effect on the activation of cell wall remodeling in response to cell wall stress caused by ethanol and Calcofluor white. Furthermore, we found that ethanol and sorbitol induced the expression of GPD1 by short-term incubation and sorbitol is involved in inducing

expression of some cell wall-related genes such as CRH1 and SED1 in response to ethanol and Calcofluor white, leading to increased tolerance to ethanol.