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A Knockout Strain of *CPR1* Induced during Fermentation of *Saccharomyces cerevisiae* KNU5377 Is Susceptible to Various Types of Stress

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To investigate the tolerance factor of *S. cerevisiae* KNU5377 against various types of environmental stress during fermentation, we identified the protein that is up-regulated at high temperatures. The highly up-regulated protein was high-score-matched as a cytoplasmic peptidyl-prolyl cis-trans isomerase, cyclophilin (Cpr1p), by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF). We constructed a *CPR1*-deleted KNU5377 strain (KNU5377Y *cpr1Δ*) to determine the roles of the protein under fermentative or stress condition. The growth of the *S. cerevisiae* KNU5377Y *cpr1Δ* strain was completely inhibited under the following conditions: heat (40°C), hydrogen peroxide (20-30 mM), menadione (0.3 mM), ethanol (16%), sulfuric acid (5 mM), and lactic acid (0.4-0.8%). However, the wild-type and *cpr1Δ* mutant of *S. cerevisiae* BY4741 as a positive control did not show differences in sensitivity to stress. It is interesting to note that the wild-type KNU5377Y and KNU5377Y *cpr1Δ* mutant showed high sensitivity against various stresses, particularly, acid stress such as in the presence of sulfuric and lactic acid. Although the alcohol fermentation rate of the KNU5377Y *cpr1Δ* mutant markedly decreased with an increase in temperature up to 40°C, we observed no decrease in that of the wild-type strain under the same conditions. These results suggest that CPR1 contributes to the stress tolerance of KNU5377 against various types of environmental stress caused during fermentation, thus leading to the physiological role of maintaining an alcohol fermentation yield, even at high temperatures such as 40°C.

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A Comparative Analyses of Genetic Diversities of *Saccharomyces cerevisiae* KNU5377 by Different DNA Fingerprinting Methods

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Saccharomyces cerevisiae, potential yeast species used in many fields of industrial fermentation, exhibits significant differences in bread, beer, and wine fermentation resulting from the genomic DNA. To survey the genetic diversity of *S. cerevisiae* KNU5377 strain, which presents the high fermentation capacity at strain levels, we performed various PCR profiling techniques. Among five *S. cerevisiae* strains analyzed, genetic differences of KNU5377 were well elucidated by PCR typing of pulse-field gel electrophoresis (PFGE), δ sequences, PCR differentiation using intron splice site primers, random amplified polymorphic DNA (RAPD) analysis with M13 primer, Sau-PCR, and microsatellite analysis, but not by restriction fragment length polymorphism (RFLP) analysis of internal transcribed spacers (ITS). Notably, minisatellite analysis and the nucleotide sequences of the *AGA1* gene (*YNR044w*), the coding a-agglutinin anchorage subunit, proved to be highly polymorphic in KNU5377. The ORF (2519 bp) of *AGA1*, which contained two blocks of tandem repeat units as serine (Ser) and/or threonine (Thr)-rich block, was longer than that (2178 bp) of *S. cerevisiae* S288C, the positive control. The current results showed that genetic patterns of KNU5377 were strongly different from the other *S. cerevisiae* strains. We suggest that KNU5377 might have the different molecular mechanisms involved in the adaptive evolution of yeast traits by the specificities of the molecular basis of the physiological properties such as the generation of evolutionary novelties resulting from significant genetic polymorphism.