# Molecular mechanism for the aldehyde tolerance of *S. cerevisiae* and its application in the fermentation industry

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**Research aims**

Acetaldehyde is a volatile organic chemical compound, which serves as an important intermediate product in several intracellular metabolic pathways, contributing to amino acid metabolism, ethanol detoxification, and ethanol fermentation. However, acetaldehyde readily forms adducts with both protein and DNA, inhibiting cell growth and interfering with a wide range of cellular metabolic activities. Therefore, despite a central role in many critical pathways, acetaldehyde also exhibits high toxicity and has irritant properties, mutability, and may be carcinogenic to living organisms.

The budding yeast *Saccharomyces cerevisiae* is likely to have evolved an especially well developed acetaldehyde tolerance system owing to the fact the species produces acetaldehyde as the predominant intermediate during anaerobic metabolism and the generation of ethanol.

In this study, we demonstrated the molecular mechanisms for acetaldehyde tolerance of *S. cerevisiae*, and apply an understanding of these mechanisms to molecular breeding of industrial yeast strains.

**Methods**

*S. cerevisiae* strain BY4741 was used as the wild type strain. The gene disrupted strains of BY4741 used in this study were purchased from Invitrogen Co. (Carlsbad, CA., USA).

Cell-free extracts from yeast strains were prepared using a method outlined in Oku *et al.*[1)]. Intracellular levels of $NADP^+$/NADPH were identified using a Prominence nano HPLC (Shimadzu)-4000QTRAP Mass spectrograph system (AB SCIEX) [(1)].

**Results**

1. **Physiological role and functions of the intracellular redox regulation system in acetaldehyde tolerance of *S. cerevisiae*.**

Under acetaldehyde stress, the intracellular $NADP^+$/NADPH ratio in the *S. cerevisiae* strain was increased. In strain *zwf1*Δ, which shows particularly high acetaldehyde sensitivity[2)], the

$NADP^+$/NADPH ratio in the cell was significantly increased under acetaldehyde stress. This indicates that the main function of the pentose phosphate pathway in acetaldehyde tolerance is to supply NADPH to the acetaldehyde tolerance system.

Additionally, under acetaldehyde stress, the intracellular levels of $NADP^+$/NADPH concentrations in *S. cerevisiae* increased. This demonstrates that *S. cerevisiae* not only controls the $NADP^+$/NADPH ratio, but also the production of $NADP^+$/NADPH under acetaldehyde stress. We observed specific phenotypes in the $NADP^+$/NADPH kinase gene-disrupted strains. Strain *pos5*Δ, in which the mitochondrial $NADP^+$/NADPH kinase gene is deleted, showed severe acetaldehyde sensitivity. Strain *yef1*Δ*utr1*Δ, in which cytosolic $NADP^+$/NADPH kinase genes were deleted, also demonstrated acetaldehyde sensitivity. Furthermore, among the $NADP^+$/NADPH kinase genes, *YEF1* was found to be acetaldehyde-inducible.

## 2. Physiological roles and functions of the glutathione degradation pathway in acetaldehyde tolerance of *S. cerevisiae*.

*S. cerevisiae* has at least three acetaldehyde tolerance pathways, each requiring NADPH for their activities [(2)]. These acetaldehyde tolerance pathways include the glutathione (GSH) synthesis and reduction pathways[2)]. We have already demonstrated that GSH forms adducts with acetaldehyde[3)], briefly reducing acetaldehyde toxicity[3)]. However, GSH alone cannot explain the whole mechanism of acetaldehyde tolerance. On the other hand, Anni *et al*. reported that Cys-Gly, a decomposition product from GSH, has an ability to form adducts with acetaldehyde[4)]. We have therefore observed a possible effect that the GSH degrading pathway has on the acetaldehyde tolerance system. Expression of Dug2/Dug3 was up regulated by acetaldehyde stress, and strain *dug2*Δ showed slightly elevated acetaldehyde sensitivity. However, intracellular Cys-Gly level was maintained despite the presence or absence of acetaldehyde.

From these results, it seems that *S. cerevisiae* uses both GSH and Cys-Gly as an acetaldehyde scavenger in the cell, although it is possible that GSH is the primary scavenger, rather than Cys-Gly.

## Conclusions

In this study, we have shown that PPP plays an important role in the acetaldehyde tolerance system of *S. cerevisiae* by supplying NADPH. $NAD^+$/NADH kinases also contribute by elevating NADPH levels in the cell. Pos5, a $NAD^+$/NADH kinase localized in mitochondria, also has an important function in acetaldehyde tolerance. Mitochondria possess the synthesis pathway for oleic acid, a key component for acetaldehyde tolerance [(2)] and are therefore key organelles in acetaldehyde tolerance.

GSH, which requires NADPH for reduction, is also involved in the acetaldehyde tolerance of *S. cerevisiae* [3]and may be more important for this function than Cys-Gly.
These findings can be applied to improve molecular breeding of industrial budding yeasts.

**References**


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