TOLERANCE TO ACETIC ACID IS IMPROVEDBY MUTATIONS OF THE TATA-BINDING PROTEIN GENE OF *SACCHAROMYCES CEREVISIAE*

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Introduction

As acetic acid (AA) is one of the major inhibitors resulting from the pretreatment of lignocellulose for bioethanol production, yeast strains that can endure a stress produced by AA are highly desirable. Improved cell growth in the presence of 0.6–0.7% AA was observed in strains with *FPS1* disruption (1) or *HAA1* overexpression (2). Here, we used the gTME technique (3) to develop AA tolerant strains.

Methods

Saccharomyces cerevisiae BY4741 was used for the construction of gTME library and isogenic strains. AA-containing media were adjusted to pH 4.5. Spot assay and susceptibility assay were routinely performed to examine the sensitivity to AA. Intracellular reactive oxygen species (ROS) was measured as described previously (4). For Northern blot analysis, total RNAs were from cells treated with 0.9% AA for 10 h.

Results

Identification of acetic acid tolerant strains. Four clones (MRRC 3248–3251), selected from a *SPT15* gTME library screen in the presence of 0.7% AA, displayed better growths than the control strain MRRC 3247 (Fig. 1A). Mutations were identified after retrieving plasmids (Fig. 1B). The enhanced tolerance was further confirmed by back-transforming two chosen mutant alleles (AA311 and AA342) (Fig. 1C).

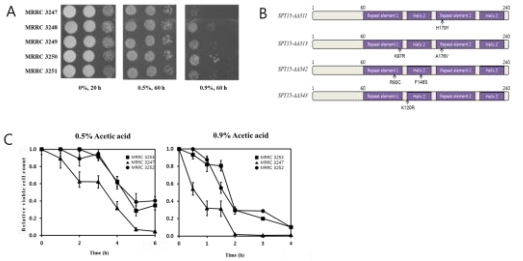

Fig. 1. (A) Enhanced acetic acid tolerance. 10-fold serially diluted cells (5 µl) were spotted onto YPD plates containing appropriate concentrations of acetic acid (pH 4.5) and incubated at 30°C for 4–6 days. (B) Position of point mutations. (C) Acetic acid susceptibility. Following the construction of MRRC 3252 and MRRC 3253 by back-transforming pSPT15-AA311 and -342 into BY4741, the acetic acid susceptibility was assayed along with the control strain MRRC 3247.

Effect of mutated *SPT15* on the accumulation of intracellular ROS. Similar to a previously developed AA-tolerant strain (1), the accumulation rate and concentration of ROS were significantly lower in our AA-tolerant strains than in the control (Fig. 2).

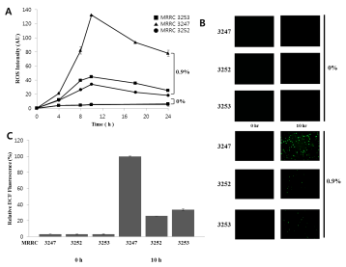

Fig. 2. ROS accumulation**.** (A) Kinetics of the intracellular ROS level. Exponential cultures of MRRC strains were treated with 0 or 0.9% acetic acid (pH 4.5) for 24 h at 30 °C. Representative images (B) and averages of fluorescence intensity (C) were taken at 10h.

Effect of mutated *SPT15* on the regulation of *FPS1* and *HAA1*. Northern analysis data shows that the expression levels of *FPS1* and *HAA1* were detectable but not changed upon exposure to 0.9% AA for 10 h (Fig 3). Neither gene is apparently associated with AA tolerance in our strains. Undetectably low expression of the *HAA1* downstream gene *PDR12* confirmed that *HAA1* was not activated by the presence of mutated *SPT15*s.

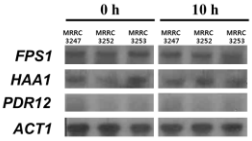

Fig.3. Effect of mutated *SPT15* on the regulation of *FPS1* and *HAA1*. Northern analysis was performed with total RNAs prepared from cells treated with none or 0.9% acetic acid for 0 or 10 h.

Conclusions

1. Two AA tolerant strains were obtained by screening a library overexpressing *SPT15* mutant alleles.
2. A remarkable decrease in the level of intracellular ROS upon exposure to AA was observed in those strains.
3. *FPS1* and *HAA1* were not associated with the enhanced AA tolerance conferred by mutated *SPT15*.

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References

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