

A Tobacco Gene *NtAsT8* is Involved in Decreasing Arsenite Accumulation in *S. cerevisiae*

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Objectives

Heavy metal pollution of soils is a critical environmental problem. Plants can be used to remove or reduce heavy metals from contaminated soils. To study the molecular mechanism of arsenite accumulation in *Saccharomyces cerevisiae*, we characterized the AsS78(arsenite-sensitive 78) related in tolerating arsenite and transformed with an yeast expression library of tobacco (*N. tabacum*).

Materials and Methods

1. Materials

- Yeast strain - Y800 (MATa/MATalpha leu2- Δ 98/leu2- Δ 98 ade2-101/ade-101 HIS3/his3- Δ 200 ura3-52/ura3-52 can1/CAN1 lys2-801/lys2-801 CYH2/cyh2 trp1-1/TRP1)
- *E. coli* strain - DH5a

2. Methods

For northern blot analysis, total RNA was extracted from yeast using glass beads method. Plasmid was introduced into yeast by the LiAc/PEG method.

Results and Discussion

To clone plant genes involved in accumulating As, arsenite-sensitive yeast AsS78 was transformed with an yeast expression library of tobacco (*N. tabacum*). Surviving transformants were selected on agar media with arsenite on which the growth of Y800 was inhibited. A plasmid was isolated from the surviving transformant, and an insert was sequenced and named as *NtAsT8*. *NtAsT8* gene has high homology to an ubiquitin conjugating enzyme. To confirm the role of *NtAsT8* it was over-expressed in AsS78 mutant and Y800(WT). AsS78 transformants expressing *NtAsT8* gene exhibited an increased tolerance to As, but a reduced accumulation of As with respect to those of AsS78. *NtAsT8*-expressing Y800 did not show any change in As-tolerance, but As level decreased compared to Y800. Taken together, we concluded that a novel gene of *N. tabacum*, *NtAsT8*, plays a role in decreasing As-accumulation in *S. cerevisiae*.