

AsS78 is Involved in Decreasing Arsenite Accumulation in *S. cerevisiae*

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Objectives

Heavy metal pollution of soils is a critical environmental problem. To study the molecular mechanism of As accumulation in *Saccharomyces cerevisiae*, we characterized the AsS78(As-sensitive 78) involved in tolerating arsenite.

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 (*supE44* Δ lac169(Φ 80lacZ Δ M15) *hsdR17recA* *lenda* *1gyrA96thi-1* *relA1*)

2. Methods

AsS78 was selected in solid medium supplemented with 400uM sodium arsenite. To isolate AsS78 mutant, we performed inverse PCR. Genomic DNA from AsS78 mutant was digested in *EcoR* I and self ligated. 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 understand the molecular mechanism of As tolerance in *Saccharomyces cerevisiae*, arsenite-sensitive mutant was isolated by screening yeast mutant pools generated by mini-Tn(*mTn*) insertion; AsS78(As-sensitive 78) grew worse than WT(Y800) in solid medium with 400uM sodium arsenite. While WT accumulated 260ppm As, AsS78 concentrated 810ppm. Therefore, the decrease in As-tolerance in AsS78 is due to an increase in As accumulation. IPCR(inverse PCR) identified that mTn-mutated gene(AsS78) has a Ser/Thr kinase domain. The disruption of AsS78 by mini-Tn insertion was confirmed by Northern hybridization. AsS78 was disrupted by single insertion of mini-Tn since Southern hybridization displayed the detection of same DNA fragment both by AsS78 and mini-Tn probes. When AsS78 was over-expressed in AsS78 mutant, As-tolerance increased, but As accumulation decreased. Over-expression of AsS78 in WT decreased As accumulation, but did not alter As tolerance. Taken together, AsS78 is involved in decreasing As accumulation in *S. cerevisiae*.