INTRODUCTION

Health impairment due to arsenic pollution of ground water is currently noted among residents worldwide, mainly in South Asia (Polizzotto et al., 2008). Arsenic compounds are also being used as therapeutic agents for some leukemia patients. However, their side effects impair their therapeutic effects (Emadi and Gore, 2010). The mechanisms underlying arsenic toxicity and the biological defense mechanisms against it are unclear. Therefore, we searched for genes involved in resistance to arsenic toxicity using budding yeast as a eukaryotic cell model.

MATERIALS AND METHODS

Yeast strains, media, and transformation

The yeast open reading frame (ORF) collection (Open Biosystems, Huntsville, AL, USA) is a gene expression library of over 4,900 Saccharomyces cerevisiae genes. These genes are expressed under the control of the GAL1 promoter in the URA3-based multicopy plasmid BG1805. This library was introduced into the BY4742 strain (MATa, his3Δ1, leu2Δ0, lys2Δ0, ura3Δ0) by the lithium acetate procedure (Hwang et al., 2009). Transformants were cultured in synthetic dextrose (SD) liquid medium without uracil (-Ura) at 30°C.

Selection of genes that confer resistance to arsenite

To induce gene expression, we incubated the Ura+ transformants in synthetic galactose (SG) medium containing 2% raffinose (+Raf) without uracil (-Ura) for 6 hr. Subsequently, Ura+ transformants were cultured (1 × 10^5 cells/200 µl/well) in SG (-Ura) liquid medium containing 2 mM arsenite in 96-well plates. This concentration of arsenite inhibits the growth of wild-type BY4742 cells. We isolated transformed cells that had proliferated rapidly and had formed aggregates in the presence of arsenite. In this way, we obtained aggregates that exhibited arsenite resistance. We isolated plasmids from the cells as described previously (Takahashi et al., 2006). The plasmids were amplified in Escherichia coli. Each plasmid was reintroduced into BY4742 cells to confirm the phenotype. We then selected the plasmid that conferred the strongest resistance to arsenite for further study. The ORF sequence in the selected plasmid was determined with an automated DNA sequencer: a CEQ™ 2000 Genetic Analysis System (Beckman Coulter, Fullerton, CA, USA).

Quantitation of arsenite-related growth inhibition of yeast cells

To induce the expression of genes, we incubated yeast cells in SG medium containing 2% raffinose (+Raf) without uracil (-Ura) for 6 hr. Yeast cells were suspended in liquid-SG medium at 1 × 10^4, 2 × 10^4, 4 × 10^4, and 8 × 10^4 cells/ml. Five microliters of each suspension of cells was spotted on agar-solidified SG medium that contained 2 mM arsenite. Plates were photographed after incubation for 48 hr at 30°C.

ABSTRACT — To elucidate mechanisms of arsenic toxicity and biological defense mechanisms against arsenic, we searched for genes that, when overexpressed, conferred arsenite resistance on yeast. Employing a Saccharomyces cerevisiae open reading frame (ORF) library, four genes associated with arsenite resistance, FAP7, MIG3, TMA19, and YLR392c, were identified.

Key words: Arsenite, Resistance, Yeast, Fap7, Mig3

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RESULTS AND DISCUSSION

To search for genes that influence arsenite sensitivity when overexpressed, an ORF-expressing plasmid library covering more than 90% of all genes of budding yeast was introduced into a wild-type yeast strain (BY4742). From this, transformants (about 30,000 colonies) were obtained. In the plasmid library, genes of interest were inserted downstream of GAL1 promoters to induce gene expression with galactose. Expression of the genes was induced by culturing the transformants in a galactose-containing SG (-Ura) medium. Subsequently, the transformants were cultured in the presence of arsenite (2 mM) to preclude the growth of the parent strain. In this way, 37 arsenite-resistant yeast clones were obtained. Subsequently, plasmids were extracted from these arsenite-resistant strains and introduced into the parent strain, BY4742, to investigate arsenite sensitivity. Six yeast strains acquired arsenite resistance after introduction of the plasmid. Then, the ORF inserted into the plasmid was sequenced, revealing four genes: FAP7, MIG3, TMA19, and YLR392c (Fig. 1). Fap7 is an NTPase involved in ribosome biosynthesis (Granneman et al., 2005). Mig3 is a transcription repressor involved in glucose metabolism (Dubacq et al., 2004). Tma19 is a ribosome-binding factor involved in translation (Rinnerthaler et al., 2006). Ylr392c is a protein of unknown function. There has been no report on the involvement of these genes in arsenic toxicity until now. In the future, the relationship between these factors and arsenite sensitivity will be investigated to elucidate uncharacterized mechanisms of arsenic toxicity.

REFERENCES


Fig. 1. Effects of overexpression of genes for FAP7, MIG3, TMA19, or YLR392c on the sensitivity of yeast to arsenite. Yeast cells that harbored BG1805 (empty vector), BG1805-FAP7, BG1805-MIG3, BG1805-TMA19, or BG1805-YLR392c were suspended in liquid-SG (-Ura) medium at 2,000, 400, and 80 cells/ml. Five microliters of each suspension of cells was spotted on agar-solidified SG (-Ura) medium prepared with and without 2 mM arsenite. Plates were photographed after incubation for 48 hr at 30°C.