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Toxicomics Report

Identification of growth-dependent genes involved in paraquat toxicity in *Saccharomyces cerevisiae*

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ABSTRACT — Using the yeast strain library we established by overexpressing each gene essential for yeast growth, we comprehensively searched for genes affecting the sensitivity of yeast to paraquat. As a result, seven novel genes, *UTP4*, *UTP25*, *SEC65*, *NDD1*, *TFB2*, *TIM23*, and *CCT6,* were identified as conferring paraquat resistance in yeast via overexpression.

Key words: Paraquat, Resistance, Yeast, *UTP4*, *UTP25*

INTRODUCTION

Paraquat is a herbicide classified within the bipyridiniums, and this compound is used widely all over the world (Raghu *et al.*, 2013). Paraquat produces free radicals *in vivo* and is well known to lead to lung dysfunction (Suntres, 2002); however, the molecular mechanisms involved in toxicity development have not been completely elucidated.

Recently, by producing budding yeast strains showing overexpression for each gene essential for yeast growth, a comprehensive screening method was established for identifying essential genes conferring resistance against chemical substances in yeast (Zhu *et al.*, 2014). In this study, we used this screening method to comprehensively search for overexpressed gene clusters affecting the sensitivity of yeast to paraquat.

MATERIALS AND METHODS

Selection of essential cell growth-related genes involved in paraquat resistance in yeast cells

Yeast cells (BY4742; a uracil-auxotrophic strain) were transformed with a yeast essential gene plasmid library designed for overexpression of 809 essential protein-coding genes related to cell growth, using the *URA3*-based high-copy plasmid pKT10 expression system (Zhu *et al*., 2014). Yeast cells carrying essential gene expression plasmids were cultured in synthetic dextrose (SD) (-ura) liquid media in 96-well plates for 48 hr at 30°C. Each culture was diluted to 1/1,600 with SD (-ura) media and added paraquat (1.5 mM) in 96-well plates. After a 48 hr incubation, yeast cells exhibiting increased growth were identified as candidates for paraquat-resistant yeast cells.

Quantification of the sensitivity of yeast cells to paraquat

BY4742 strains were cultured (1×10^4 cells/200 μL) in SD liquid media containing paraquat at various concentrations in 96-well plates. After incubation for 48 hr at 30°C, absorbance at 600 nm was measured using a spectrophotometer.

RESULTS AND DISCUSSION

For 809 genes (among approximately 1,000 genes considered essential for yeast cell growth), yeast strains showing overexpression of each essential gene were individually cultured for 48 hr in the presence of paraquat concentration levels (1.5 mM) that inhibit the growth of yeast with empty vectors introduced (control yeast). Those strains showing cell growth were subsequently considered as candidates for paraquat-resistant yeast cells. As a result, yeast strains showing overexpression of seven genes (*UTP4*, *UTP25*, *SEC65*, *NDD1*, *TFB2*, *TIM23*, and *CCT6*) demonstrated increased paraquat resistance com-

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pared to the control yeast (Fig. 1).

Both *UTP4* and *UTP25* are genes encoding for proteins involved in ribosome biosynthesis (Fromont-Racine *et al.*, 2003; Goldfeder and Oliveira, 2010). Other gene products are as follows: *SEC65* encodes for a subunit of a signal recognition particle (SRP) related to protein binding at the endoplasmic reticulum (Hann *et al.*, 1992), *NDD1* encodes for a transcription activation factor associated with nuclear division (Loy *et al.*, 1999), *TFB2* encodes for a subunit of the general transcription factor TFIIH (Feaver *et al.*, 1997), *TIM23* encodes for a protein involved in protein transportation to the matrix or endosporium of mitochondria (Popov-Celeketic *et al.*, 2008), and *CCT6* encodes for a subunit of the cytosolic chaperonin Cct ring complex (Kabir *et al.*, 2005). None of these seven genes have previously been examined for their association with paraquat toxicity. The literature reports that paraquat confers oxidative stress to cells (Suntres, 2002); however, in this study, overexpression of the seven paraquat resistance genes did not affect yeast susceptibility to substances inducing oxidative stress (hydrogen peroxide and tert-butyl hydroperoxide; data not shown). This suggests that the enhancement of intracellular antioxidant mechanisms is not correlated with the acquisition of paraquat resistance by overexpression of these genes. Since all proteins encoded by the seven genes identified in this study are essential for yeast growth, it is possible that paraquat exerts cytotoxicity by restraining the functions of these proteins. By examining the relationship between these genes and paraquat toxicity, we can expect to obtain new insights elucidating the mechanisms for the development of paraquat toxicity.

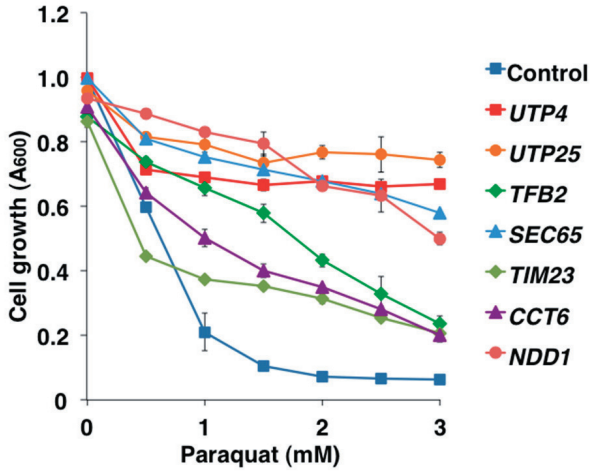

Fig. 1. Effect of overexpression of essential genes on the sensitivity of yeast cells to paraquat. Yeast cells carrying pKT10 (control) or pKT10 containing indicated essential genes were grown in SD (-ura) liquid media containing paraquat at the indicated concentration. After incubation for 48 hr, absorbance was measured at 600 nm. Values represent the mean ± SD of three cultures. The absence of a bar indicates that the SD falls within the symbol.

Conflict of interest---- The authors declare that there is no conflict of interest.

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