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Effect of textile wastewaters on *Saccharomyces cerevisiae* using DNA microarray as a tool for genome-wide transcriptomics analysis

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ABSTRACT

Textile mill effluents (TMEs) discharged from the textile industry can be considered as one class of hypothetical toxicants in the environment. To investigate the potential toxicity of TMEs, we applied cDNA microarray technology to examine the genome-wide expression profiles in model eukaryote, *Saccharomyces cerevisiae*. The results revealed a rich source of genetic information for the yeast cells that were exposed to the untreated and treated TMEs. Among the 5956 valid genes, 275 genes were up-regulated and 40 genes were down-regulated for the untreated TMEs. On the other hand, only 90 genes were up-regulated, and 29 genes were down-regulated upon exposure to the treated TMEs. The changes in gene expression were also confirmed by RT-PCR. The potent up- and down-regulation of genes suggest that yeast cells undergo genome-wide changes in mRNA expression, indicative of a stress response. Additionally, a classification into specific functional gene categories indicated that untreated and even treated TMEs still had toxicity. Especially, the genes related to oxidative stress, such as *AHP1, ATX1, GRX1, TRX1* and *TRX2*, were up-regulated in treated TMEs that can directly reach to surface and ground waters, and sediments.

1. Introduction

Textile mill effluents (TMEs) are one kind of the industrial wastewaters consisting of complex chemical mixtures, such as linear alkyl benzene sulfonates, discharged from textile mills. The wastewater from textile mills are involved in a multitude of wet processes, such as scouring, neutralizing, desizing, mercerizing, carbonizing, fulling, bleaching, dyeing, printing, and finishing activities (Abrahams, 1987). The wet processes of textile mills involve the use of a wide range of chemicals, pH, temperature, color and oxygen demand characteristics (Correia et al., 1994). Therefore, usually the TMEs are transferred into the municipal wastewater collection system involving some forms of wastewater treatment.

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Finally, the treated effluent (treated TMEs) is checked on the base of a simple criteria for water quality, and diverted into the river. However, the possibility of toxicity in the treated TMEs cannot be ruled out, and which serves as the basis of our present investigation. Actually, in a number of studies, the hypothetical toxicity of TMEs has been proposed (Walsh et al., 1980; Moore et al., 1987; Wells et al., 1994; Haniffa and Selvan, 1991).

Advances in genomics have helped spur a sub-discipline of toxicology, toxicogenomics that applies the expanding knowledge of genomics to identify and evaluate genome-wide effects of environmental xenobiotics (Afshari et al., 1999; Nuwaysir et al., 1999; Pennie et al., 2000; Murata et al., 2003). Especially, DNA microarray technology using a 'DNA chip' has provided a revolutionary approach to monitor the genome-wide gene expression (Schena et al., 1995; DeRisi et al., 1996; Duggan et al., 1999; Bowtell, 1999; Lipshutz et al., 1999). From several early reports, we can confirm the application of microarray to identify potential toxicity of pharmaceutical compounds, industrial chemicals, and environmental toxicants (Afshari et al., 1999; Lovett, 2000; Bartosiewicz et al., 2001; Momose and Iwahashi, 2001; Kitagawa et al., 2002; Kitagawa et al., 2003). In particular, during the late 1990s, two research groups (Afshari et al., 1999; Nuwaysir et al., 1999) had focused on the use of microarray technology as a bioassay for risk and environmental assessment. We used a yeast *Saccharomyces cerevisiae*, as a simple and unicellular eukaryote developed to a unique powerful model system for microarray. For the past two decades *S. cerevisiae* has been the model system for much of molecular genetic research because the basic cellular mechanics of replication, recombination, cell division and metabolism are generally conserved between yeast and larger eukaryotes, including mammals. Its prominent features are easy cultivation, short generation times, the detailed genetic and biochemical knowledge accumulated in many years of research. Moreover, this organism provides a highly suitable system to study basic toxico-genetical metabolisms that are relevant for higher eukaryotes, including humans. Recently, our group has focused on examining the toxicity of anionic detergents (Sirisattha et al., 2004), and environmental water containing burned ash (Kim et al., 2004) on yeast cells using microarray. In the case of anionic detergents, sodium *n*-dodecyl benzene sulfonate (LAS) and sodium dodecyl sulfate (SDS), mRNA expression profiles suggested damage to membranes and alterations in carbon metabolism, and induction of oxidative stress response. Moreover, LAS and SDS were also found to induce the pleiotropic drug-resistance network, indicating that LAS and SDS may be pumped out of yeast cells by this network (Sirisattha et al., 2004). On the other hand, the study on burned ash discovered a lot of evidences for the action of cell homeostasis and stress response, etc., against the toxic effects on yeast cells. Significantly, the toxicities, caused by reactive oxygen species, metals and the other xenobiotics, and the possibility of mutagenicity were also indicated in burned ash.

Our present yeast microarray analysis using the highly polluting textile wastewaters reveals alterations of gene expression for each treated and untreated TMEs that were examined on a basis of the MIPS gene database. We believe that an approach targeting the specific functional categories, which are related to the stress response, detoxification, and DNA repair and processing, will be able to give us an important insight for toxicity and its hypothetical metabolism.

2. Materials and methods

2.1. Experimental strain

S. cerevisiae S288C IFO1136 (*SUC2 mal mel gal2 CUP1*), the strain used as the probes in a Kuhara DNA chip (DNA Chip Research, Yokohama, Japan), was cultured and used for DNA microarray. Yeast cells were grown at 25 °C in YPD medium with a base of 1% yeast extract, 2% polypeptone, and 2% glucose.

2.2. Source of untreated and treated TMEs

Substances for the toxicity test were obtained from one of the public facilities for textile wastewater treatment. In this facility a complex mixture of TMEs that are discharged through the pipeline from the various textile mills are processed by treatment with a variety of chemical (such as precipitation of waste materials with chlorine compound ferric chloride that acts as a flocculant) and biological processes involving use of sewage microorganisms. It should be noted that TMEs mainly consist of linear alkyl benzene sulfonates, which are wildly used in the textile mill process as a surfactant. The untreated TMEs were sampled at several positions of the influent basin, and the treated TMEs were sampled from the final effluent basin after secondary wastewater treatment. Both the untreated and treated TMEs were taken as only the soluble materials after filter-sterilization by a 0.2 μm pore size (Steritop™ Filter Units, Millipore).

2.3. Exposure to untreated/treated TMEs and cell harvest

Yeast cells were cultured in YPD medium until OD_{660} reached ca. 1.0 (1×10^7 cells/ml), and collected by centrifugation. For a microarray experiment, samples should be prepared in pairs; one is for control, and the other is for experimental sample. We directly used the TMEs, instead of distilled water, with 1% yeast extract, 2% polypeptone, and 2% glucose, for the purpose of exposing the cells to potential toxicity of the TMEs. The exposure to the TMEs was done at 25 °C for 2 h, after which the cells were pelleted by centrifugation, and washed twice with distilled water. The finally pelleted cells were stored at −80 °C until used for extraction of total RNA. The viability of treated cells was measured from the relative alteration of colony-forming units (CFU) by comparing with the control.

2.4. DNA microarray procedure

2.4.1. DNA chip preparation

Yeast DNA chip for the microarray was purchased from DNA Chip Research, Inc. (Yokohama, Japan). The DNA chip is a glass slide, with mounted cDNA probes for the yeast genomic