INTRODUCTION

Tributyltin chloride (TBT) is a widespread environmental pollutant. TBT has several uses, such as in wood preservation and in antifouling paints for marine vessels. It is also used as a biocide in cooling system and as an organometallic chemical in diverse applications (Antizar-Ladislao, 2008). The use and production of TBT are now strictly regulated, but TBT and its degradation products will persist in aquatic sediments causing widespread contamination of the environment (Iwata et al., 1995; Tanabe et al., 1998). TBT is toxic to mammals, causing diseases in various organs as well as in the nervous, endocrine and immune systems (Antizar-Ladislao, 2008). Many studies have demonstrated that TBT induced apoptosis, a programmed cell death (Aw et al., 1995; Grondin et al., 2007; Thompson et al., 1996). TBT is harmful to eukaryotic microorganisms such as fungi. Studies on the marine yeast such as Debaryomyces hansenii (Laurence et al., 1989), Rhodotorula ferulica (Veiga et al., 1997), Saccharomyces cerevisiae (Golin et al., 2000; Masia et al., 1998) or Candida maltosa (White and Tobin, 2004) indicated that TBT induced cellular K⁺ leakage as well as a defect in ATP production. Recently we found that TBT induced apoptosis of S. cerevisiae via production of reactive oxygen species and caspase-like Yca1p-dependent pathway (Chahomchuen et al., 2009). Interestingly, at low concentration, this compound did not induce cell death but arrested the cell growth of S. cerevisiae, suggesting a concentration-dependent action of TBT in cell cycle and cell death. Since the molecular events of cell cycle has been well studied in the fission yeast Schizosaccharomyces pombe (Le Goff et al., 1999), further investigation for the action of TBT on this fungus will be as well valuable. For understanding the action of TBT, it is important to estimate the cellular concentration of this compound. In this context, we should consider the role of ATP-binding cassette (ABC) transporter because it is primarily involved in the cellular levels of a broad range of organic compounds, i.e., xenobiotics. In S. cerevisiae it has been reported that ABC transporter Pdr5p, one of the pleiotropic drug resistance (PDR) gene products, is involved in efflux of organic compounds (Golin et al., 2000). S. pombe has eleven ABC transporters and the intracellular localization of all these transporters has been elucidated (Iwaki et al., 2006). However, it is still unclear whether ABC transport-

Bfr1p is responsible for tributyltin resistance in Schizosaccharomyces pombe

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ABSTRACT — ATP-binding cassette (ABC) transporter plays an important role for resistance against xenobiotics. There are eleven ABC transporter genes in the genome of fission yeast Schizosaccharomyces pombe. We examined the role of ABC transporter against the toxicity of tributyltin chloride (TBT), a widespread environmental pollutant, in cell growth. Among individual ABC transporter mutants, the growth of a mutant deficient in Bfr1p, a plasma membrane-embedded transporter, was extremely sensitive to TBT. The lethal TBT concentration inducing 50% of cell death (LC₅₀) was 25 μM for the parent strain and 10.2 μM for the bfr1Δ mutant. Thus, Bfr1p was responsible for TBT resistance in S. pombe.

Key words: Schizosaccharomyces pombe, ATP-binding cassette (ABC) transporter, Tributyltin
er is involved in sensitivity of S. pombe to TBT. Here we examined the effect of TBT on the growth of ABC transporter mutants of S. pombe.

MATERIALS AND METHODS

Yeast strains, culture medium and cell death assessment
The S. pombe strains used in this study were the laboratory strain ARC039 (hr leu1-32 ura4-C190T) and its isogenic knockout mutants of eleven ABC transporters (Iwaki et al., 2006). Yeast cells were grown aerobically at 30°C in rich medium (YES) as described in Iwaki et al. (2006). For cell death assessment, yeast cells were grown in YES medium, harvested at the mid-exponential phase, resuspended in YES medium and treated with TBT (Supelco, Sigma-Aldrich, St. Louis, MO, USA) at various concentrations. For the control, cells were treated with 0.1% dimethylsulfoxide (DMSO) in similar conditions. After 30 min of incubation at 30°C, cells were harvested, plated on YES agar and incubated for 3 days at 30°C. The survival rates were calculated based on the number of colonies obtained with DMSO treatment in relation with the number of colonies observed after TBT treatment. The lethal TBT concentration inducing 50% of cell death (LC50) was calculated from the number of colonies obtained with DMSO treatment at various concentrations.
cell viability of *S. pombe* by TBT at high concentrations (Fig. 1B) represents apoptosis and/or necrosis is yet to be examined. It has also been reported that cell cycle is blocked by TBT at G2/M phase in murine erythroleukemic cells (Zucker et al., 1989). However, the concentration of TBT used in this study was enough to induce cell death and, therefore, interpretation of the action of TBT on cellular events should be complicated. Our finding in this study will be helpful for investigating the molecular mechanism of TBT action specific for cell cycle arrest in addition to cell death. The interfering effect of TBT on cell cycle of *S. pombe* is now under investigation by Flow cytometry analysis.

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**REFERENCES**


**Fig. 1.** Comparison of TBT sensitivity between the parent and bfr1Δ mutant of *S. pombe*. (A) Effect of TBT on cell growth. *S. pombe* cells of the parent strain (upper panel) and bfr1Δ (lower panel) were cultured in YES medium in the presence or absence of TBT, and the cell growth was monitored by measuring the optical density at 660 nm. Closed circle, no TBT (control); open circle, 0.3 μM TBT; closed triangle, 0.63 μM TBT; open triangle, 1.3 μM TBT; closed square, 2.5 μM TBT; open square, 5 μM TBT. (B) Effect of TBT on cell survival. *S. pombe* cells were harvested at mid-exponential phase, and treated with TBT as described in Materials and Methods. Survival rates were calculated as the number of colonies compared with those treated with DMSO as control. Open circle: parent strain, closed circle: bfr1Δ. Error bars indicate the S.D. of three independent experiments.