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RESEARCH ARTICLE

Biosorption and bioaccumulation of thallium by thallium-tolerant fungal isolates

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Abstract Little is known about the biosorption and bioaccumulation capacity of thallium (Tl) by microorganisms that occur in Tl-polluted soil. The present study focused on characterizing the biosorption and bioaccumulation of Tl by Tl-tolerant fungi isolated from Tl-polluted soils. Preliminary data showed a positive correlation between the biomass and the biosorbed Tl content. The Tl-tolerant strains were capable of bioaccumulating Tl, up to 7189 mg kg^{-1} dry weight. The subcellular distribution of Tl showed obvious compartmentalization: cytoplasm ≫ cell wall > organelle. The majority of Tl (up to 79 %) was found in the cytoplasm, suggesting that intracellular compartmentalization appeared to be responsible for detoxification. These findings further suggest the applicability of the fungal isolates for cleanup of Tl in Tl-polluted water and soil.

Keywords Thallium · Biosorption · Bioaccumulation · Fungal isolates · Subcellular distribution

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Introduction

Thallium (Tl) is a toxic trace metal included in the US EPA list of priority metal pollutants, although it is generally present at low concentrations in soil. Thallium has attracted increasing environmental concerns due to its high toxicity (De Albuquerque et al. 1972; Oves et al. 2013; Tremel et al. 1997; Xiao et al. 2004, 2007; Zitko 1975). Thallium pollution in soils, either from natural mineralization or human activities, has been shown to be a major environmental problem (Jia et al. 2013; Turner and Pilsbury 2013; Wang et al. 2013; Xiao et al. 2012). A few researchers have addressed the environmental effects of Tl on the microbial community in Tl-polluted soils (Sun et al. 2012). The paucity of detailed knowledge of the microbial response to high Tl is a major concern.

Microbial ecosystems composed of fungi, bacteria, and algae have been successfully applied as useful adsorbing agents for the removal of heavy metals (Babu et al. 2014; Kalin et al. 2004). Microorganisms can oxidize the Tl ion to Tl_2O_3 in mitochondria (Lindegren and Lindegren 1973), promoting dispersion of Tl compounds in the environment (Skłodowska and Matlakowska 2004). Besides the focus on the Tl-tolerant bacteria (Bao et al. 2014), some fungal isolates such as *Aspergillus niger* were applied to remove Tl from aqueous solutions (Peter and Viraraghavan 2008). At a higher initial concentration of Tl (47.6 mg L^{-1}), the amount of bioaccumulated Tl correlated with the biovolatilized amount by filamentous fungus *Scopulariopsis brevicaulis* (Boriová et al. 2014). Previous studies have shown the existence of culturable bacteria, filamentous fungi, and Actinomyces in long-time Tl-contaminated soils. Some fungal groups can grow in the presence of high Tl concentrations, up to 1000 mg kg^{-1} (Sun et al. 2012). This suggests that certain fungal isolates could endure high Tl dosages in polluted soil and still maintain their metabolic activities and resistances. In

view of this phenomenon, an overall assessment of the biosorption, bioaccumulation, and associated detoxification of Tl by such fungal isolates is critical before they can be used for the bioremediation of Tl-contaminated sites.

The present study characterized the biosorption and bioaccumulation of Tl by fungal isolates from the long-time Tl-polluted soils in Guizhou Province, Southwest China. In addition, the subcellular distribution of Tl was investigated to understand the bioaccumulation mechanism of Tl. These findings will help us to understand how microbes adapt to high Tl concentrations and possibly provide us with potential microbes for use in the bioremediation of Tl-polluted water and soils.

Materials and methods

Sample preparation and analysis

Nine Tl-tolerant fungal strains isolated from the Tl-polluted soils in our previous study (Sun et al. 2012) were employed for the study of biosorption and bioaccumulation of Tl (Table 1). The biosorption and bioaccumulation experiment was processed in liquid culture with corresponding Tl concentrations. Each test unit was supplemented with $TlNO_3$ (Merck, Germany) at concentrations of 1000, 1200, and 1500 mg L^{-1}, respectively. A culture without Tl addition was also used as control. All tests were performed in triplicate.

Each Tl-tolerant fungal culture was incubated in a 250-mL conical flask containing 75 mL potato dextrose (PD) broth at 26 °C for 6 days on a rotary shaker (150 rpm). Note that the same experimental conditions (26 °C and incubation time of 144 h) were used for determining the biosorption and bioaccumulation capacities and subcellular distribution of Tl. After being harvested by vacuum filtration, the biomass (mycelia) was thoroughly washed three times with 200 mL distilled water containing 0.05 % Tween 80. This was designed to wash off any Tl adsorbed to the fungal mycelial cell walls.

Table 1 Tl-tolerant fungal strains isolated from Tl-contaminated soil (Sun et al. 2012)

Strains	Taxon
T01	*Trichoderma koningiopsis*
T02	*Trichoderma koningiopsis*
T03	*Mariannaea* sp.
T04	*Trichoderma viride*
T05	*Paecilomyces farinosus*
T06	*Penicillium ochrochloron*
T07	*Penicillium ochrochloron*
T08	*Trichoderma* sp.
T09	*Trichoderma asperellum*

The biomass was dried for 72 h at 80 °C and then ground in a mortar for geochemical analysis.

To analyze the subcellular distribution of Tl, the harvested biomass was treated with snailase (20 mg mL^{-1}) (Ameresco, USA, mycelia:snailase=1:10) at 30 °C for 3 h on a rotary shaker (150 rpm) after being washed with distilled water. Then, the separation of the subcellular components was carried out at 4 °C by differential centrifugation (Srinivas et al. 2004). First, the mixture was transferred to 5-mL centrifuge tubes and centrifuged at 1600 rpm for 15 min. The resulting sediments were designated as the cell wall fraction. Then, the supernatants were centrifuged at 18,000 rpm for 45 min to sediment the cytoplasmic organelle fraction. The supernatant solutions were designated as the cytoplasmic supernatant fraction.

Approximately 100 mg of the powdered samples was digested with a 10-mL mixture of strong acids (8 mL of 15 M HNO_3 and 2 mL of 12 M $HClO_4$) for Tl determination. Milli-Q water (18.2 MΩ cm) was used for all experiments, and reagents used were of super pure grades. The Tl content of the samples was determined using inductively coupled plasma optical emission spectrometry (ICP-OES, iCAP 6500, Thermo Scientific, Germany) at the Institute of Geochemistry, Chinese Academy of Sciences. Quality control of Tl determination was assessed using standard references of plant material GBW07603 (GSV-2, National Institute of Standard Materials, China), as well as blank and duplicate samples. The measured Tl concentrations in the standard material, GSV-2, averaged 0.37±0.03 mg kg^{-1} (n=3), which was consistent with the certified value of 0.38 mg kg^{-1}. The recovery of standard additions ranged from 91 to 112 %.

Statistical analysis

Statistical analysis was carried out using the SPSS statistical package (version 16.0 for Windows, SPSS Inc., USA), and all the plots were analyzed by Origin (version 8.5 for Windows, Origin Lab Corp., USA). Mean values were calculated from three replicates. One-way analysis of variance (ANOVA) was performed for multiple comparisons, and the significance of different treatments was determined using a least significant difference (LSD) test, where a p value of less than 0.05 was considered statistically significant.

Results and discussion

Adsorption of Tl by the mycelia of the Tl-tolerant fungal isolates

The growth of nine fungal isolates exposed to Tl in the PD broth test units was expressed as grams of dried mycelia during the culture period (Fig. 1). In the Tl-spiked treatments, the

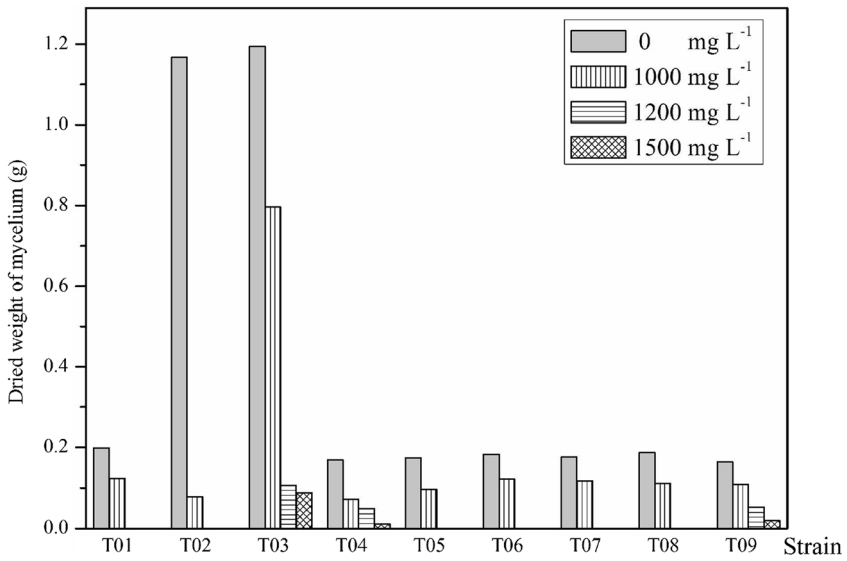

Fig. 1 Growth stress of fungal isolates in the Tl-spiked treatments. Each *point* represents an average of three replicates

growth of all nine fungal strains was affected adversely by the elevated Tl levels in the culture. The biomass of the strains exposed to 1000, 1200, and 1500 mg L^{-1} were in the following ranges, respectively: 0.0782 to 0.7960, 0.0490 to 0.1065, and 0.0195 to 0.0880 g. Moreover, only three strains (T03, T04, and T09) grew slightly in the 1200- and 1500-mg L^{-1} treatments, suggesting that the effects of spiked Tl on the growth differed.

The adsorption ability of the fungal isolates was defined as the ratio of the total Tl in the washed filtrate to the initial Tl in the culture (Fig. 2). All nine fungal isolates generally exhibited adsorption rates of less than 17 % and even lower rates in higher-Tl-level treatments, for example, 4.63 % in the 1500-mg L^{-1} treatment. Interestingly, adsorption rates were statistically significantly different between the 1000- and the 1200-mg L^{-1} treatments by LSD analysis. Whereas, there was no significant difference between the 1200- and the 1500-mg L^{-1} treatments, which revealed that Tl addition had only a limited effect above a certain threshold.

From Figs. 1 and 2, it could be concluded that the biomass (growth) and adsorption capacity of the fungal strains tended to decrease with higher concentrations of Tl. These further indicate that the Tl adsorption ability by each fungal strain is dependent on the larger surface area generated by higher growth, and that each concentration had specific equilibrium, after which there was no significant effect on adsorption by increasing the time of incubation.

Metal uptake by microorganisms from contaminated media can be divided into two categories (Pan et al. 2009). One is biosorption by non-living or non-growing biomass, which is a metabolism-independent and passive uptake process (Olguín and Sánchez-Galván 2012; Volesky and Holan 1995), and the

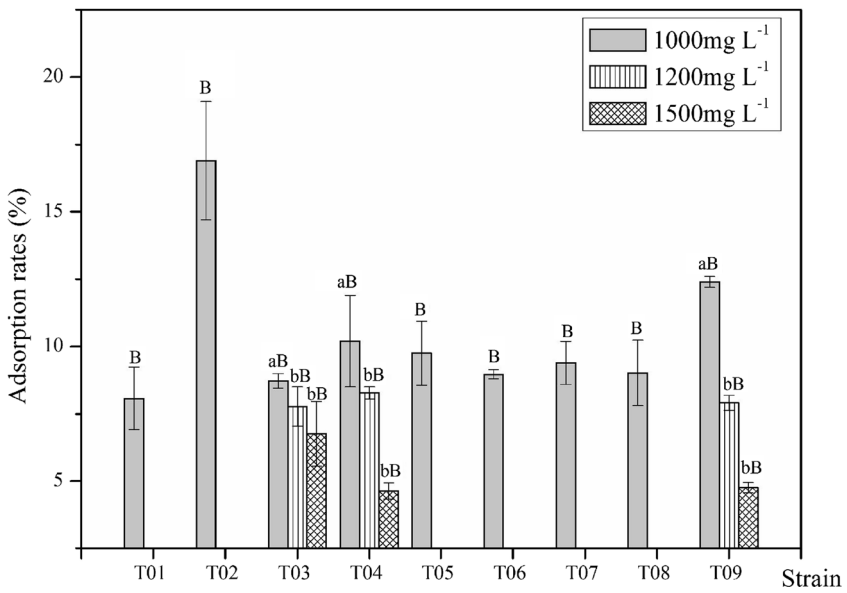

Fig. 2 Adsorption rates of Tl by the fungal isolates. Average± standard deviation from three separate experiments. *Values for a given Tl treatment labeled with different lowercase letters* differ significantly from the other strains in the same Tl treatment ($p<0.05$). *Values for a given strain labeled with different uppercase letters* differ significantly ($p<0.05$)

other is bioaccumulation by living and growing cells, which is mainly an intracellular accumulation. Biosorption, which encompasses trace metal uptake by live or dead microorganisms, is an emerging and attractive technology for heavy metal pollution control (Bhargava et al. 2012; Firdaus-e-Bareen et al. 2012). Moreover, the biosorption of heavy metals to microbes depends not only on the metal employed but also on the microbial species tested. Among the tested isolates, the Tl-tolerant strains, especially *Mariannaea* sp., *Trichoderma viride*, and *Trichoderma asperellum*, exhibited greater Tl tolerance, surviving in cultures with up to 1500 mg L^{-1}. Our results suggested that the Tl biosorption capacities decreased with increasing Tl concentration. It has been observed that an increase in the initial metal concentration results in a reduction in the metal removal capacity of the absorbent. This clearly reveals the existence of a finite, heavy metal reduction capacity, possibly due to heavy metal toxicity toward cells and the filling of the adsorption sites on the cell walls. Similar results were also obtained in the case of microalgae for Cr^{3+} (Pereira et al. 2013), *Phomopsis* sp. for Cu^{2+} and Pb^{2+} (Saiano et al. 2005), and *Phanerochaete chrysosporium* for Cd^{2+} and Pb^{2+} (Li et al. 2004).

Intracellular accumulation of Tl by the Tl-tolerant fungal isolates

Figure 3 summarizes the Tl bioaccumulation capacities of all nine fugal isolates. The intracellularly accumulated Tl concentrations ranged from 255.9 to 7189 mg kg^{-1} in the 1000-mg L^{-1} treatment, 930.7 to 6165 mg kg^{-1} in the 1200-mg L^{-1} treatment, and 189.6 to 2915 mg kg^{-1} in the 1500-mg L^{-1} treatment, respectively. The highest Tl concentration was observed at 7189 mg kg^{-1} from T09 strain in the 1000-mg L^{-1} treatment, which was also consistent with the high adsorption rate. Bioaccumulated Tl decreased with increasing Tl concentrations, probably as a result of the reduced biomass at the higher Tl concentrations.

The intracellular bioconcentration factor (BCF) is defined as the ratio of an element concentration in an organism to its concentration in culture, and is employed to evaluate an organism's ability to accumulate the element (Olguín and Sánchez-Galván 2012). In the present study, BCF values greater than 1 were only observed by fungal strains in the 1000-mg L^{-1} treatment. The highest BCF was produced by strain T09 (7.19), which was far higher than for other strains and treatments, indicating that the T09 strain has a much stronger resistance to Tl toxicity.

In contrast to biosorption, intracellular bioaccumulation of Tl by Tl-tolerant fungi may be attributed to detoxification mechanisms and cellular metabolisms that assist Tl regulation. These metal-resistant fungi can tolerate metals by accumulation, efflux, or other mechanisms (Cao et al. 2015; Li et al. 2014).

In living cells, the main source of accumulation is active intracellular uptake of metals through the cell membrane. An efflux mechanism may also function at a certain metal concentration to prevent further accumulation (Velásquez and Dussan 2009). Therefore, the metal removal efficiency by living cells is constrained by the ambient metal concentration. In the present study, the Tl bioaccumulation process predominated at 1000 mg L^{-1}. When the initial Tl concentrations increased to 1200 and 1500 mg L^{-1}, efflux rather than bioaccumulation may have contributed to the decline in bioaccumulation. This suggested that such spiked Tl levels to critically toxic points may start to inhibit the rate of metabolically dependent influx of Tl.

To develop an effective bioremediation system using microbes or plant-microbe interactions, one of the important steps is to identify microorganisms which can survive and grow in heavily metal-contaminated sites (Oves et al. 2013). Such living microorganisms have high potential in metal bioremoval because of their abilities of self-replenishment, continuous metabolic uptake of metals after physical adsorption, and no need for separated biomass production processes. High metal resistance of microbes is directly related to microbial survival and growth in metal-contaminated soil or water. In comparison to other reported metal-resistant strains (Babu et al. 2014; Li et al. 2014; Oves et al. 2013), our fungal isolates exhibited relatively high metal resistance. Although we did not investigate the tolerance mechanism(s), our results nonetheless elucidated the potential applicability of using the isolated fungal strains for thallium bioremediation, especially important in sites where Tl contamination reaches very high values.

Subcellular distribution of Tl in the Tl-tolerant fungal isolates

Figure 4 shows the subcellular distribution of Tl in the mycelia by the Tl-tolerant fungal isolates. Significant differences were observed in the distribution of Tl in cell fractions from the three concentration treatments. The average fraction of Tl in the cytoplasm, composed of the cytosol and vacuoles, was 56.8 % in the 1000-mg L^{-1} treatment, 71.3 % in the 1200-mg L^{-1} treatment, and 79.5 % in the 1500-mg L^{-1} treatment, respectively. The average fraction of Tl in the cell wall was 31.3 % in the 1000-mg L^{-1} treatment, 21.1 % in the 1200-mg L^{-1} treatment, and 14.4 % in the 1500-mg L^{-1} treatment, respectively. Similarly, the average share of Tl in the organelle fraction was 14.9 % in the 1000-mg L^{-1} treatment, 7.6 % in the 1200-mg L^{-1} treatment, and 5.1 % in the 1500-mg L^{-1} treatment, respectively. The results showed that the subcellular distribution of Tl decreased in the following order: cytoplasm ≫ cell wall > organelles.

Cell wall deposition and vacuolar compartmentalization of toxic metals in organisms have been recognized as metal

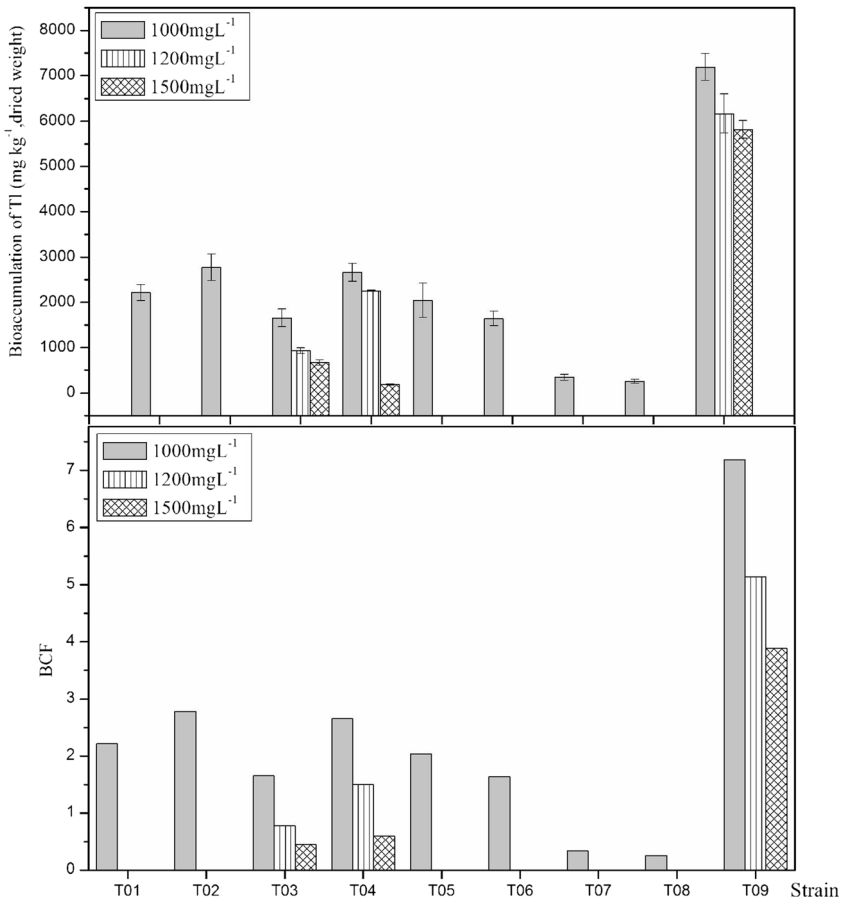

Fig. 3 Bioaccumulation of Tl by the fungal isolates. Average± standard deviation from three separate experiments

detoxification mechanisms (Qiu et al. 2011; Weng et al. 2012; Zhang et al. 2014). Our results for the distribution of Tl among the subcellular compartments in the fungal mycelia showed obvious compartmentalization. In addition, the Tl proportion in the cytoplasm fraction rose with increasing Tl treatment strength, whereas the contrary trend occurred in the cell wall and the organelle fractions. It could be concluded that accumulation of Tl in cells mainly occurred in the cytoplasm compartment, in which the presence of functionally active vacuole may result in detoxification for Tl, a role of compartmentation.

Similar compartmentalization was also observed by Kwan and Smith (1991) who found that 80 % of the Tl taken up by *Lemna minor* occurs in the cell vacuoles. The vacuolar system of the Tl accumulator *Iberis intermedia* has also been implicated in increasing its tolerance to Tl (Scheckel et al. 2004). Therefore, intracellular compartmentalization may play an important role in the detoxification and tolerance of Tl in the fungal isolates.

The cell wall is considered to be the first line of defense, protecting the protoplast from metal poisoning, particularly in low concentrations and short periods of metal threat (Wójcik

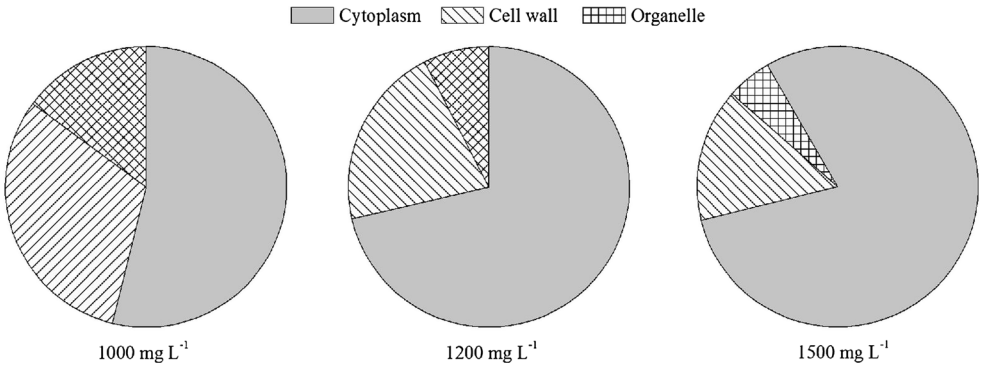

Fig. 4 Average fraction of Tl in the subcellular compartments of the fungal mycelia in the three thallium treatments

et al. 2005). When the cell wall binding sites reach saturation, most intracellular metals tend to be transported to vacuoles and then chelated with citric, malic, oxalic, and other organic acids to achieve metal compartmentalization (Kramer 2000). In the present study, the average proportion of Tl bound to cell walls ranged from 14 to 31 % (Fig. 3), which indicated that the cell wall also serves as a storage site for Tl at low Tl concentrations. Moreover, the lowest concentration of Tl in all treatments was bound to cytoplasmic organelles (5 to 14 %). It thus appears that the ability of the cytoplasm to preferentially compartmentalize Tl helps the fungal isolates to avoid Tl toxicity damage to their vital organelles.

Conclusions

In the present study, we have demonstrated that some of the fungal strains isolated from the Tl-contaminated soils grow well and have a high Tl biosorption and bioaccumulation ability under Tl pollution threat. Tl-tolerant isolates, especially *Mariannaea* sp., *T. viride*, and *T. asperellum*, can survive in thallium concentrations up to 1500 mg L^{-1}. According to the data, intracellular compartmentalization is critical to the detoxification of Tl. Most of the thallium is stored in the cytoplasm, either in the cytosol or vacuoles. Our work has demonstrated that the isolated fungal strains have a potential application for the decontamination of Tl-polluted media. The next step is to see how the growth of these fungal strains can be scaled up to remediate the Tl-contaminated soils of Guizhou Province. Further studies also need to be carried out on an environmentally friendly method of disposal.

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