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Short communication

Cadmium and zinc response of the fungi *Heliscus lugdunensis* and *Verticillium* cf. *alboatrum* isolated from highly polluted water

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Abstract

The aquatic hyphomycete *Heliscus lugdunensis* and the terrestrial fungus *Verticillium* cf. *alboatrum*, both isolated from a highly polluted surface water, were investigated for their tolerance against Cd and Zn. *Hl*-H4 showed a 50% growth inhibition at 0.1 mM Cd, whereas at 0.7 mM Cd the growth of *Va*-H4 was only reduced by 30%. The fungi also showed a remarkable difference in their Zn-tolerance. The growth of *Va*-H4 was not inhibited at 1 mM Zn, whereas for *Hl*-H4 no growth occurred above 0.3 mM Zn. The biosorption and accumulation capacities for Cd or Zn of both fungi differed between the fungal species. In a 0.1 mM Cd-medium *Hl*-H4 biosorbed 15-fold and accumulated 39-fold more Cd than *Va*-H4. Exposure to 0.3 mM Zn resulted in a 13-fold higher biosorption and 11-fold higher accumulation for *Hl*-H4 than *Va*-H4. As glutathione (GSH) is known to be involved in the phytochelatin synthesis and other stress related processes we investigated its synthesis. Both fungi increased their synthesis of GSH in response to Cd. For *Hl*-H4 a concentration of 0.0125 mM Cd, corresponding to an intracellular Cd content of 2.1 nmol Cd mg^{-1} dw, increased the GSH content, whereas *Va*-H4 only responded with a higher production of GSH at 1 mM Cd and a concomitant intracellular Cd content of 22.5 nmol Cd mg^{-1} dw. An increased GSH synthesis under Zn-stress was only detectable for *Va*-H4 (20 mM).

Keywords: Fungi; Aquatic hyphomycete; *Heliscus lugdunensis*; *Verticillium* cf. *alboatrum*; Cd/Zn tolerance; Glutathione

1. Introduction

Biotopes rich in heavy metals represent extreme environments, where the speciation of heavy metals and their bioavailability exert the main selective pressure on microbial communities. A selective advantage is conferred to those organisms that have

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developed mechanisms to tolerate the toxic effects of high heavy metal concentrations (Prasad, 2001).

Like bacteria, fungi affect the mobility and environmental fate of metals (Gadd and Sayer, 2000). Aquatic fungi are essential intermediaries in the transfer of nutrients between the different trophic levels of the ecosystem (Bärlocher, 1992, 2005). As recently demonstrated fungal communities in aquatic habitats are impoverished in the presence of heavy metal pollution (Krauss et al., 1998, 2001, 2003a,b, 2005a,b; Sridhar et al., 2000, 2001). Over time, local populations may evolve in response to the specific selection pressure of their habitat. The ability of some fungi to survive in environments with extremely high levels of heavy metals depends on a range of tolerance/resistance mechanisms. From a heavily polluted spring (over 2 g of dissolved Zn L^{-1}, 3 mg Cd L^{-1} among other cations and anions), emerging from a mining and smelting waste dump, a *Heliscus lugdunensis* strain (Krauss et al., 1998, 2001) and *Verticillium* cf. *alboatrum* strain were isolated. Both species belong to the mitosporic fungi. As shown in diversity studies, the aquatic hyphomycete *H. lugdunensis* was consistently one of the most abundant species occurring at heavy metal polluted sites (Krauss et al., 2001; Sridhar et al., 2000). *V.* cf. *alboatrum*, a fungus with presumed ascomycetous affiliation (Seiffert and Gams, 2001; Marvanová, personal communication), was isolated from an extremely contaminated habitat for the first time. Heavy metal tolerance in filamentous fungi and their adaptation to polluted waters are not fully understood. The aim of this work was to compare two fungi in their physiological and biochemical response to high Cd and Zn concentrations.

2. Material and methods

The *H. lugdunensis* strain H4-2-4 (*Hl*-H4) strain was maintained on malt agar (Krauss et al., 2001). Since *V.* cf. *alboatrum* strain H4-P-3 (*Va*-H4) did not grow under such conditions, it was maintained on heavy metal-containing malt agar (1 mM $ZnCl_2$, 0.01 mM $PbCl_2$, 0.12 mM $CuCl_2$, 0.025 mM $CdCl_2$) found to facilitate its growth. To minimize the effects of heavy metals possibly contaminating the malt agar plate-derived inocula used for liquid culture experiments, liquid cultures in heavy metal-free medium (0.5% malt extract, Merck; 0.1% peptone, Difco) were first established to produce the inocula for subsequent cultivations in heavy metal-containing media. For this, 15 agar plugs (∅ 7 mm) overgrown with fungal mycelium were homogenized in 15 mL liquid medium (0.5% malt extract, Merck; 0.1% peptone, Difco). 1 mL of mycelial homogenate of *Hl*-H4 and *Va*-H4 was used to inoculate 75 mL and 40 mL liquid medium, respectively, in 100 mL Erlenmeyer flasks. After 4 days of incubation (14 °C on a rotary shaker, 120 rpm) in the dark, the mycelium was homogenized aseptically. The main culture containing different amounts of heavy metals (Cd: 0.0125–1 mM; Zn: 0.025–1 mM) was started at the conditions described above by inoculation with mycelium corresponding to 7 μg dry weight and was cultured for 5 days. To determine growth the mycelium was harvested on a Whatman filter 3, washed with distilled water, dried overnight at 80 °C and weighed. To determine biosorbed and accumulated heavy metal the mycelium was harvested on a nitrocellulose filter (Millipore 0.45 μm HA). Mycelium of Cd treated samples of each flask was washed for each 5 minutes with distilled water (100 mL), 3 times with 20 mM $NiCl_2$ (100 mL) solution to remove extracellular Cd and again with distilled water (100 mL) (Brown and Wells, 1988). The Zn exposed mycelium was washed 3 times with 20 mL distilled water, 4 times with 20 mM $NiCl_2$ solution (100 mL) and again with distilled water (100 mL). Each washing step was applied during 5 min. The mycelium was then removed from the filter and dried overnight at 80 °C. 50 mg of the mycelium was digested with 4 mL 65% HNO_3 and 2 mL 30% H_2O_2 in a microwave (CEM MDS 2100). Heavy metal concentrations in the $NiCl_2$ washings (for biosorption) and in the mycelium digests (for accumulation) were determined by an atomic absorption spectrometer (ATI Unicam). To extract glutathione (GSH) freshly harvested mycelium was rinsed twice with distilled water and dabbed dry between filter paper. The mycelium was crushed in liquid N_2 and approximately 50 mg was homogenized in 1.5 mL of 0.1 N HCl (Gallego et al., 1996). The GSH concentration of the extract was determined enzymatically in the presence of NADPH and DTNB (Anderson, 1985). Data were analysed with SPSS (Statistical Package for the Social Science version 11.0). All the values reported in this