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Cadmium removal capacities of filamentous soil fungi isolated from industrially polluted sediments, in La Plata (Argentina)

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Summary

Aspergillus terreus, *Cladosporium cladosporioides*, *Fusarium oxysporum*, *Gliocladium roseum*, *Penicillium* spp., *Talaromyces helicus* and *Trichoderma koningii* were isolated from heavily polluted streams near an industrial area in La Plata, Argentina. The fungi were obtained from sediments with 0.25–0.50 mg Cd/l and they were isolated in cadmium-basal medium. They were then cultivated to evaluate their Cd detoxification abilities. The biomass developed in static assays represented 5–53% of the yield of stirred cultures, for the different fungal species, although the cadmium absorption were similar in both cases. These soil fungi represented 50% of the total isolates and their mycelial growth was conspicuous in these polluted sediments. Although bacteria have been mentioned as active microorganisms against heavy metals, the mycelial fungi were able to develop a significantly higher mass to sequestrate more metals. Thus, they could be used in remediation biotechnology to improve the Cd detoxification of chronically contaminated habitats.

Introduction

Heavy metals are a common cause of environmental pollution and they mostly accumulate in soils (Hiroki 1992). Extensive data suggest that cadmium is the most toxic heavy metal and it is listed as a priority pollutant by the US Environmental Protection Agency (US EPA, 1979). Cd is extensively used for a number of applications, including electroplating, protection against corrosion and stabilizing plastic (Lebrun *et al.* 1994). Cadmium is carcinogenic, embryotoxic, teratogenic and mutagenic and may cause hyperglycaemia, reduced immunopotency and anaemia, due to its interference with iron metabolism (Sanders 1986).

Several fungal species have developed a high resistance to heavy metals and have developed a variety of mechanisms to remove ions, such as adsorption to cell surfaces (Mullen *et al.* 1989), complexation by exopolysaccharides (Ariff *et al.* 1999), intracellular accumulation (Laddaga & Silver 1985) or precipitation (Aiking *et al.* 1985).

The artificial and natural streams in La Plata, Argentina, receive industrial and urban effluents that produce a high level of contamination. This situation allowed us to isolate filamentous Cd-resistant fungal species from sediment samples, with the purpose of evaluating their abilities to remove cadmium, in relationship to their biomass development.

Materials and methods

Isolation and identification of the fungal species

Sediment samples were taken from streams near an oil refinery in La Plata, Argentina. A 500 g surface sediment samples were taken from the sites over 2 years, every 3 months. The following chemical characteristics of the sampled sites were determined: pH, humidity, textural features, organic carbon, organic nitrogen, organic phosphorus, organic matter and Cd level. Three decimal dilutions of the sediment samples were inoculated on to agar plates with a selective basal medium for filamentous fungi (KH_2PO_4 0.2 g, K_2HPO_4 0.8 g, $MgSO_4 \cdot 7H_2O$ 0.2 g, $CaSO_4$ 0.1 g, $(NH_4)_2SO_4$ 5.0 g, yeast extract 0.1 g, distilled water 1.0 l), amended with 75 ml/l of an antibiotic stock solution (streptomycin 5.0 g, chloramphenicol 2.5 g, distilled water 1.0 l).

Subsequently, pure colonies of the fungi were isolated in the above medium with 15 mg Cd/l as $CdSO_4$. The identification of the different species was carried out on the basis of micromorphology, colonial features and reproductive forms in different media and culture conditions (with or without light, u.v. exposure, 20–28 °C, pH 5.5–6.5). The assayed fungal species are maintained in selective medium in the LPS Culture Collection of 'Instituto Spegazzini', UNLP, Argentina.

Fungal growth

The filamentous soil fungi were grown in 250 ml Erlenmeyer flasks with 100 ml liquid medium, containing (per l) KH_2PO_4 0.2 g, K_2HPO_4 0.6 g, $MgSO_4 \cdot 7H_2O$ 0.2 g, $CaSO_4$ 0.1 g, $(NH_4)_2SO_4$ 5.0 g, yeast extract 1.0 g, glucose 20.0 g, pH 6.0. To this medium 10 mg Cd/l, as $CdSO_4$ was added; and it was sterilized at 121 °C, for 20 min.

The culture medium was inoculated with two loops of each filamentous fungus and cultured at 27 °C in an orbital shaker (130 rev/min) and in static conditions, for 13 days, in triplicate. The biomass of the fungal assays were determined by gentle filtration with nitrocellulose disc filters (MSI Micron Sep; ø 47 mm, pore 0.45 μm). The filters were then dried at 60 °C for 4 days, to constant weight. The Cd concentrations of the filtrates were measured by atomic absorption spectrometry EPA 3010 (US EPA 1990).

Data processing

The results were presented as percentage of Cd removal in relation to the initial Cd levels of the assays. The removal efficiencies were calculated by the ratio between the fungal biomass and the Cd removed, for each fungal species and cultural conditions.

Results and discussion

The sampled sites were natural and artificial channels that drained into the Río Santiago and they are located in an industrialized area near the YPF-Refinery, La Plata, Argentina; with more than 20 years of petrochemical contamination (Figure 1). The chemicals and textural features of the samples are shown in Table 1.

Table 1. Characteristics of the sites of sampling.

	Zanjón (1)	Copetro (2)	Prosul (3)
pH	6.6	6.2	6.1
Humidity (%)	8.26	5.82	9.02
Organic matter (%)	10.8	11.5	25.4
C (%)	6.3	6.7	14.4
N (%)	0.51	0.95	1.58
P (mg/kg)	6.82	7.03	1.25
Sand (%)	47	69	48
Lime (%)	21	7	28
Clay (%)	32	24	24
Cd (ppm)	0.50	0.25	0.25

In this study, seven filamentous fungal species were isolated from the sediments and identified. *Aspergillus terreus* Thom, *Cladosporium cladosporioides* (Fresenius) de Vries, *Gliocladium roseum* Bainier, *Fusarium oxysporum* Schlecht, *Penicillium* spp., *Talaromyces helicus* (Raper & Frenelli) C.R. Benjamin and *Trichoderma koningii* Oudem. were able to grow on Cd plates.

The biomass developed was very different between stirred and static conditions, being greater for all the cultured species in the shaker (Figure 2), probably due to greater availability of oxygen. In this study, both culture conditions were examined. The static assays showed values that varied from 53, 34, 29, 21, 16, 9 to 5%, for *C. cladosporioides*, *Penicillium* spp., *F. oxysporum*, *T. koningii*, *G. roseum*, *A. terreus* and *T. helicus* respectively, in relation to the shaken cultures. Some authors have found higher biomass in fungal static cultures, such as Coates *et al.* (1996) and Ascom-Cabrera & Lebeault (1993).

Although a significantly greater mass was obtained in stirred conditions, the cadmium removal was higher in static conditions, and the values fluctuated between 0 and 27% in all the fungi tested (Figure 3).

In all cases, the static cultures showed greater Cd-removal efficiencies than the shaken ones**.** The removal abilities of *G. roseum*, *T. helicus*, *A. terreus* and *T. koningii*, were higher in the static cultures than the corresponding values for *C. cladosporioides*, *F. oxysporum* and *Penicillium* spp (Figure 4).

There are a only few reports on cadmium removal by filamentous soil fungi. Most of researchers have focused their interest on bacteria: Cunningham & Lundie (1993),

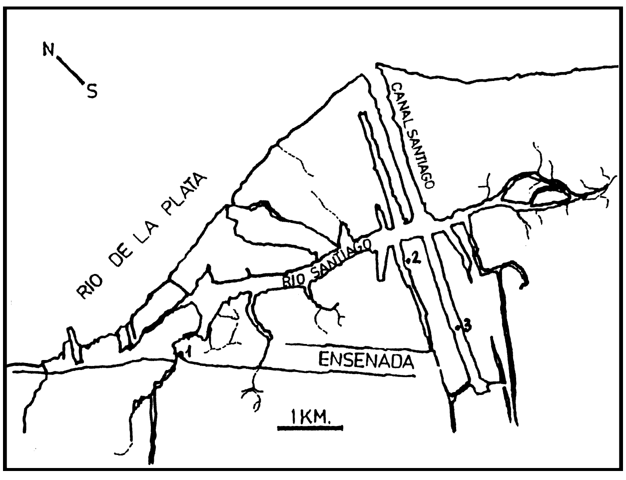

Figure 1. Sites of sampling: (1) Zanjón, (2) Copetro, (3) Prosul.

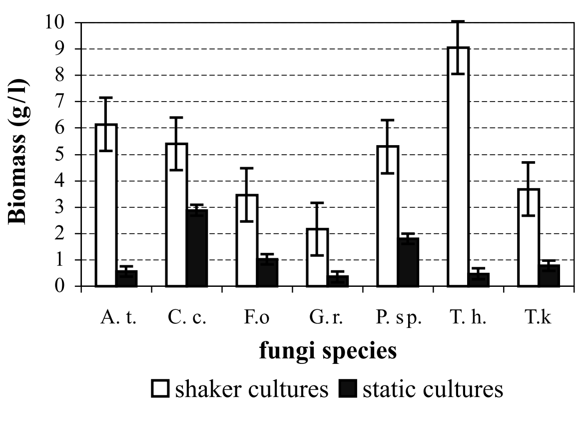

Figure 2. Biomass developed in shaken (130 rev/min) and static conditions *(A. t., A. terreus*; *C. c., C. cladosporioides*, *F. o., F. oxysporum*; *G. r., G. roseum*; *P.* spp., *Penicillium* spp.; *T. h., T. helicus*; *T. k., T. koningii*).

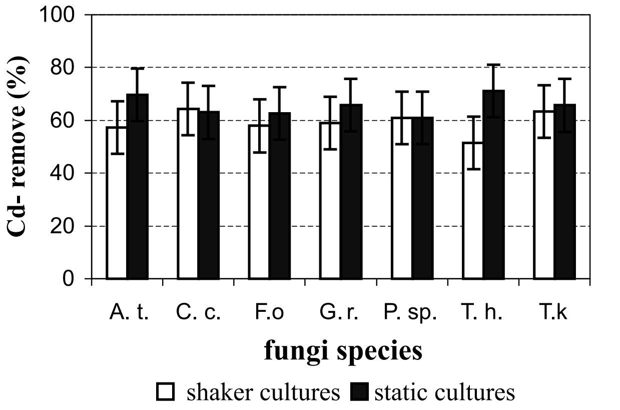

Figure 3. Cadmium removal (%) in shaken (130 rev/min) and static conditions (*A. t.*, *A. terreus*; *C. c.*, C. cladosporioides; F. o., *F. oxysporum*; *G. r.*, G. roseum; *P.* spp., *Penicillium* spp.; *T. h.*, *T. helicus*; *T. k.*, *T. koningii*).

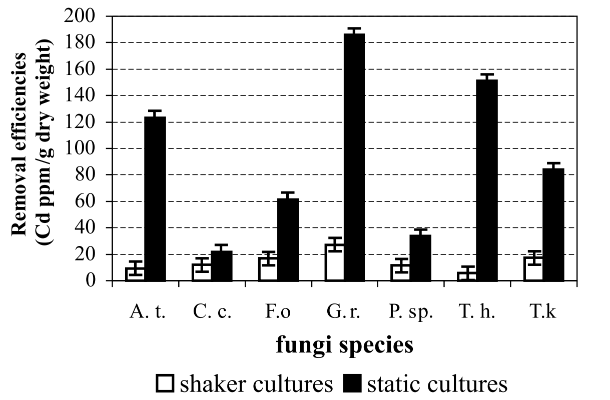

Figure 4. Cd-removal efficiencies in shaken (130 rev/min) and static conditions (*A. t.*, *A. terreus*; *C. c.*, *C. cladosporioides*; *F. o.*, *F. Oxysporum*; *G. r.*, *G. roseum*, *P. spp.*, *Penicillium* spp.; *T. h.*, *T. helicus*; *T. k.*, *T. koningii*).

Puranick *et al.* (1995), Riazul Haq *et al.* (1999). Only some investigations have pointed out the role of yeasts and wood-rotting fungi in metal ion removal (Berdicevsky *et al.* 1993; Brady & Duncan 1994; Volesky & May-Phillips 1995; Gabriel *et al.* 1996).

The detoxification abilities of filamentous fungal species with other heavy metals have been mentioned and the kinetics of lead sequestration have been studied by Ariff *et al.* (1999) and uranium biosorption by Bengtsson *et al.* (1995) and Bhainsa *et al.* (1999).

Talaromyces spp., *Aspergillus* spp. and *Penicillium* spp. have already been mentioned as heavy metal tolerant (Galun *et al.* 1987; Niu *et al.* 1993), but this is the first report of metals absorption by *G. roseum*, *F. oxysporum*, *C. cladosporioides* and *T. koningii*.

Metal accumulation depends on diverse factors, such as pH, temperature, organic matter, soil texture and the presence of other ions (Scheuhammer 1991; Fourest & Roux 1992). Although the natural Cd concentrations were low, all the fungal species grew and removed a 100-fold higher Cd level.

The removal efficiencies obtained in all the fungal cultures showed promising immobilization of cadmium in 13 days incubation time. Furthermore, these results point to the possible application of these fungi as metallic bioabsorbents for polluted sites. In conclusion, from an environmental point of view, the assayed fungi developed a significant biomass, moreover, the fungal strains removed more cadmium in static conditions, similar to the ones found in natural habitats.

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