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IN VITRO UPTAKE OF CADMIUM BY BASIDIOMYCETES *PLEUROTUS OSTREATUS*

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SUMMARY

The mycelium of Basidiomycetes *Pleurotus ostreatus* was grown in liquid cultures of malt broth enriched with increasing amounts of cadmium also in the presence of copper and glutathione. Cadmium, up to 150 μg/ml gradually inhibited mycelial development but never blocked it completely. Cadmium accumulated to a higher degree (20 mg/g dry wt) when administered alone and was mostly bound (80%) to hyphal cell walls. Interactions with copper may play an important role in cadmium tolerance.

INTRODUCTION

Much effort has been spent in the last few decades to investigate heavy metal accumulation in the higher fungi, mostly because of their possible entry into the terrestrial food chain (Byrne *et al.*, 1976; Seeger, 1978; Lepsova and Kral, 1988; Vetter, 1989;). There are remarkable differences between species in the ability to concentrate heavy metals; however it may be stated that metal concentrations depend particularly on the trophic pattern. Saprophytic species show the highest ability in accumulating metals; those producing mycorrhizae and species saprophitic on wood show the lowest (Lepsova and Mejstric, 1988). According to these considerations, we verified that the wood-decaying Basidiomycetes *P.ostreatus*, intensively used for bioconversion of agro-industrial wastes into edible products of higher quality (Bisaria *et al*, 1987; Ginterova, 1989), is able to control cadmium (Cd) intracellular uptake from metal-contaminated substrates. A Concentration Factor (CF) lower than 1 was observed in fruit bodies grown in the presence of increasing Cd exposures (Favero *et al.*, 1990 a). We also hypothesized that the metal could be firmly complexed within the substrate, probably in the vegetative structure, since substrate Cd concentration remained unchanged during fruit body production in spite of periodical waterings.

These experimental results led us to study the mechanisms of Cd uptake by the mycelium of *P.ostreatus* grown in a liquid culture, to verify if intracellular uptake occurs together with biosorption on the hyphal cell-wall. Heavy metal accumulation by microorganisms may in fact be due both to metabolism-independent extracellular binding (biosorption) and to energy-dependent intracellular transfer and binding (Gadd, 1990). These two mechanisms may be active at successive moments, as has been observed in yeasts (Mowll & Gadd, 1983) and algae (Ting *et al.*, 1989).

Cd uptake from the liquid medium was also studied in the presence of copper (Cu), an essential metal, since it had been observed that Cd exposures can induce Cu uptake corresponding to an increase in Cu-binding peptides in fruit bodies (Favero *et al.*, 1990 b). Exposure to Cd was also tested in the presence of glutathione (GSH) a ubiquitous tripeptide and the most abundant naturally occurring low molecular thiol (Meister and Anderson, 1983). Our purpose was to verify the possible role of thiolic compounds in the growth of fungi exposed to heavy metals, probably through biosynthesis of thiol-endowed chelating molecules (Mehra and Winge, 1991).

MATERIALS AND METHODS:

Culture conditions

The mycelium of *P.ostreatus*, used for fruit body production on solid substrates, was inoculated by means of a wooden stick in a liquid medium consisting of a sterile 2,5% malt solution (pH 6.3) in deionized water.

Cultures were grown statically at 25 °C in 1-lt flasks containing 200 ml of medium, for 30 days. At the end of the experiment, the mycelium was separated by filtration and repeatedly washed with ultrapure deionized water (Milli Q). The whole biomass was then lyophilized to determine growth levels on the basis of biomass dry wt (Darlington and Rauser, 1988).

Exposure to Cd and metal uptake

Exposure to Cd was performed by supplementation of malt broth with a solution of Cd chloride to final concentrations of 5, 10, 20, 50, 100, 150 μg Cd/ml. Cd was administered: (a) alone, (b) in the copresence of a fixed Cu dose (5 μg/ml as $CuSO_4$), (c) in the copresence of the fixed Cu dose and of GSH (2mM). The selected Cu dose is not toxic; it produces an increase of mycelial growth in control conditions. Each treatment was performed in triplicate.

Metal concentrations in mycelium and pellets derived from centrifugations (each sample in triplicate) were determined by atomic absorption spectrophotometry (Perkin-Elmer model 4000) after digestion with 65% HNO_3 (Aristar BDH) in Teflon bombs at 160 ° for 2 h.

Aliquots of 120 mg of dry mycelium where homogenized in the cold in 10 mM Tris-HCl pH 7 with an Ultra Turrax homogenizer and centrifuged at 45,000 g for 60 min. Supernatants were direcly monitored for Cd content. Avarage values from three cultures are reported for each parameter.

RESULTS AND DISCUSSION

P.ostreatus mycelium exposed to Cd in a liquid culture reveals that it can tolerate high concentrations of the toxic metal; this conclusion agrees with evidence from fruit body cultures.

Mycelial growth differs according to Cd treatment conditions, the greatest development occurring in the copresence of Cu and GSH (Fig.1). Differences among these three exposure conditions also occur in controls, suggesting that Cu and GSH act as growth factors independently of the presence of Cd. The benefit produced by Cu availability may be due to the improved biosynthesis of some Cu enzymes, e.g. laccase, a blue-oxidase, particularly abundant in wood-destroying white-rot fungi which can degrade lignin (Lontie, 1984). This hypothesis is being verified. Instead, GSH represents a rich metabolic reservoir. With increasing levels of Cd exposure, mycelial development decreases, although complete growth inhibition never occurs at the observed doses. In the copresence of GSH, inhibition of mycelial development proceedes slowly, suggesting that Cd toxicity is reduced in the presence of thiols. Fruit body production on solid substrates was never affected up to the dose of 100 μg/g wet wt; consequently, the mycelium of *P. ostreatus* appears to be more sensitive to Cd salts in solution.

Cd accumulation reaches its highest values (more than 20,000 μg/g dry wt) in the absence of both Cu and GSH (Fig 2). Data on metal uptake into the vegetative structure of higher fungi are lacking in the literature; however *P.ostreatus* mycelium shows a remarkable ability in binding Cd relative to other microorganisms (Gadd, 1990). Examination of the CF (Tab. 1) reveals the similar response of mycelia grown in the presence of Cu, mainly at higher exposure levels, suggesting that Cu other than GSH can limit Cd accumulation.

Table 2 describes the concentration and percentage distribution of Cd in the soluble and granular fractions of the mycelium exposed to Cd in the three examined conditions. The cytosoluble compartment accounts for about 20% of the total metal uptake; consequently, Cd appears to be mainly accumulated in the cell walls. This result explains the limited tranfer of toxic metal into *P.ostreatus* fruit bodies and, if verified for other species, may suggest that binding of heavy metals by cell wall may be the main mechanism involved in metal tolerance in higher fungi. Cytosoluble Cd increases according to exposure dose but it levels off in the presence of Cu. Cu may modify the situation of

binding sites on the cell wall, thus limiting Cd translocation across the membrane and, consequently, its intracellular uptake as in the case of competitive inhibition. Interactions with Cu may play an important role in Cd tolerance by *P.ostreatus*; they may develop both extracellularly and intracellularly by induction of cytosoluble metal-binding peptides, as described in a previous paper (Favero *et al.*, 1990 b).

In our opinion the biosorption produces a reservoir of metals which may gradually be tranferred into the developing fruit bodies. This may also explain the permanence of dangerous levels of radioactive metals in mushrooms, years after a contamination event (Elstner *et al.*, 1989; Bakken and Olsen, 1990).

Investigations on mycelial chelating ability in higher fungi may be useful in explaining the different intracellular uptakes of the various species.

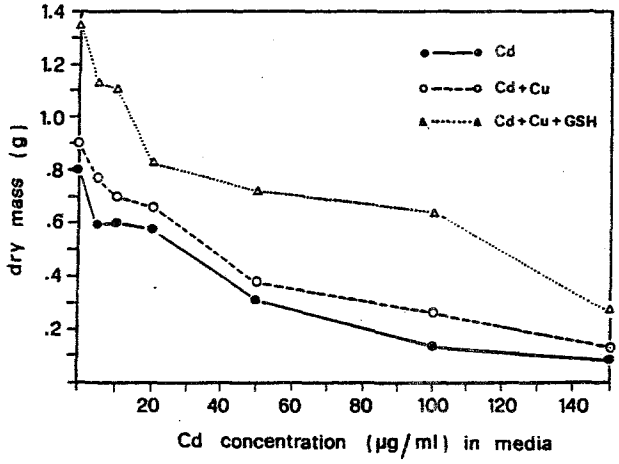

Fig. 1 Growth of the mycelium of *P.ostreatus* exposed to increasing Cd levels in different conditions.

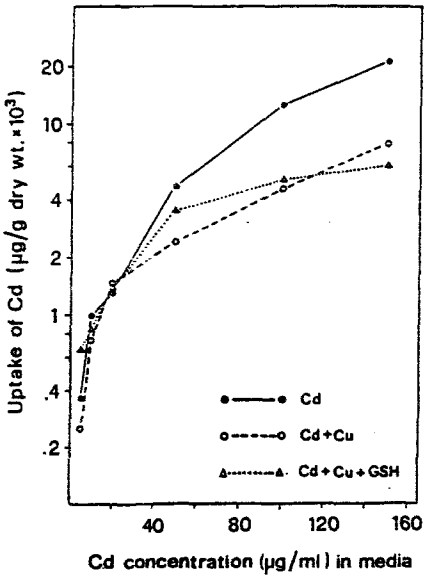

Fig. 2 Uptake of Cd by the mycelium of *P. osreatus* exposed to increasing Cd levels in different conditions.

EXPOSURE Cd (µg/ml)	A	B	C
5	7.28	4.96	13.16
10	9.89	7.35	8.35
20	6.45	7.29	7.02
50	9.31	4.83	7.14
100	12.59	4.49	4.94
150	14.00	5.22	4.03

Tab. 1 Cd Concentration Factor, (CF) = (µgCd/g of wet mycelium) / (µgCd/ml of medium), in the mycelium of *P.ostreatus* exposed to increasing Cd concentrations in different conditions:
A = Cd, B = Cd + Cu, C = Cd + Cu + GSH.

	EXPOSURE Cd (μg/ml)					
	5	10	20	50	100	150
A						
Supernatant	69 (19)	89 (9)	232 (18)	932 (20)	2518 (20)	3581 (17)
Pellet	295 (81)	900 (91)	1058 (82)	3724 (80)	10,072 (80)	17,485 (83)
Whole homogenate	364	989	1290	4656	12,590	21,067
B						
Supernatant	50 (20)	95 (13)	219 (15)	629 (26)	1168 (26)	1331 (17)
Pellet	198 (80)	640 (87)	1240 (85)	1790 (74)	3326 (74)	6498 (83)
Whole homogenate	248	735	1459	2419	4494	7829
C						
Supernatant	112 (17)	142 (17)	239 (17)	821 (23)	1286 (26)	1088 (18)
Pellet	546 (83)	693 (83)	1166 (83)	2750 (77)	3659 (74)	4956 (82)
Whole homogenate	658	835	1405	3571	4945	6044

Tab. 2 Concentration (μg/g dry wt) and percentage distribution (in parentheses) of Cd in supernatant and pellets from the mycelium of *P.ostreatus* exposed to increasing Cd concentrations in different conditions (see Tab. 1).

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