Biometals (2007) 20:797–805
DOI 10.1007/s10534-006-9043-x

ORIGINAL PAPER

Enhancement of emulsifier production by *Curvularia lunata* in cadmium, zinc and lead presence

Katarzyna Paraszkiewicz · Aleksandra Frycie · Mirosława Słaba · Jerzy Długoński

Received: 19 June 2006 / Accepted: 18 October 2006 / Published online: 22 November 2006

Abstract The influence of cadmium, zinc and lead on fungal emulsifier synthesis and on the growth of filamentous fungus *Curvularia lunata* has been studied. Tolerance to heavy metals established for *C. lunata* was additionally compared with the sensitivity exhibited by strains of *Curvularia tuberculata* and *Paecilomyces marquandii*—fungi which do not secrete compounds of emulsifying activity. Although *C. lunata*, as the only one out of all studied fungi, exhibited the lowest tolerance to heavy metals when grown on a solid medium (in conditions preventing emulsifier synthesis), it manifested the highest tolerance in liquid culture - in conditions allowing exopolymer production. Cadmium, zinc and lead presented in liquid medium up to a concentration of 15 mM had no negative effect on *C. lunata* growth and stimulated emulsifier synthesis. In the presence of 15 mM of heavy metals, both the emulsifier and 24-h-old growing mycelium exhibited maximum sorption capacities, which were determined as 18.2 ± 2.67, 156.1 ± 10.32 mg g^{-1} for Cd^{2+}, 22.2 ± 3.40, 95.2 ± 14.21 mg g^{-1} for Zn^{2+} and 51.1 ± 1.85, 230.0 ± 28.47 mg g^{-1} for Pb^{2+} respectively. The results obtained by us in this work indicate that the emulsifier acts as a protective compound increasing the ability of *C. lunata* to survive in heavy metal polluted environment. Enhancement of exopolymer synthesis in the presence of Cd^{2+}, Zn^{2+} and Pb^{2+} may also suggest, at least to some extent, a metal-specific nature of emulsifier production in *C. lunata*. Due to accumulation capability and tolerance to heavy metals, *C. lunata* mycelium surrounded by the emulsifier could be applied for toxic metal removal.

Keywords *Curvularia lunata* · Emulsifier · Exopolymers · Filamentous fungi · Heavy metals · Tolerance

K. Paraszkiewicz · A. Frycie · M. Słaba · J. Długoński (✉)
Department of Industrial Microbiology and Biotechnology, University of Łódź, Banacha 12/16, PL-90237 Lodz, Poland
e-mail: jdlugo@biol.uni.lodz.pl

Introduction

The increasing problem of heavy metal contamination has stimulated a search for mechanisms of microbial metal-binding capacity as well as factors affecting cell surviving in the presence of high concentrations of heavy metals (Gharieb 2001; Zhang et al. 2002; Malik 2004; Gadd 2004; Jarosz-Wilkołazka et al. 2006). Filamentous fungi are common and important residues of soil biota, and also many of them have been used for bioremediation of heavy metal contaminated wastes (Gadd 2001; Wainwright and Gadd 1997).

Describing the ability to grow at high metal concentrations, Wainwright and Gadd (1997) distinguished fungi tolerant and resistant to heavy metals. Resistance involves detoxification mechanisms produced in direct response to the toxic metal, whereas tolerance is defined as the ability to cope with toxicity by means of intrinsic properties of the organism including impermeable, usually pigmented, cell walls, as well as excretion of various extracellular high-molecular-mass substances (Nies 1999). Melanins which are dark pigments located in the fungal cell wall (but also exist as extracellular polymers) can reduce the toxic effect of heavy metals due to the presence of various groups of high affinity to metal ions (Fogarty and Tobin 1996; Ledin 2000). The strains of *Aureobasidium pullulans* synthesize a mixture of extracellular polysaccharides (pullulan, β-glucan), whose presence increases metal resistance of the yeast and plays an important role in the processes of heavy metal detoxification (Breierová et al. 2004). Although many microbial exopolymers exhibit high affinity to bind heavy metals, thus protecting cells from metals toxicity (Gutnick and Bach 2000; Freire-Nordi et al. 2005), the role of these compounds in the tolerance of producing microorganisms remains still uncertain. Biosynthesis of EPS (exopolysaccharide) was not responsible for increased tolerance to aluminium in *Rhizobium lequminosarum* (Kingsley and Bohlool 1992). Gellan producing strain of *Sphingomonas paucimobilis* R40 was less tolerant to copper than nonmucoid variant RP10 (Richau et al. 1997).

The genus *Curvularia* contains several, worldwide distributed species mostly recognized as typical saprophitic fungal residues of agriculture soils. Reports of human diseases caused by these microorganisms are relatively uncommon and mainly concern certain cases of mycosis and allergy disorders (Rinaldi et al. 1987; Safdar 2003). Some of these fungi expecially strains of *Curvularia lunata* exhibit a biotechnologically important feature of various cyclic compounds transformation including steroids and terpens (Fernandes et al. 2003; Collins and Reese 2001).

The wild saprophitic strain of *C. lunata* (IM 2901) used in this study is known for its ability of cortexolone 11β-hydroxylation with a high efficiency in hydrocortisone production—a pharmauceutical corticosteroid of significant value (Wilmańska et al. 1992; Paraszkiewicz and Długoński 1998). This fungus produces also with high efficiency an extracellular, slime polymer capable of stabilising oil in water emulsions—mixtures in which hydrophobic phase is dispersed as microscopic droplets in water phase (Kanwal et al. 2001; Paraszkiewicz et al. 2002). Similarly to other fungi within *Curvularia* genus studied by us, *C. lunata* strain is able to form melanin. However, it should be emphasized that synthesis of this pigment by mycelium cultured in liquid media occurs only in the late stationary phase of growth.

Our present study was aimed to estimate the influence of heavy metals on the *C. lunata* growth and exopolymer synhesis as well as Cd^{2+}, Zn^{2+} and Pb^{2+} sorption capacity of the mycelium and emulsifier of this fungus. To investigate a possible physiological role of *C. lunata* emulsifier we compared the sensitivity of *C. lunata* strain to heavy metals when this fungus was grown on solid and liquid medium (in conditions preventing and stimulating emulsifier production, respectively).

The assessement of *C. lunata* growth was additionally contrasted with the results obtained for other two fungal strains which were cultured in the same conditions but had no ability to synthesize any emulsifying agents. For this comparison we have chosen another fungus belonging to genus *Curvularia*—strain of *C. tuberculata* isolated from a sample of agricultural soil. The third fungus, *Paecilomyces marquandii* has been selected due to its confirmed ability to tolerate and bind Zn and Pb (Słaba and Długoński 2004; Słaba et al. 2005).

Materials and methods

Strains and maintenance

Three strains of filamentous fungi: *Curvularia lunata* (Wakker) Boedijn IM 2901, *Curvularia tuberculata* (Jain) IM 4417 (phyllum: *Ascomycota*, order: *Pleosporales*, family: *Pleosporaceae*) and *Paecilomyces marquandii* (Massee) Hughes IM 6003 (phyllum: *Ascomycota*, order: *Eurotiales*,

family: *Trichocomaceae)* from the collection of the Department of Industrial Microbiology and Biotechnology, University of Łódź were used in this study. In contrast to the tested strains of *Curvularia* originating from unpolluted environments, strain of *P. arquandii* was selected from postflotation dumps of non-ferrous metal works (Silesia, Poland) (Słaba and Długoński 2004; Słaba et al. 2005). The fungal strains were maintained on ZT slants [g l^{-1}: glucose, 4; Difco yeast extract, 4; agar, 25; malt extract 6° Blg, up to 11 (1° Balling = 1 g of soluble substances extracted from the grain per 100 ml malt extract); pH 7.0], at 4°C and transferred at 2-month intervals.

Screening for tolerance on agar PL-2 medium

The nutrient-rich PL-2 medium (g l^{-1}: glucose, 20; peptone, 5; Difco yeast extract, 5; KH_2PO_4, 5; malt extract 12°Blg, 100 ml) was used first and foremost because, as shown in former studies (Kanwal et al. 2001; Paraszkiewicz *et al*. 2002) it is the most convenient for the emulsifier synthesis by *C. lunata*. The effects of the heavy metals (in initial concentration of 10 mM) on the colony growth were investigated on the PL-2 medium solidified with agar (2%). The PL-2 medium was amended with filter-sterilized solutions of cadmium, zinc and lead acetates in deionized water. The metal ions were tested individually. Petri plates (90 mm diameter) were inoculated with agar discs (5 mm diameter) collected from the growing edge of a stock plate. Plates were incubated at 28°C, for 6 days and then radial mycelial growth was measured.

Liquid cultures studies

The liquid PL-2 medium amended with 10 and 15 mM of appropriate heavy metal solution was inoculated with 10% homogenous second-step preculture (24-h-old) prepared according to the method of Paraszkiewicz and Długoński (1998). Stirred cultures (100-ml Erlenmeyer flasks, 20 ml broth volume) were incubated on a rotatory shaker (180 rpm), at 28°C, for 48 h. At 24 and 48 h of cultivation, samples were withdrawn for analyses. During *C. lunata* incubation with heavy metals a microscope Axiovert 200 M with confocal scanning module LSM 5 Pascal (Zeiss, Germany) with Nomarski differential interference contrast was used for microscopic inspection.

Fungal biomass and emulsifier isolation

Culture samples were centrifuged at 5,000 × *g* for 10 min at room temperature. Pellets of the fungal biomass were washed several times with deionized water by filtration through a nitrocellulose filter with a pore size of 0.65 μm (Millipore) and dried at 105°C to the constant weight. For preparation of *C. lunata* emulsifier 1 volume of acetone was added carefully to 1 volume of cell-free culture broth. The resulting white precipitate was collected by centrifugation (5,000 × *g* for 10 min), then dissolved in deionized water and finally lyophilized.

The effect of heavy metals on the fungal growth

Fungal tolerance to heavy metals was calculated on the basis of dry weight of biomass and the radius of the colonies (for experiments conducted in liquid and on solid PL-2 medium, respectively) and then compared with the growth of control (in the absence of the heavy metals) taken as 100%. It ought to be emphasized that heavy metal acetates, when added to nutrient-rich Pl-2 medium, precipitated immediately and concentrated around the mycelia but in the following hours of cultivation gradual decay of metal precipitants in liquid cultures was observed.

Metal estimation

Metal concentration in the mycelia and emulsifier was determined in a Varian atomic absorption spectrophotometer (Spectra 300). Mineralization was carried out in a thermal oven, with a mixture of concentrated acids HNO_3 and $HClO_4$ for 1 h, at 100°C and next at 140°C. The quantity of metal adsorbed by mycelia was determined on a dry-weight biomass and emulsifier basis, and expressed as mg metal accumulated per gram of dry weight.

Statistical analysis

All data are presented as the mean of four replicates. An average standard deviation (±SD) was calculated.

Results and discussion

Influence of emulsifier presence on *C. lunata* sensitivity to heavy metals

As shown in Fig. 1 Cd^{2+} and Zn^{2+} presented at 10 mM completely prevented the growth of *C. lunata* colonies. The only metal tolerated in this concentration was Pb^{2+}, however the growth of *C. lunata* was limited by about 85.0 ± 0.72%. In the same conditions of culture diameters of *C. tuberculata* colonies achieved in the presence of Cd^{2+}, Zn^{2+} and Pb^{2+} respectively 7.0 ± 1.23%, 12.2 ± 1.51% and 60.1 ± 3.42% of control samples diameter. Strain of *P. marquandii* manifested a higher level of tolerance to heavy metals than the examined fungi from genus *Curvularia* and was slightly inhibited only by cadmium and zinc (to 83.2 ± 2.22% and 86.0 ± 2.03% compared to the growth intensity of the control). The same mycelium of *C. lunata* cultured on the solid medium (in conditions preventing synhesis of the emulsifier) appeared to be more sensitive to the toxic effect of metal ions than mycelia of *C. tuberculata* and *P. marquandii*. Cd^{2+} presented in liquid medium in the concentration of 15 mM caused at around 78.6 ± 2.97% growth inhibition of *C. tuberculata* (Fig. 2b). For *P. marquandii* a

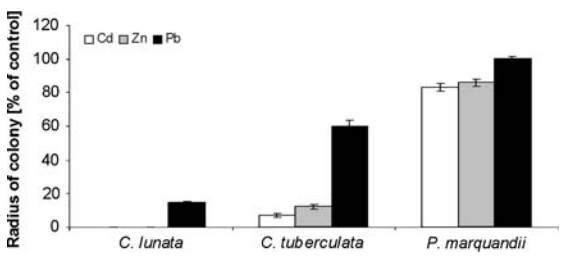

Fig. 1 The effect of Cd, Zn and Pb at concentration 10 mM on the growth of *C. lunata*, *C. tuberculata* and *P. marquandii* conducted on the solid PL-2 medium, monitored from the radius of 6-d-old colonies. Data are mean ± SD (n = 4)

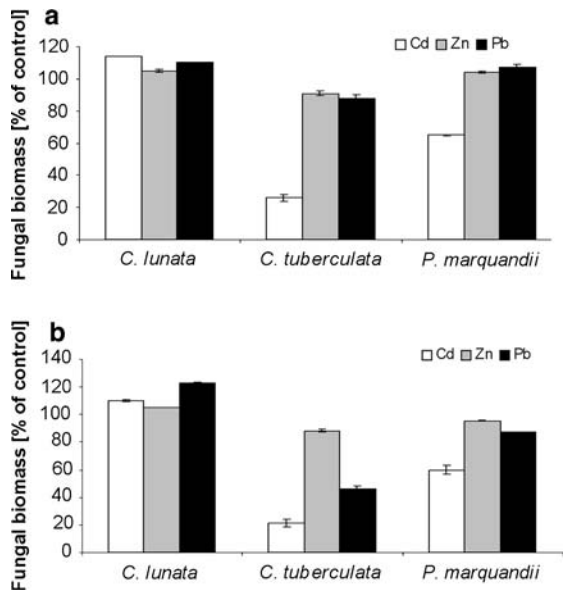

Fig. 2 The comparison of growth intensity of *C. lunata*, *C. tuberculata* and *P. marquandii* after a period of 48 h cultivation in liquid PL-2 medium at presence of (a) 10 and (b) 15 mM of cadmium, zinc or lead. Data are mean ± SD (n = 4)

40.0 ± 3.18% growth inhibition was observed in the presence of 15 mM of Cd^{2+} (Fig. 2b). In liquid medium no negative influence of Cd^{2+}, Zn^{2+},and Pb^{2+} on fungal growth of *C. lunata* was observed at the concentration of 10 as well as 15 mM of heavy metal ions (Fig. 2a, b). According to our observations, it was not connected with a possible protective role of melanin due to the absence of this pigment from examinated liquid cultures of *C. lunata*.

Interestingly, among the tested strains the highest ability to bind heavy metals was expressed by *C. lunata* and the lowest one by *P. marquandii*. Table 1 presents the results of cadmium, zinc and lead uptake by 48-h-old mycelia cultivated in liquid PL-2 medium, amended with 10 mM of heavy metals. In case of cadmium the tested fungi showed the biggest differences in the level of loaded metal. Mycelium of *C. lunata* accumulated 64.6 ± 1.39 mg of cadmium per g of weight, whereas *C. tuberculata* and *P. marquandii* only 20.6 ± 2.65 and 8.5 ± 0.74 mg Cd^{2+} per g of mycelium, respectively. However, the most effective uptake of zinc and lead by *P. marquandii* (206.2 and 324.5 mg of heavy metal per g of

Table 1 The capacity of heavy metals sorption of *C. lunata, C. tuberculata* and *P. marquandii* mycelia after 48 h of cultivation in liquid PL-2 medium amended with 10 mM of cadmium, zinc or lead. Data are mean ± SD (n = 4)

Fungal strain	Metal accumulation by mycelium [mg g^{-1}]		
	Cadmium	Zinc	Lead
C. lunata	64.62 ± 1.39	45.77 ± 1.50	169.57 ± 5.01
C. tuberculata	20.63 ± 2.65	41.40 ± 2.64	144.05 ± 5.29
P. marquandii	8.50 ± 0.74	14.89 ± 0.57	65.14 ± 1.59

mycelium, respectively) occurred in the absence of nutrient compounds (Słaba and Długoński 2004).

Additionally, microscopic inspections (Fig. 3a) showed that cadmium (similarly to the other metals) precipitated immediately and concentrated around the mycelia. In following hours of cultivation gradual decay of metal precipitants in liquid cultures was observed. As shown in Fig. 3b the 48 h growth of *C. lunata*, in the presence of cadmium (the most toxic tested metal) resulted in the formation of short filaments with numerous branches.

Microorganisms isolated from environments contaminated with heavy metals often evince a high level of tolerance as a result of adaptation to pollutants. Strain of *Trichoderma atroviride*, isolated from a sludge sample polluted with heavy metals was capable of surviving in liquid Sabouraud medium amended with zinc up to 750 mg l^{-1} (11.5 mM) and stopped growing at 300 mg l^{-1} (2.7 mM) of cadmium (López Errasquin and Vázquez 2003). The choice of the medium used in the presented study had probably some impact on neutralizing the toxic effect of heavy metals. Nevertheless, the results obtained by us indicate that *C. lunata* mycelium in the presence of emulsifier exhibits a very high tolerance to cadmium, zinc and lead as compared to data in literature.

It turns out from our study that cadmium occurred to be more toxic than zinc and lead. Similar findings were reported by Hatvani and Mécs (2003) who established for *Lentinula edodes* (*Basidiomycota*) the following sequence of decreasing toxicity: Cd^{2+} (0.0087), Zn^{2+} (0.62) and Pb^{2+} (2.7) mM causing 50% inhibition of mycelial growth. Fungal strains of *Heliscus lugdunensis* and *Verticillium* cf *alboatrum* exhibited a higher tolerance against zinc than against cadmium (Jaeckel *et al.* 2005). The studies of lead and cadmium tolerance of *Corollospora lacera* and *Monodictys pelagica* proved than Cd^{2+} has a stronger toxic effect than Pb^{2+} (Taboski et al. 2005).

Increased *C. lunata* mycelium tolerance to heavy metals which was observed in liquid medium in the presence of the emulsifier, was probably related to the limitation of the contact

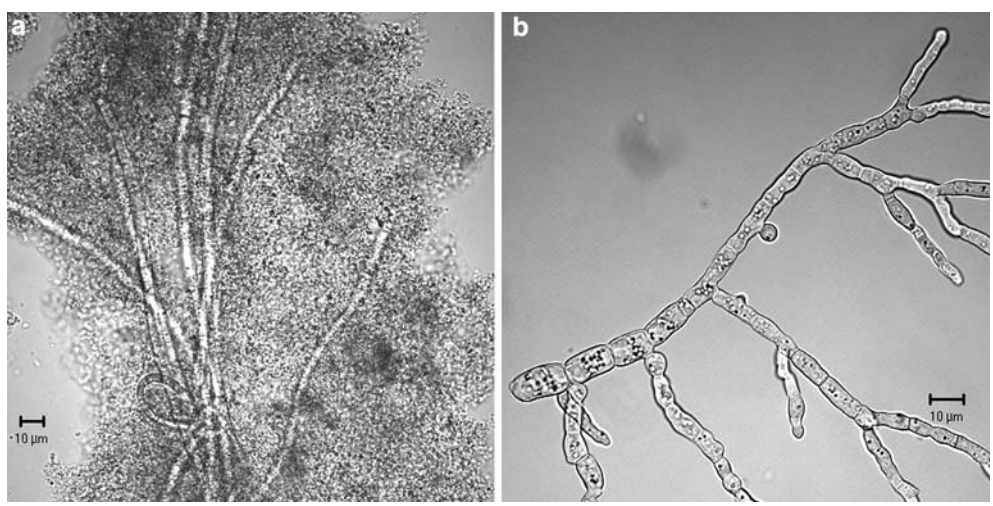

Fig. 3 Hyphae of *C. lunata* (a) directly after the inoculation to liquid medium PL-2 amended with 10 mM of cadmium and (b) after 48 h of cultivation in the presence of this metal ions

between toxic metals and the fungal cell surface as a result of metal ions uptake by exopolymer. This process may have been very significant during the first hours of mycelium cultivation. It should be stressed, however, that the liquid inoculation sample of *C. lunata* contained the emulsifier produced by the mycelium at the stage of second-step preculture incubation (during growth of inoculum). Based on the above results, it can be suggested that *C. lunata* emulsifier protects this fungus especially against the toxic effect of cadmium. It is worth mentioning that in specific ecological niches the ability of exopolymers synthesis may become a significant advantage increasing the chance of survival.

Production of *C. lunata* emulsifier under heavy metal stress

The synthesis of the emulsifier after first 24 h of *C. lunata* cultivation in PL-2 liquid medium was not inhibited either at the concentration of 10 or 15 mM of the heavy metals (Fig. 4). Moreover, the presence of Cd^{2+}, Zn^{2+} and Pb^{2+} increased the intensity of biopolymer synthesis. This effect, however, was not caused by the inhibition of biomass growth, but resulted from the increase in polymer concentration in culture medium. Lead at 10 and 15 mM of the initial used metal concentration increased intensity of emulsifier production from 0.15 ± 0.007 to 0.20 ± 0.007 and to 0.20 ± 0.014 mg of polymer per mg of mycelium respectivly. Maximum stimulation effect caused by cadmium (0.30 ± 0.007 mg of emulsifier per mg of mycelium) was observed when the metal ions were added to the medium in concentration of 10 mM. The most efficient stimulation of emulsifier production was achieved in the presence of Zn^{2+}. In the presence of 10 and 15 mM of zinc, the intensity of emulsifier secretion by *C. lunata* increased to 0.36 ± 0.021 and 0.40 ± 0.007 mg polymer per mg of mycelium, respectively.

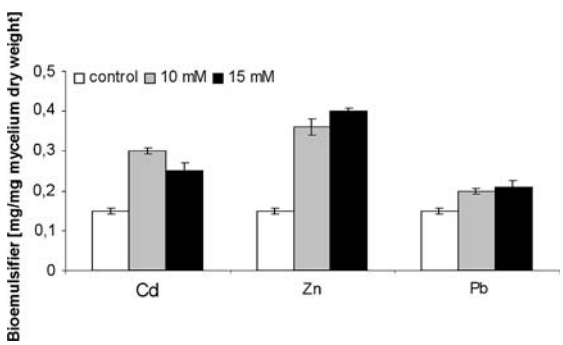

Fig. 4 Bioemulsifier production intensity exhibited by *C. lunata* after the period of 24 h of cultivation in liquid medium PL-2 amended with 10 and 15 mM of heavy metal. Data are mean ± SD (n = 4)

A similar stimulation by heavy metal is reported by Iyer et al. (2004) who observed that in the presence of chromium (VI) the strain of *Enterobater cloaceae* showed an enhanced growth of biomass as well as exopolysaccharide production. Metal-specific nature of exopolymer synthesis in *Nostoc spongiaeforme* (a cyjanobacterium) has been reported by Singh et al. (1999). The stimulation of this exopolysaccharide production occurred in the presence of Ni, Cu and Hg ions. Although increasing concentrations of copper in growth medium decreased the biomass of *S. paucimobilis* R40 as well as the amount of gellan, nevertheless, these concentrations caused the increase in the intensity of exopolymer production (Richau et al. 1997). Increased production of extracellular compounds in the presence of heavy metals as a response of yeast to stressed conditions have also been reported (Breierová et al. 2004; Soares et al. 2002).

Uptake of metals by emulsifier and mycelium of *C. lunata*

It has been established that the emulsifier of *C. lunata* can bind all heavy metals used in this experiment (Fig. 5). It has also occurred that the sorption level mainly depends on the type of heavy metal. The examined emulsifier binds Cd^{2+} and Zn^{2+} on a similar level and demonstrates a higher intensity of Pb^{2+} sorption. Exopolymer isolated from a 24-h-old culture, grown in the presence of 15 mM of heavy metal accumulated cadmium, zinc and lead with the intensity of 18.2 ± 2.67, 22.2 ± 3.40 and 51.1 ± 1.85 mg of these metals per gram of mycelium respectively.

Cell-bound polysaccharide from *Zooglea* spp. accumulated cadmium, iron, lead and chromium (Kong et al. 1998). Exopolysaccharide of marine

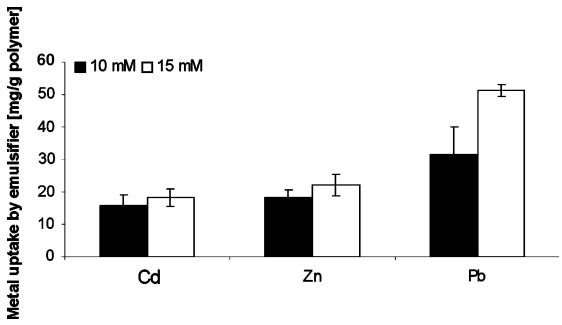

Fig. 5 Cd, Zn and Pb sorption capacity of *C. lunata* emulsifier isolated from a 24-h-old culture conducted in liquid PL-2 medium amended with 10 and 15 mM of heavy metal. Data are mean ± SD (n = 4)

bacterium *Enterobacter cloaceae* can act as cadmium, copper and hexavelant chromium adsorbent (Iyer et al. 2004). Salenizadeh and Shojaosadati (2003) reported on the efficient Pb^{2+}, Cu^{2+} and Zn^{2+} sorption by the acidic exopolysaccharide of *Bacillus firmus*. It should also be mentioned that microbial exopolymers differ widely both in the affinity to heavy metals and in their metal-binding capacity. For example, exopolysaccharide produced by *Alteromonas macleodii* subsp. *fijiensis* (bacterium isolated from a deep sea-hydrotermal field) binds Pb^{2+}, Cd^{2+} and Zn^{2+} with the uptake capacities of 316, 154 and 77 mg heavy metal g^{-1} of polymer, respectively (Loaëc et al. 1997). However, zooglan, heteropolysaccharide produced by *Zooglea ramigera* 115, binds Cd^{2+} with the intensity as low as 1.9 mg g^{-1} of polymer (Park et al. 1999).

It is widely accepted that carboxylic acids and amine groups are mainly responsible for the metal capacity of extracellular polymeric compounds. Nevertheless, complexes of metal ions with neutral carbohydrates (rich in hydroxyl groups) are also well documented (Angyal 1989). As reported in the previous paper (Paraszkiewicz et al. 2002), the emulsifier of *C. lunata* is a complex of protein and polysaccharide. Sugar component was identified as a polymer of D-glucose, and the amino-acids analysis demonstrated a relatively high content of Asp and Glu (11 and 16.7% respectively). We assume that the presence of these amino acids may be responsible for the anionic character of *C. lunata* emulsifier, and, by the same token, for the affinity of this polymer to heavy metal cations. For emulsifier secreted by the studied strain of *C. lunata*, lead exhibits greater affinity than cadmium or zinc. These results are in good agreement with Angyal (1989) who postulated that anionic polysaccharides prefer to bind cations of large ionic radii. The Pb^{2+} ions are in aqueous solution, much higher (112 pm) than Cd^{2+} and Zn^{2+} (97 pm and 74 pm, respectively).

As shown in Fig. 6, the uptake of heavy metal ions by 24-h-old growing mycelium of *C. lunata* depends on the kind and initial concentration of the heavy metal in the medium. The metal-binding capacity of *C. lunata* biomass was observed to follow the order Pb > Cd > Zn. The maximum binding intensity of Pb^{2+}, Cd^{2+} and Zn^{2+} was calculated for biomass cultured in the presence of 15 mM of the heavy metal. In this configuration the uptake of Pb^{2+}, Cd^{2+} and Zn^{2+} was found to be 230.0 ± 28.47, 156.1 ± 10.32 and 95.2 ± 14.21 mg g^{-1} of mycelium.

The aforementioned results of heavy metals depend on the characteristics of the microorganism applied, the medium and the metal concentration. Fungi were shown to bind up to 190 mg of cadmium, 206 mg of zinc and 350 mg of lead per 1 g of mycelium (Wainwright and Gadd 1997; Słaba and Długoński 2004; Taboski et al. 2005; Voleski and Holan 1995). When compared to data in literature, the sorption level achieved by *C. lunata* mycelium shows good biosorption qualities.

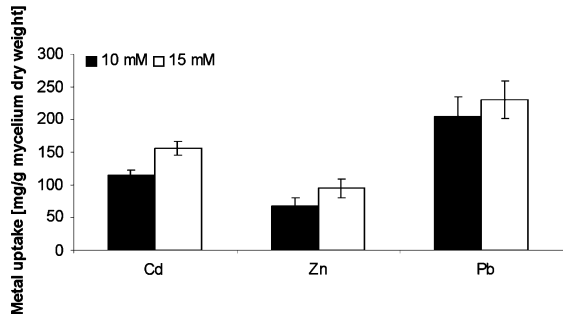

Fig. 6 Concentrations of Cd, Zn and Pb in *C. lunata* mycelium after 24 h of cultivation in liquid PL-2 medium amended with 10 and 15 mM of heavy metal. Data are mean ± SD (n = 4)

Conclusion

The present observation allows suggesting that the emulsifier of *C. lunata* acts as a protective substance probably by cadmium, zinc and lead capturing (especially during the first hours of contact of growing mycelium with heavy metals). Results shown above also indicate that the exopolymer *of C. lunata* is produced with a higher intensity as a response to the toxicity of heavy metal ions. Our results add new information on the effects of heavy metals as environmental stress factors, on mechanisms involved in fungal emulsifier synthesis. Although the microbial exopolymers presence is recognized as a nonspecific mechanism of tolerance to heavy metals, but some microorganisms (such as studied by us strain of *C. lunata*) in nutrient-rich conditions may control the intensity of these compounds production. The data obtained in this study revealed also the potential of *C. lunata* biomass for the removal of Cd^{2+}, Zn^{2+} and Pb^{2+} from aqueos solutions.

Acknowledgement This study was supported by the University of Lodz, grant (No. 505/390)

References

Angyal SJ (1989) Complexes of metal cations with carbohydrates in solution. Ad Carbohydr Chem Biochem 47:1–43

Breierová E, Gregor T, Juršíková N, Stratilová E, Fišera M (2004) The role of pullulan and pectin in the uptake of Cd^{+2} and Ni^{+2} ions by *Aureobasidium pullulans*. Ann Microbiol 54:247–255

Collins DO, Reese PB (2001) Biotransformation of cedrol by *Curvularia lunata* ATTCC 12017. Phytochemistry 56:417–421

Fernandes P, Cruz A, Angelova B, Pinheiro HM, Cabral JM (2003) Microbial conversion of steroid compounds: recent developments. Enzyme Microb Technol 32:688–705

Fogarty RV, Tobin JM (1996) Fungal melanins and their interactions with metals. Enzyme Microb Technol 19:311–317

Freire–Nordi CS, Vieira AAH, Nascimento OR (2005) The metal binding capacity of *Anabaena spiroides* extracellular polysaccharide: an EPR study. Process Biochem 40:2215–2224

Gadd GM (2001) Metal transformation. In: Gadd G (eds) Fungi in bioremediation. Cambridge University Press, Cambridge, pp 359–382

Gadd GM (2004) Microbial influence on metal mobility and application for bioremediation. Geoderma 122:109–119

Gharieb MM (2001) Pattern of cadmium accumulation adn essential cations during growth of cadmium-tolerant fungi. BioMetals 14:143–151

Gutnick DL, Bach H (2000) Engineering bacterial biopolymers for the biosorption of heavy metals; new products and novel formulations. Appl Microbiol Biotechnol 54:451–460

Hatvani N, Mécs I (2003) Effects of certain heavy metals on the growth, dye decolorization, and enzyme activity of *Lentinula edodes*. Ecotoxicol Environ Saf 55:199–203

Iyer A, Mody K, Iha B (2004) Accumulation of hexavalent chromium by an exopolysaccharide producing marine *Enterobacter cloaceae*. Mar Pollut Bull 49:974–977

Jaeckel P, Krauss GJ, Krauss G (2005) Cadmium and zinc response of the fungi *Heliscus lugdunensis* and *Verticillium* cf *alboatrum* isolated from highly polluted water. Sci Tot Environ 346:274–279

Jarosz-Wilkołazka A, Grąż M, Braha B, Menge S, Schlosser D, Krauss GJ (2006) Species-specific Cd-stress response in the white rot basidiomycetes *Abortiporus biennis* and *Cerrena unicolor*. BioMetals 19:39–49

Kanwal A, Paraszkiewicz K, Długoński J (2001) Transformation of *Curvularia lunata* IM 2901 with pAN7-1 influences selected physiological properties of the fungus. Microbios 104:27–38

Kingsley MT, Bohlool BB (1992) Extracellular polysaccharide is not responsible for aluminium tolerance of *Rhizobium leguminosarum* bv. *Phaseoli* CIAT899. Appl Environ Microbiol 58:1095–1101

Kong JY, Lee HW, Hong JW, Kang YS, Kim JD, Chang MW, Bae SK (1998) Utilization of a cell-bound polysaccharide produced by marine bacterium *Zoogloea* sp. New biomaterial for metal adsorption and enzyme immobilization. J Mar Biotechnol 6:99–103

Ledin M (2000) Accumulation of metals by microorganisms – processes and importance for soil systems. Earth-Sci Rev 51:1–31

Loaëc M, Olier R, Guezennec J (1997) Uptake of lead cadmium and zinc by a novel bacterial exopolysaccharide. Water Res 31:1171–1179

López Errasquin E, Vázquez C (2003) Tolerance and uptake of heavy metals by *Trichoderma atroviride* isolated from sludge. Chemosphere 50:137–143

Malik A (2004) Metal bioremediation through growing cells. Environ Int 30:261–278

Nies DH (1999) Microbial heavy-metal resistance. Appl Microbiol Biotechnol 51:730–750

Paraszkiewicz K, Długoński J (1998) Cortexolone 11β-hydroxylation in protoplasts of *Curvularia lunata*. J Biotechnol 65:217–224

Paraszkiewicz K, Kanwal A, Długoński J (2002) Emulsifier production by steroid transforming filamentous fungus *Curvularia lunata* Growth and product characterization. J Biotechnol 92:287–294

Park JK, Jin YB, Chen JH (1999) Reusable biosorbents in capsules from *Zooglea ramigera* cells for cadmium removal. Biotechnol Bioeng 63:116–121

Richau JA, Choquenet D, Fialho AM, Moreira LM, Sá-Correia I (1997) The biosynthesis of the exopolysaccharide gellan results in the decrease of *Sphingomonas paucimobilis* tolerance to copper. Enzyme Microb Technol 20:510–515

Rinaldi MG, Phillips P, Schwartz JG, Winn RE, Holt GR, Shagets FW,Elrod J, Nishioka G, Aufdemorte TB (1987) Human *Curvularia* infections Report of five cases and review of the literature. Diag Microbiol Infect Dis 6:27–39

Safdar A (2003) *Curvularia* – favorable response to oral itraconazole therapy in two patients with locally invasive phaeohyphomycosis. Clin Microbiol Infect 9:1219–1223

Salehizadeh H, Shojaosadati SA (2003) Removal of metal ions from aqueus solution by polysaccharide produced from *Bacillus firmus*. Water Res 37:4231–4235

Singh N, Asthana RK, Kayastha AM, Pandey S, Chaudhary AK, Singh SP(1999) Thiol and exopolysaccharide production in a cyanobacterium under heavy metal stress. Process Biochem 35:63–68

Słaba M, Bizukojć M, Pałecz B, Długoński J (2005) Kinetic study of the toxicity of zinc and lead ions to the heavy metals accumulating fungus *Paecilomyces marquandii*. Bioprocess Biosyst Eng 28:185–197

Słaba M, Długoński J (2004) Zinc and lead uptake by mycelium and regenerating protoplasts of *Verticillium marquandii*. World J Microbiol Biotechnol 20:323–328

Soares EV, Duarte APRS, Boeventura RA, Soares HMVM (2002) Viability and release of complexing compounds during accumulation of heavy metals by brewer's yeast. Appl Microbiol Biotechnol 58:836–841

Taboski MAS, Rand TG, Piórko A (2005) Lead and cadmium uptake in the marine fungi *Corollospora lacera* and *Monodictys pelagica*. FEMS Microbiol Ecol 53:445–453

Volesky B, Holan ZR (1995) Biosorption of heavy metals. Biotechnol Prog 11:235–248

Wainwright M, Gadd GM (1997) Fungi and Industrial Pollution. In: Wicklow DT, Söderström BE (eds) The Mycota, IV, Environmental and microbial relationships. Springer–Verlag, Berlin Heidelberg, pp 85–97

Wilmańska D, Milczarek K, Rumijowska A, Bartnicka K, Sedlaczek L (1992) Elimination of by-products in 11β-hydroxylation of Substance S using *Curvularia lunata* clones regenerated from NTG-treated protoplasts. Appl Microbiol Biotechnol 37:626–630

Zhang D, Duine JA, Kawai F (2002) The extremely high Al Resistance of *Penicillium janthineleum* F-13 is not caused by internal or external sequestration of Al. BioMetals 15:167–174