TOLERANCE AND BIOACCUMULATION OF LEAD BY FUNGAL ENDOPHYTES

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Introduction. Lead (Pb) is one of the most common toxic elements found in polluted soils. Bioremediation is an option to recover these soils, through the decrease in the metal toxicity, either by biological transformation or by influencing its bioavailability. Microorganisms transform metals by changing their oxidation state, and also accumulate them. Root-associated fungi can mobilize, incorporate or transform Pb to either translocate it towards phytoextracting plants, or immobilize it leading to plant protection (1). A type of plant-associated fungi, are those that cause asymptomatic infections in plant tissues, which are called endophytic. Many endophytic fungi have been shown to be highly tolerant and can sequester significant amounts of metals, also promoting the plant growth in polluted soils (2). A variety of mechanisms, both active and incidental, contribute to their metal tolerance (3). Since the endophyte-assisted phytoremediation has been documented as a promising technology for remediation of metal-polluted soils (2), it is important to seek tolerant fungal species able to associate with phytoremediating plants.

The aim of this study was to characterize the tolerance and Pb bioaccumulation ability by three species of endophytic fungi.

Methods. Three fungal endophytes (*Penicillium* sp. (*Pen*), *Cladosporium* sp. (*Cla*) and *Aspergillus* sp. (*Asp*)) were isolated from surface-sterilized roots of shrubs growing in a Pb-polluted soil. Fungi were grown (x3) in Petri dishes with Czapek agar added with 40 g/L of sucrose (C/N = 36) and Pb (0, 1, 2 and 3 g Pb/L). Strains were grown for 10-14 days at 25°C. Tolerance index (TI) was estimated as the ratio of the radial growth rate (RGR) in media + Pb to the RGR in controls. As a measure of tolerance, we estimated the half inhibitory concentration (IC_{50}). Pb in fungal biomass was quantified by atomic absorption spectrometry and the concentration was used to estimate the bioconcentration factor (BCF), as the ratio of Pb in biomass to Pb in media. Data were compared by ANOVA and a Duncan test ($P<0.05$).

Results. The strain *Pen* was the more tolerant to Pb up to 2000 mg/L (Fig. 1). Although ITs for *Asp* and *Cla* decreased with increasing Pb concentrations, both strains showed IC_{50} values higher than *Pen*, exceeding the range of Pb levels tested (Table 1). Then, based on the TI values, the strain more tolerant to Pb was *Cla*, since registered a decrease of only 26% regarding the control, when it grew with 3000 mg/L. The genus *Cladosporium* is a dark septate endophyte (DSE), whose high metal-tolerance and beneficial effects on plant growth have been demonstrated (4).

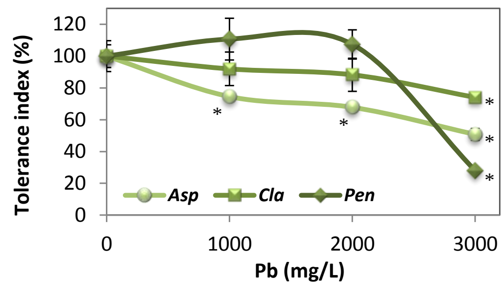

Fig. 1. Tolerance index to Pb for endophytic fungi of genera *Aspergillus* (*Asp*), *Cladosporium* (*Cla*) and *Penicillium* (*Pen*). Significant differences regarding to controls are indicated with an asterisk (n = 3).

The highest Pb bioaccumulation capacity was found for *Asp* and *Cla*, reaching BCFs as high as 76 and 68, respectively, when grown with 3000 mg Pb/L (Table 1). In contrast, *Pen* showed a low bioaccumulation ability, attaining a maximum BCF of 22. Typically, the higher the BCF, the greater the accumulating ability (5).

Table 1. IC_{50} values and Pb-bioconcentration factors (BCF) for *Aspergillus* sp. (*Asp*), *Cladosporium* sp. (*Cla*) and *Penicillium* sp. (*Pen*)*

Strain	IC_{50} (mg Pb/L)	BCF		
		1 g Pb/L	*2 g Pb/L*	*3 g Pb/L*
Asp	3045	18 ± 1 [c]	37 ± 4 [b]	76 ± 11 [a]
Cla	4695	26 ± 2 [b]	19 ± 5 [b]	68 ± 23 [a]
Pen	2655	16 ± 5 [b]	25 ± 4 [a]	22 ± 4 [a]

* Different letter by row indicates significant differences.

Conclusions. Pb tolerance by the studied strains was found in the following order: *Cla* > *Asp* > *Pen*. Pb bioaccumulation ability was found as follows: *Asp* > *Cla* > *Pen*. The strains *Cla* and *Asp* have great potential for use in DSE-assisted phytoremediation.

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