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Diversity and heavy metal tolerance of endophytic fungi from six dominant plant species in a Pb–Zn mine wasteland in China

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ABSTRACT

The diversity and metal tolerance of endophytic fungi from six dominant plant species in a Pb–Zn mine wasteland in Yunnan, China were investigated. Four hundred and ninety-five endophytic fungi were isolated from 690 tissue segments. The endophytic fungal colonization extent and isolation extent ranged from 59 % to 75 %, and 0.42–0.93, respectively, and a positive correlation was detected between them. Stems harboured more endophytic fungi than leaves in each plant species, and the average colonization extent of stems was 82 %, being significantly higher than that of leaves (47 %) ($P \leq 0.001$, chi-square test). The fungi were identified to 20 taxa in which *Phoma*, *Alternaria* and *Peyronellaea* were the dominant genera and the relative frequencies of them were 39.6 %, 19.0 % and 20.4 %, respectively. Metal tolerance test showed that 3.6 mM Pb^{2+} or 11.5 mM Zn^{2+} exhibited the greatest toxicity to some isolates and they did not grow on the metal-amended media. In contrast, some isolates were growth stimulated in the presence of tested metals. The isolates of *Phoma* were more sensitive to Zn^{2+} than the isolates of *Alternaria* and *Peyronellaea*. However, the sensitivity of isolates to Pb^{2+} was not significantly different among *Phoma*, *Alternaria*, *Peyronellaea* and other taxa ($P > 0.05$, chi-square test). Our results suggested that fungal endophyte colonization in Pb–Zn polluted plants is moderately abundant and some isolates have a marked adaptation to Pb^{2+} and Zn^{2+} metals, which has a potential application in phytoremediation in this area.

Introduction

Contamination of soils by heavy metals, resulting from anthropogenic activities such as ore mining and smelting, is a serious problem in many areas around the world (Gadd 1993). The remediation of soils contaminated with HM is gaining considerable momentum. Phytoremediation is an emerging and ecologically benign technology for decontamination of soils. However, most plants including hyperaccumulators are not suitable for phytoremediation owing to their small biomass and slow growth in heavy metals contaminated soils (Prasad *et al.* 2010). The potential for metal phytoextraction is highly restricted and it is necessary to develop some strategies to promote plant growth and increase their biomass.

Fungi causing asymptomatic infections in living plant tissues have been called endophytic fungi (Arnold *et al.* 2000). They are ubiquitous and comprise a diverse group of fungi. It has been reported that some endophytes could promote host plant growth in HM contaminated soils (Monnet *et al.* 2001; Sun *et al.* 2010; Zhang *et al.* 2010). However, the diversity and ecological roles of endophytic fungi in Pb–Zn contaminated ecosystems are almost unknown (Zhang *et al.* 2008; Guo *et al.* 2010; Xiao *et al.* 2010).

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Huize Pb–Zn mine, located in the Northeast of Yunnan Province, Southwest China, is an important Pb–Zn source in China. More than 300 yr continuous mine exploitation has resulted in large-scale and serious soil HM pollution. In the present study, we investigated the endophytic fungal diversity and then Pb–Zn tolerance of 6 dominant plant species grown in a wasteland of Huize Pb–Zn mine, in which the concentration of Pb and Zn in the soils ranged between 2 620–8 384 and 42 340–61 260 mg kg^{-1}, and in the plant shoots/leaves were between 100–2 023 and 158–5 632 mg kg^{-1}, respectively (Zu *et al.* 2005; Liang *et al.* 2007; Fang & Cao 2009). Our results will not only give a clearer insight into the endophytic fungal diversity in Pb–Zn contaminated environments, but also can supply Pb–Zn tolerant isolates for phytoremediation in this area.

Materials and methods

Sampling site and plants

The Huize Pb–Zn mine (103°03′–103°55′E, 25°48′–27°04′N) is located in the Northeast of Yunnan Province, Southwest China. The sampling site was a Pb–Zn mine wasteland about 2 km^2 located in Zhehai town, Huize County. The vegetation in the sampling site was very scarce containing only a little dwarf shrub and herb growth.

Six dominant plant species *Arabis hirsuta*, *Acacia decurrens*, *Symplocos paniculata*, *Rabbosia eriocalyx*, *Arenaria serpyllifolia* and *Rosa longicuspis* were selected for investigation. Ten healthy plants of each plant species were collected randomly and taken, in sterile polythene bags, to the laboratory and processed within 24 hr, in Apr. 2009.

Fungal endophyte isolation, culture and identification

For isolation of endophytic fungi, 20 healthy leaves and 20 healthy stem segments were selected from each plant species at random, washed in running tap water and processed as follows: samples were cut into segments (about 5 × 5 mm) and surface-sterilized by sequentially dipping into 0.5 % sodium hypochlorite (2 min) and 70 % ethanol (2 min) (Arnold *et al.* 2000). Then, 66 leaf segments and 66 stem segments of each plant species were placed on a Petri dish containing PDA (potato dextrose agar; Guangzhou Huankai Microbiol Sci & Tech. Co. Ltd., Luogang, Guangzhou) medium amended with 0.5 g l^{-1} streptomycin sulphate. Plates were incubated at 25 °C and checked every other day for 30 d. Fungi growing out of the plant tissues were then transferred to other PDA plates.

Endophytic fungal identification was based on morphology, the mechanism of spore production and spore characteristics (some isolates were further identified based on their rDNA ITS sequence analysis, not shown in the present paper) (Barnett & Hunter 1987; Ellis 1988). Sterile isolates were identified as sterile mycelia and sorted into different groups on the basis of colony surface texture, hyphal pigmentation and growth rates. All isolates were stored in the Faculty of Life Sciences and Technology, Kunming University of Science and Technology.

Metal tolerance

The endophytic fungi were inoculated into 100 ml PDB (Potato Dextrose Broth, pH 5.1–5.3; Guangzhou Huankai Microbiol Sci & Tech. Co. Ltd., Luogang, Guangzhou), in which the concentration of Pb^{2+} or Zn^{2+} was 3.6 or 11.5 mM achieved by adding $Pb(NO_3)_2$ or $ZnSO_4$. PDB without Pb^{2+} or Zn^{2+} was used for controls. Cultures were incubated at 28 °C for 7 d, 150 rpm. Then, the fermentation broths were filtered and the mycelia were washed 3 times with pure water and dried at 80 °C for 10 hr until reaching constant weight. Finally, the dry weights of fungal biomass were measured. The experiments were carried out in triplicate.

Data analyses

Colonization extent (CE) was calculated as the total number of segments colonized by endophytic fungi divided by the total number of segments incubated for that plant sample, and expressed as percentage. Isolation extent (IE) was calculated as the number of isolates obtained from segments divided by the total number of segments, but not expressed as percentage. Relative frequency of isolation was calculated as the number of isolates of one species divided by the total number of isolates, and also expressed as a percentage (Su *et al.* 2010; Yuan *et al.* 2010).

Metal tolerance results were expressed in terms of a tolerance index (TI) based on the dry weights of fungal biomass (DW): TI_{DW} = (DW of treated mycelium/DW of control mycelium) × 100 (%) (Fomina *et al.* 2005). SPSS 13.0 was used for statistical analysis. The chi-square test was used to compare the difference in CE of endophytes between stem and leaf.

Table 1 – Number, colonization extent (CE) and isolation extent (IE) of endophytic fungi (EF) isolated from 6 dominant plant species in Huize Pb–Zn mine wasteland

Host plant	No. of segments plated (No. of segments colonized by EF)			No. of EF isolated			CR% (IE)		
	Leaf	Stem	Total	Leaf	Stem	Total	Leaf	Stem	Total
Arabis hirsuta	60 (39)	54 (37)	114 (76)	35	37	72	65 (0.58)	69 (0.69)	67 (0.63)
Acacia decurrens	36 (6)	54 (53)	90 (59)	5	70	75	17 (0.14)	98 (1.30)	66 (0.83)
Symplocos paniculata	60 (30)	66 (65)	126 (95)	19	86	105	50 (0.32)	98 (1.30)	75 (0.83)
Rabbosia eriocalyx	54 (24)	66 (64)	120 (88)	23	89	112	44 (0.43)	97 (1.35)	73 (0.93)
Arenaria serpyllifolia	66 (29)	66 (49)	132 (78)	24	62	86	44 (0.36)	74 (0.94)	59 (0.65)
Rosa longicuspis	54 (27)	54 (27)	108 (54)	21	24	45	50 (0.39)	50 (0.44)	50 (0.42)
Total	330 (155)	360 (295)	690 (450)	127	368	495	47 (0.38)	82 (1.02)	65 (0.72)