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REGULAR ARTICLE

Inoculation of seed-borne fungus in the rhizosphere of *Festuca arundinacea* promotes hydrocarbon removal and pyrene accumulation in roots

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Abstract
Background and aims The selective inoculation of specific hydrocarbon-degrading microbes into the plant rhizosphere offers a useful means for remediating hydrocarbon-contaminated soils. The effect of inoculating a seed-borne filamentous fungus (*Lewia* sp.) on hydrocarbon removal by *Festuca arundinacea* and its growth was studied on perlite (model soil) and soil, both spiked with hydrocarbons.
Methods A hydrocarbon mixture (1,500 mg kg^{-1}) of two polycyclic aromatic hydrocarbons (PAH), phenanthrene and pyrene, blended with hexadecane (1.0:0.5:0.5 weight) was used. Greenhouse experiments were carried out for 45 days. Inoculated and non-inoculated plants were grown in dark cylindrical glass pots containing perlite or soil.
Results Inoculation with *Lewia* sp. stimulated (100 %) root growth in spiked perlite. Inoculated plants showed higher phenanthrene removal (100 %) compared to non-inoculated plants in perlite and soil. Pyrene removal by inoculated plants was 37-fold higher than that by non-inoculated plants in perlite; in soil, pyrene removal by inoculated plants (97.9 %) differed significantly from that of non-inoculated plants (91.4 %). Accumulation of pyrene in roots (530.9 mg kg^{-1} of dry roots) was promoted in perlite.
Conclusions Our results demonstrate that *Lewia* sp. (endophytic fungus) improved the efficiency of PAH removal by *F. arundinacea*, on both perlite and soil, stimulating pyrene accumulation in roots.

Keywords Seed-borne filamentous fungus · Endophytic fungus · *Lewia* sp. · PAH · *Festuca arundinacea*

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Introduction

Polycyclic aromatic hydrocarbons (PAH) composed of two or more condensed aromatic rings of carbon and hydrogen atoms, are chemically stable at room temperature, and due to their hydrophobic structures, are almost or totally insoluble in water and are highly lipophilic (Gan et al. 2009). PAH are found in the environment, soil included, as a result of the incomplete combustion of organic matter, motor vehicle, stationary matter (e.g. coal-fired, electricity generating power plants), domestic matter (e.g. tobacco smoke and residential wood or coal combustion), area source

matter (e.g. forest fires and agricultural burning), waste incinerator, oil refining, asphalt production, and aluminium production (Sudip et al. 2002; Srogi 2007). Some PAH have been found to be carcinogenic, cytotoxic, or ecotoxic (Srogi 2007) and are persistent organic pollutants. Soils contaminated with PAH pose potential risks to human and ecological health (Sudip et al. 2002). Recently, interest in developing *in situ* remediation strategies for environmental contaminants with PAH has increased (Gan et al. 2009; Ndimele 2010). A variety of plants, including the grass family, have been considered for contaminated soil phytoremediation purposes and are an economic and effective technology for abating contaminants in soil (Chiapusio et al. 2007). Phytoremediation involves the use of plants and their associated microbes for environmental clean-up (Pilon-Smits 2005). There are three possible mechanisms for the phytoremediation of organic pollutants: (1) phytovolatilization, the evaporation of absorbed volatile compounds through plant leaves, (2) phytodegradation, the accumulation and subsequent metabolism of contaminants by plants after uptake by roots, (3) phytostimulation or rhizodegradation, the degradation or transformation of contaminants in soil by the microbial community induced by root exudates (Pilon-Smits 2005; Lazcano et al. 2010). A variety of rhizosphere microorganisms have been investigated for the degradation of petroleum hydrocarbons. Many bacteria have been discovered that degrade PAH and diverse fungi capable of utilizing PAH have been also investigated. For example, Cerniglia and Sutherland (2010) reported fungi able to metabolize PAH with enzymes that include peroxidase, laccase and hydrolase. Current phytoremediation research suggests that endophytic fungi will play an increasingly important role (Schardl et al. 2004; Mohsenzadeh et al. 2010). Endophytes are defined as microbes that colonize the living internal tissues of a plant without causing any immediate and obvious negative effect (Tarkka et al. 2008). These fungi benefit from the association through the provision of nutrients by root exudates, whereas the plants benefit through enhanced nutrient uptake and the reduced toxicity of soil contaminants (Chaudhry et al. 2005). Further developments in this area are expected to focus on ways of providing conditions that promote plant growth in the soil, and accelerate the remediation of hydrocarbon-contaminated soil through selective inoculation with specific hydrocarbon-degrading microbes in the plant rhizosphere. This study was conducted to evaluate the effect of *Lewia* sp. (a seed-borne filamentous fungus) as an inoculum on hydrocarbon removal and accumulation by *Festuca arundinacea*. Since soil is a complex system made up of a heterogeneous mixture of solid, liquid, and gaseous components, matrices such as perlite and peat moss (Escalante-Espinosa et al. 2005) or glass beads (Acevedo et al. 2011) have been used in addition to soil in order to understand the role of rhizosphere microorganisms. In this work, perlite and soil spiked with hydrocarbons were used.

Materials and methods

Plant material

Seeds of tall fescue (*Festuca arundinacea* Schreb.) were obtained from a wholesale food market in Mexico City and kept at room temperature. The seeds were surface-sterilized. Damaged and small seeds were removed by hand and selected seeds were placed into a filter paper envelope, submerged into a commercial powder soap solution (60 g soap L^{-1} water) for 20 min with constant agitation, and rinsed with tap water for 10 min. The envelope containing the seeds was then submerged in 70 % (v/v) ethanol for 30 s in a laminar flow hood, followed by immersion in 10 % (v/v) sodium hypochlorite containing 0.1 mL of Tween-20 for 25 min, and finally in 70 % (v/v) ethanol for 1 min with constant agitation. The envelope was washed three times with 100 mL of deionized sterile water. The envelope was opened under aseptic conditions and surface-sterilized seeds (disinfested) were stored in sterile empty Petri dishes for subsequent use.

Fungus

Isolation and preparation of inoculant

The fungal strain used was isolated from surface-sterilized commercial seeds of *F. arundinacea*. This fungus was propagated on Petri dishes containing potato dextrose agar (30°C, 10 days) and maintained at 4°C. Several fungal disks (5 mm) were aseptically

punched out from the Petri dishes. Individual disks were transferred to Petri dishes and Erlenmeyer flasks containing 25 and 50 mL of medium, respectively. The medium used for plant aseptic cultures was also used for fungal culture, and comprised 4.4 gL^{-1} Murashige and Skoog (MS), including micro- and macro-nutrient stock solutions (Murashige and Skoog 1962) and 30 g L^{-1} sucrose. pH was adjusted with 0.1 N NaOH to 5.8 and the mixture was autoclaved (15 min at 1.2 kg cm^{-2}). Two g L^{-1} of Phytagel (agar substitute, Sigma Chemical, St. Louis, MO, USA) was added to the medium destined for Petri dishes. After incubation (14 days, 30°C), one fungal disk was used as inoculum in phytopathogenic assays.

Finally, the pellets that formed in the Erlenmeyer flasks after 10 days incubation at 200 rpm, 30°C, were disrupted with glass beads to achieve a homogeneous cell suspension. The cell suspensions were mixed in equal volumes with an isotonic solution and used as the inoculum for hydrocarbon removal experiments.

Molecular identification

The isolated fungus was grown in liquid medium (50 mL): sucrose, 40; $NaNO_3$, 3; yeast extract, 2; KCl, 0.5; $MgSO_4 \cdot 7H_2O$, 0.5; $FeSO_4 \cdot 7H_2O$, 0.1 (g L^{-1}) at 30°C, 200 rpm for 48 h. Fresh mycelium was collected and disrupted with liquid nitrogen. Fungal DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega) according to the manufacturer's instructions. PCR amplification was carried out using ITS5 and ITS4b primers for 30 cycles of 94°C for 45 s; 55°C for 1 min; and 72°C for 45 s. ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′) and ITS4b (5′-TCCTCCGCTTATTGATATGC-3′) are primers for conserved sequences of fungal 18S rDNA and 28S rDNA, respectively (Gardes and Bruns 1993; Larena et al. 1999). The amplified product thus included a partial sequence of the 18S rDNA gene, the internal transcribed spacer 1 (ITS1), the complete 5.8S rDNA gene, the internal transcribed spacer 2 (ITS2), and a partial sequence of the 28s rDNA gene. The PCR reaction was separated on a 1.5 % (w/v) agarose gel and the expected product of 600–650 base pairs was excised from the gel and purified using a QIAquick Gel Extraction Kit (Qiagen). The nucleotide sequence of the product was determined using AmpliTaq DNA Polymerase FS and the ABIPrism® BigDye™ Terminator Cycle Sequencing Ready Reaction Kit on an ABIPRISM 3100 system (Applied Biosystems). The nucleotide sequence of the amplified product was analysed using the BLAST (NCBI) programme (http://www.ncbi.nlm.nih.gov/BLAST).

Model soil

Perlite (Dicalite, México) was used to simulate soil. Perlite is an amorphous volcanic glass that is used as a soil amendment or as a hydroponics medium. The perlite was sieved through 4.76-mm and 1.19-mm meshes. The remaining fraction was washed with hot tap water, air-dried, and stored at room temperature for further use.

Soil sample

A non-contaminated soil sample (250 kg) was obtained from Hueypoxtla, in Mexico State (19°54′ 10.6″N, 99°05′16.0″W). The soil sample was collected from a depth of 0 to 20 cm, transported to our laboratory and immediately air-dried and passed through a 2 mm sieve to remove stones and roots. The original particle size distribution (54.8 % sand, 25.2 % silt, and 24.6 % clay) allowed us to identify the soil sample as a sandy-clay-loam soil. The organic matter content was 4.2 % and the pH was 7.53.

Hydrocarbons

Hexadecane (HXD), phenanthrene (PHE) (purity >96 %), and pyrene (PYR) (purity >98 %) were purchased from Sigma-Aldrich Chemie (Steinheim, Germany). The substances were dissolved in acetone (Mallinckrodt Baker, Edo. Mex., Mexico). The blend ratio of polycyclic aromatic hydrocarbons (PAH) was: PHE and PYR (1:1; w/w). HXD and the PAH blend (1:1; w/w) were combined to form the hydrocarbon mixture (HM).

Artificially spiked soil and model soil

Perlite and soil were artificially contaminated with acetone-dissolved HM up to a concentration of 1,500 mg kg^{-1}. Excess acetone was air-evaporated off; the spiked soils were placed in dark plastic bags and stored at room temperature for 6 weeks. The initial

concentrations of HXD, PHE and PYR in the treated soils were measured before transfer to experimental pots. Control soil, without HM, was treated in the same way.

Seed germination and phytopathogenic assays

These experiments were performed under aseptic conditions. MS medium (10 mL), 10 g L^{-1} sucrose and 2 g L^{-1} Phytagel (agar substitute, Sigma Chemical, St. Louis, MO, USA) were added to culture tubes (diameter 2.5 cm, height 15 cm), prior to sterilizing for 15 min at 1.2 kg cm^{-2}. One disinfested seed was aseptically placed into each culture tube. The culture tube was closed with a plastic cap and sealed with Parafilm. Sealed culture tubes were kept at 25±2°C, with a 16-h photoperiod. All seeds were cultivated during 20 days and then fungus-free seedlings were inoculated with the isolated fungus, under aseptic conditions; one fungal disk (5 mm) was placed into each culture tube with one seedling. After 60 days, the phytopathogenic response was evaluated as positive or negative. It was negative when inoculated plants were green and healthy. Fungal growth within the roots tissues was evaluated. The plants were harvested, separated into roots and shoots, and the roots were surface-sterilized to eliminate remaining fungal coloniser from the root surface. Under a laminar flow hood, roots were submerged into 70 % ethanol for 30 s, 10 % sodium hypochlorite for 10 min, 70 % ethanol for 30 s, and washed with sterile water. The surface-sterilized roots were cut with sterile scalpel (5 mm length) and cultivated on Petri dishes containing potato dextrose agar (30°C, 10 days). Hyphae colonisation was periodically observed using visual methods with Zoom Stereo Microscope (Olympus SZ61).

Hydrocarbon removal

The effect of the filamentous fungus on hydrocarbon degradation by *F. arundinacea* was evaluated in dark cylindrical glass pots containing spiked perlite (40 g) or soil (400 g). After 30 days of seed germination under aseptic conditions, six or three *F. arundinacea* seedlings were transplanted to each pot of perlite or soil, respectively. Four sets of treatments (three pots per treatment) were assayed simultaneously: (1) plants inoculated with the fungus, (2) non-inoculated plants, (3) an unplanted control with only the fungus, and (4) a control without fungus or plants. Fungal inoculation (mycelium suspension; 1 mL) of planted and unplanted perlite and soil was performed after 20 days (this was considered the start of the removal process). A modified Long-Ashton solution was added as a nutrient solution twice a week (in g L^{-1}): KNO_3, 808; $Ca(NO_3)_2{\cdot}4H_2O$, 944; $NaH_2PO_4{\cdot}H2O$, 184; and $MgSO_4{\cdot}7H_2O$, 368; 1 mL of oligoelement solution was also added. The oligoelement solution comprised (in mg L^{-1}): $MnSO_4{\cdot}4H_2O$, 2.23; $CuSO_4{\cdot}5H_2O$, 0.25; $ZnSO_4{\cdot}7H_2O$, 0.29; H_3BO_3, 3.10; NaCl, 5.90; $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 0.088; $FeSO_4{\cdot}7H_2O$, 0.02. Pots were maintained at 23–28°C, with a 16-h photoperiod. After 45 days, the plants were harvested, separated into roots and shoots, and the roots were washed to remove perlite or soil. Root and shoot length was measured using a graduated 30-cm transparent plastic ruler. Total root and shoot dry weights were determined upon reaching a constant weight after drying at 60°C. Perlite and soil were carefully collected from each pot, air-dried and homogenized. Plant and soil samples were stored at room temperature for further analysis.

Analytical methods

Spiked soil and model soil hydrocarbon extraction

Residual hydrocarbons were extracted using a MARS-Solvent Extraction Microwave. Samples of dry perlite or soil (5 g dry weight) were mixed with 30 mL of dichloromethane:acetone (1:1) in MARS vessels. Perlite and soil samples were extracted at 175–200 psig and 150°C for 30 min. The extraction efficiencies of the method were: 87.6 %±8.4 for HXD, 96.7 %±3.2 for PHE, and 99.6 %±2.2 for PYR. The average recovery from the samples was 82.2 %±9.6 for HXD, 82.0 %±10.2 for PHE, and 84.6 %±9.5 for PYR in perlite, and 60.2 %±3.6 for HXD, 75.8 %±5.6 for PHE, and 84.8 %±2.2 for PYR in soil, 40 days after spiking.

Plant hydrocarbon extraction

The root and shoot samples were ground in liquid nitrogen using a mortar. Shoots were treated with 30 mL of KOH (1 N) in methanol: H_2O (80:20; v/v) for 30 min at 60°C in order to saponify the

chlorophylls. Saponification allows a good elimination of the interfering chlorophylls from the extracts containing the PAH (Dugay et al. 2002). The hydrocarbons were extracted with dichloromethane (DCM) (30 mL). Each sample was filtered and reduced to 10 mL by evaporation for further chromatographic analyses.

Hydrocarbon quantification

Hydrocarbons were quantified using a Varian 3900 gas chromatograph, flame ionization detector (FID), and AT-1HT (15 m, 0.25 mm i.d. × 0.10 μm film thickness) column; helium was the carrier gas, and the gas flow rate was 2.0 mL min^{-1}. Aliquots of 2 μL were injected in splitless mode. Injection was performed using a Varian CP-8410 auto injector. The initial oven temperature was 100°C (hold for 2 min). Temperature was increased to 200°C at 20°C min^{-1} with a final holding time of 1 min. The injector and transfer lines were heated to 300°C.

Statistical analysis

A completely randomized factorial design was implemented with and without fungus with three replicates. An analysis of variance (one-way ANOVA) was conducted for all treatments using the NCSS programme 2007. Statistical differences among individual treatments were assessed using the Tukey-Kramer test (α=0.05).

Results

Molecular identification

Fungal identification was based on nucleotide sequence analysis of enzymatically amplified ITS rDNA, after comparison with deposited fungal sequences through a standard nucleotide BLAST homology search. The strain was found to be similar to the genus *Lewia* (EF432279; 100 % similarity in 555 bp). Therefore, the filamentous fungus is hereafter named *Lewia* sp. ACH-4. The nucleotide sequence was registered in the GenBank nucleotide database (accession number GU296022).

Phytopathogenic assays and plant growth

Pathogenic responses derived from *Lewia* sp. were evaluated after 60 days of culture in aseptic MS medium (Fig. 1). It should be noted that we successfully used the same plant medium (MS) to grow *Lewia* sp. *F. arundinacea* showed no pathogenic symptoms. In contrast, *Lewia* sp. had a positive effect on plant growth: healthy shoot and tiller numbers were enhanced, as shown in Fig. 1a. Figure 1c shows, morphologically changes produced by the presence of *Lewia* sp. Root thickness was higher than that of non-inoculated roots, as shown in Fig. 1d. In addition, Fig. 2 shows a clear, hyphae growth within roots tissues. Since *F. arundinacea* showed no pathogenic responses derived from *Lewia* sp. under aseptic conditions, we decided to evaluate the effect of *Lewia* sp. inoculation on plant growth, using different hydrocarbon-contaminated matrices. Plant growth was evaluated after 45 days of culture in either contaminated perlite or soil (Figs. 3 and 4). In contaminated perlite,

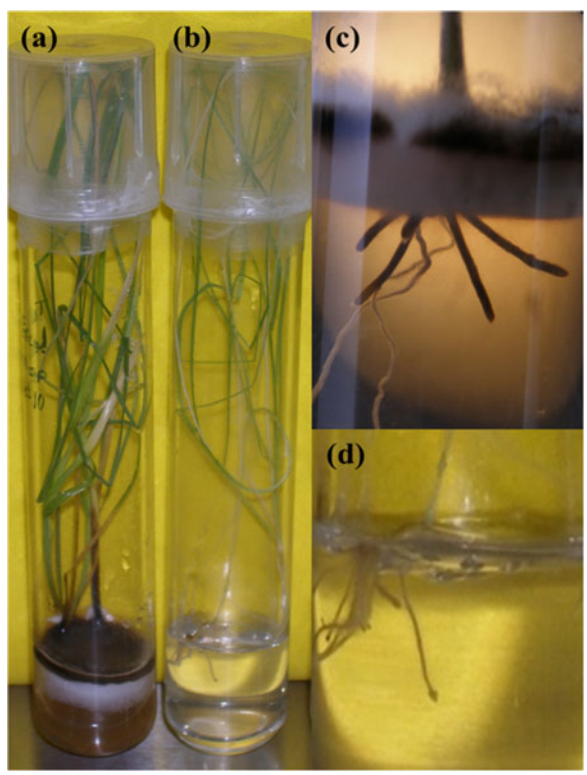

Fig. 1 *F. arundinacea* growing on MS medium: **a** inoculated, **b** non-inoculated with *Lewia* sp. Roots in MS medium: **c** inoculated, **d** non-inoculated with *Lewia* sp. after 60 days of culture, in all cases

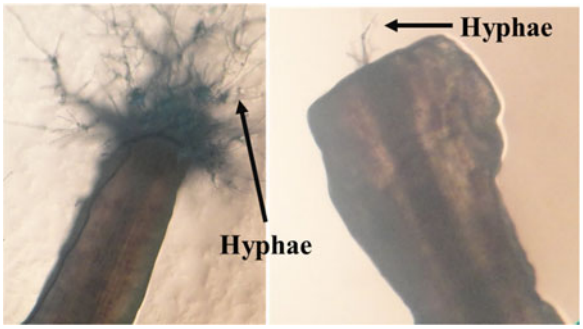

Fig. 2 *Lewia* sp. colonisation within the tissues of *F. arundinacea* roots, after 60 days of culture, under aseptic conditions

the plants produced shoots with a 31 % reduction in length compared to the control (without HM). In contaminated soil, there were no significant differences in shoot length due to the HM. The presence of *Lewia* sp. had no significant effect on the shoot length of plants grown in either perlite or soil (Fig. 3). Root and shoot biomass was lower in contaminated perlite or soil, for both inoculated and non-inoculated plants. *Lewia* sp. clearly stimulated root biomass production when *F. arundinacea* was grown in contaminated perlite (Fig. 4).

Hydrocarbon removal and accumulation

The efficiency of removal of HXD, PHE, and PYR by inoculated and non-inoculated *F. arundinacea* grown in perlite or soil after 45 days of culture is shown in Table 1. Inoculated plants showed higher PHE removal compared to non-inoculated plants in perlite and soil. In soil, PYR removal by inoculated *F. arundinacea* (97.9 %) was also significantly higher than that by non-inoculated plants (91.4 %). In perlite, PYR removal by inoculated *F. arundinacea* was 37-fold higher than that by non-inoculated plants. Root accumulation of PYR was only detected in *F. arundinacea* inoculated with *Lewia* sp. Shoot accumulation of PYR and PHE was not detected in either inoculated or non-inoculated plants grown in soil.

Discussion

The filamentous fungus isolated from surface-sterilized *F. arundinacea* seeds was found to be similar to the genus *Lewia.* Kwasna and Kiosak (2003) isolated other species of the genus *Lewia* from surface-sterilized oat seeds, identified as *L. avenicola*. Similarly, Kwasna et al. (2006) isolated *L. hordeicola* from barley seeds. The use of *Lewia* strains for phytoremediation purposes can be questioned because *Alternaria* sp. is the anamorph of *Lewia* sp. and *Alternaria* includes many species of plant pathogens (Kwasna et al. 2006). Nevertheless, recent studies have described a non-phytopathogenic *Lewia* species, *L. eureka*, isolated from the stem tissue of *Theobroma*

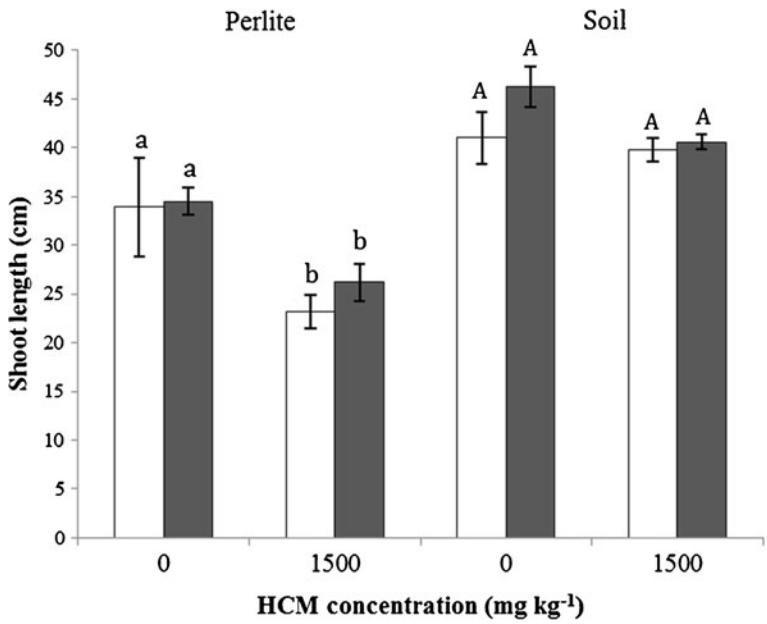

Fig. 3 Shoot length of *F. arundinacea* after 45 days of growth in contaminated perlite and soil with or without inoculation with *Lewia* sp. Different *letters* (*uppercase* soil and *lowercase* perlite) represent significant differences according to the Tukey-Kramer test (α=0.05). Mean of three replicates ± SD. *Open columns* non-inoculated and *filled columns* inoculated

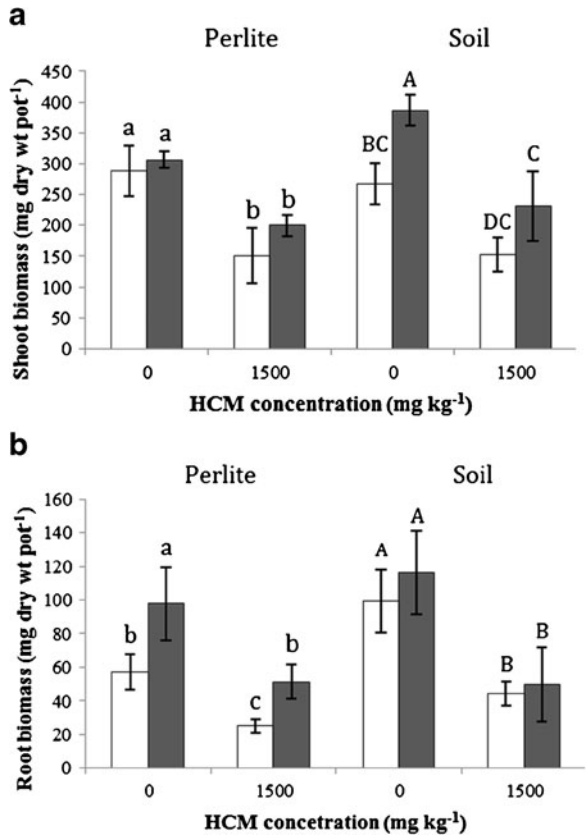

Fig. 4 Shoot (**a**) and root (**b**) biomass of *F. arundinacea* after 45 days of growth in contaminated perlite and soil with or without inoculation with *Lewia* sp. Different *letters* (*uppercase* soil and *lowercase* perlite) represent significant differences according to the Tukey-Kramer test (α=0.05). Mean of three replicates ± SD. *Open columns* non-inoculated and *filled columns* inoculated

gileri and characterized as a fungal endophyte (Thomas et al. 2008).

F. arundinacea is tolerant to petroleum hydrocarbons, as demonstrated by its frequent occurrence on polluted road-sides (Gawronski and Gawrosnska 2007). The genus *Festuca* belongs to the *Poaceae* family, one of the most important plant families for phytoremediation of organic compounds (Gawronski and Gawrosnska 2007; Cheema et al. 2009; Soleimani et al. 2010). This family and certain fungal genera show evidence of establishing endophytic associations. According to Lugtenberg et al. (2002), and

Table 1 Hexadecane (HXD), phenanthrene (PHE) and pyrene (PYR) removal by *F. arundinacea,* with or without inoculation with *Lewia* sp., after 45 days of culture in perlite and soil

	Perlite			Soil		
	Removal (%)			Removal (%)		
	PYR	PHE	HXD	PYR	PHE	HXD
Control	2.7±2.4 b	80.1±3.4 b	76.0±1.2 b	66.8±3.7 C	66.0±0.6 D	65.6±3.1 B
Lewia sp.	9.5±5.6 b	82.2±4.0 b	78.8±1.3 b	69.9±1.2 C	73.7±0.2 C	60.4±7.8 B
F. arundinacea	1.9±0.7 b	85.2±4.1 b	74.5±6.8 ba	91.4±1.2 B	91.9±0.1 B	94.2±1.4 A
F. arundinacea - *Lewia* sp.	70.3±7.2 a	100±0.0 a	91.7±6.9 a	97.9±0.8 A	99.9±0.1 A	95.1±0.8 A
Initial mass (mg pot^{-1})	12.7±1.4	12.3±1.5	24.6±2.8	124.8±3.9	111.6±8.9	176.4±8.0

Different *letters* (*uppercase* soil and *lowercase* perlite assays) represent significant differences among treatments: *Lewia* sp.; *F. arundinacea* and *F. arundinacea* and *Lewia* sp., and control (perlite or soil) for each hydrocarbon. The means were compared at a significance level of α=0.05. Mean of three replicates ± SD

based on their effects on the plant, *Lewia* sp. that interacts with *F. arundinacea* can be considered as beneficial microorganism. Commonly, endophytic fungi are vertically transmitted via host seeds in most mutualistic associations (Schardl et al. 2004). In our work, *Lewia* sp. was isolated from previously surface-sterilized seeds and was probably vertically transmitted via *F. arundinacea* seeds. However, endophytic microbes may also be parasitic, *i.e.* detrimental to their hosts (Paparu et al. 2007). For this reason, our first goal was to rule out any pathogenic responses derived from *Lewia* sp. in *F. arundinacea*. As shown in Fig. 1, *Lewia* sp. had positive effects; in contrast, plant growth increased in the presence of *Lewia* sp. compared with non-inoculated plants (Fig. 1). *Lewia sp.* established an endophytic interaction with *F. arundinacea* (Fig. 2). It was transmitted via *F. arundinacea* seeds and consequently it had a positive effect on plant growth. Therefore, it is not surprising that *Lewia* sp. had a significant effect on the growth of both roots and shoots under aseptic conditions. Similar results were observed when *F. arundinacea* was grown on spiked perlite; root biomass production was higher in inoculated compared to non-inoculated plants (Fig. 4). These results may be attributed to the high porosity (>80 %) of perlite (Martínez et al. 2006), which provides the appropriate aeration and moisture for fungal growth. The positive effect on plant growth detected in the presence of *Lewia* sp. was not observed when *F. arundinacea* was grown on contaminated soil, suggesting possible competition with native microbes.

Plant–microbe interactions in the rhizosphere offer a useful means for remediating hydrocarbon-contaminated soils (Cheema et al. 2009; Mohsenzadeh et al. 2010). Certain plant roots release substances that are nutrients for microorganisms like bacteria and fungi (Sinha et al. 2007). In some cases, the degradation process can be promoted by rhizospheric microorganisms (Kuiper et al. 2004). In the present study, a mycelial suspension was inoculated in the rhizosphere of *F. arundinacea* in order to promote hydrocarbon removal. *F. arundinacea* inoculated with *Lewia* sp. was able to remove all of the initial PHE from both perlite and soil (Table 1). Our results are consistent with those reported by Soleimani et al. (2010), who found that *Neotyphodium coenophialum*-infected *F. arundinacea* was able to remove all of the initial PHE from aged soil. In our work, the removal of PYR, one of the most potent carcinogenic PAH, by *F. arundinacea* was significantly improved by inoculation with *Lewia* sp. in perlite. Beneficial microorganisms are often used as inoculants in the phytoremediation of organic contaminants (rhizoremediators). Most environmental contaminants with organic compounds can be degraded by beneficial rhizoremediators (Lugtenberg et al. 2002). Our results suggest that *Lewia* sp. can be considered as a beneficial rhizoremediator, by stimulating hydrocarbon uptake. Plant uptake is one of the steps in the phytoremediation of soil contaminated with organic compounds. Information about the final plant PAH concentration is essential to predict the effectiveness of a phytoremediation operation (Gao and Ling 2006). Recent studies have

Table 2 Final concentration of hexadecane (HXD), phenanthrene (PHE) and pyrene (PYR) in root and shoot, with or without inoculation with *Lewia* sp., after 45 days of culture in perlite and soil

	Perlite			Soil		
	PYR mg kg^{-1}	PHE	HXD	PYR mg kg^{-1}	PHE	HXD
Roots	n.d.	n.d.	n.d.	694.7±246	n.d.	1,603.4±84.7
Roots (inoculated)	318±162	n.d.	490±300	1,603.4±336	n.d.	1,404.3±56
Shoots	n.d.	n.d.	445±11	n.d.	n.d.	309±103
Shoots (inoculated)	20.7±16.9	n.d.	358.8±26	n.d.	n.d.	136.5±79
Initial conc. *mg kg^{-1}	357.8±27	328.5±38.5	639±96.2	317.9±8.0	284.2±20.9	451.7±26.9

n.d. not detected

mg hydrocarbons kg^{-1} dry tissue, roots or shoots

*mg hydrocarbons kg^{-1} dry support, perlite or soil

The values are means of three replicates ± CI

demonstrated that *F. arundinacea* is able to accumulate and degrade PAH by using phytodegradation mechanism (Su et al. 2008; Reynoso-Cuevas et al. 2011). However, it is not yet clear how interactions between plants and specific microorganisms stimulate the same phytoremediation mechanisms. In our opinion, the simplest way to elucidate such an interesting association would be to investigate the final fate of each of the assayed hydrocarbons. According to Reynoso-Cuevas et al. (2011), after 90 days of growth in MS medium under aseptic conditions, *F. arundinacea* was able to accumulate 46.7 and 15.95 mg PYR (kg dry tissue)$^{-1}$ in roots and shoots, respectively. In the present study, after 45 days of culture in spiked perlite, the root accumulation of PYR was 6.8-fold higher in *F. arundinacea* inoculated with *Lewia* sp. than reported by Reynoso-Cuevas et al. (2011). When soil was used as the growth medium, there were no significant differences in the concentration of PYR in roots of inoculated and non-inoculated plants. This difference could be explained by two different features: (i) the presence of native microorganisms in the soil that may be involved in the degradation of hydrocarbons and (ii) soil organic matter that plays a crucial role in the fate and transport of many organic contaminants like hydrocarbons (Karthikeyan and Kulakow 2003). Su et al. (2008) reported that PYR was not detected in the shoots of *F. arundinacea* after 8 weeks of growth in aged contaminated soil. In this work, PYR was not detected in shoots when *F. arundinacea* was grown in inoculated or non-inoculated soil (Table 2). Our results suggest that the transport of PYR from roots to shoots was restricted to the interaction between *F. arundinacea–Lewia* sp., these results suggest that our technological approach in the laboratory could also be applied in the field, because the absence of translocation of contaminants from the roots to the shoots means that toxic organic contaminants are not transferred into the food chain (Gao and Ling 2006). As shown in this study, *Lewia* sp. inoculation in the rhizosphere promotes the accumulation of pyrene in roots and hydrocarbon removal by *F. arundinacea* in contaminated perlite and soils.

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