Journal of Hazardous Materials 186 (2011) 1568–1575

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Soil type affects plant colonization, activity and catabolic gene expression of inoculated bacterial strains during phytoremediation of diesel

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ARTICLE INFO

Article history:
Received 3 November 2010
Accepted 9 December 2010
Available online 15 December 2010

Keywords:
Soil type
Pseudomonas
Pantoea
alkB
CYP153
GENE expression

ABSTRACT

The combined use of plants and associated microorganisms has great potential for cleaning up soils contaminated with petroleum hydrocarbons. Apart from environmental conditions the physicochemical properties of the soil are the main factors influencing the survival and activity of an inoculated strain as well as the growth of plants. This study examined the effect of different soil types (sandy, loamy sand and loam) on the survival, gene abundance and catabolic gene expression of two inoculated strains (*Pseudomonas* sp. strain ITRI53 and *Pantoea* sp. strain BTRH79) in the rhizosphere and shoot interior of Italian ryegrass vegetated in diesel contaminated soils. High colonization, gene abundance and expression in loamy soils were observed. By contrast, low colonization, gene abundance and absence of gene expression in sandy soil were found. The highest levels of genes expression and hydrocarbon degradation were seen in loamy soil that had been inoculated with BTRH79 and were significantly higher compared to those in other soils. A positive correlation was observed between gene expression and hydrocarbon degradation indicating that catabolic gene expression is necessary for contaminant degradation. These results suggest that soil type influences the bacterial colonization and microbial activities and subsequently the efficiency of contaminant degradation.

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1. Introduction

Petroleum hydrocarbons are frequently occurring pollutants and an increasing number of sites is seriously polluted by these contaminants world-wide [1,2]. Phytoremediation is a promising technology for the removal of pollutants from contaminated environments. It employs plants and their associated microorganisms to degrade, transform, assimilate, metabolize, or detoxify hazardous pollutants from soils [3–5]. Although the use of plants alone in bioremediation was successful in some cases [6–8], the combined use of plants and biodegradative and/or plant growth-promoting bacteria is particularly promising for the decontamination of polluted soils [9–11].

The degradation of toxic organic compounds in soil by plant-associated bacteria can be mediated by endophytic and/or rhizosphere bacteria. Endophytic bacteria colonize in the internal tissues of plants and do not confer pathogenic effects to their host [12]. They have been reported to have a high potential to promote plant growth and contribute to enhanced biodegradation of pollutants [13–15]. Rhizosphere bacteria colonize the root environment, where root exudates act as substrate for microorganisms supporting also the degradation of organic contaminants [16,17]. Many plant-associated bacteria have the ability to deaminate 1-amino-cyclopropane-1-carboxylic acid (ACC), a precursor of ethylene in plants, which is particularly produced under stress conditions. Bacteria exhibiting ACC deaminase activity have the potential to promote plant growth and alleviate plant stress such as that induced by the presence of toxic contaminants [18].

For efficient phytoremediation of organic soil contaminants, the soil has to provide the appropriate environment for both, the plant and the associated microorganisms. Soil properties including texture, organic matter content, pH, particle size, cation exchange capacity and structure have been shown to affect not only plant growth and microbial colonization [19–21], but also the degradation of organic contaminants [22–24]. Moreover, the bioavailability of hydrocarbons may be influenced by soil properties [25–27]. Hydrocarbons strongly bind to humic substances and clay minerals [28]. However, it has been also shown that sandy soils may bind hydrocarbons by adsorption despite the absence of silty material or significant amounts of organic matters. This was explained by high microporosity [29].

In bioremediation studies, the quantitative analysis of functional genes such as alkane hydroxylase genes has provided a

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doi:10.1016/j.jhazmat.2010.12.040

Table 1
Physicochemical properties of three different types of soil (standard soils from LUFA, Speyer, Germany).

Parameters	Soil type (according to USDA)		
	Sand (LUFA 2.1)	Loamy sand (LUFA 2.2)	Loam (LUFA 2.4)
Organic carbon (%)	0.74 ± 0.14	2.09 ± 0.40	2.99
Particles < 0.02 mm (%)	7.9 ± 1.1	13.5 ± 1.1	51.5
pH-value (0.01 M $CaCl_2$)	5.1 ± 0.5	5.5 ± 0.1	7.2
Cation exchange capacity (meq/100 g)	4.0 ± 1.0	10.0 ± 0.5	33.6
Particle sizes according to USDA (%)			
<0.002 mm	2.9 ± 0.8	6.4 ± 0.9	27.2
0.002–0.05 mm	9.1 ± 1.4	11.6 ± 0.7	40.6
0.05–2.0 mm	88.0 ± 1.0	82.0 ± 0.7	32.2
Water holding capacity (g/100 g)	31.8 ± 3.0	46.5 ± 6.0	45.7
Weight per volume (g/100 ml)	1430 ± 57	1220 ± 78	1310

Mean values of different batch analysis according to good laboratory practice (GLP) ± standard deviation. All values refer to dry matter. Soil was collected, analyzed and characterized by LUFA Speyer, Germany.

valuable tool for studying the relationship between specific microbial populations or strains and the performance of degradation processes [30–32]. The abundance of alkane degrading genes has been assessed in the soil and plant interior [33–35], but the activity of alkane degrading bacteria in situ and under natural conditions by e.g. gene expression has been rarely addressed [31,36]. However, for efficient degradation not only the abundance of plant-associated bacteria and/or applied inoculants strains but also the degrading activity is highly important [37]. Characteristics of the soil environment such as soil organic matter or particle sizes may influence the colonization process but may also have pronounced influence on the expression of degrading genes. Therefore, the objective of this study was to determine the effect of the soil type and the associated properties on the performance of hydrocarbon degrading inoculants strains.

2. Materials and methods

2.1. Soils

The physicochemical properties of the three soils used for this study are presented in Table 1. Standard soils were purchased from LUFA Speyer, Germany, that were characterized as sand (LUFA 2.1), loamy sand (LUFA 2.2) and loam (LUFA 2.4) according to USDA. Apart from their differences in particle size, these soils showed also different values in pH, organic carbon, and cation exchange capacity. They were used directly without any addition of macro- or micronutrients.

2.2. Bacterial strains

Two bacterial strains, which were previously isolated from the endosphere and rhizosphere of Italian ryegrass and birdsfoot trefoil [38], were used in this study. These included *Pseudomonas* sp. strain, ITRI53 (a root endophyte isolated from Italian ryegrass carrying an alkane monooxygenase (*alkB*) gene), and *Pantoea* sp. strain BTRH79 (a rhizosphere strain isolated from birdsfoot trefoil carrying a cytochrome P450 alkane hydroxylase gene (CYP153)). Both strains have the capacity to degrade alkanes [38], while only the *Pantoea* sp. strain shows ACC deaminase activity. Strains were cultivated in 10% Luria Bertani broth amended with 1% (v/v) filter-sterilized diesel at 30 °C. Cells were harvested by centrifugation and resuspended in sterile 0.9% NaCl solution.

2.3. Plant experiment

For the plant experiment Magenta boxes were filled with 300 g soil and sterilized by 30 kGy-rediation. Before sowing, the soil was amended with 1% (v/v) filter-sterilized diesel and then mixed with 50 ml inoculant suspension (app. 10^{10} cfu/ml) containing either strain ITRI53, strain BTRH79 or sterile 0.9% NaCl solution. Control treatments with and without the amendment of diesel but without

Table 2
Effect of soil type on seed germination (SG) and shoot length (SL).

Soil type/treatment	SG (%)	SL (cm)		
		1st harvest	2nd harvest	3rd harvest
Sandy soil				
Control (vegetated) (-diesel)	81^{d} (3.6)	9^{cd} (0.7)	17^{d} (0.6)	20^{e} (0.7)
Control (vegetated)	65^{f} (2.3)	7^{e} (0.8)	12^{f} (0.4)	14^{g} (0.8)
Pseudomonas sp. ITRI53	68^{ef} (2.6)	7^{e} (0.6)	13^{ef} (0.3)	15^{g} (0.5)
Pantoea sp. BTRH79	72^{e} (3.3)	8de (0.5)	14^{e} (0.2)	17^{f} (0.3)
Loamy sand soil				
Control (vegetated) (-diesel)	89^{ab} (3.9)	12^{ab} (0.3)	25^{ab} (0.4)	30^{b} (0.8)
Control (vegetated)	78^{d} (2.9)	8de (0.2)	17^{d} (0.7)	21^{e} (0.6)
Pseudomonas sp. ITRI53	83^{cd} (3.3)	10^{bc} (0.4)	20^{d} (0.5)	26^{d} (0.4)
Pantoea sp. BTRH79	87^{bc} (2.6)	11^{b} (0.6)	23^{c} (0.8)	28^{c} (0.9)
Loamy soil				
Control (vegetated) (-diesel)	94^{a} (2.3)	13^{a} (0.7)	26^{a} (0.4)	32^{a} (0.6)
Control (vegetated)	81^{d} (2.9)	9^{cd} (0.5)	18^{d} (0.5)	21^{e} (0.4)
Pseudomonas sp. ITRI53	89^{ab} (2.6)	11^{b} (0.4)	23^{c} (0.9)	27^{d} (0.5)
Pantoea sp. BTRH79	91^{ab} (2.9)	12^{ab} (0.3)	25^{ab} (0.7)	30^{b} (0.3)

1st harvest, 2nd harvest and 3rd harvest were after one week, four weeks and eight weeks of seed germination, respectively. Each value is the mean of three replicates, means in the same column followed by the same letter are not significantly different at a 5% level of significance, the standard error of three replicate is presented in parentheses.

Table 3
Effect of soil type on shoot and root dry weight (DW).

Soil type/treatment	1st harvest		2nd harvest		3rd harvest	
	Shoot DW (g)	Root DW (g)	Shoot DW (g)	Root DW (g)	Shoot DW (g)	Root DW (g)
Sandy soil						
Control (vegetated) (-diesel)	1.9^{f} (0.14)	ND	2.2^{g} (0.12)	0.8^{f} (0.07)	2.8^{h} (0.21)	1.0^{f} (0.08)
Control (vegetated)	1.4^{g} (0.08)	ND	1.6^{h} (0.10)	0.6^{f} (0.05)	2.0^{i} (0.16)	0.7^{g} (0.03)
Pseudomonas sp. ITRI53	1.5^{g} (0.12)	ND	1.7^{h} (0.12)	0.6^{f} (0.09)	2.2^{i} (0.14)	0.8^{fg} (0.02)
Pantoea sp. BTRH79	1.6^{g} (0.13)	ND	1.8^{h} (0.15)	0.7^{f} (0.10)	2.4^{hi} (0.18)	0.9^{fg} (0.05)
Loamy sandy soil						
Control (vegetated) (-diesel)	2.9^{c} (0.18)	ND	6.8^{b} (0.23)	2.0^{bc} (0.13)	9.1^{c} (0.45)	3.1^{bc} (0.24)
Control (vegetated)	2.1^{f} (0.13)	ND	4.6^{f} (0.31)	1.4^{e} (0.08)	6.5^{g} (0.37)	2.3^{e} (0.14)
Pseudomonas sp. ITRI53	2.4^{e} (0.17)	ND	5.8^{d} (0.34)	1.6^{de} (0.06)	8.2^{e} (0.28)	2.6^{d} (0.19)
Pantoea sp. BTRH79	2.7^{cd} (0.23)	ND	6.4^{c} (0.36)	1.8^{cd} (0.12)	8.9^{cd} (0.31)	2.9^{c} (0.16)
Loamy soil						
Control (vegetated) (-diesel)	3.5^{a} (0.24)	ND	7.3^{a} (0.35)	2.7^{a} (0.16)	10.1^{a} (0.42)	3.5^{a} (0.23)
Control (vegetated)	2.6^{de} (0.16)	ND	5.2^{e} (0.26)	1.8^{cd} (0.11)	7.8^{f} (0.27)	2.5^{de} (0.16)
Pseudomonas sp. ITRI53	2.8^{cd} (0.13)	ND	6.0^{d} (0.30)	2.1^{bc} (0.13)	8.7^{d} (0.34)	2.9^{c} (0.15)
Pantoea sp. BTRH79	3.2^{b} (0.29)	ND	6.9^{b} (0.34)	2.4^{b} (0.14)	9.6^{b} (0.37)	3.3^{ab} (0.11)

1st harvest, 2nd harvest and 3rd harvest were after one week, four weeks and eight weeks of seed germination, respectively. Each value is the mean of three replicates, means in the same column followed by the same letter are not significantly different at a 5% level of significance, the standard error of three replicate is presented in parentheses. ND, not determined

bacterial inoculation were included. One hundred seeds of Italian ryegrass (*Lolium multiflorum*) were surface-sterilized in a 5% (v/v) NaOCl for 10 min, washed five times with sterilized water and were sown in each box and each treatment was triplicated. Plants were grown at 25 °C in a sterile environment and subjected to a cycle of 16 h light and 8 h dark for 2 months. Plants were watered with sterile distilled water when needed.

2.4. Sampling and extraction of DNA and RNA

After 1 week, 2 weeks and 8 weeks, shoots were cut from 2 cm above soil and weighed. The remaining plants were harvested to obtain root and rhizosphere samples. Rhizosphere soil was collected by gently sampling the soil closely attached to the root surface. The roots were washed, dried and weighed. The remaining soil was mixed and stored at −80 °C for total hydrocarbon analysis. Shoots were surface-sterilized as described earlier [39]. Sterility was checked by plating on Tryptic Soy Agar plates (TSA, Merck).

DNA from rhizosphere was extracted by using the FastDNA Spin Kit for soil (Qbiogene), whereas RNA was isolated with the FastRNA Pro Soil-Direct Kit (MP Biochemicals) as described by the manufacturers. Shoots were briefly ground in liquid N_2 and microbial cells were lysed by beat-beating [40]. For isolation of DNA and RNA the DNeasy Plant Mini Kit and RNeasy Plant Mini Kit (Qiagen) were used. In RNA preparations genomic DNA was eliminated by DNase I enzyme (Ambion) digestion and the potential presence of contaminating DNA was checked by PCR amplification of 16S rDNA [39].

2.5. Quantification of inoculant strains by cultivation

Surface-sterilized shoots (1 g) were cut into small pieces. Rhizosphere soil as well as shoot were resuspended in 2 ml of 0.9% (w/v) NaCl solution and shaken at 180 rpm for 30 min. After plant and soil particles were settled, the aqueous phase (100 μl) of 10^{-3} dilutions were plated on 10% TSA in duplicates and incubated at 30 °C for two days to determine CFU/g dry soil or plant material. Ten colonies were randomly picked and the identity of isolates with the inoculant strain was confirmed by restriction fragment length polymorphism (RFLP) analysis of the 16S–23S rRNA intergenic spacer region (IGS) [41]. Similarly, *alkB* and CYP153 genes were amplified, digested with *Alu*I (Invitrogen), electrophoresed on 1.5% agarose gels and compared with the profiles obtained from the inoculant strains. Isolates and inoculant strains had identical restriction patterns [39].

2.6. Quantitative analysis of the abundance and expression of alkB and CYP153 genes

Reverse transcription was performed with 10–20 ng RNA, the specific primers PpalkB-for and P450-for and Superscript II Reverse Transcriptase (Invitrogen) according to manufacturer's instructions. Abundance and expression of both genes were quantified by real-time PCR using an iCycler (IQ) (Biorad) as described by Andria et al. [39]. Besides melting curve analysis, PCR products were examined on 2% agarose gels. No primer-dimers were detected. Serial dilutions of DNA and cDNA were spiked with 10^6 copies of amplified *alkB* and CYP153 genes to check for real-time PCR inhibition [42]. Highly linear standard curves (r^2 values >0.95, PCR efficiency >98%) over the dilution range and a detection limit of 10^1 copies were obtained indicating no PCR inhibition. The *alkB* and CYP153 gene copy numbers were quantified relative to a standard curve of positive control [39].

2.7. ACC deaminase activity

ACC deaminase activity of two strains were tested on the minimal medium containing 0.7 g ACC l^{-1} as sole nitrogen source, as described earlier [43].

2.8. Hydrocarbon analysis of soil samples

Total hydrocarbon content (THC) of the soil was measured employing infrared spectroscopy as described previously [44].

2.9. Statistical analysis

All statistical analyses were performed using SPSS software package (SPSS In., U.S.A.) and Excel (Microsoft, U.S.A.). The data were subjected to analysis of variance, and significant differences between means were determined by Duncan's multiple range test ($p < 0.05$).

Table 4
Effect of soil type on colony forming unit (cfu), *alkB* and CYP153 genes abundance and gene expression in the rhizosphere of Italian ryegrass inoculated either with *Pseudomonas* sp. ITRI53 or with *Pantoea* sp. BTRH79, respectively.

Soil type/treatment	Cfu/g dry weight × 10^5			Genes abundance (copies/g dry weight) × 10^5			Gene expression (copies/g dry weight) × 10^5		
	1st harvest	2nd harvest	3rd harvest	1st harvest	2nd harvest	3rd harvest	1st harvest	2nd harvest	3rd harvest
Sandy soil									
Pseudomonas sp. ITRI53	1.5^{d} (0.7)	0.61^{e} (0.08)	0.48^{e} (0.09)	0.68^{e} (0.1)	0.25^{d} (0.04)	0.19^{e} (0.04)	0	0	0
Pantoea sp. BTRH79	3.2^{d} (0.4)	0.72^{e} (0.08)	0.53^{e} (0.03)	0.79^{e} (0.12)	0.37^{d} (0.09)	0.24^{e} (0.037)	0	0	0
									Loamysandysoil
Pseudomonas sp. ITRI53	560^{c} (45)	85^{d} (17)	46^{d} (2.2)	160^{d} (26)	34^{c} (4.9)	78^{c} (15)	53^{c} (4.7)	8.4^{c} (0.62)	17^{c} (5.7)
Pantoea sp. BTRH79	1600^{b} (830)	630^{b} (88)	140^{b} (31)	530^{b} (86)	270^{b} (87)	94^{b} (7.4)	240^{ab} (48)	79^{b} (4.2)	27^{b} (6.6)
									Loamysoil
Pseudomonas sp. ITRI53	550^{c} (34)	380^{c} (84)	86^{c} (23)	330^{c} (82)	34^{c} (8.2)	37^{d} (3.9)	94^{b} (6.8)	12^{c} (5.3)	6.9^{d} (1.6)
Pantoea sp. BTRH79	4700^{a} (790)	3400^{a} (680)	590^{a} (84)	570^{a} (79)	850^{a} (56)	460^{a} (4)	270^{a} (75)	460^{a} (86)	130^{a} (82)

Means in the same column followed by the same letter are not significantly different at a 5% level of significance, $n = 3$; the standard error of three replicate is presented in parentheses. 1st harvest, 2nd harvest and 3rd harvest were after one week, four weeks and eight weeks of seed germination, respectively; the standard error of three replicate is presented in parentheses.

Table 5
Effect of soil type on colony forming unit (cfu), *alkB* and CYP153 genes abundance and gene expression in the shoot of Italian ryegrass inoculated either with *Pseudomonas* sp. ITRI53 or with *Pantoea* sp. BTRH79, respectively.

Soil type/treatment	Cfu/g dry weight × 10^5			Genes abundance (copies/g dry weight) × 10^5			Gene expression (copies/g dry weight) × 10^5		
	1st harvest	2nd harvest	3dr harvest	1st harvest	2nd harvest	3rd harvest	1st harvest	2nd harvest	3rd harvest
Sandy soil									
Pseudomonas sp. ITRI53	0.48^{c} (0.06)	7.1^{e} (0.83)	1.8^{e} (0.46)	0.06^{c} (0.02)	0.39^{e} (0.06)	0.037^{e} (0.01)	0.037^{c} (0.007)	0.24^{d} (0.06)	0.026^{e} (0.008)
Pantoea sp. BTRH79	0.25^{e} (0.05)	0.58^{e} (0.09)	0.22^{e} (0.06)	0.02^{d} (0.006)	0.52^{e} (0.07)	0.014^{e} (0.004)	0.008^{d} (0.003)	0.28^{d} (0.06)	0.009^{e} (0.007)
Loamy sandy soil									
Pseudomonas sp. ITRI53	3.2^{b} (0.38)	680^{b} (65)	420^{a} (84)	0.37^{b} (0.06)	8.1^{c} (0.75)	6.4^{a} (0.41)	0.29^{b} (0.08)	5.4^{b} (0.82)	3.7^{a} (0.06)
Pantoea sp. BTRH79	0.24^{e} (0.06)	25^{d} (6.2)	38^{d} (7.2)	0.023^{d} (0.004)	1.3^{d} (0.52)	0.86^{d} (0.09)	0.016^{d} (0.01)	0.75^{d} (0.07)	0.39^{d} (0.08)
Loamy soil									
Pseudomonas sp. ITRI53	5.2^{a} (0.83)	790^{a} (78)	250^{b} (51)	0.45^{a} (0.07)	76^{a} (7.2)	5.3^{b} (0.64)	0.31^{a} (0.07)	39^{a} (4.9)	3.2^{b} (0.8)
Pantoea sp. BTRH79	0.37^{d} (0.07)	81^{c} (9.6)	53^{c} (6.8)	0.056^{c} (0.01)	9.4^{b} (0.24)	1.8^{c} (0.35)	0.038^{c} (0.008)	3.8^{c} (0.64)	0.86^{c} (0.2.5)

Means in the same column followed by the same letter are not significantly different at a 5% level of significance, $n = 3$; the standard error of three replicate is presented in parentheses; 1st harvest, 2nd harvest and 3rd harvest were after one week, four weeks and eight weeks of seed germination, respectively.

Table 6
Effect of soil type and bacterial inoculants on hydrocarbon content at the tree dates of harvest.

Soil type/treatment	Hydrocarbon concentration ($g\,kg^{-1}$ soil)			
	Initial value	1st harvest	2nd harvest	3rd harvest
Sandy soil				
Control (vegetated)	10.0	9.4^{d} (0.52)	9.2^{f} (0.46)	8.8^{f} (0.47)
Pseudomonas sp. ITRI53	10.0	8.9^{c} (0.37)	8.0^{de} (0.50)	7.8^{d} (0.38)
Pantoea sp. BTRH79	10.0	8.7^{c} (0.46)	7.9^{d} (0.35)	7.6^{d} (0.35)
				Loamysandsoil
Control (vegetated)	10.0	8.8^{c} (0.48)	8.5^{e} (0.42)	8.3^{e} (0.41)
Pseudomonas sp. ITRI53	10.0	7.8^{b} (0.35)	6.4^{c} (0.55)	5.2^{c} (0.30)
Pantoea sp. BTRH79	10.0	7.6^{b} (0.43)	5.8^{b} (0.38)	4.3^{b} (0.60)
				Loamysoil
Control (vegetated)	10.0	8.5^{c} (0.49)	8.2^{de} (0.35)	8.0^{de} (0.48)
Pseudomonas sp. ITRI53	10.0	7.5^{b} (0.38)	5.5^{b} (0.36)	4.4^{b} (0.37)
Pantoea sp. BTRH79	10.0	7.1^{a} (0.33)	4.9^{a} (0.41)	3.8^{a} (0.28)

Means in the same column followed by the same letter are not significantly different at a 5% level of significance, $n = 3$; the standard error of three replicate is presented in parentheses. 1st harvest, 2nd harvest and 3rd harvest were after one week, four weeks and eight weeks of seed germination, respectively.

3. Results

3.1. Plant biomass

Growth parameters (seed germination, shoot length and shoot and root biomass) were determined to evaluate the effect of the soil type on plant development (Tables 2 and 3). In the absence of the contaminant, there was significantly more seed germination, shoot length and biomass (shoot and root) in loamy soils than in sandy soil. In soils containing diesel, plants without bacterial inoculation displayed significantly less seed germination, shoot length and plant biomass than the plants grown in unpolluted soils. Soils inoculated with both, *Pseudomonas* sp. strain ITRI53 and *Pantoea* sp. strain BTRH79, exhibited a significantly higher percentage of seed germination, shoot length and plant biomass as compared to un-inoculated soils. The strain with ACC deaminase activity (BTRH79) induced higher shoot and particularly root biomass compared to the uninoculated diesel soil than the strain (ITRI53) lacking this activity. In uninoculated as well as in inoculated sandy soil plant growth was very slow. Plant biomass production gradually increased with time.

3.2. Abundance of inoculant strains and expression of degrading genes

The abundance of the two inoculant strains was followed by cultivation and by a cultivation-independent approach. Both approaches clearly showed that stain BTRH79 better colonized the rhizosphere of Italian ryegrass in all three soil types than strain ITRI53 (Table 4), whereas strain ITRI53 better colonized the shoot interior of Italian ryegrass (Table 5).

Inoculated strains did not only colonize the rhizosphere and shoot interior of plants grown in loamy sand and loamy soils, but also expressed alkane degradation genes indicating an active role in the degradation of the pollutant. The highest gene abundances and gene expression levels were found in the loamy soil, where also plant growth and hydrocarbon degradation (compare Section 3.3 and Table 6) were the highest. However, in sandy soil both strains showed comparatively low survival and abundance in the rhizosphere and shoot interior, and expression of alkane degrading genes was only detected in the shoot but not in the rhizosphere. Similarly, hydrocarbon degradation was lower in sandy soil. The comparison between samples taken at different harvests showed that the total number of bacteria, measured via both CFU counting and real-time PCR, decreased with time. Maximum hydrocarbon degradation was observed at the second harvest, which correlated well ($r = 0.7$) with high *alkB* and CYP153 gene expression levels. These results showed that bacterial abundance and expression of alkane degrading genes was affected by the soil type and decreased with time. When the degradation potential was high, gene numbers were high as well. Control non-inoculated soils did not show colonies at the beginning of the experiment, however a few colonies were detected after eight weeks presumably due to cross-contamination (data not shown). In the same soil, none of the functional genes (*alkB* and CYP153) were detected, and the degradation of hydrocarbon was very low.

3.3. Hydrocarbon degradation

In order to determine the effect of soil type on degradation of diesel, the residual amount of hydrocarbon in soil was determined. In soils that had been planted without inoculation, hydrocarbon degradation was very low and bacterial inoculation enhanced hydrocarbon degradation (Table 6). Generally, both un-inoculated and inoculated plants in loamy soil displayed more efficient hydrocarbon degradation than in sandy and loamy sandy soils. Soils inoculated with *Pantoea* sp. strain BTRH79 showed significantly more hydrocarbon degradation than those inoculated with *Pseudomonas* sp. strain ITRI53. Among inoculated soils, highest hydrocarbon degradation (62%) was observed in loamy soil inoculated with BTRH79. This was significantly more than the 20% in the uninoculated control and also significantly more than the degradation rate in loamy sand (57% after 8 weeks) and in the sandy soils (24% after 8 weeks). In sandy soil the degradation was generally smallest with 12% in uninoculated treatment and 22% in the treatment with strain ITRI53. In vegetated, inoculated soils 11–29%, 20–51% and 22–62% hydrocarbon degradation was observed at the first, second and third harvest time, respectively.

4. Discussion

Optimal plant growth is an important factor influencing rhizodegradation of hydrocarbons by supporting the colonization of degrading microorganisms and increasing oxygen availability. In the present study we obtained reduced seed germination and biomass (shoot and root) production of Italian ryegrass grown in sandy soil as compared to both loamy soils. Loamy soil provided the best habitat for plant growth, and sandy soil the worst. This may be due to differences in organic carbon content and cation exchange capacity. High cation exchange capacity generally indicates high nutrient levels potentially leading to better plant growth [45–47]. Sandy soils are usually more porous, warmer, drier, and less fertile than soils with a finer texture thus limiting plant growth [47,48].

Seed germination, shoot length and biomass of plants grown in soils containing 1% diesel were significantly lower than of those

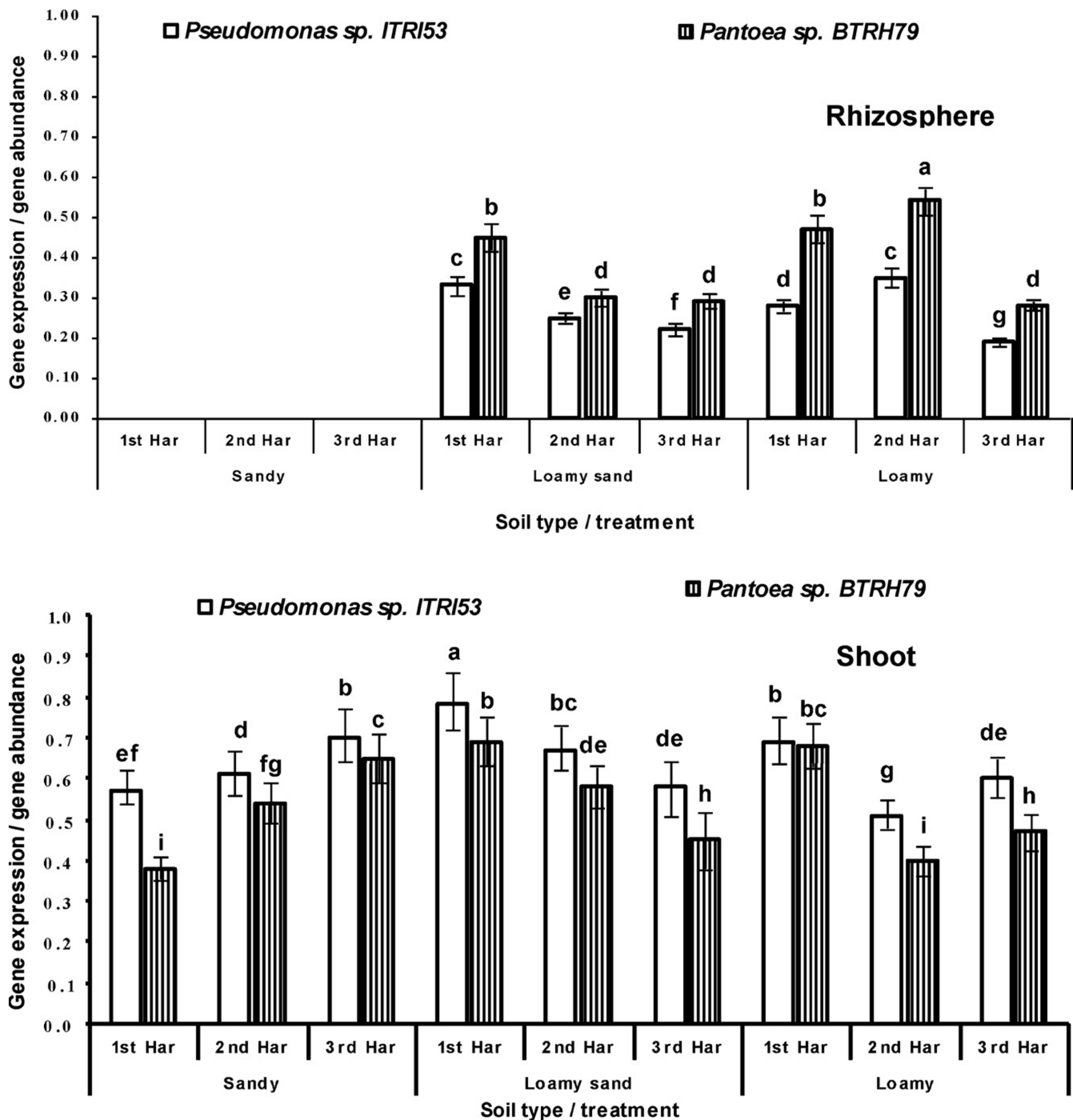

Fig. 1. Ratio of gene expression and gene abundance in the rhizosphere and shoot interior of Italian ryegrass vegetated in three different types of soil spiked with 1% diesel.

grown in non-polluted soils. Hydrocarbons are known to inhibit plant growth [21,39,44]. The primary inhibiting factors are considered to be the toxicity of low molecular weight compounds and the hydrophobic properties that limit the ability of plants to absorb water by decreasing the field capacity of soils (Reichenauer, personal communication) and nutrient contents [49]. Inoculation appears to have protected plants from the phytotoxic effects of diesel. In inoculated soils, shoot height and plant biomass increased by 8% and 41% and 7% and 38%, respectively, as compared to non-inoculated soils. Particularly strain BTRH79 exhibiting alkane degradation capacity as well as ACC-deaminase activity was highly efficient in enhancing plant biomass (especially root biomass) and hydrocarbon degradation and performed better than strain ITRI53 lacking ACC deaminase activity. This is in line with previous reports showing that the bacterial enzyme ACC-deaminase alleviates plant stress symptoms [18,50,51]. Similarly, Gurska et al. [52] previously showed that inoculation with ACC deaminase-containing rhizobacteria enhanced root growth and hydrocarbon degradation.

Very low reduction (12–20% after eight weeks) of hydrocarbon was observed in all three types of non-inoculated, vegetated soil. As the soil was initially sterilized adsorption of the hydrocarbons to soil organic matter and possibly to roots is likely to have been the reason for the measured reduction of the contaminant. Among inoculated soils, total hydrocarbon reduction was higher in loamy soil (62%) and loamy sandy soil (57%) than in sandy soil (24%). This indicates that mostly the inoculated bacteria were responsible for the degradation of hydrocarbons. Better developed roots and more root exudates, particularly in loamy soils, probably contributed to better colonization of the inoculants strains and more efficient degradation. In an earlier study, more hydrocarbons were

degraded in a loamy and a clayey soil than in a sandy soil [53]. The concentration of basic nutrients are low in sandy soils [20] and no biodegradation of hydrocarbon was observed after 30 days without fertilization. Furthermore, Davis and Madsen [54] reported that degradation of toluene was affected by soil type, soil organic matter content and inorganic nitrogen availability. They observed very slow toluene degradation in sandy soil due to low organic content (0.8%) as compared to sandy loam and clay soils containing high organic content, 4% and 5.5%, respectively.

Strain BTRH79 showing ACC deaminase activity in vitro was more efficient in hydrocarbon degradation than strain ITRI53 lacking this activity. Strain BTRH79 favoured shoot and root growth, the latter provided an increased surface area for bacterial colonization resulting also in higher degradation. This strain was also previously reported to efficiently colonize plants and degrade hydrocarbons [44].

The ability of *Pseudomonas* sp. strain ITRI53 and *Pantoea* sp. strain BTRH79 to colonize the rhizosphere and shoot interior of Italian ryegrass was assessed using cultivation and DNA-based methods. In contrast to the high abundance of the inoculant strains in loamy sand and loamy soils, rather poor survival was observed in sandy soil. Similarly, bacterial gene abundance and expression were higher in loamy soils than in sandy soil. In sandy soil, low organic carbon, low cation exchange capacity and a limited surface area available for bacterial attachment might be underlying the lower bacterial colonization as well as lower or no activity. However, genes involved in the degradation of hydrocarbons were expressed in loamy soils throughout the experiment. The abundance of degrading genes (*alkB* and CYP153) showed positive correlations with gene expression ($r = 0.82$) and hydrocarbon degradation ($r = 0.74$). Catabolic genes may serve as markers of actual function: in the case of hydrocarbon degrading communities, strong positive correlations have previously been found between gene copies and transcripts [56,57] indicating that the presence of genes is related to their activity. In contrast to the relationship between gene abundance and gene expression, it seems that inoculated bacteria were inactive in the sandy soil. In loamy sandy and loamy soils, a rapid decrease in soil hydrocarbon concentrations was observed within only four weeks of treatment (loamy sand, 32%; loamy soil, 40%). This could partly be due to the release of root exudates enhancing bacterial growth and hydrocarbon degradation. Gene expression results also showed that this decrease in hydrocarbons in loamy sand and loam was caused by bacterial degradation, since the expression level of measured biodegradation genes (*alkB* and CYP153 genes) was higher at the first and second harvest than at the third harvest. The fact that the abundance and expression of inoculated bacteria/genes decreased throughout the study period may result from insufficient nutrient availability and sub-optimal environmental conditions such as matric water potential, pH and ionic strength [58]. The competition between plant roots and microbes for nutrients may have influenced microbial activities, especially in sandy soil characterized by a low organic matter content [22]. Secondly, the decrease in gene abundance and expression of hydrocarbon degraders may be due to a decline in easily biodegradable hydrocarbons [36].

Cultivation-dependent and cultivation-independent analysis showed that the inoculated strains, ITRI53 and BTRH79, well colonized the rhizosphere and shoot interior of Italian ryegrass. However, strain BTRH79, a rhizosphere bacterium, better colonized the rhizosphere than strain ITRI53, whereas strain ITRI53, a root endophyte, was more successful in colonizing the shoot interior. Furthermore, the endophyte ITRI53 was more active (higher transcript numbers/gene abundance) in the shoot interior, whereas the rhizosphere strain BTRH79 was constantly more active in the rhizosphere. Similar observations were made by Rosenblueth and Martínez-Romero [59] and Andria et al. [39], who postulated that endophytes are generally better able to colonize plant interior than the rhizosphere. Both strains also expressed functional genes in the plant interior indicating an active role in this environment. However, for both strains, *alkB* and CYP153 gene abundance and overall expression were the highest in rhizosphere indicating that root exudates provide nutrients for bacterial growth and co-metabolites for alkane degradation [60]. Nevertheless, as the average activities (*alkB*/CYP153 transcripts/numbers) were higher in the shoot interior than in the rhizosphere (Fig. 1), we assume that a larger cell fraction of this population was active inside the plant than in the rhizosphere. This suggests that growth conditions were more favourable in the plant interior and more nutrients and/or activating substances might have been present. Similarly, Andria et al. [39] reported higher activity levels in the shoot interior.

In conclusion we showed that inoculation with suitable bacterial strains has the potential to support plant growth and enhance phytoremediation of hydrocarbons, however, the process is strongly influenced by soil characteristics such as the soil type, particle sizes or organic matter content. The soil type did not only affect plant growth but also substantially influenced microbial colonization and activities. The importance of such parameters should be considered in the design of bioremediation applications.

Acknowledgements

M. Afzal would like to thank NIBGE and Higher Education Commission, Govt. of Pakistan, for funding. We are thankful to A. Grahsl, who assisted in performing the greenhouse experiment.

References

[1] R. Margesin, D. Labbe, F. Schinner, C.W. Greer, L.G. Whyte, Characterization of hydrocarbon-degrading microbial populations in contaminated and pristine alpine soils, Appl. Environ. Microbiol. 69 (2003) 3085–3092.
[2] S. Heiss-Blanquet, Y. Benoit, C. Maréchaux, F. Monot, Assessing the role of alkane hydroxylase genotypes in environmental samples by competitive PCR, J. Appl. Microbiol. 99 (2005) 1392–1403.
[3] T.G. Reichenauer, J.J. Germida, Phytoremediation of organic pollutants in soil and groundwater, Chem. Sus. Chem. 1 (2008) 708–719.
[4] P. Agamuthu, O.P. Abioye, A.A. Aziz, Phytoremediation of soil contaminated with used lubricating oil using *Jatropha curcas*, J. Hazard. Mater. 179 (2010) 891–894.
[5] Z. Cai, Q. Zhou, S. Peng, K. Li, Promoted biodegradation and microbiological effects of petroleum hydrocarbons by *Impatiens balsamina* L. with strong endurance, J. Hazard. Mater. 183 (2010) 731–737.
[6] P.E. Olson, A. Castro, M. Joern, N.M. Duteau, E. Pilon-Smits, K.F. Reardon, Effects of agronomic practices on phytoremediation of an aged PAH-contaminated soil, J. Environ. Qual. 37 (2008) 1439–1446.
[7] S. Peng, Q. Zhou, Z. Cai, Z. Zhang, Phytoremediation of petroleum contaminated soils by *Mirabilis Jalapa* L. in a greenhouse plot experiment, J. Hazard. Mater. 168 (2009) 1490–1496.
[8] K.E. Gerhardt, X.D. Huang, B.R. Glick, B.M. Greenberg, Phytoremediation and rhizoremediation of organic soil contaminants: Potential and challenges, Plant Sci. 176 (2009) 20–30.
[9] A. Alarcón, F.T. Davies Jr., R.L. Autenrieth, D.A. Zuberer, Arbuscular mycorrhiza and petroleum-degrading microorganisms enhance phytoremediation of petroleum-contaminated soil, Int. J. Phytoremediation 10 (2008) 251–263.
[10] H. Al-Awadhi, I. El-Nemr, H. Mahmoud, N.A. Sorkhoh, S.S. Radwan, Plant-associated bacteria as tools for the phytoremediation of oily nitrogen-poor soil, Int. J. Phytoremediation 11 (2009) 11–27.
[11] B.R. Glick, Using soil bacteria to facilitate phytoremediation, Biotechnol. Adv. 28 (2010) 367–374.
[12] S. Compant, C. Clément, A. Sessitsch, Plant growth-promoting bacteria in the rhizo- and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization, Soil Biol. Biochem. 42 (2010) 669–678.
[13] A. Sessitsch, B. Reiter, U. Pfeifer, E. Wilhelm, Cultivation-independent population analysis of bacterial endophytes in three potato varieties based on eubacterial and Actinomycetes-specific PCR of 16S rRNA genes, FEMS Microbiol. Ecol. 39 (2002) 23–32.
[14] K.J. Germaine, E. Keogh, D. Ryan, D.N. Dowling, Bacterial endophyte-mediated naphthalene phytoprotection and phytoremediation, FEMS Microbiol. Lett. 296 (2009) 226–234.
[15] N. Weyens, D. van der Lelie, S. Taghavi, L. Newman, J. Vangronsveld, Exploiting plant-microbe partnerships to improve biomass production and remediation, Trends Biotechnol. 27 (2009) 591–598.

[16] I. Alkorta, C. Garbisu, Phytoremediation of organic contaminants in soils, Bioresour. Technol. 79 (2001) 273–276.
[17] M.R.T. Palmroth, J. Pichtel, J.A. Puhakka, Phytoremediation of subarctic soil contaminated with diesel fuel, Bioresour. Technol. 84 (2002) 221–228.
[18] B.R. Glick, Phytoremediation, Synergistic use of plants and bacteria to clean up the environment, Biotechnol. Adv. 21 (2003) 383–393.
[19] S. Labana, O.V. Singh, A. Basu, G. Pandey, R.K. Jain, A microcosm study on bioremediation of p-nitrophenol-contaminated soil using *Arthrobacter protophormiae* RKJ100, Appl. Microbiol. Biotechnol. 68 (2005) 417–424.
[20] E. Kaimi, T. Mukaidani, M. Tamaki, Effect of rhizodegradation in diesel-contaminated soil under different soil conditions, Plant Prod. Sci. 10 (2007) 105–111.
[21] T.-R. Sun, L. Cang, Q.-Y. Wang, D.-M. Zhou, J.-M. Cheng, H. Xu, Roles of abiotic losses, microbes, plant roots, and root exudates on phytoremediation of PAHs in a barren soil, J. Hazard. Mater. 176 (2010) 919–925.
[22] J. Kaakinen, P. Vähäoja, T. Kuokkanen, K. Roppola, Studies on the effects of certain soil properties on the biodegradation of oils determined by the manometric respirometric method, J. Autom. Methods Manage. Chem. 2007 (2007) 1–7.
[23] H. Jung, K.-D. Sohn, B. Neppolian, H. Choi, Effect of soil organic matter (SOM) and soil texture on the fatality of indigenous microorganisms in intergrated ozonation and biodegradation, J. Hazard. Mater. 150 (2008) 809–817.
[24] A.K. Haritash, C.P. Kaushik, Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): a review, J. Hazard. Mater. 169 (2009) 1–15.
[25] D. Sijm, R. Kraaij, A. Belfroid, Bioavailability in soil or sediment: exposure of different organisms and approaches to study it, Environ. Pollut. 108 (2000) 113–119.
[26] N. Chung, M. Alexander, Effect of soil properties on bioavailability and extractability of phenanthrene and atrazine sequestered in soil, Chemosphere 48 (2002) 109–115.
[27] Z.D. Parrish, M.K. Banks, A.P. Schwab, Assessment of contaminant lability during phytoremediation of polycyclic aromatic hydrocarbon impacted soil, Environ. Pollut. 137 (2005) 187–197.
[28] H.H. Richnow, R. Seifert, M. Kästner, B. Mahro, B. Horsfield, U. Tiedgen, S. Böhm, W. Michaelis, Rapid screening of PAH-residues in bioremediated soils, Chemosphere 31 (1995) 3991–3999.
[29] C. Löser, H. Seidel, P. Hoffmann, A. Zehnsdorf, Bioavailability of hydrocarbons during microbial remediation of a sandy soil, Appl. Microbiol. Biotechnol. 51 (1999) 105–111.
[30] R. Piskonen, M. Nyyssönen, T. Rajamäki, M. Itävaara, Monitoring of accelerated naphthalene-biodegradation in a bioaugmented soil slurry, Biodegradation 16 (2005) 127–134.
[31] G. Panicker, N. Mojib, J. Aislabie, A. Bej, Detection, expression and quantitation of the biodegradative genes in Antarctic microorganisms using PCR, Antonie van Leeuwenhoek 97 (2010) 275–287.
[32] J. Salminen, P. Tuomi, K. Jørgensen, Functional gene abundances (*nahAc*, *alkB*, *xylE*) in the assessment of the efficacy of bioremediation, Appl. Biochem. Biotechnol. 151 (2008) 638–652.
[33] S.D. Siciliano, N. Fortin, A. Mihoc, G. Wisse, S. Labelle, D. Beaumier, D. Ouellette, R. Roy, L.G. Whyte, M.K. Banks, P. Schwab, K. Lee, C.W. Greer, Selection of specific endophytic bacterial genotypes by plants in response to soil contamination, Appl. Environ. Microbiol. 67 (2001) 2469–2475.
[34] L. Whyte, A. Schultz, J. van Beilen, A. Luz, V. Pellizari, D. Labbé, C. Greer, Prevalence of alkane monooxygenase genes in Arctic and Antarctic hydrocarbon-contaminated and pristine soils, FEMS Microbiol. Ecol. 41 (2002) 141–150.
[35] E. Yergeau, M. Arbour, R. Brousseau, D. Juck, J.R. Lawrence, L. Masson, L.G. Whyte, C.W. Greer, Microarray, Real-Time, PCR analyses of the responses of high-arctic soil bacteria to hydrocarbon pollution and bioremediation treatments, Appl. Environ. Microbiol. 75 (2009) 6258–6267.
[36] S. Powell, S. Ferguson, J. Bowman, I. Snape, Using real-time PCR to assess changes in the hydrocarbon-degrading microbial community in Antarctic soil during bioremediation, Microb. Ecol. 52 (2006) 523–532.
[37] J. Juhanson, J. Truu, E. Heinaru, A. Heinaru, Survival and catabolic performance of introduced *Pseudomonas* strains during phytoremediation and bioaugmentation field experiment, FEMS Microbiol. Ecol. 70 (2009) 446–455.
[38] S. Yousaf, V. Andria, T.G. Reichenauer, K. Smalla, A. Sessitsch, Phylogenetic and functional diversity of alkane degrading bacteria associated with Italian ryegrass (*Lolium multiflorum*) and birdsfoot trefoil (*Lotus corniculatus*) in a petroleum oil-contaminated environment, J. Hazard. Mater. 184 (2010) 523–532.
[39] V. Andria, T.G. Reichenauer, A. Sessitsch, Expression of alkane monooxygenase (*alkB*) genes by plant-associated bacteria in the rhizosphere and endosphere of Italian ryegrass (*Lolium multiflorum* L.) grown in diesel contaminated soil, Environ. Pollut. 157 (2009) 3347–3350.
[40] B. Reiter, N. Wermbter, S. Gyamfi, H. Schwab, A. Sessitsch, Endophytic *Pseudomonas* spp. populations of pathogen-infected potato plants analysed by 16S rDNA- and 16S rRNA-based denaturating gradient gel electrophoresis, Plant Soil 257 (2003) 397–405.
[41] F. Rasche, H. Velvis, C. Zachow, G. Berg, J.D. Van Elsas, A. Sessitsch, Impact of transgenic potatoes expressing anti-bacterial agents on bacterial endophytes is comparable with the effects of plant genotype, soil type and pathogen infection, J. Appl. Ecol. 43 (2006) 555–566.
[42] J.C. López-Gutiérrez, S. Henry, S. Hallet, F. Martin-Laurent, G. Catroux, L. Philippot, Quantification of a novel group of nitrate-reducing bacteria in the environment by real-time PCR, J. Microbiol. Methods 57 (2004) 399–407.
[43] M. Kuffner, M. Puschenreiter, G. Wieshammer, M. Gorfer, A. Sessitsch, Rhizosphere bacteria affect growth and metal uptake of heavy metal accumulating willows, Plant Soil 304 (2008) 35–44.
[44] S. Yousaf, K. Ripka, T. Reichenauer, V. Andria, M. Afzal, A. Sessitsch, Hydrocarbon degradation and plant colonization by selected bacterial strains isolated from Italian ryegrass and birdsfoot trefoil, J. Appl. Microbiol. 109 (2010) 1389–1401.
[45] I.L.C. Weissenhorn, Spore germination of arbuscular mycorrhizal fungi in soil differing in heavy metal content and other parameters, Eur. J. Soil Biol. 32 (1996) 165–172.
[46] W.R. Eason, J. Scullion, E.P. Scott, Soil parameters and plant responses associated with arbuscular mycorrhizas from contrasting grassland management regimes, Agric. Ecosyst. Environ. 73 (1999) 245–255.
[47] R. Carrenho, S.F.B. Trufem, V.L.c.R. Bononi, E.S. Silva, The effect of different soil properties on arbuscular mycorrhizal colonization of peanuts, sorghum and maize, Acta Bot. Brasilica 21 (2007) 723–730.
[48] D.M. Sylvia, S.S. Williams, Vesicular-arbuscular mycorrhizae and environmental stress, in: G.H. Bethlenfalvayn, R.G. Linderman (Eds.), Mycorrhizae in Sustainable Agriculture, ASA Special Publication, Madison, 1992, pp. 101–124.
[49] J.L. Kirk, J.N. Klironomos, H. Lee, J.T. Trevors, The effects of perennial ryegrass and alfalfa on microbial abundance and diversity in petroleum contaminated soil, Environ. Pollut. 133 (2005) 455–465.
[50] X.D. Huang, Y. El-Alawi, J. Gurska, B.R. Glick, B.M. Greenberg, A multi-process phytoremediation system for removal of polycyclic aromatic hydrocarbons from contaminated soils, Environ. Pollut. 130 (2004) 465–476.
[51] X.D. Huang, Y. El-Alawi, J. Gurska, B.R. Glick, B.M. Greenberg, A multi-process phytoremediation system for decontamination of persistent total petroleum hydrocarbons (TPHs) from soils, Microchem. J. 81 (2005) 139–147.
[52] J. Gurska, W. Wang, K.E. Gerhardt, A.M. Khalid, D.M. Isherwood, X.-D. Huang, B.R. Glick, B.M. Greenberg, Three year field test of a plant growth promoting rhizobacteria enhanced phytoremediation system at a land farm for treatment of hydrocarbon waste, Environ. Sci. Technol. 43 (2009) 4472–4479.
[53] M. Molnár, É. Fenyvesi, K. Gruiz, L. Leitgib, G. Balogh, A. Murányi, J. Szejtli, Effects of RAMEB on bioremediation of different soils contaminated with hydrocarbons, J. Inclusion Phenom. Macrocycl. Chem. 44 (2002) 447–452.
[54] J.W. Davis, S. Madsen, Factors affecting the biodegradation of toluene in soil, Chemosphere 33 (1996) 107–130.
[56] J. Sanseverino, C. Werner, J. Fleming, B. Applegate, J.M.H. King, G.S. Sayler, Molecular diagnostics of polycyclic aromatic hydrocarbon biodegradation in manufactured gas plant soils, Biodegradation 4 (1993) 303–321.
[57] J.M. DeBruyn, C.S. Chewning, G.S. Sayler, Comparative quantitative prevalence of mycobacteria and functionally abundant *nidA*, *nahAc*, and *nagAc* dioxygenase genes in coal tar contaminated sediments, Environ. Sci. Technol. 41 (2007) 5426–5432.
[58] J.D. Van Elsas, L.S. van Overbeek, Bacterial response to soil stimili, in: S.E. Kjelleberg (Ed.), Starvation in Bacteria, Plenum Press, New York, 1993, pp. 55–79.
[59] M. Rosenblueth, E. Martínez-Romero, Bacterial endophytes and their interactions with hosts, Mol. Plant Microbe Interact. 19 (2006) 827–837.
[60] P.E. Olson, K.F. Reardon, E.A.H. Pilon-Smits, Ecology of rhizosphere bioremediation, in: S.C. McCutcheon, J.L. Schnoor (Eds.), Phytoremediation: Transformation and Control of Contaminants, John Wiley & Sons, NJ, 2003, pp. 317–353.