Appl Microbiol Biotechnol (2017) 101:5199–5212
DOI 10.1007/s00253-017-8247-z

ENVIRONMENTAL BIOTECHNOLOGY

Inoculation of a phenanthrene-degrading endophytic bacterium reduces the phenanthrene level and alters the bacterial community structure in wheat

Juan Liu[1] · **Yanbing Xiang**[1] · **Zhiming Zhang**[1] · **Wanting Ling**[1] · **Yanzheng Gao**[1]

Received: 3 January 2017 / Revised: 6 March 2017 / Accepted: 12 March 2017 / Published online: 28 March 2017
© Springer-Verlag Berlin Heidelberg 2017

Abstract Colonization by polycyclic aromatic hydrocarbon (PAH)-degrading endophytic bacteria (PAHDEB) can reduce the PAH contamination risk in plant. However, little information is available on the impact of PAHDEB colonization on the endophytic bacterial community of inner plant tissues. A phenanthrene-degrading endophytic bacterium (PDEB), *Massilia* sp. Pn2, was inoculated onto the roots of wheat and subjected to greenhouse container experiments. The endophytic bacterial community structure in wheat was investigated using high-throughput sequencing technology. The majority of endophytic bacteria in wheat were *Proteobacteria*, and the dominant genus was *Pseudomonas*. Phenanthrene contamination clearly increased the diversity of endophytic bacteria in wheat. The cultivable endophytic bacteria counts in wheat decreased with increasing the level of phenanthrene contamination; the endophytic bacterial community structure changed correspondingly, and the bacterial richness first increased and then decreased. Inoculation of strain Pn2 reduced the phenanthrene contamination in wheat, enlarged the biomass of wheat roots, changed the bacterial community structure and enhanced the cell counts, diversity and richness of endophytic bacteria in phenanthrene-contaminated wheat in a contamination level-dependent manner. The findings of this investigation provide insight into the responses of endophytic bacterial community in plant to external PAH contamination and PAHDEB colonization.

Keywords Polycyclic aromatic hydrocarbons · Phenanthrene degradation · Endophytic bacteria · Bacterial community · Colonization

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of persistent organic contaminants commonly found in soils worldwide (Kim et al. 2013). According to China's first nationwide soil quality survey, released by the Ministry of Environmental Protection (MEP) and the Ministry of Land Resources (MLR) in April 2014, 0.2% of the collected farmland soil samples in China is heavily polluted by PAHs (MEP and MRL 2014). PAHs in soil can be taken up and accumulated by plants and then enter the food chain, which represents a serious threat to human health and the ecosystem (Glick 2003; Johnsen et al. 2005; Gao et al. 2013). Therefore, methods for regulating and controlling the uptake and metabolism of PAHs in plants have been a focus of interest.

Endophytic bacteria are bacteria that colonize the internal tissues of plants, resulting in no external sign of infection or negative effects on their host (Harish et al. 2008). Endophytic bacteria have been found in virtually all plant species studied and can form relationships with their host plants, including symbiotic, mutualistic, commensalistic and trophobiotic interactions (Lodewyckx et al. 2002; Ryan et al. 2008). Previous studies have shown that some endophytic bacterial strains possess the capacity to degrade organic contaminants, which can be applied to reduce organic contamination in soils and plants (Rajkumar et al. 2009; Khan and Doty 2011). For

Juan Liu and Yanbing Xiang contribute equality to this paper.

Electronic supplementary material The online version of this article (doi:10.1007/s00253-017-8247-z) contains supplementary material, which is available to authorized users.

✉ Yanzheng Gao
gaoyanzheng@njau.edu.cn

[1] Institute of Organic Contaminant Control and Soil Remediation, College of Resource and Environmental Sciences, Nanjing Agricultural University, Nanjing, China

instance, compared with no inoculation control, the inoculation of plants with the poplar endophyte *Pseudomonas putida* VM1441 facilitated higher (40%) naphthalene removal efficiency from contaminated soil (Germaine et al. 2009). An endophytic pyrene-degrading bacterium, *Staphylococcus* sp. BJ06, was isolated from *Alopecurus aequalis* grown in PAH-contaminated soil and inoculated onto the root of ryegrass (*Lolium multiflorum* Lam). BJ06 inoculation reduced the pyrene concentrations in ryegrass roots and shoots by 31.0 and 44.2%, respectively, compared with endophyte-free treatment (Sun et al. 2014). Hence, inoculation of PAH-degrading endophytic bacteria (PAHDEB) into plant tissues facilitated PAH removal from the inner tissues of plants.

It has been documented that organic contamination could affect the population characteristics of endophytic bacteria in plants. Siciliano et al. (2001) found that the number of contaminant-degrading bacteria in plants was increased by exposure to the corresponding contaminant. Ho et al. (2009) isolated 188 endophytic strains from three plant species and observed that among these strains, 29 not only proliferated in the presence of naphthalene, catechol and phenol but were also able to utilize these pollutants as the sole carbon source for growth. Peng et al. (2013) reported that the endophytic bacterial populations in plant tissues varied according to total PAH concentrations, as did the proportion of each endophytic bacterial genus. However, there is little information available on the influence of PAHDEB inoculation on the endophytic microbial communities of plant grown in PAH-contaminated soils. Since endophytic bacteria participate directly in PAH metabolism in inner plant tissues, elucidating the role of inoculated PAHDEB could help to reveal the mechanism of PAH removal from plants.

The objectives of this work were to explore the bacterial community in inner wheat tissues according to the level of phenanthrene contamination and investigate the effects of the inoculation of a phenanthrene-degrading endophyte on the endophytic bacterial community in wheat. To this end, greenhouse container experiments were performed, and the endophytic bacterial community in wheat was analysed using high-throughput sequencing technology. The phenanthrene-degrading endophytic bacterium, *Massilia* sp. Pn2, was induced to colonize wheat by root inoculation in soil contaminated with various levels of phenanthrene. After 30-day cultivation, the effects of phenanthrene contamination and Pn2 inoculation on the endophytic bacterial community of wheat were evaluated. The Pn2 counts and phenanthrene concentrations of wheat were determined to assess the effect of colonization by this endophyte on plant phenanthrene contamination. The results of this study showed that the colonization by strain Pn2 significantly enhanced the phenanthrene degradation in wheat and increased the diversity and richness of endophytic bacteria in a contamination level-dependent manner.

Materials and methods

Chemicals and media

Phenanthrene (purity >98%) was obtained from Aldrich Chemical Co., Milwaukee, WI, USA. Its molecular weight, water solubility in water at 25 °C and log-transformed octanol–water partitioning coefficient (log K_{ow}) are 178.2 g mol^{-1}, 1.18 mg L^{-1} and 4.46 (Yaws 1999). All solvents used were of analytical grade.

The mineral salts medium (MSM) and Luria–Bertani (LB) medium were the same as those used in the previous investigation by our lab (Sun et al. 2014). One-tenth strength Luria–Bertani (1:10 LB) medium was produced by diluting LB medium 1:10 with MSM. Solid media were obtained by adding 18 g L^{-1} agar to the above liquid media.

Bacterial strain and plant

Massilia sp. Pn2 (CGMCC 1.12927), a phenanthrene-degrading endophytic bacterium, was isolated from amur foxtail (*A. aequalis* Sobol) grown in PAH-contaminated soil, as detailed in our previous work (Liu et al. 2014). Wheat (*Triticum aestivum* L. cv. Yangmai-16) was used as the host plant because of its rapid growth and tolerance to high levels of phenanthrene in soil (Sheng and Gong 2006).

Greenhouse container experiments

Yellow-brown surface soil (0–20 cm), with a pH of 6.02, 14.3 g kg^{-1} soil organic carbon, 24.7% clay and 67.9% sand, was collected from a suburb of Nanjing, China. Soils were sieved through a 20-mm mesh and artificially contaminated with phenanthrene at four different initial concentrations of 0 mg kg^{-1} (S0), 5 mg kg^{-1} (S1), 50 mg kg^{-1} (S2) and 200 mg kg^{-1} (S3), respectively. Phenanthrene was dissolved in acetone and mixed thoroughly with a portion of soil samples, and then, the treated soils were thoroughly mixed with the rest of soils. As a control, soil samples were sprayed with the same volume of acetone. After 30-day ageing period in the dark at room temperature (Hwang and Cutright 2002), the phenanthrene-contaminated soils, "S0", "S1", "S2" and "S3", harboured phenanthrene concentrations of 0, 2.23, 30.24 and 134.64 mg kg^{-1}, respectively, and each was planted with wheat. Wheat not inoculated with Pn2 and planted in phenanthrene-contaminated soils was designated CP, and wheat inoculated with Pn2 and planted in phenanthrene-contaminated soils was designated CPB. All treatments were replicated three times.

Each pot was filled with 250 g of soil, and 12 surface-sterilized wheat seeds (Hwang and Cutright 2002) were vegetated at a 2-cm depth. Strain Pn2 was inoculated in 1:10 LB medium and incubated at 30 °C with shaking at 180 r min^{-1}

until reaching an OD_{600} of 1.0. Bacteria were collected by centrifugation, washed twice with the same volume of MSM and finally resuspended in MSM to a density of 3.3–4.6 × 10^8 colony-forming units (CFU) mL^{-1}. Bacterial suspensions (20 mL·pot^{-1}) were inoculated (CPB) with wheat roots through root-drenching method (Ahmed et al. 2000) 10 days after seedling emergence, and an inactivated bacterial suspension in MSM was inoculated (CP) as a control. Wheat was grown for 30 days in a greenhouse at 25–30 °C during the day and 16–20 °C at night at a relative humidity of 50–60%.

Phenanthrene concentration in wheat and soil

Phenanthrene in soil and wheat samples was extracted, purified and detected according to the methods described by Gao and Zhu (2004) and Gao et al. (2017). In brief, soil and plant samples were freeze-dried, crushed and homogenized. Then, samples were extracted three times by ultrasonication using a solution of acetone and dichloromethane (DCM) (1:1, *v*/*v*). Extracts were filtered through silica gel and eluted using DCM and hexane (1:1, *v*/*v*). The solvent fractions were evaporated and exchanged with methanol. After filtration through a 0.22-mm polytetrafluoroethylene (PTFE) filter, phenanthrene was measured by high-performance liquid chromatography (HPLC). The HPLC detection conditions were given in the previous investigation (Gao et al. 2017). The phenanthrene recovery from the soil and plant samples was ≥95% (n = 5, relative standard deviation (RSD) ≤ 2.52%).

To investigate the phenanthrene removal by strain Pn2 in wheat roots, shoots and soils, the enhancement ratio (E; %) was estimated according to the following equation: E (%) = (C_{CP} − C_{CPB}) / C_{CP} × 100, where C_{CPB} and C_{CP} are the phenanthrene concentrations in wheat roots, shoots or soils with and without Pn2 inoculation, respectively.

Phenanthrene accumulation in wheat roots and shoots (A, μg·pot^{-1}) was calculated using the following equation: $A = C_p \times M$, where C_p (mg kg^{-1}) is the phenanthrene concentration in wheat root or shoot and M (μg·pot^{-1}, on a dry weight basis) is the biomass of wheat root or shoot in each pot. The plant concentration factor (PCF) describes the capacity of plants to accumulate contaminants from the environment, which in this study was calculated as the ratio of the phenanthrene concentration in wheat root and shoot (C_p) to that in soil (C_s): PCF = C_p / C_s. The plant translocation factor (TF) is defined as the ratio of the shoot phenanthrene concentration factor (SCF) to the root phenanthrene concentration factor (RCF), which was equal to the ratio of phenanthrene concentration in wheat shoot (C_{shoot}) to that in root (C_{root}): TF = SCF / RCF = C_{shoot} / C_{root}.

Enumeration of Pn2 and cultivable aboriginal endophytic bacteria in wheat

After being collected, wheat samples were rinsed three times with deionized water and subsequently sterilized by sequential immersion in 75% (*v*/*v*) ethanol for 3–5 min, 2% sodium hypochlorite (*v*/*v*) for 3 min and 70% ethanol for 30 s. Then, wheat samples were washed three times with sterilized distilled water to remove residual surface sterilization agents (Sobral et al. 2005). Wheat samples were pressed onto fresh LB plates to detect any remaining epiphytic bacteria to determine whether the surface disinfection process was successful.

Shoots and roots were separated, cut into pieces and triturated in 5 mL of sterile distilled water. One hundred microlitre aliquots of the 10^{-1}, 10^{-2} and 10^{-3} dilutions were spread onto MSM plates (20 mg L^{-1} ampicillin, 20 mg L^{-1} chloramphenicol and 100 mg L^{-1} phenanthrene) and incubated at 30 °C for 5–7 days to enumerate strain Pn2 (Liu et al. 2014). Bacterial colonies were randomly selected to undergo 16S ribosomal RNA (rRNA) gene sequencing, and the phenanthrene-degrading ability of the isolated bacterial strains was investigated to confirm their identification as strain Pn2. Numbers of strain Pn2 in wheat roots and shoots were determined by enumerating CFU on MSM plates.

In addition, 100 μL aliquots of the 10^{-3}, 10^{-4} and 10^{-5} dilutions were spread onto LB plates and incubated at 30 °C for 5–7 days to enumerate total cultivable endophytic bacteria. The numbers of cultivable endophytic bacterial cells in wheat roots and shoots were determined by enumerating CFU on LB plates. The cell count of cultivable aboriginal endophytic bacteria in wheat was calculated as the difference in value (D value) of the number of cultivable endophytic bacterial cells and the number of Pn2 cells.

DNA extraction and PCR amplification

Directly sequencing the entire endophytic bacterial community is more accurate for analysis of the community composition in plants than cultivation method (Huse et al. 2008). The total DNA of the entire endophytic bacterial community in wheat samples was extracted according to the protocol detailed by Hung and Annapurna (2004). The V4–V5 region of the 16S rRNA gene in endophytic bacteria was amplified by PCR using the primers 799F (5′-AACMGGATTAGATACCCKG-3′) (Chelius and Triplett 2001) and 1115R (5′-AGGG TTGCGCTCGTTG-3′) (Reysenbach and Pace 1995); the barcode was an eight-base sequence unique to each sample. PCR reactions were performed in triplicate 25 μL mixtures containing 5 μL of 5× FastPfu buffer, 2.5 μL of 2.5 mM dNTPs, 1 μL of each primer (5 μM), 0.5 μL of FastPfu polymerase and 10 ng of template DNA. The three amplicons of each sample were mixed together and extracted from 1.5% agarose gels, purified using the AxyPrep DNA Gel

Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions.

Library construction and sequencing

Purified PCR products were quantified using Qubit® v. 3.0 (Invitrogen, Carlsbad, CA, USA), and every 24 amplicons with different barcodes were equally mixed. The PCR products were pooled and used to construct an Illumina pair-end library following the Illumina genomic DNA library preparation procedure. The amplicon library was paired-end sequenced (2 × 250) on an Illumina MiSeq platform (Shanghai Biozeron Co., Ltd., Shanghai, China) using standard protocols.

Processing of sequencing data

Raw fastq files were demultiplexed and quality-filtered using QIIME (v. 1.17) (Campbell et al. 2010) with the following criteria: (i) The 250-bp reads were truncated at any site with an average quality score <20 over a 10-bp sliding window, discarding truncated reads shorter than 50 bp. (ii) Exact barcode matching, two-nucleotide mismatches in primer matching and reads containing ambiguous characters were removed. (iii) Only sequences that overlapped by at least 10 bp were assembled according to their overlap sequence. Reads that could not be assembled were discarded. The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database with accession number of SRP 080266.

Operational taxonomic units (OTUs) were clustered with a 97% similarity cut-off using UPARS (v. 7.1; http://drive5.com/uparse/), and chimeric sequences were identified and removed using UCHIME (Edgar 2010). The phylogenetic affiliation of each 16S rRNA gene sequence was analysed by RDP Classifier (http://rdp.cme.msu.edu/) against the Silva (SSU123) 16S rRNA gene database using a confidence threshold of 70% (Amato et al. 2013).

Alpha and beta diversity analyses

Chao index is used due to its accuracy for bacterial richness analyses (Gotelli and Colwell 2011). Shannon index is selected for bacterial diversity analyses due to its simple operation and little effect of sample size on the observation of bacterial diversity (Spellerberg 2008). The two indices were performed using Mothur v. 1.21.1 (Schloss et al. 2009). To examine dissimilarities in community composition, a principal coordinate analysis (PCoA) was performed in QIIME. PCoA, in which a distance matrix is used to plot n samples in an $(n - 1)$-dimensional space, was used to compare groups of samples based on unweighted and weighted UniFrac distance metrics (Lozupone et al. 2011). Venn diagrams were generated using VennDiagram (Chen and Boutros 2011), and Heatmap figures were created using the Vegan package in R (R Development Core Team 2013). Clustering of genera obtained from the RDP Classifier was performed by means of the complete linkage hierarchical clustering technique using the R package HCLUST (http://sekhon.berkeley.edu/stats/html/hclust.html).

Statistical analysis

All data were processed by Microsoft Excel 2013 (Liengme 2015). Each data point is the mean of three replicates, and error bars are standard deviations (SD). The data were subjected to analyses of variance (ANOVA) using the statistical software package SPSS 20.0 (IBM, Armonk, NY, USA). Differences were considered significant at p values <0.05. Spearman's rank correlations were calculated using SPSS ver. 20.0. Correlations were considered significant at p values <0.05 and highly significant at p values <0.01.

Results

Phenanthrene contamination of wheat was reduced by strain Pn2

Phenanthrene uptake by wheat roots and shoots from contaminated soils after 30 days is shown in Fig. 1a. As the level of phenanthrene contamination increased, the phenanthrene concentrations in wheat roots and shoots generally enlarged. The phenanthrene concentration in wheat roots was markedly higher than that in wheat shoots. Meanwhile, the phenanthrene concentrations in wheat roots and shoots with versus without Pn2 inoculation were significantly lower ($p < 0.05$) when soil phenanthrene contamination levels were higher (S2 and S3 treatments). The enhancement ratios (E; %) of phenanthrene removal by Pn2 inoculation in wheat roots and shoots were 15.5–28.4 and 20.8–30.6%, respectively.

The phenanthrene concentrations in wheat-vegetated soils S1, S2 and S3 without Pn2 inoculation after 30-day cultivation were 0.74, 7.20 and 69.70 mg kg^{-1}, while those in the corresponding soils inoculated with strain Pn2 were 0.63, 6.93 and 67.18 mg kg^{-1}, respectively (Fig. 1b). No significant difference ($p < 0.05$) was observed between the phenanthrene concentrations in soils with no Pn2 control and with Pn2 inoculation under S1 and S2. Although the phenanthrene concentration in soil with Pn2 inoculation was a little lower ($p < 0.05$) than that with no Pn2 control under S3, the efficacy was much weaker than that in wheat roots or shoots.

The accumulation of phenanthrene in wheat roots and shoots (A, μg·pot^{-1}) was also reduced by Pn2 inoculation. As shown in Table 1, phenanthrene accumulation mass in wheat roots and shoots colonized with strain Pn2 was 0.30–2.07 and 0.17–1.11 μg·pot^{-1}, respectively, which were 6.3–

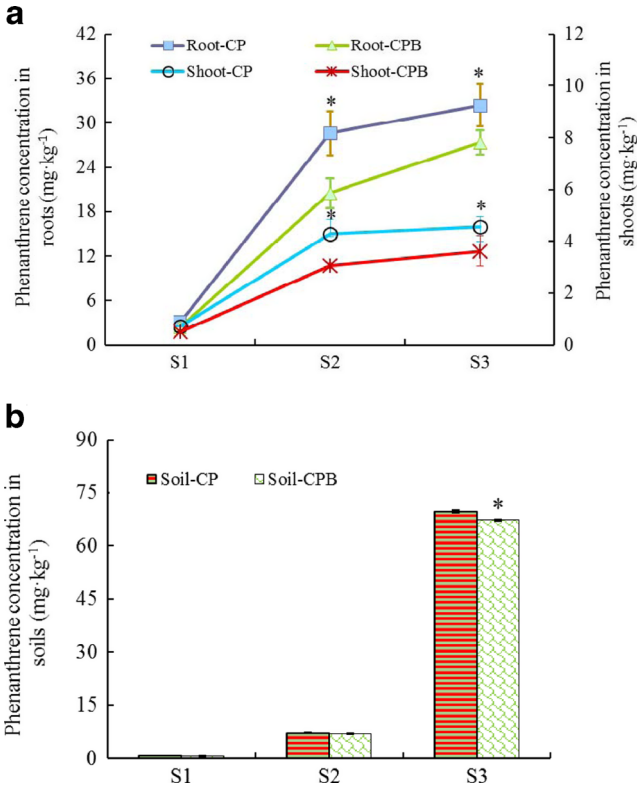

Fig. 1 The concentrations of phenanthrene in wheat (**a**) and soil (**b**) under different levels of phenanthrene contamination. *S1*, *S2* and *S3* are phenanthrene-spiked soils aged for 30 days, and their phenanthrene concentrations before vegetation were 2.23, 30.24 and 134.64 mg kg^{-1}, respectively. *CP* means treatment with wheat but no Pn2 inoculation in soil, and *CPB* means treatment with wheat and Pn2 inoculation. Error bars are ±standard deviation (n = 3). *p < 0.05, significant difference between treatments with and without Pn2 inoculation

17.9 and 21.8–29.2% lower than those controls without Pn2 inoculation. One notes that growth of wheat roots was significantly inhibited (p < 0.05) when the level of soil phenanthrene contamination was higher (S3). Inoculation of strain Pn2 significantly promoted the growth of wheat roots in the soils S0–S2 (p < 0.05).

A higher TF value indicates the greater quantity of phenanthrene translocated from the plant root to shoot. The RCF and SCF of phenanthrene for wheats inoculated with strain Pn2 (0.41, 2.96 and 3.76 and 0.05, 0.44 and 0.79, respectively) were lower than those for the control wheats without Pn2 inoculation (0.46, 3.98 and 4.18 and 0.07, 0.59 and 0.97, respectively) after 30-day cultivation. The TF values of phenanthrene for Pn2-colonized wheats grown in soils S1–S3 after 30 days were the same or a little lower than those for control wheats without Pn2 inoculation (Table 1).

Numbers of strain Pn2 and cultivable aboriginal endophytic bacteria in wheat

Wheat harboured large numbers of cultivable aboriginal endophytic bacteria, and strain Pn2 colonized and proliferated in the interior of wheat. As shown in Table 2, the cultivable aboriginal endophytic bacteria counts in wheat were 5.56 to 7.21 log CFU g^{-1} fresh weight (FW) of plant and were higher in roots than in shoots. Wheat tissue contained 3.74 to 4.53 log CFU g^{-1} FW of Pn2 in roots and 2.88 to 3.38 log CFU g^{-1} FW of Pn2 in shoots.

As the phenanthrene concentration increased, the number of cultivable aboriginal endophytic bacteria in wheat decreased significantly, particularly in roots (p < 0.05), while Pn2 counts increased gradually with increasing levels of phenanthrene contamination. As a consequence, the proportion of Pn2 in the total cultivable endophytic bacteria in wheat

Table 1 Accumulation mass, plant concentration factors (PCFs) and translocation factors (TFs) of phenanthrene in wheat planted in yellow-brown soil after 30 days

Treatment		Plant biomass (dry weight, mg·pot^{-1})		Accumulation mass (μg·pot^{-1})		Plant concentration factor (PCF)		Translocation factor (TF)
		Root	Shoot	Root	Shoot	Root	Shoot	
S0	CP	86.70 ± 16.66 bc	299.30 ± 29.88 a	ND	ND	–	–	–
	CPB	101.60 ± 7.47 cd	314.50 ± 33.95 a	ND	ND	–	–	–
S1	CP	117.60 ± 12.08 de	333.90 ± 42.27 a	0.36	0.24	4.18	0.97	0.23
	CPB	126.30 ± 10.2 4e	339.90 ± 33.68 a	0.30	0.17	3.76	0.79	0.21
S2	CP	77.10 ± 4.76 ab	307.63 ± 35.38 a	2.21	1.32	3.98	0.59	0.15
	CPB	101.00 ± 12.40 cd	324.43 ± 37.74 a	2.07	0.99	2.96	0.44	0.15
S3	CP	65.23 ± 4.35 a	311.90 ± 22.63 a	2.11	1.42	0.46	0.07	0.14
	CPB	67.73 ± 2.37 a	305.40 ± 40.13 a	1.85	1.11	0.41	0.05	0.13

S0 is the control soil without phenanthrene addition. S1, S2 and S3 are phenanthrene-spiked soils aged for 30 days, and their phenanthrene concentrations before vegetation were 2.23, 30.24 and 134.64 mg kg^{-1}, respectively. CP means treatment with wheat but no Pn2 inoculation in soil, and CPB means treatment with wheat and Pn2 inoculation. Data after "±" are standard deviations (n = 3). ND means undetectable. – means unavailable. Values in the same columns followed by the same letter are not significantly different at p < 0.05

increased from 0.38 to 5.37‰ in roots and 1.53 to 6.30‰ in shoots with increases in soil phenanthrene concentration from 0 to 134.64 mg kg^{-1}.

As shown in Table 3, a significantly negative correlation was observed between the cultivable aboriginal endophytic bacteria count in inner wheat tissues and the phenanthrene concentration in soil or in wheat ($p < 0.01$), while the Pn2 count in inner wheat tissues correlated positively with the phenanthrene contamination level ($p < 0.01$). A significantly negative correlation was also given between the cell counts of cultivable aboriginal endophytic bacteria and those of strain Pn2 ($p < 0.01$).

The colonization of internal wheat tissues by strain Pn2 increased the aboriginal endophytic bacteria count significantly compared with the absence of Pn2 at moderate and high levels of phenanthrene contamination (S2 and S3, $p < 0.05$). For example, as shown in Table 2, the cultivable aboriginal endophytic bacteria count in Pn2-inoculated wheat roots in S2 soil (6.98 log CFU g^{-1} FW) was significantly higher than that in Pn2-free soil subjected to S2 treatment (6.76 log CFU g^{-1} FW).

Alpha diversity of endophytic bacteria in wheat

The richness (Chao index) and diversity (Shannon index) of endophytic bacteria in internal wheat tissues at each level of phenanthrene contamination were calculated based on Illumina PE250 sequencing analysis (Table 4).

In general, as the level of phenanthrene contamination increased, the richness of endophytic bacteria in wheat increased in the presence of low and moderate levels of phenanthrene contamination (S0–S2) and decreased in the presence of a high level of phenanthrene contamination (S3). The diversity of endophytic bacteria in wheat roots followed a similar pattern with increasing levels of phenanthrene contamination. However, the diversity of endophytic bacteria in wheat shoots did not differ significantly among the phenanthrene treatments (S1–S3, $p < 0.05$); all values were higher than that of uncontaminated soil (S0).

As seen in Table 3, the diversity of endophytic bacteria in inner wheat tissues correlated positively with the phenanthrene concentration in wheat ($p < 0.01$ in roots and $p < 0.05$ in shoots, respectively). Meanwhile, significantly positive correlation was also observed between the richness of endophytic bacteria in inner wheat tissues and the level of phenanthrene contamination ($p < 0.01$).

Furthermore, inoculation of strain Pn2 increased the diversity and richness of endophytic bacteria in wheat at a given level of phenanthrene contamination. For example, the richness of endophytic bacteria in wheat inoculated with Pn2 was higher ($p < 0.05$) than that of wheat not inoculated with Pn2 in S1 and S3 soils. The diversity of endophytic bacteria in wheat roots inoculated with Pn2 was higher ($p < 0.05$) than that in wheat roots without Pn2 inoculation in S1 and S2 soils. In addition, the diversity and richness of endophytic bacteria in wheat were positively correlated with the cell count of Pn2 ($p < 0.05$, Table 3).

Dissimilarities in endophytic bacterial community composition in wheat

PCoA was used to evaluate the effects of various levels of phenanthrene contamination and Pn2 inoculation on the endophytic bacterial community of the inner tissue of wheat

Table 2 The cell counts of Pn2 and cultivable aboriginal endophytic bacteria in wheat roots and shoots planted in yellow-brown soil after 30 days

Treatments		Pn2 (log CFU g^{-1} FW)		Cultivable aboriginal endophytic bacteria (log CFU g^{-1} FW)		Proportion of Pn2 to total bacteria (‰)	
		Root	Shoot	Root	Shoot	Root	Shoot
S0	CP	ND	ND	7.21 ± 0.03d	5.73 ± 0.04c	–	–
	CPB	3.74 ± 0.03a	2.88 ± 0.15a	7.16 ± 0.01d	5.71 ± 0.02c	0.38	1.53
S1	CP	ND	ND	7.17 ± 0.06d	5.73 ± 0.05c	–	–
	CPB	4.09 ± 0.08b	3.05 ± 0.10a	7.14 ± 0.06d	5.74 ± 0.01c	0.89	2.10
S2	CP	ND	ND	6.76 ± 0.10b	5.57 ± 0.04ab	–	–
	CPB	4.33 ± 0.03c	3.27 ± 0.05b	6.98 ± 0.05c	5.63 ± 0.02b	2.24	4.43
S3	CP	ND	ND	6.59 ± 0.16a	5.56 ± 0.03a	–	–
	CPB	4.53 ± 0.03d	3.38 ± 0.04b	6.80 ± 0.10b	5.59 ± 0.02ab	5.37	6.30

S0 is the control soil without phenanthrene addition. S1, S2 and S3 are phenanthrene-spiked soils aged for 30 days, and their phenanthrene concentrations before vegetation were 2.23, 30.24 and 134.64 mg kg^{-1}, respectively. CP means treatment with wheat but no Pn2 inoculation in soil, and CPB means treatment with wheat and Pn2 inoculation. Data after "±" are standard deviations ($n = 3$). ND means undetectable. – means unavailable. Values in the same columns followed by the same letter are not significantly different at $p < 0.05$

Table 3 Spearman's rank correlations (r) between phenanthrene concentration in soil and wheat, cell count of Pn2, cultivable aboriginal endophytic bacteria in wheat and the Chao and Shannon indices of endophytic bacteria in wheat

	Root	C_{Soil}	C_{Plant}	Pn2	Cultivable	Shannon	Chao	Shoot	C_{Soil}	C_{Plant}	Pn2	Cultivable	Shannon	Chao
Spearman's rank correlation	C_{Soil}	1						C_{Soil}	1					
	C_{Plant}	0.957**	1					C_{Plant}	0.943**	1				
	Pn2	0.944**	0.958**	1				Pn2	0.894**	0.937**	1			
	Cultivable	−0.892**	−0.847**	−0.806**	1			Cultivable	−0.724**	−0.753**	−0.779**	1		
	Shannon[a]	0.986**	0.928**	0.886*	−0.543	1		Shannon	0.928**	0.870*	1.000**	−0.493	1	
	Chao[b]	0.928**	0.986**	0.829*	−0.486	0.943**	1	Chao	0.928**	0.986**	0.886*	−0.464	0.886*	1

C_{Soil} is the phenanthrene concentration in soil. C_{Plant} is the phenanthrene concentration in wheat. Pn2 is the cell count of strain Pn2 in wheat. Cultivable is the cell count of cultivable aboriginal endophytic bacteria in wheat. Shannon is the diversity of endophytic bacteria in wheat. Chao is the richness of endophytic bacteria in wheat

[a,b] The data from S0 to S2 treatments were used for correlation analysis between Shannon index or Chao index and other parameters

**$p < 0.01$, *$p < 0.05$

(Fig. 2). The first coordinate (PC1) explained 63.69% of the difference, and the second coordinate (PC2) explained 17.03% of the difference in the endophytic bacterial community in wheat roots (Fig. 2a). As the level of phenanthrene contamination increased, the magnitude of difference in the endophytic bacterial communities in wheat roots grown in S0, S1, S2 and S3 soils without Pn2 treatment (CP) increased (Fig. 2a). Furthermore, the endophytic bacterial community in wheat roots inoculated with Pn2 (CPB) was distinct in S1 soil compared with those in S0, S2 and S3 soils, whereas the endophytic bacterial communities of wheat roots grown in S2 and S3 soils were distinct, but overlapped slightly (Fig. 2a).

In wheat shoots, the first coordinate (PC1) explained 81.26% of the difference, and the second coordinate (PC2) explained 10.73% of the difference in the endophytic bacterial community (Fig. 2b). As the level of phenanthrene contamination increased, the endophytic bacterial community in wheat shoots in S0 soil not inoculated with Pn2 (CP) became increasingly distinct from those in S1–S3 soils (Fig. 2b). However, the endophytic bacterial community in wheat shoots inoculated with Pn2 (CPB) in S0 soil was distinct from those grown in S1–S3 soils, whereas the endophytic bacterial communities in wheat shoots grown in S1 and S2 soils differed, but overlapped slightly (Fig. 2b).

Venn diagrams were used to identify shared and unique endophytic bacterial communities. As shown in Fig. 3, up to 311 OTUs were shared in wheat roots (Fig. 3a) and 54 OTUs were shared in wheat shoots (Fig. 3b) under different levels of phenanthrene contamination not inoculated with Pn2. Moreover, most of the OTUs were similar following CP and CPB treatments under different levels of phenanthrene contamination in wheat roots (Fig. 3c) and shoots (Fig. 3d). These results indicated that, although phenanthrene contamination and Pn2 inoculation influenced the endophytic bacterial community, the proportions of the majority of the endophytic bacterial taxa were unaffected.

Endophytic bacterial community structure in wheat

The endophytic bacteria in wheat roots belonged to 18 phyla (Fig. 4a) and those in wheat shoots to 7 phyla (Fig. 4b). Endophytic bacteria that could not be categorized into any known group were classified as bacteria-unclassified. Interestingly, all of the bacterial phyla in wheat shoots were also found in wheat roots. *Proteobacteria* was the dominant endophytic bacterial population in wheat, accounting for >84% in roots and >95% in shoots, respectively. *Actinobacteria* was the second-most dominant endophytic bacterial population in wheat, accounting for 5.56–11.47% in roots and 0.22–0.81% in shoots (Table S1 in Supplementary materials). Each of the other bacteria phyla had relative abundances of <1% (Fig. 4).

Table 4 Alpha diversity of the entire endophytic bacterial community in wheat roots and shoots planted in yellow-brown soil after 30 days

Treatments		Chao index		Shannon index	
		Root	Shoot	Root	Shoot
S0	CP	381.00 ± 5.65 bc	89.00 ± 5.65 a	2.37 ± 0.07 a	0.89 ± 0.03 a
	CPB	362.00 ± 16.97 ab	111.50 ± 3.54 ab	2.36 ± 0.12 a	0.76 ± 0.03 a
S1	CP	354.00 ± 0.00 a	98.50 ± 2.12 ab	2.71 ± 0.13 abc	1.25 ± 0.16 b
	CPB	387.50 ± 2.12 c	140.00 ± 8.49 c	2.77 ± 0.06 bc	1.15 ± 0.01 b
S2	CP	445.00 ± 11.31 d	171.50 ± 10.61 d	2.68 ± 0.34 abc	1.29 ± 0.0.03 b
	CPB	466.50 ± 3.53 d	172.50 ± 17.68 d	2.99 ± 0.21 c	1.28 ± 0.09 b
S3	CP	353.50 ± 16.26 a	118.00 ± 9.90 bc	2.55 ± 0.07 ab	1.31 ± 0.16 b
	CPB	365.00 ± 8.49 abc	140.50 ± 9.19 c	2.39 ± 0.04 ab	1.26 ± 0.10 b

S0 is the control soil without phenanthrene addition. S1, S2 and S3 are phenanthrene-spiked soils aged for 30 days, and their phenanthrene concentrations before vegetation were 2.23, 30.24 and 134.64 mg kg^{-1}, respectively. CP means treatment with wheat but no Pn2 inoculation in soil, and CPB means treatment with wheat and Pn2 inoculation. Data after "±" are standard deviations (n = 3). Values in the same columns followed by the same letter are not significantly different at $p < 0.05$

The relative abundance of *Proteobacteria* in wheat roots grown in phenanthrene-contaminated soil was lower than that in wheat roots grown in uncontaminated soil, while the relative abundance of *Actinobacteria* in wheat roots grown in phenanthrene-contaminated soil was markedly higher than that in wheat roots grown in uncontaminated soil (Table S1 in Supplementary materials). However, no such tendency was detected in wheat shoots. Furthermore, inoculation of strain Pn2 influenced the relative abundance of several bacterial phyla. For example, Pn2 inoculation increased the relative abundance of *Firmicutes* compared with that in the absence of Pn2 inoculation irrespective of the contamination level (Table S1 in Supplementary materials).

Using an average relative abundance >1% to define dominance, the dominant endophytic bacterial genus in wheat roots was *Pseudomonas* (accounting for 6.88–50.71%), followed by *Stenotrophomonas*, *Arthrobacter*, *Halomonas*, *Rhizobium* and *Nocardioides* (Fig. S1A and Table S2 in Supplementary materials). In wheat shoots, the dominant endophytic bacterial genus was also *Pseudomonas* (accounting for 10.44–74.31%), followed by *Halomonas*, *Stenotrophomonas*, *Rhizobium* and *Arthrobacter* (Fig. S1B and Table S2 in Supplementary materials).

The relative abundances of the majority of the dominant bacterial genera (*Pseudomonas*, *Arthrobacter*, *Halomonas*, *Rhizobium* and *Nocardioides*; Table S2) in wheat roots grown in phenanthrene-contaminated soil were higher than those in wheat roots grown in uncontaminated soil, suggesting that these genera were capable of using phenanthrene as a carbon source and so might participate in phenanthrene degradation in internal wheat tissues. However, no such tendency was detected in wheat shoots. Furthermore, inoculation of Pn2 influenced the relative abundance of the majority of bacteria genera. For example, the relative abundance of *Pseudomonas* sp. in wheat roots inoculated with Pn2 was higher than that in those not inoculated with Pn2 in S0–S2 soils. However, the relative abundance of *Pseudomonas* sp. in wheat shoots inoculated with Pn2 was markedly lower than that in wheat shoots not inoculated with Pn2 in S0–S3 soils (Table S2 in Supplementary materials).

A hierarchical cluster dendrogram of endophytic bacterial community was generated based on the Bray–Curtis distance indices calculated using OTUs at a distance of 3% to further examine the similarity of endophytic bacterial community in wheat samples (Fig. 5). As shown in Fig. 5b, clustering of samples according to the 100 most dominant OTUs in wheat shoots showed greater similarity among wheat samples not inoculated with Pn2 in S0 soil than among samples subjected to other treatments. Furthermore, there was greater similarity among samples inoculated or not with Pn2. These results indicated that either phenanthrene contamination or Pn2 inoculation affected the bacterial community structure in wheat shoots.

Discussion

Phenanthrene can be absorbed by plants and translocated from roots to shoots in inner plant bodies (Gao and Zhu 2004; Collins et al. 2006). Cheema et al. (2010) evaluated the uptake capability of phenanthrene by four plant species, including tall fescue, ryegrass, alfalfa and rape seed, in spiked soil with 200.0 mg kg^{-1} of phenanthrene. The RCFs and SCFs of phenanthrene for these plants after 65-day growth were 0.38–1.37 and 0.12–0.49, respectively. In this study, after 30-day growth in soils contaminated with 134.64 mg kg^{-1} of phenanthrene, the RCFs for wheat uptake of phenanthrene were 0.41–0.46, while the SCFs were a little lower (0.05–0.07) than those reported by Cheema et al. (2010). The reason might be that high concentration of phenanthrene in soil S3 restrained wheat

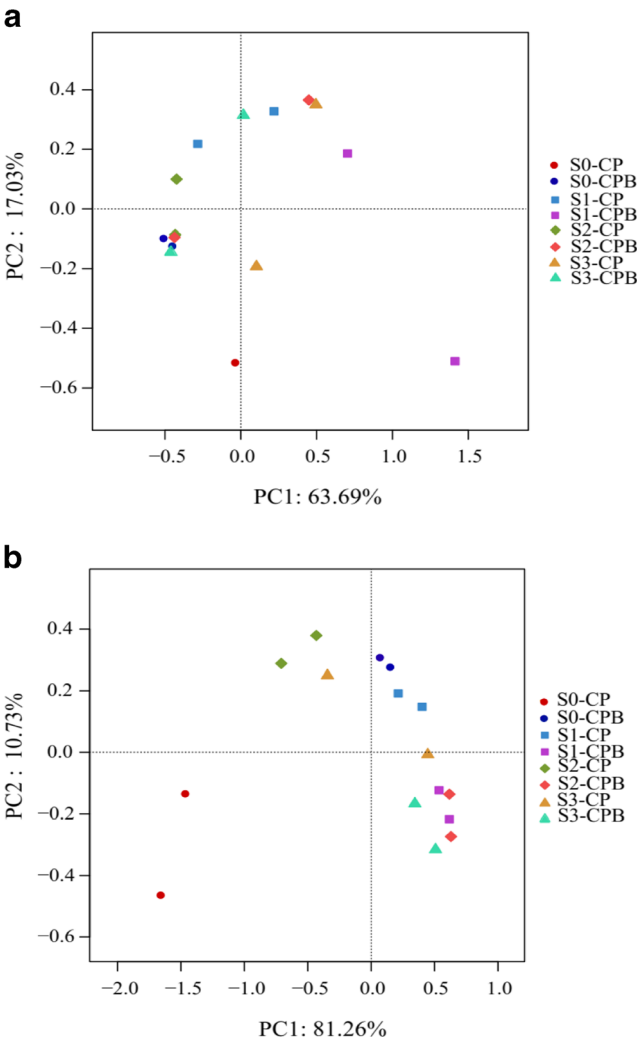

Fig. 2 Principal coordinates analysis of the endophytic bacterial community in wheat roots (**a**) and shoots (**b**) based on OTUs at a distance of 3%. *S0* is the control soil without phenanthrene addition. *S1*, *S2* and *S3* are phenanthrene-spiked soils aged for 30 days, and their phenanthrene concentrations before vegetation were 2.23, 30.24 and 134.64 mg kg^{-1}, respectively. *CP* means treatment with wheat but no Pn2 inoculation in soil, and *CPB* means treatment with wheat and Pn2 inoculation

root growth (Table 1), thus interfering with the translocation of phenanthrene from roots to shoots. The phenanthrene uptake by wheat could be accumulated in inner plant bodies, causing plant phenanthrene contamination risk.

Studies have attempted to enhance the removal of organic pollutants from inner plant tissues by exploiting the plant–endophyte interaction (Afzal et al. 2014). Bacteria colonized on root surfaces or into plant tissues have better chance to survive and proliferate than bacteria in soils, because the host plants can offer stable residence, protect them from the harsh environments and provide sustainable nutrients for their growth (Morrissey et al. 2004). Meanwhile, plant-associated bacteria may promote plant growth by increasing the availability of growth-limiting elements, producing phytohormones, exerting ACC deaminase activity and overcoming contaminant-induced stress responses (Weyens et al. 2009a,b). In this study, the phenanthrene-degrading endophytic bacterium Pn2, isolated from amur foxtail grown in PAH-contaminated soil, was successfully colonized into wheat tissues. Since both wheat and amur foxtail belong to the *Gramineae* family, the antagonistic effects of aboriginal endophytes to strain Pn2 in wheat should be weak, which endow strain Pn2 the potential to perform good phenanthrene-degrading ability in inner wheat tissues. In addition, the colonization of strain Pn2 in wheat overcomes the phenanthrene-induced stress to the growth of wheat roots under moderate levels of phenanthrene contamination in soils (Table 1).

The effective use of endophytic bacteria to ameliorate the contamination risk of plants with organic contaminants requires the presence of large numbers of contaminant-degrading bacteria within plant tissues (Afzal et al. 2014). PAH-degrading endophytes can colonize plant interiors and promote the PAH degradation in inner plant tissues (Compant et al. 2010; Khan et al. 2014). For instance, following colonization by the pyrene-degrading endophytic strain BJ06 of ryegrass via root inoculation for 15 days, BJ06 counts in ryegrass tissues were $>10^4$ CFU g^{-1} FW, and the pyrene concentrations in ryegrass roots and shoots in soil inoculated with endophytes were reduced by 31.0 and 44.2%, respectively, compared with those in endophyte-free soil (Sun et al. 2014). In this study, the respective Pn2 counts in wheat roots and shoots were 3.74 to 4.53 log CFU g^{-1} FW and 2.88 to 3.38 log CFU g^{-1} FW and increased with the enhanced level of phenanthrene contamination. Therefore, Pn2 was capable of using phenanthrene in inner wheat tissues as the carbon source for growth. Moreover, the phenanthrene concentration in inner wheat tissues inoculated with Pn2 was lower than that in wheat not inoculated with Pn2, indicating that the presence of large numbers of Pn2 in plant tissues reduced the level of phenanthrene contamination.

It has well been documented that large numbers of endophytic bacteria present in inner plant tissues. For instance, Garbeva et al. (2001) obtained 10^3–10^5 CFU g^{-1} FW of endophytic bacterial cells from potato, while Araújo et al. (2002) obtained 10^3–10^4 CFU g^{-1} FW of endophytic bacteria from citrus. Peng et al. (2013) found that the endophytic populations in amur foxtail and creeping oxalis growing at a PAH-contaminated site were 7.84×10^4–1.70×10^7 and 4.40×10^5–3.06×10^6 CFU g^{-1} FW, respectively. Similar results were observed in this investigation. The cultivable aboriginal endophytic bacterial counts in wheat ranged from 5.59 to 7.16 log CFU g^{-1} FW, and the cell counts decreased with the enhanced level of phenanthrene contamination in soils, showing the opposite tendency with strain Pn2. These results indicated that the phenanthrene absorbed from soil and accumulated in wheat inhibited the growth of phenanthrene-sensitive aboriginal endophytic bacteria but benefited that of the strain Pn2 in

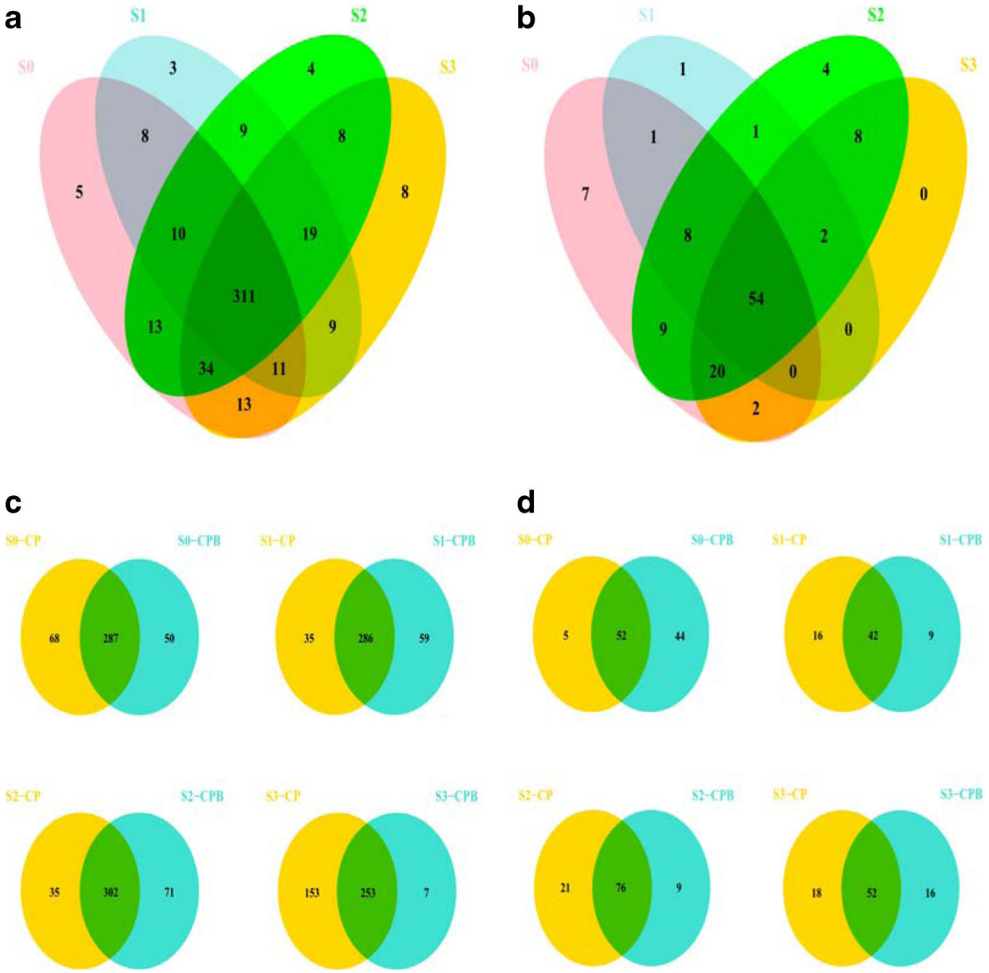

Fig. 3 Venn diagram showing shared and unique OTUs in wheat based on OTUs at a 97% similarity level. **a** Shared and unique OTUs in wheat roots without Pn2 inoculation under different levels of phenanthrene contamination. **b** Shared and unique OTUs in wheat shoots without Pn2 inoculation under different levels of phenanthrene contamination. **c** Shared and unique OTUs in wheat roots with and without Pn2 inoculation under different levels of phenanthrene contamination. **d** Shared and unique OTUs in wheat shoots with and without Pn2 inoculation under different levels of phenanthrene contamination. *S0* is the control soil without phenanthrene addition. *S1*, *S2* and *S3* are phenanthrene-spiked soils aged for 30 days, and their phenanthrene concentrations before vegetation were 2.23, 30.24 and 134.64 mg kg^{-1}, respectively. *CP* means treatment with wheat but no Pn2 inoculation in soil, and *CPB* means treatment with wheat and Pn2 inoculation

the inner tissue of wheat. The inoculation of Pn2 significantly increased the cell counts of aboriginal endophytic bacteria, indicating that strain Pn2 degraded phenanthrene in inner plant tissues and thus ameliorated the phenanthrene-inhibited growth of aboriginal endophytic bacteria.

Endophytic bacteria in plants are of great diversity, among which *Proteobacteria* has been reported to be the dominant phylum (Hallmann et al. 1997; Sun et al. 2008; Xie et al. 2015) and *Pseudomonas* is the dominant genus (Gardner et al. 1982; Tanprasert and Reed 1997; Huse et al. 2008). For instance, the majority of endophytic populations in amur foxtail growing at a PAH-contaminated site were *Proteobacteria* and *Firmicutes*; the dominant genus was *Pseudomonas* according to PCR-denaturing density gradient electrophoresis (DGGE) analysis (Peng et al. 2013). Moore et al. (2006) analysed the cultivable endophytic bacteria community composition in poplar grown at a site contaminated with benzene, toluene, ethylbenzene and xylene (BTEX) and observed the presence of diverse taxa; the dominant genus was *Pseudomonas*. Similarly, this investigation found that the dominant phylum in phenanthrene-contaminated wheat was *Proteobacteria* and the dominant genera were *Pseudomonas* sp. and *Stenotrophomonas* sp., indicating these taxa to be highly tolerant to PAHs.

In general, plant samples and pollution levels determine the distribution and diversity of endophytic bacterial populations (Oliveira et al. 2014). Peng et al. (2013) reported that the endophytic bacterial community profiles varied markedly depending on the plant origin, tissue and PAH level in soil, and the Shannon diversity indices differed among individual plants and according to PAH pollution level. Qing et al. (2006) documented the similar findings. In this study,

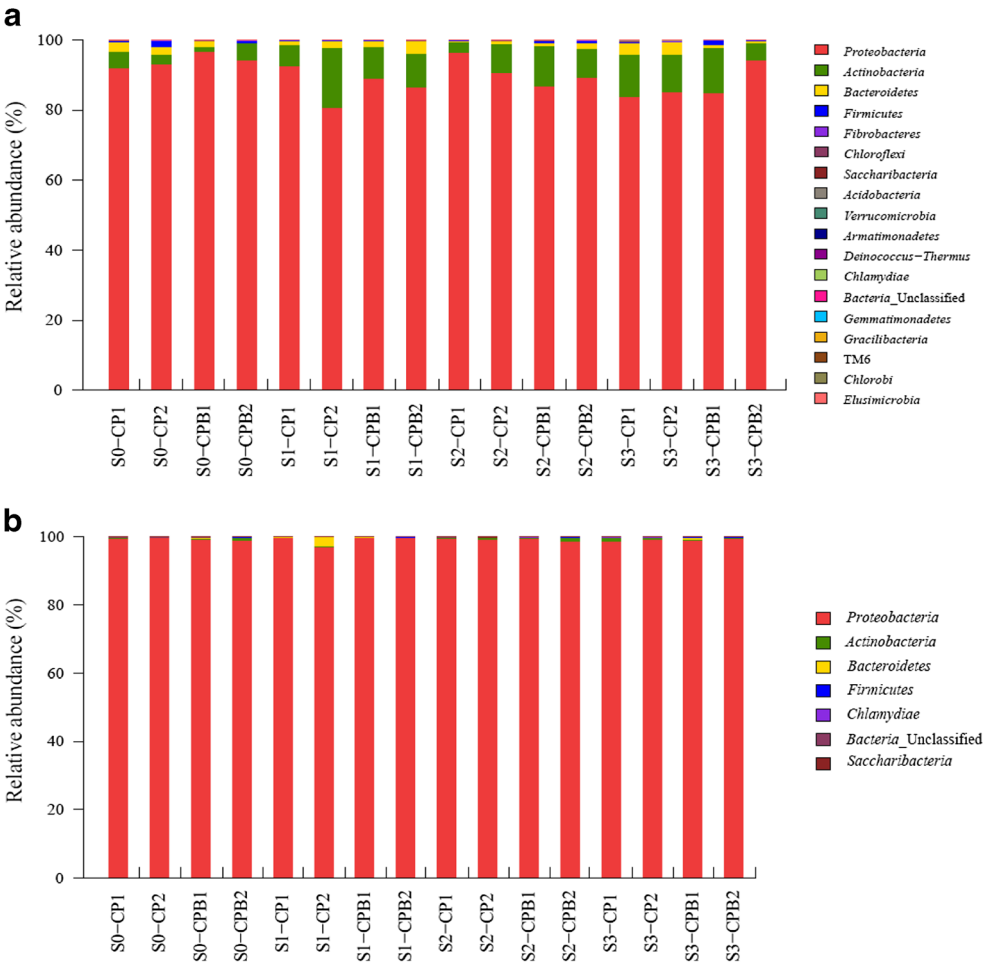

Fig. 4 Composition and relative abundance of endophytic bacterial phyla in wheat roots (**a**) and shoots (**b**). *S0* is the control soil without phenanthrene addition. *S1*, *S2* and *S3* are phenanthrene-spiked soils aged for 30 days, and their phenanthrene concentrations before vegetation were 2.23, 30.24 and 134.64 mg kg^{-1}, respectively. *CP* means treatment with wheat but no Pn2 inoculation in soil, and *CPB* means treatment with wheat and Pn2 inoculation

phenanthrene contamination increased obviously the diversity of endophytic bacteria in wheat. Low level of phenanthrene contamination enhanced, while high contamination level reduced the richness of endophytic bacterial populations in wheat. When the phenanthrene contamination increased, the relative abundance of *Proteobacteria* in wheat roots decreased, while that of *Actinobacteria* increased. This suggests that some taxa of *Actinobacteria* in wheat roots have the potential for phenanthrene degradation.

Little information is available on the impact of colonization by PAHDEB on aboriginal endophytic populations in plants contaminated with PAHs. In this study, the endophytic strain Pn2 was demonstrated to colonize and proliferate in wheat tissues. Furthermore, inoculation of Pn2 increased the cell counts, diversity and richness of endophytic bacteria in wheat, depending on the level of phenanthrene contamination. These results revealed that not only plant species and pollution level determine the count and diversity of endophytic bacteria but also that colonization by functional endophytic bacterium influences the biomass, diversity and richness of the endophytic bacterial population.

Moreover, inoculation of wheat with Pn2 affected the relative abundances of the dominant endophytic bacterial taxa, which may influence the capacity of the plant to metabolize phenanthrene. Phillips et al. (2008) analysed the relationship between the endophytic bacterial community and the organic pollutant degradation capacity of alfalfa. The results showed that when *Pseudomonas* sp. predominated, the capacity of plants to metabolize alkane pollutants increased, whereas when *Brevundimonas* sp. and *Pseudomonas rhodesiae* predominated, the capacity of the plant to metabolize aromatic hydrocarbons increased (Phillips et al. 2008). In this study, inoculation of Pn2 influenced the relative abundances of the majority of bacterial genera. For example, Pn2 inoculation increased and decreased the relative abundance of *Pseudomonas* sp. in wheat roots and shoots, respectively. These results suggest that inoculation of plant tissues with PDEB could affect the frequency and composition of the endophytic bacterial communities of inner plant tissues. However, the effect of the change in the dominant endophytic bacterial taxa due to Pn2 inoculation on the capacity of the plant to metabolize phenanthrene warrants further research.

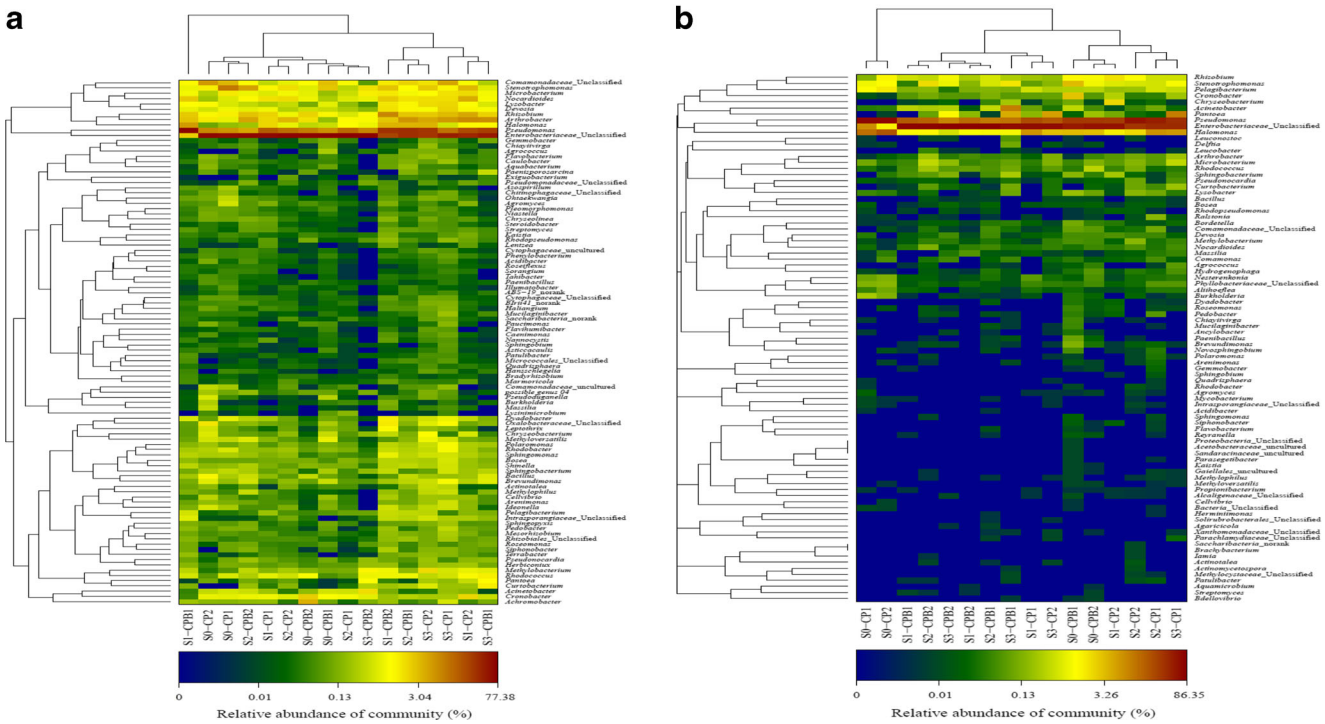

Fig. 5 The distribution of the top 100 abundant endophytic bacterial genera in wheat roots (**a**) and shoots (**b**). *S0* is the control soil without phenanthrene addition. *S1*, *S2* and *S3* are phenanthrene-spiked soils aged for 30 days, and their phenanthrene concentrations before vegetation were 2.23, 30.24 and 134.64 mg kg^{-1}, respectively. *CP* means treatment with wheat but no Pn2 inoculation in soil, and *CPB* means treatment with wheat and Pn2 inoculation

In summary, this is a primary investigation into the effect of colonization by a PDEB, strain Pn2, on the endophytic bacterial community structure in a phenanthrene-contaminated plant. Phenanthrene in soil was absorbed by wheat, and the phenanthrene accumulation increased the diversity of endophytic bacteria in inner wheat tissues. Inoculation of strain Pn2 changed the bacterial community structure and enhanced the cell counts, diversity and richness of endophytic bacteria in wheat in a phenanthrene contamination level-dependent manner. Although this study is limited in making broad conclusions due to the use of a single bacterial strain and plant host, and the interaction between the endophytic bacterial community and the plant capacity of phenanthrene metabolism requires further investigation, the results of this investigation provide insight into the responses of endophytic bacterial community in plant to external phenanthrene contamination and prove that the impacts of PDEB colonization on the bacterial community in inner plant tissues depend on the level of phenanthrene contamination.

Acknowledgements This work was supported by the Major State Basic Research Development Program of China (973 Program, 2015CB150505), the Fundamental Research Funds for the Central Universities (KYZ201516), the Natural Science Foundation of Jiangsu Province (BK20161454), the Special Fund for Agro-Scientific Research in Public Interest, China (201503107) and a project funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

Compliance with ethical standards

Ethical statement This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest The authors declared that they have no conflict of interest.

References

Afzal M, Khan QM, Sessitsch A (2014) Endophytic bacteria: prospects and applications for the phytoremediation of organic pollutants. Chemosphere 117:232–242

Ahmed AS, Sánchez CP, Candela ME (2000) Evaluation of induction of systemic resistance in pepper plants (*Capsicum annuum*) to *Phytophthora capsici* using *Trichoderma harzianum* and its relation with capsidiol accumulation. Eur J Plant Pathol 106:817–824

Amato KR, Yeoman CJ, Kent A, Righini N, Carbonero F, Estrada A, Gaskins HR, Stumpf RM, Yildirim S, Torralba M, Gillis M, Wilson BA, Nelson KE, White BA, Leigh SR (2013) Habitat degradation impacts black howler monkey (*Alouatta pigra*) gastrointestinal microbiomes. The ISME J 7:1344–1353

Araújo WL, Marcon J, Maccheroni W, Elsas JD, Vuurde JW, Azevedo JL (2002) Diversity of endophytic bacterial populations and their interaction with *Xylella fastidiosa* in citrus plants. Appl Environ Microbiol l68:4906–4914

Campbell BJ, Polson SW, Hanson TE, Mack MC, Schuur EA (2010) The effect of nutrient deposition on bacterial communities in Arctic tundra soil. Environ Microbiol 12:1842–1854

Cheema SA, Khana MI, Shen C, Tang X, Farooq M, Chen L, Zhang C, Chen Y (2010) Degradation of phenanthrene and pyrene in spiked soils by single and combined plants cultivation. J Hazard Mater 177: 384–389

Chelius MK, Triplett EW (2001) The diversity of Archaea and Bacteria in association with the roots of *Zea mays* L. Microb Ecol 41:252–263

Chen H, Boutros PC (2011) VennDiagram: a package for the generation of highly-customizable Venn and Euler diagrams in R. BMC Bioinformatics 12(1):35

Collins CD, Fryer M, Grosso A (2006) Plant uptake of non-ionic organic chemicals. Environ Sci Technol 40:45–52

Compant S, Clement C, Sessitsch A (2010) Plant growth-promoting bacteria in the rhizo and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. Soil Biol Biochem 42:669–678

Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26:2460–2461

Gao YZ, Zhu LZ (2004) Plant uptake, accumulation and translocation of phenanthrene and pyrene in soils. Chemosphere 55:1169–1178

Gao YZ, Zhang Y, Liu J, Kong HL (2013) Metabolism and subcellular distribution of anthracene in tall fescue (*Festuca arundinacea* Schreb.) Plant Soil 365:171–182

Gao YZ, Hu XJ, Zhou ZY, Zhang W, Wang YZ, Sun BQ (2017) Phytoavailability and mechanism of bound PAH residues in filed contaminated soils. Environ Pollut. doi:10.1016/j.envpol.2016.11.076

Garbeva P, Overbeek VL, Vuurde VJ, Elsas VJ (2001) Analysis of endophytic bacterial communities of potato by plating and denaturing gradient gel electrophoresis (DGGE) of 16S rDNA based PCR fragments. Microb Ecol 41:369–383

Gardner JM, Feldman AW, Zablotowicz M (1982) Identity and behavior of xylem-residing bacteria in rough lemon roots of Florida citrus trees. Appl Environ Microbiol 43:1335–1342

Germaine KJ, Keogh E, Ryan D, Dowling DN (2009) Bacterial endophyte-mediated naphthalene phytoprotection and phytoremediation. FEMS Microbiol Lett 296:226–234

Glick BR (2003) Phytoremediation: synergistic use of plants and bacteria to clean up the environment. Biotechnol Adv 21:383–393

Gotelli NJ, Colwell RK (2011) Estimating species richness. In: Magurran AE, McGill BJ (eds) Biological diversity: frontiers in measurement and assessment. Oxford University Press, Oxford, pp 39–54

Hallmann J, Quadt-Hallmann A, Mahafee WF, Kloepper JW (1997) Bacterial endophytes in agricultural crops. Can J Microbiol 43: 895–914

Harish S, Kavino M, Kumar N, Saravanakumar D, Soorianathasundaram K, Samiyappan R (2008) Biohardening with plant growth promoting rhizosphere and endophytic bacteria induces systemic resistance against banana bunchy top virus. Appl Soil Ecol 39:187–200

Ho YN, Shih CH, Hsiao SC, Huang CC (2009) A novel endophytic bacterium, *Achromobacter xylosoxidans*, helps plants against pollutant stress and improves phytoremediation. J Biosci Bioeng 108: S75–S95

Hung PQ, Annapurna K (2004) Isolation and characterization of endophytic bacteria in soybean (*Glycine* sp.) Omonrice 12:92–101

Huse SM, Dethlefsen L, Huber JA, Mark WD, Relman DA, Sogin ML (2008) Exploring microbial diversity and taxonomy using SSU rRNA hypervariable tag sequencing. PLoS Genet 4:e1000255

Hwang S, Cutright TJ (2002) Biodegradability of aged pyrene and phenanthrene in a natural soil. Chemosphere 47:891–899

Johnsen AR, Wick LY, Harms H (2005) Principles of microbial PAH-degradation in soil. Environ Pollut 133:71–84

Khan Z, Doty S (2011) Endophyte-assisted phytoremediation. Curr Top Plant Biol 12:97–105

Khan Z, Roman D, Kintz T, delas Alas M, Yap R, Doty S (2014) Degradation, phytoprotection and phytoremediation of phenanthrene by endophyte *Pseudomonas putida*, PD1. Environ Sci Technol 48:12221–12228

Kim KH, Jahan SA, Kabir E, Brown RJ (2013) A review of airborne polycyclic aromatic hydrocarbons (PAHs) and their human health effects. Environ Inter 60:71–80

Liengme BV (2015) Guide to Microsoft Excel 2013 for scientists and engineers. Academic Press, London

Liu J, Liu S, Sun K, Sheng YH, Gu YJ, Gao YZ (2014) Colonization on root surface by a phenanthrene-degrading endophytic bacterium and its application for reducing plant phenanthrene contamination. PLoS One 9:e108249

Lodewyckx C, Vangronsveld J, Porteous F, Moore ER, Taghavi S, Mezgeay M, der Lelie DV (2002) Endophytic bacteria and their potential applications. Crit Rev Plant Sci 21:583–606

Lozupone C, Lladser ME, Knights D, Stombaugh J, Knight R (2011) UniFrac: an effective distance metric for microbial community comparison. ISME J 5:169

Ministry of Environmental Protection and Ministry of Land and Resource of the People's Republic of China (MEP and MLR) (2014) The bulletin of nationwide soil pollution status survey April 14. Index No. 000014672/2014–00351

Moore FP, Barac T, Borremans B, Oeyen L, Vangronsveld J, van der Lelie D, Campbell CD, Moore ER (2006) Endophytic bacterial diversity in poplar trees growing on a BTEX-contaminated site: the characterisation of isolates with potential to enhance phytoremediation. Syst Appl Microbiol 29:539–556

Morrissey JP, Dow JM, Mark GL (2004) Are microbes at the root of a solution to world food production? EMBO Rep 5:922–926

Oliveira V, Gomes N, Almeida A, Silva A, Simões MM, Smalla K, Cunha Â (2014) Hydrocarbon contamination and plant species determine the phylogenetic and functional diversity of endophytic degrading bacteria. Mol Ecol 23:1392–1404

Peng A, Liu J, Gao YZ, Chen ZY (2013) Distribution of endophytic bacteria in *Alopecurus aequalis* Sobol and *Oxalis corniculata* L. from soils contaminated by polycyclic aromatic hydrocarbons. PLoS One 8:e83054

Phillips LA, Germida JJ, Farrell RE (2008) Hydrocarbon degradation potential and activity of endophytic bacteria associated with prairie plants. Soil Biol Biochem 40:3054–3064

Qing W, Zhao X, Zhao SY (2006) Application of PCR-DGGE in research of bacterial diversity in drinking water. Biomed Environ Sci 19:371–374

R Development Core Team (2013) R: a language and environment for statistical computing. Available on line at: http://www.r-project.org/

Rajkumar M, Ae N, Freitas H (2009) Endophytic bacteria and their potential to enhance heavy metal phytoextraction. Chemosphere 77: 153–160

Reysenbach A, Pace N (1995) Reliable amplification of hyperthermophilic archaeal 16S rRNA genes by the polymerase chain reaction. In: Robb F (ed) Archaea: a laboratory manual. Cold Spring Harbor Laboratory Press, New York, pp p101–p107

Ryan RP, Germaine K, Franks A, Ryan DJ, Dowling DN (2008) Bacterial endophytes: recent developments and applications. FEMS Microbiol Lett 278:1–9

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Sahl JW (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7537–7541

Sheng XF, Gong JX (2006) Increased degradation of phenanthrene in soil by *Pseudomonas* sp. GF3 in the presence of wheat. Soil Biol Biochem 38:2587–2592

Siciliano SD, Fortin N, Mihoc A, Wisse G, Labelle S, Beaumier D, Schwab P (2001) Selection of specific endophytic bacterial genotypes by plants in response to soil contamination. Appl Environ Microbiol 67:2469–2475

Sobral JK, Araujo WL, Mendes R, Kleiner AAP, Joao LA (2005) Isolation and characterization of endophytic bacteria from soybean (*Glycine max*) grown in soil treated with glyphosate herbicide. Plant Soil 273:91–99

Spellerberg IF (2008) Shannon–Wiener index. Lincoln University Press, Lincoln, pp 3249–3252

Sun L, Qiu F, Zhang X, Dai X, Dong X, Song W (2008) Endophytic bacterial diversity in rice (*Oryza sativa* L.) roots estimated by 16S rDNA sequence analysis. Microb Ecol 55:415–424

Sun K, Liu J, Jin L, Gao YZ (2014) Utilizing pyrene-degrading endophytic bacteria to reduce the risk of plant pyrene contamination. Plant Soil 374:251–262

Tanprasert P, Reed BM (1997) Detection and identification of bacterial contaminants from strawberry runner explants. In Vitro Cell Dev Biol 33:221–226

Weyens N, van der Lelie D, Taghavi S, Newman L, Vangronsveld J (2009a) Exploiting plant-microbe partnerships to improve biomass production and remediation. Trends Biotechnol 27:591–598

Weyens N, van der Lelie D, Taghavi S, Vangronsveld J (2009b) Phytoremediation: plant-endophyte partnerships take the challenge. Curr Opin Biotechnol 20:248–254

Xie WY, Su JQ, Zhu YG (2015) Phyllosphere bacterial community of floating macrophytes in paddy soil environments as revealed by Illumina high-throughput sequencing. Appl Environ Microbiol 81: 522–532

Yaws CL (1999) Chemical properties handbook. McGraw-Hill, New York