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PGPR enhanced phytoremediation of petroleum contaminated soil and rhizosphere microbial community response

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HIGHLIGHTS

- PGPR can enhance C21–C34 fraction of TPH removal during phytoremediation.
- Petroleum removal was irrelative with bacterial community diversity.
- Specific degraders and biosurfactant producers were the driving factor of TPH removal.

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ABSTRACT

The aim of this study was to investigate petroleum phytoremediation enhancement by plant growth promoting bacteria (PGPR), specifically the correlation between petroleum hydrocarbon fractions and bacterial community structure affected by remediation and PGPR inocula. Aged petroleum contaminated soil was remediated by tall fescue (*Testuca arundinacea* L.) inoculated with two PGPR strains. Hydrocarbon degradation was measured by GC–MS (Gas-chromatography Mass-spectrometer) based on carbon fraction numbers (C8–C34). Changes in bacterial community structure were analyzed by high-throughput pyrosequencing of 16s rRNA. PGPR inoculation increased tall fescue biomass and petroleum hydrocarbons were removed in all the treatments. Maximum hydrocarbon removal, particular high molecular weight (C21–C34) aliphatic hydrocarbons (AHs) and polycyclic aromatic hydrocarbons (PAHs), was observed in tall fescue inoculated with PGPR. The relative abundance of phyla *γ-proteobacteria* and *Bacteroidetes* increased after different treatments compared with controls. Moreover, a bacterial guild mainly comprising the genera *Lysobacter*, *Pseudoxanthomonas*, *Planctomyces*, *Nocardioides*, *Hydrogenophaga*, *Ohtaekwangia* was found to be positively correlated with C21–C34 petroleum hydrocarbons fractions removal by RDA analysis, implying that petroleum degradation was unrelated to bacterial community diversity but positively correlated with specific petroleum degraders and biosurfactant producers.

1. Introduction

Petroleum contamination continues to be of serious environmental concern due to the sustained growth of crude oil extraction and associated production (Jurelevicius et al., 2013). This has led to a search for sustainable methods for the remediation of contaminated environments (Pizarro-Tobías et al., 2015). Phytoremediation for the *in situ* remediation for petroleum contaminated soil is being seen as an efficient, sustainable and cost-effective remediation technique compared to conventional physical–chemical techniques (Pizarro-Tobías et al., 2015). During phytoremediation, biodegradation of petroleum organic components is promoted by the synergy between plants and the microorganisms present in the rhizosphere, the region of soil that is directly influenced by root secretions (Yateem, 2013).

Successful phytoremediation is dependent on the survival and growth of plants on contaminated sites, as well as the ability of the rhizosphere to support an active soil microbial population (Cook and Hesterberg, 2013). In contaminated areas, soil microbial populations are often restricted by petroleum toxins and nutrient deficiency (Wang et al., 2011). To promote phytoremediation, contaminant-tolerant plant species with rapid growth characters and vigorous root system, like tall fescue, have been utilized

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(Liu et al., 2014). Plant growth promoting rhizobacteria (PGPR) have also been used as inocula to further increase plant growth, reduce environmental stress and promote degradation by rhizosphere-associated microorganism (Khan et al., 2013).

Numerous pot experiments and field trials have reported success in total petroleum hydrocarbon removal utilizing PGPR inocula in association with tolerant plants (Agarry et al., 2013; Liu et al., 2013). The survival of introduced PGPR, and subsequent changes to the rhizosphere associated microbial community structure, are considered to be key factors for successful phytoremediation (Agarry et al., 2013). However, studies reporting changes in the microbial community have been mostly performed by traditional techniques such as T-RFLP and DGGE (Ribeiro et al., 2013; Wei et al., 2014). These techniques often have a low taxonomic resolution and do not provide information on low abundance microbial species in the rhizosphere. In recent years, high-throughput sequencing technologies with the advantages of high throughput and low cost, like the Illumina platform, are becoming more widely used and allow analysis of microbial community diversity through 16s rRNA analysis (Das and Kazy, 2014).

Considerable attention has been paid to total petroleum hydrocarbons (TPHs) removal efficiency during phytoremediation. However, this may not be suitable as petroleum hydrocarbon is a very complex mixture composed mainly of aliphatic hydrocarbons (AHs) and polycyclic aromatic hydrocarbons (PAHs), each with different carbon chain lengths and structures with different physicochemical and toxicity properties. The variation in the relative abundance of these components is potentially important in influencing the degradation efficiency and bacterial activity (Hamamura et al., 2013). Therefore, more comprehensive analysis of petroleum hydrocarbon is required and fractionation, in terms of aliphatic and aromatic compounds, and Equivalent Carbon Number (EC), determined by GC–MS, would be useful in such studies.

To better elucidate the mechanisms of phytoremediation enhancement by PGPR, particularly the correlation between bacterial community and components of petroleum hydrocarbons, a pot experiment using tall fescue plants was designed in weathered petroleum contaminated soil. The affects of fertilization, planting only, and planting with two PGPR strains were studied using GC–MS, Illumina sequencing and multivariate statistic analysis to monitor the different fractions of petroleum hydrocarbons and bacterial community to elucidate their relationship in the PGPR assisting phytoremediation process.

2. Material and methods

2.1. Soil and bacterial strains

Weathered contaminated soil was collected near an individual oil production well at Shengli oilfield, Shandong province, China (available nitrogen, 34.0 mg kg^{-1}; available P, 28.0 mg kg^{-1}; pH, 8.7; salt content, 0.17%).

PGPR strains *Klebsiella* sp. D5A and *Pseudomonas* sp. SB were obtained from the Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing, China. They had been deposited in the Chinese Culture Collection Management Committee General Microbiology Center (CGMCC), accession numbers are CGMCC Nos. 7248 and 7246, respectively.

2.2. Pot experiment

Tall fescue seeds were sterilized in 70% ethanol for 2 min and in 1% sodium hypochlorite for 10 min then rinsed twice with sterile water before planting. Experimental design consisted of 5 treatments in 3 replicates. The five treatments were: (1) no-fertilizer (Control); (2) fertilizer (F); (3) fertilizer + tall fescue (FP); (4) fertilizer + tall fescue + *Klebsiella* sp. D5A (FP + D5A); and (5) fertilizer + tall fescue + *Pseudomonas* sp. SB (FP + SB). Plant pots were filled with 2.0 kg 4-mm sieved air-dry soil and water content of the soil was maintained at about 60% field capacity during the experimental period. Inorganic nutrients $(NH_4)_2SO_4$ and K_2HPO_4 were added to all treatments except control to give a final rates of 250 mg N kg^{-1} and 100 mg P kg^{-1}.

PGPR inoculation treatments were prepared by centrifugation at 9000 rpm for 15 min at 4 °C of exponential growth phase cultures grown in Luria Broth medium. Cell pellets were washed with sterile distilled water before resuspension in sterile distilled water to form inocula with a density of 10^9 colony-forming units (cfus) mL^{-1}. PGPR inocula (15 mL pot^{-1}) were mixed with soil before planting to give a final inoculation of 10^7 cfus kg^{-1} soil in accordance with Bashan (1986). Plants were grown for 4 months in a sunlight greenhouse. At harvest, the roots and shoots of plants were collected and washed with deionized water. Soil from each pot was sampled and stored at 4 °C for TPH analysis or −80 °C for microbial community analysis. Dry weights of the shoots and roots were measured.

2.3. Quantification of petroleum hydrocarbons

Soil samples were lyophilized and passed through a 0.25-mm sieve before TPH analysis. Aliquots of 2 g of the soil sample were mixed with 2 g of anhydrous sodium sulfate and extracted using 70 mL dichloromethane in a Soxhlet extractor for 24 h. The extracts were rotary-evaporated while being solvent-exchanged into 10 mL of hexane. The hexane extract was centrifuged and 3 mL of supernatant was chromatographed on a 0.5 cm (i.d.) × 20 cm column containing 0.5 g anhydrous sodium sulfate, 2 g aluminum oxide and 3 g activated silica gel (200–325 mesh size) from top to bottom. The fraction of aliphatic hydrocarbons was eluted by 30 mL of hexane before the fraction of polycyclic aromatic hydrocarbons was eluted by 15 mL of a 2/1 mixture of dichloromethane/hexane from the column. The eluates were concentrated to 2 mL for GC–MS analysis.

GC–MS analysis was performed using an Agilent 7890 GC-5975 MSD fitted with a capillary column (RESTEK, USA) DB-5 ms (30 m × 0.25 mm i.d., 0.25 μm). For fractions of aliphatic hydrocarbons, the carrier gas was He at 1.4 mL min^{-1}; the injection temperature was 300 °C; the temperature program was: 50 °C (hold 5 min) to 45 °C at 2 °C min^{-1} and then to 310 °C (hold 10 min) at 20 °C min^{-1}. For fractions of polycyclic aromatic hydrocarbons, the carrier gas was He at 1.4 mL min^{-1}; the injection temperature was 280 °C; the temperature program was: 50 °C (hold 3 min) to 40 °C at 2 °C min^{-1} and then to 300 °C (hold 5 min) at 25 °C min^{-1}. The setup of external standard calibrations and quantity analysis of different fractions were following the WSDE (1997) method.

2.4. Soil DNA extraction and PCR amplification

Microbial DNA was extracted from 2 g of soil from each replicate of the five treatments using a soil DNA kit (Fast DNA SPIN for soils, MP Biomedicals, Solon, OH). The V4-V5 region of the bacteria 16s ribosomal RNA gene were amplified by PCR (95 °C for 2 min, followed by 25 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s and a final extension at 72 °C for 5 min) using primers 515F 5′-barcode- GTGCCAGCMGCCGCGG-3′ and 907R 5′-CC GTCAATTCMTTTRAGTTT-3′, where the barcode is an eight-base sample-specific sequence. PCR reactions were performed in triplicate 20 μL mixture containing 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu

Polymerase, and 10 ng of template DNA. Paired-end sequenced (2 × 250) Sequencing was conducted on Illumina MiSeq platform by Majorbio Bio-pharm Technology Co., Ltd. (Shanghai, China).

2.5. Data analysis

Sequence reads were trimmed by Trimmomatic (Bolger et al., 2014); after trimming, reads were assembled by Flash software (Magoč and Salzberg, 2011) for further analysis by MOTHUR 1.33.0 (Kozich et al., 2013). Unique sequences were aligned to the reference SILVA database by default settings and chimeric sequences were removed. Sequences passing these screens were classified using a Ribosomal Database Project naïve Bayesian rRNA classifier with a confidence of 80%. At each taxonomic level, the proportion of sequence identities was calculated as a percent of all sequences classified in that sample. OTUs (operational taxonomic units) were classified at similarities of 97% after normalized to 7369 sequences per sample. The α-diversity indices, including observed OTUs (Sobs), Chao, Ace, InvSimpson and Shannon, were calculated using MOTHUR. Bacterial community structure, based on the OTU composition, was preformed by principal coordinates analysis (PCoA) conducted in R (Version 3.1.2). The correlation between the frequencies of top 30 abundant genera of each treatment, treatment type and degradation of petroleum hydrocarbon fractions was compared by RDA with Monte Carlo permutation test in R software. The Monte Carlo tests were based on 1000 random permutations of the data. The nucleotide sequences produced in the current study have been deposited in the European Nucleotide Archive under accession number PRJEB8995.

2.6. Statistical analysis

Statistical analysis was conducted with SAS 9.1 software. Duncan's multiple range tests were used to compare the means of treatments; variability in the data was expressed as the standard errors. All analyses were performed at the $p < 0.05$ level.

3. Results

3.1. Plant biomass and soil properties

Shoot biomass showed no significant difference between treatments, however, tall fescue inoculated with D5A had a significantly greater root biomass than other treatments (Table 1). Of the four soils physicochemical properties determined (Table 1), only available phosphorus had significant difference between treatments. Available phosphorus was significantly lower in the control and reduced in the FP compared to the other three treatments after four months phytoremediation.

3.2. Petroleum hydrocarbon removal

The AHs and PAHs fractions of the original contaminated soil (before phytoremediation) were reduced after four months of phytoremediation (Fig. 1). The concentration of TPH (sum of AHs and PAHs) was 4407 mg kg^{-1}, 3316 mg kg^{-1}, 2533 mg kg^{-1}, 2097 mg kg^{-1}, 1674 mg kg^{-1}, 1443 mg kg^{-1} in original soil, treatment control, F, FP, FP + D5A, FP + SB respectively. The total AHs decreased by 28.5%, 43.1%, 52.9%, 62.6%, 67.9% and PAHs by 34.7%, 38.7%, 49.6%, 58.1%, 62.9% in control, F, FP, FP + D5A, FP + SB respectively. The concentration of AHs (C8–C16) was higher in F, FP + D5A and FP + SB compared with original

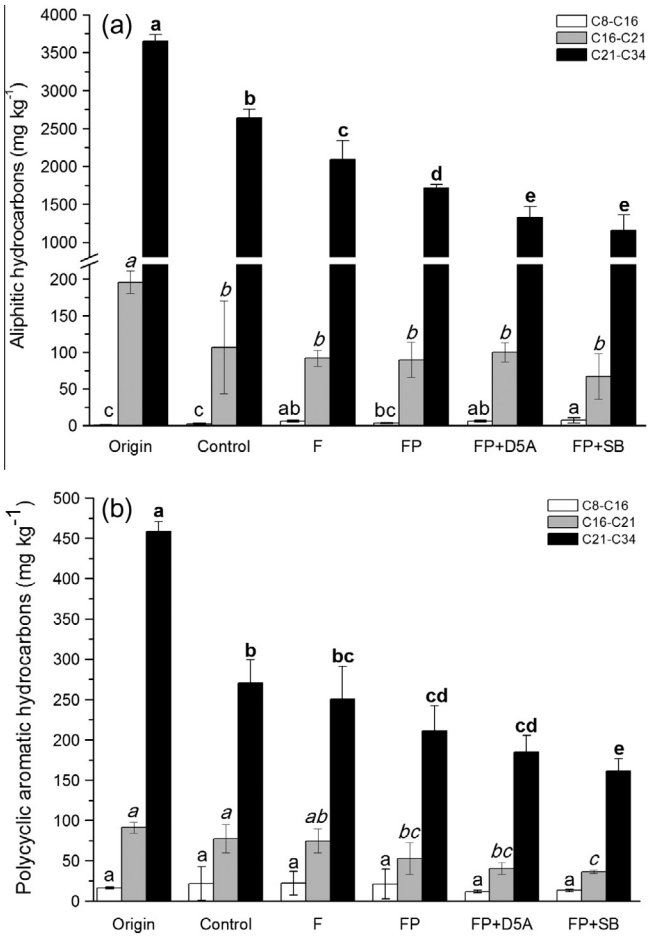

Fig. 1. Aliphatic hydrocarbon (a) and polycyclic aromatic hydrocarbon concentration (b) of different treatments. Vertical bars represent standard deviation ($n = 3$). Letters on columns indicate statistically significant differences for each petroleum fraction among treatments at $p < 0.05$ according to Duncan test.

Table 1
Plant biomass and soil properties of different treatments.

	Treatments				
	Control	F	FP	FP + D5A	FP + SB
Shoot biomass (dry weight g pot^{-1})	–	–	5.51 ± 0.13a	6.09 ± 0.59a	6.24 ± 0.57a
Root biomass (dry weight g pot^{-1})	–	–	7.65 ± 0.94b	10.0 ± 1.1a	8.39 ± 1.07ab
Total organic matter (g kg^{-1} soil)	20.0 ± 1.3a	20.0 ± 0.4a	19.0 ± 1.3a	20.5 ± 1.8a	18.4 ± 1.9a
Available phosphorus (mg kg^{-1}soil)	4.90 ± 0.82c	21.5 ± 2.9a	13.0 ± 7.0b	20.0 ± 4.2ab	19.2 ± 1.2ab
Available potassium (mg kg^{-1} soil)	118 ± 0a	113 ± 29a	108 ± 9 a	111 ± 10a	110 ± 5a
Available nitrogen (mg kg^{-1} soil)	30.9 ± 8.2b	67.3 ± 3.7a	41.9 ± 10.6b	37.3 ± 11.6b	38.2 ± 8.0b

Each treatment is reported as the mean ± standard error of three replicates. Means followed by a different letter within each row are significantly different at $p < 0.05$ according to the Duncan test.

contaminated soil and control treatment (Fig. 1a) while there was no significant difference in PAHs (C8–C16) between treatments and original contaminated soil (Fig. 1b). The concentration of AHs (C16–C21) showed no significant difference between different treatments, but was lower than that in original soil (Fig. 1a). For PAHs (C16–C21), concentrations were lower in FP, FP + D5A and FP + SB while no significant difference was observed between F, original soil and control (Fig. 1b). The concentration of high molecular weight AHs (C21–C34), which accounted for approximately 81% of the total petroleum hydrocarbons, was significantly lower ($p < 0.05$) in three treatments with plants compared with that of the control, and the inoculation of SB and D5A further decreased AHs contents. Similar with AHs fraction content, high molecular weight PAHs (C21–C34) decreased more in three planted treatment and the maximum was found in tall fescue + SB.

3.3. Pyrosequencing and sequence analysis

A total of 233,924 sequences were obtained after a sequence optimization process, with an average number of 11,615 sequences per sample (ranging from 7639 to 16,358). A total of 7639 reads were subsampled from each replicate for further analysis.

PCoA analysis on Bray–Curtis dissimilarity matrices of OTUs at 97% cutoff was utilized to further investigate the microbial community differences between the treatments. Analysis showed that samples from the same treatment clustered together and those from different treatments separated (Fig 2). The PCoA plot revealed community level differences between the control, F and plant (FP, FP + D5A and FP + SB) microbiota. The first component differentiated treatments based on fertilized and non-fertilized regimes while the second component differentiated treatments based on the presence or absence of plants.

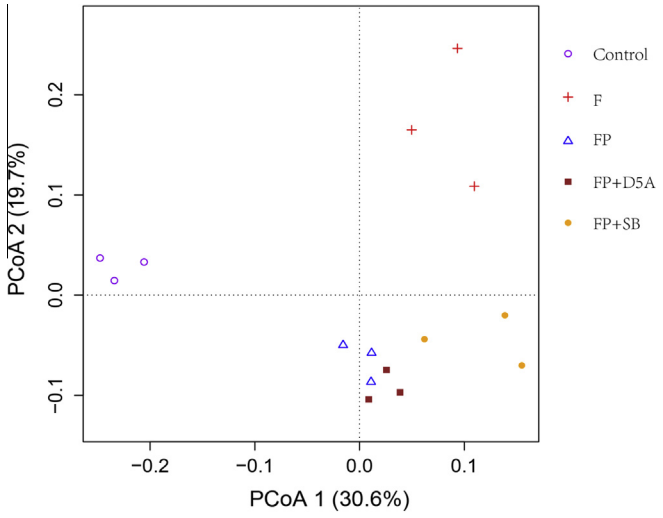

Fig. 2. Comparison of bacterial 16s rRNA communities by different treatments using principle coordination analysis. The percentages in *parentheses* indicate the proportions of variation by each ordination axis.

The calculated bacterial community richness index showed no significant differences in Sobs, Chao and ACE indexes in different treatments Table 2). However, The InvSimpson and Shannon index were significantly lower in the fertilizer treatments than in the control and other treatments with plant (FP, FP + D5A, FP + SB).

3.4. Taxonomic composition analysis

Amplified sequences were classified into 23 phyla (Bacteria 21 and Archaea 2). The overall bacterial composition of the different treatments was similar, while the distribution of each phylum or group varied (Fig. 3). In all treatments, *Proteobacteria*, *Actinobacteria*, *Acidobacteria* and *Bacteroidetes* were the four most dominant phyla, except for the unclassified group, accounting for >60% of the sequences in all the treatments. *γ-proteobacteria* was the most predominant subclass of *Proteobacteria* accounting for 46.71–66.99% of the proteobacterial sequences. In all the detected phyla *γ-proteobacteria* and *Bacteroidetes* increased after fertilization and planting. Inoculation of the *Klebsiella* sp. strain D5A and *Pseudomonas* sp. SB, in the rhizosphere soil correlated to an increase in relative abundance at genus level (Fig. 4). Relative abundance of other dominant bacteria, belonging to genus *Lysobacter*, *Pseudoxanthomonas*. *Perlucidibaca*, *Ohtaekwangia*, *Planctomyces*, *Nocardioides*, *Hydrogenophaga*, increased compared with control and the last five genera also increased compared with F. Redundancy analysis (RDA) indicated these genus positively correlated with high removal efficiency of C8–C16 PAHs, C21–C34 AHs and C21–C34 PAHs in P + PGPR (Fig. 5). C8–C16 AHs, C21–C34 AHs and C21–C34 PAHs were three determined factors that contributed significantly ($p < 0.05$) to explaining the variations in community composition in five treatments.

4. Discussions

Phytoremediation enhanced by PGPR has been widely implemented to remove organic pollutants such as petroleum hydrocarbons from contaminated soil with great success (Fester et al., 2014). However, the enhancement of the petroleum removal in this process has been frequently shown through measurements of bulk properties like TPH concentration. Such measurements may not be a suitable measure because petroleum is assemblage of complex compounds varying in relative abundance and toxicity, bulk properties analysis cannot provide detailed compositional characteristics of petroleum required to understand its fate (Kim et al., 2013). There are no uniform soil TPH levels in Environmental Quality Standards of many countries range from 1000 to 5000 mg kg^{-1} (0.1–0.5%). Therefore, a more appropriate evaluation approach, which combined column chromatography with GC–MS to analyze different petroleum hydrocarbon divisions (AHs and PAHs) and carbon number based fractions (C8–C34), was used in this study.

Table 2
Richness and diversity of 16s rRNA gene sequences from different treatments.

Treatments	Sobs	Chao	Ace	InvSimpson	Shannon
Control	2523 ± 101a	8764 ± 1000a	15722 ± 2048a	295 ± 30a	6.80 ± 0.06a
F	2279 ± 238a	7819 ± 902a	15006 ± 2189a	64 ± 36d	6.16 ± 0.42b
FP	2516 ± 288a	10122 ± 3486a	20522 ± 8807a	233 ± 25b	6.70 ± 0.14a
FP + D5A	2416 ± 137a	9380 ± 1619a	17507 ± 4126a	220 ± 21b	6.62 ± 0.02a
FP + SB	2325 ± 94a	9530 ± 703a	18245 ± 1578a	136 ± 31c	6.42 ± 0.14ab

Each treatment is reported as the mean ± standard error of three replicates. Means followed by a different letter within each row are significantly different at $p < 0.05$ according to the Duncan test.

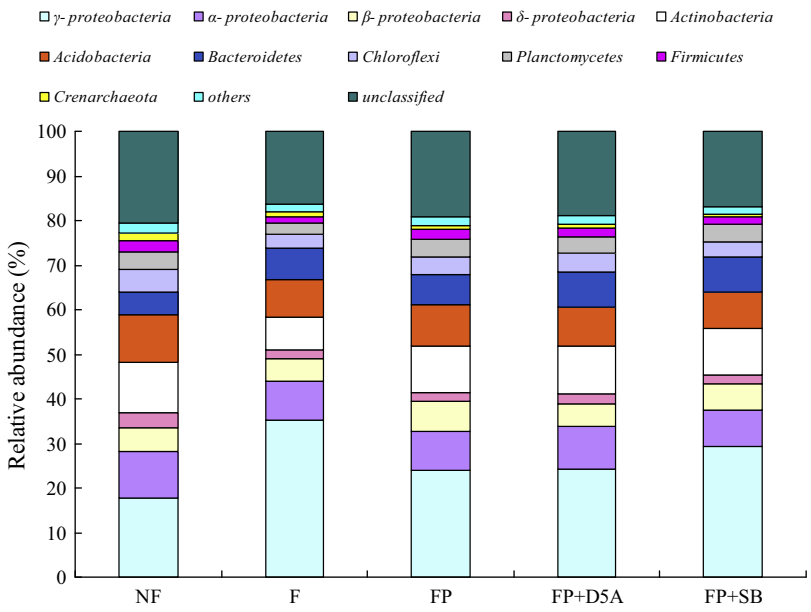

Fig. 3. Comparison of the bacterial community at the phylum level and classes of *Proteobacteria*. Relative read abundance of different bacterial phyla within the different communities. The phyla names in the legend at the top appears in order with the phylum in the graph, from left to right. Phyla that compose less than 0.1% of the bacteria and archaea in all treatments are grouped into "Others". Sequences that could not be classified into any known group were assigned as "unclassified".

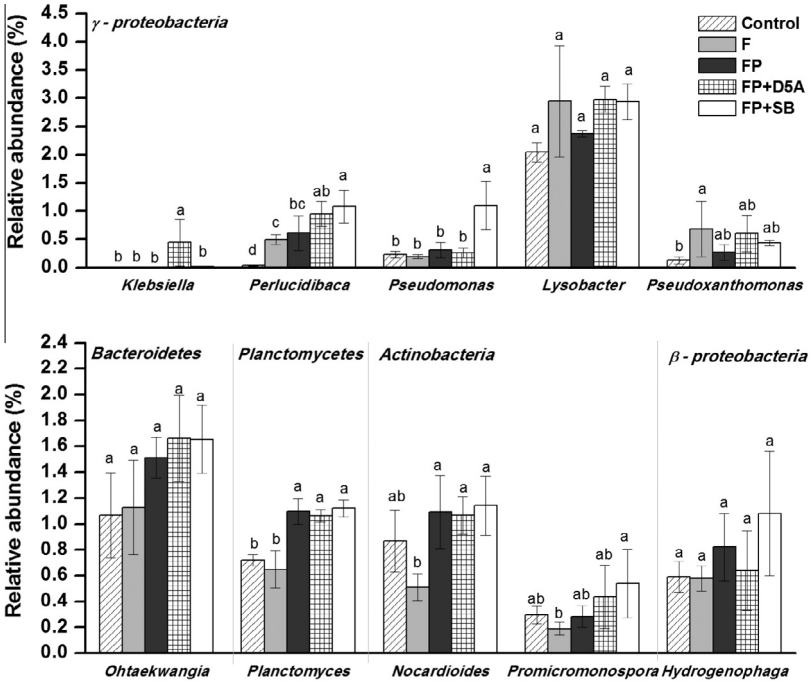

Fig. 4. The relative abundance of tall fescue enriched bacterial taxa at genus level. Vertical bars represent standard deviation ($n = 3$). Letters on columns indicate statistically significant differences in relative abundance at $p < 0.05$ according to Duncan test.

GC–MS analysis revealed that C21–C34 AHs and C21–C34 PAHs were the dominant components of petroleum in the soil used in this study. This indicates that the soil has been weathered for a long period before sampling for this study due to the relative low molecular weight (C8–C21) petroleum hydrocarbons that had likely been volatilized or been degraded. Significant degradation of AHs and PAHs occurred in all treatments after four months phytoremediation. Interestingly the amount of the most recalcitrant fraction to degradation, C21–C34, was found reduced more in phytoremediation treatments (FP, FP + D5A and FP + SB). C21–C34 AHs significantly reduced in both FP + SB and FP + D5A compared with FP while C21–C34 PAHs was removed more from FP + SB than in FP + D5A. The C16–C21 AHs fractions showed no obvious change between treatments. In China there is no standard for soil TPH but Control Standards for TPH in Sludges from Agricultural Use (China, GB 4284-84) recommend levels bellow 3000 mg kg^{-1}.

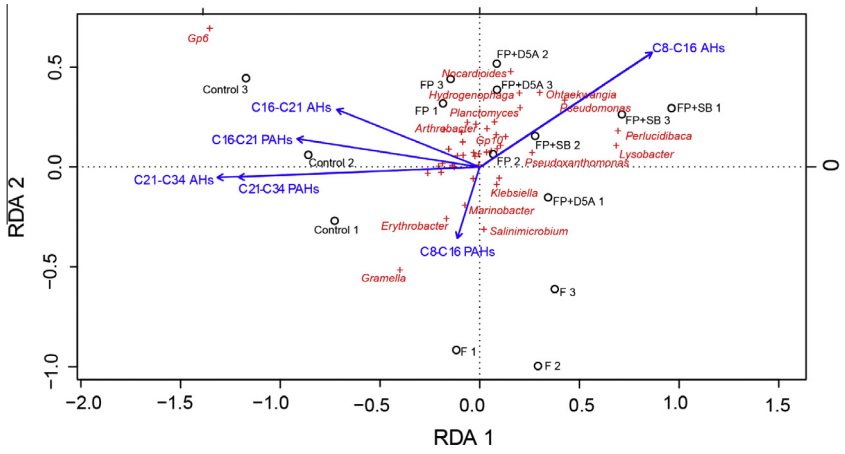

Fig. 5. Redundancy analysis (RDA) plot depicting the relationship between the petroleum fractions and bacterial diversity of the five treatments. The blank circles represent the soil samples and the red crosses mean bacterial taxa at genus level. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

After bioremediation F, FP, FP + D5A and FP + SB treatments were all below this level. This study elucidated that PGPR inoculation could enhance petroleum removal efficiency during phytoremediation, especially in the recalcitrant fractions. D5A and SB successfully survived in the rhizosphere as assessed by 16s rRNA analysis at genus level. Plant growth, particularly root biomass, (Table 1) was promoted through their beneficial effects of PGPR, which may include production of phytohormones and solubilization of minerals (Liu et al., 2014, 2013). Root biomass may also have other beneficial effects such as increasing pollutant bioavailability, providing habitat for bacterial growth of petroleum degraders as well as co-metabolism process of recalcitrant pollutant, such as PAHs, with the rhizosphere associated microbiota (Martin et al., 2014). Therefore, the biodegradation of the recalcitrant fractions may be enhanced by increasing root biomass and partially responsible for the increased petroleum degradation observed in this study. SB produces biosurfactant (Liu et al., 2013) which may increase solubilization of PAHs, a more hydrophobic compounds than n-alkanes with equivalent carbon numbers, and further enhanced the removal of these recalcitrant hydrocarbons.

The degradation of petroleum hydrocarbons in phytoremediation is general attributed to indigenous microbes, microbial community analysis was preformed by high throughput pyrosequencing to reach a more in-depth understanding of the biological processes of petroleum degradation during rhizoremediation. PCoA analysis (Fig. 2) based on OTU composition showed that bacterial community was observably differentiated between F and tall fescue planting treatments (FP, FP + D5A and FP + SB) whereas the communities of FP, FP + D5A and FP + SB did not diverge significantly. Similarly, the diversity index (Table 2) showed that InvSimpson decreased significantly in F, FP, FP + D5A and FP + SB compared with control and increased in FP, FP + D5A, FP + SB treatments compared with F treatment. Both Shannon and InvSimpson index were lowest in treatment F, which may be due to the enrichment effect on fast growing bacteria by the nutrient added as N and P source. However, the decreased diversity in F treatment did not slow the removal rate of petroleum hydrocarbon compared with control which is consistent with the study of Bell et al. (2013b). Bell et al. (2013b) suggested that diversity might be important in the initial selection of organisms, but less important for actual hydrocarbon degradation. Not all the microorganisms in soil have the same capacity for nutrient assimilation and subsequent growth. Selective stimulation of fast growing petroleum degraders is more important than general stimulation of the entire community in bioremediation of contaminated soils (Bell et al., 2013a). Therefore, for successful and rapid bioremediation such a dramatic shift in the microbial population was expected and desired.

In this study, a few groups of bacteria appeared to be enriched in planting treatments (FP, FP + D5A and FP + SB), especially FP + D5A and FP + SB. A comparison at the phylum level identified bacteria belonging to the *Proteobacteria* was the most abundant, and abundance significantly increased after fertilization and planting treatments (Fig. 3). *Proteobacteria* comprised a group of Gram-negative bacteria that has been extensively reported to degrade petroleum hydrocarbons (Jurelevicius et al., 2013). In the proteobacteial community, γ- and α-*proteobacteia* predominated in a manner similar to most studies regarding petroleum contaminated soil. This further highlights their importance during such processes (Matsui et al., 2014). In this study *Pseudoxanthomonas,* within the γ-*proteobacteria*, was enriched in the rhizosphere and showed a relative higher abundance in both PGPR inoculation treatments. Strains of this genus have been previously detected in polluted sites and identified as high molecular weight PAHs degraders (Tejeda-Agredano et al., 2013). The genus *Lysobacter* dominated γ-*proteobacteria* in this study and was favored by fertilizer and planting treatments. It has been reported to produce biosurfactant (Hayward et al., 2010) and reinforce the bioavailability of high lipophilic petroleum hydrocarbons and enhanced their degradation. Some other genera belong to β-*proteobacteria,* such as *Hydrogenphaga,* are also potential petroleum degraders. *Hydrogenphaga* has been reported could degrade a wide range of contaminants including PAHs (Aburto and Peimbert, 2011).

In addition, the dominated bacterial phyla following proteobacteria were *Actinobacteria*, *Acidobacteria* and *Bacteroidetes* containing hydrocarbon-degrading bacteria (Røberg et al., 2011). *Bacteroidetes* are Gram-negative heterotrophic bacteria which are common in ecosystems and are known to degrade high-molecular-weight organic compounds, including petroleum hydrocarbons (Drury et al., 2013). *Bacteroidetes* has been reported to be fast growing and fast acting decomposers of organic matter and increased in abundance after planting. Another dominant phylum, *Acidobacteria*, has been reported as a slow-acting decomposer tended to decrease in the fertilizer and plant treatments (Tian and Gao, 2014). Other genus enriched in two PGPR inoculation treatments (Fig. 4), such as *Ohtaekwangia*, *Planctomyces*, *Nocardioides*, *Hydrogenophaga,* have also been researched as potential petroleum hydrocarbon degraders and reported to be involved in PAH degradation (McGenity et al., 2012). A strain of *Hydrogenophaga* has been

reported to degrade naphthalene (Song et al., 2006) and *Nocardioides* can degrade crude oil including PAHs (Schippers et al., 2005). RDA analysis (Fig.5) demonstrated that all aforementioned tall fescue enriched bacteria were positively correlated with the removal of high molecular petroleum hydrocarbons (C21–C34 AHs and PAHs) in this study and confirmed that petroleum degradation was enhanced by a distinct bacterial community.

5. Conclusion

In this study, a pot experiment was designed to study the micro-ecological mechanism of PGPR enhanced phytoremediation. Tall fescue biomasses increased by PGPR while petroleum hydrocarbons was removed at the highest level by two PGPR inoculation treatments, particular high molecular weight fractions (C21–C34) of AHs and PAHs. In addition, the removal efficiency was not relative with bacterial diversity but with the selective effect of phytoremediation on specific bacterial communities. Then RDA analysis revealed that *Lysobacter, Pseudoxanthomonas, Planctomyces, Nocardioides, Hydrogenophaga*, *Ohtaekwangia* with high relative abundance in FP and FP + PGPR were positively correlated with high molecular weight petroleum hydrocarbons (C21–C34 AHs and C21–C34 PAHs). These results provide us a more in-depth understanding of the biological processes of petroleum degradation during rhizoremediation and indicate that specific bacteria group deserves more attention than bacterial diversity during phytoremediaton of petroleum for better remediation effectiveness.

Acknowledgements

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