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Synergistic Effect of Rhamnolipids and Inoculation on the Bioremediation of Petroleum-Contaminated Soils by Bacterial Consortia

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Abstract

Crude oil is a serious soil pollutant, requiring large-scale remediation efforts. Bacterial consortia in combination with rhamnolipids can be an effective bioremediation method. However, the underlying mechanisms and associated changes in soil bacterial composition remain uncharacterized. Therefore, this study sought to evaluate the effectiveness of rhamnolipids in petroleum hydrocarbon removal, and the associated bacterial community dynamics during bioremediation of petroleum-contaminated soils. Contaminated soils were subjected to natural attenuation, bioremediation with rhamnolipids, bioremediation with bacterial consortia, or bioremediation with bacterial consortia supplemented with rhamnolipids (BMR). High-throughput sequencing of bacterial sample partial 16S rRNA sequences was performed. Additionally, the *n*-alkanes and aromatic fractions were analyzed by gas chromatography-mass spectroscopy. The results showed that rhamnolipid supplementation increased the rate and extent of total petroleum hydrocarbon biodegradation to a maximum of 81% within 35 days. Further, phylogenetic analysis revealed that the bacterial community was composed of 14 phylotypes (similarity level = 97%). Actinobacteria and Proteobacteria were the two core phyla in all samples, accounting for 63–89%, but Proteobacteria was the most dominant phylum in the BMR sample (~53%). Among the top 20 genera, *Pseudomonas*, *Pseudoxanthomonas*, *Cavicella, Mycobacterium*, *Rhizobium*, and *Acinetobacter* were more abundant in BMR samples compared to other samples. Predicted functional profiles revealed that rhamnolipid addition also induced changes in gene abundance related to hydrocarbon metabolic pathways. This study provided comprehensive insights into the synergistic effect of rhamnolipids and bacterial consortia for altering bacterial populations and specific functional traits, which may serve to improve bacteria-mediated petroleum hydrocarbon biodegradation in contaminated soils.

Introduction

Crude oil is an important strategic resource that remains closely linked with the development of the global economy. However, the petroleum industry generates more than one billion tons of contamination waste worldwide every year [1]. Crude oil contains many different hydrocarbons, therein saturated and aromatic hydrocarbons make up 80% of the crude oil content [2]. It is well known that petroleum hydrocarbon is a sort of important pollutant with many harmful components that belong to the families of carcinogens and neurotoxic organic compounds, causing devastating damage to habitats with serious economic implications [3, 4]. Therefore, the persistence of petroleum hydrocarbons in the environment is of great concern requiring effective methods of remediation [5].

Bioremediation has been highlighted as an eco-friendly and economic approach for the removal of petroleum hydrocarbons, although its effectiveness has thus far been limited by the low bioavailability of crude oil components for in-situ applications [6–8]. Surfactant-mediated bioremediation has been proposed as a promising technology for enhancing the removal of these contaminants [9]. Various studies have shown that the addition of surfactants facilitates the removal of hydrocarbons by reducing the surface/interfacial tension

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and increasing the apparent solubility of hydrocarbons [10–13]. Moreover, surfactants have been shown to modulate the membrane transport and intracellular metabolism of hydrocarbons, further promoting their degradation [13, 14].

Biosurfactants are preferable to the use of chemical surfactants in bioremediation, owing to benefits of environmental compatibility along with their remarkable physicochemical and biological properties [15]. Rhamnolipids are the most extensively studied and applied biosurfactants in bioremediation, which was mostly produced by *Pseudomonas* or *Burkholderia*, with demonstrated equivalent or superior performance to synthetic counterparts for enhancing the aqueous solubility of hydrocarbons [16–18]. In this regard, the various properties of rhamnolipids have been extensively investigated [19–21]. Application of rhamnolipids to bioremediation processes (especially for bioaugmentation) had a significant influence on the degradation capacity and transport of bacteria in soils [13, 22]. However, most related studies have focused on accelerating the uptake and biodegradative rate of hydrocarbons, whereas the associated changes of bacterial populations with the use of bacterial consortia supplemented with rhamnolipids during the bioremediation process are not yet fully understood [23].

Therefore, the purpose of this study was to characterize the dynamic changes in the bacterial composition and diversity, along with their associated functions, during the biodegradation of petroleum hydrocarbons using bacterial consortia supplemented with rhamnolipids. Crude oil-contaminated soil samples were subjected to natural attenuation as a control, bioremediation with rhamnolipids only, bioremediation with a bacterial consortium only, and bioremediation with a bacterial consortium supplemented with rhamnolipids. We then assessed and compared the bacterial communities among groups using a high-throughput sequencing approach. These results can provide a theoretical basis to explain the synergistic effect of rhamnolipids and bacterial consortia on hydrocarbon biodegradation, as a crucial aspect of the monitoring process of bioremediation.

Materials and Methods

Soil Samples

The petroleum-contaminated soils polluted for several decades were obtained from an oil field in northern ShaanXi province of China, which weighed 30 kg. The soils were sampled at a 30-cm depth in a simple random design, according to procedures described by the US-EPA (1996). The soil samples were dried, homogenized, and separated with a 2-mm test sieve. The original soil samples were then spiked with 35,000 mg kg^{-1} total petroleum hydrocarbons (TPHs), containing 24,500 mg kg^{-1} of *n*-alkanes and 4820 mg kg^{-1} of aromatic hydrocarbons.

Bacteria, Media, and Culture Conditions

The bacterial consortia used in this study included *Pseudomonas aeruginosa* DN1 and *Bacillus subtilis* QHQ110 at a 1:1 ratio, which were respectively isolated from petroleum-contaminated soils in the oil field of northern ShaanXi, China [18, 24]. It was the same site at which the soils were sampled, and the bacterial consortia were proved with its capability to utilize petroleum hydrocarbons and crude oil in our previous reports [24–26].

The bacterial strains were grown in lysogeny broth containing 10 g tryptone, 5 g yeast extract, and 5 g NaCl per liter of distilled water for seed culture. Biosurfactant Production Liquid Medium (BPLM, pH 7.4) supplemented with palm oil as the carbon source was chosen to evaluate rhamnolipid productivity, consisting of the following composition: 5 g of $NaNO_3$, 1.5 g of KH_2PO_4, 1.2 g Na_2HPO_4, 0.6 g of $MgSO_4$, 3 mL of 1% $CaCl_2$, 50 μL of 1% $FeCl_3$ and 1 mL minor elements solution in 1 L distilled water. Rhamnolipids were produced by the engineered *P. aeruginosa* strain DNAB that resulted in a high yield of 22.9 g L^{-1} after shake-flask cultivation for 7 days, and culture broth was centrifuged at 4 °C for 20 min at 8000 rpm to remove the cells. Then the supernatant was acidified and extracted to obtain 100 mL crude extract with a rhamnolipid yield of 20 g L^{-1} at the very least, which was harvested by centrifugation and washed three times with acidic water [25, 26].

Experimental Design and Sample Collection

The crude oil-contaminated soils were dried, ground, and sieved through a 2-mm sieve prior to use, and then subjected to the four different treatments: natural attenuation (NA), bioremediation with the addition of 10 mL of 20 g L^{-1} rhamnolipid solution (BR), bioremediation with bacterial consortia inoculated with 10^6 CFU g^{-1} of each bacterial strain (BM), and bioremediation by means of bacterial consortia supplemented with rhamnolipids (BMR) under the same condition. For each treatment, approximately 1 kg of petroleum-contaminated soils was subjected to microcosm solid culture using a sterile solution containing $NaNO_3$ and KH_2PO_4 at an N/P ratio of 10, which serves as a biostimulation agent to promote the growth of hydrocarbon-utilizing microorganisms [27]. Sterile water was used to maintain 20% water content of the soil. Triplicate microcosm tests were prepared in a $50 \times 30 \times 20$ cm plastic box without a lid and allowed to incubate for 5 weeks with weekly stirring to maintain aerobic conditions at ambient temperature. All treatments were sampled at days 0 (post-inoculation), 5, 10

15, 20, 25, 30, and 35 for hydrocarbon quantification. All of assays were carried out in triplicate.

Degradation of Crude Oil

The TPH concentrations in the soil samples were measured by the ultrasonic-Soxhlet extraction gravimetric method [28]. The solutions of *n*-alkane and aromatic hydrocarbons were obtained by eluting with hexane and dichloromethane respectively, and their concentrations were detected by gas chromatography/mass spectrometry (GC–MS) (Agilent Technologies, Palo Alto, CA) as reported previously [29].

DNA Extraction, Amplification of 16S rRNA Genes, and Sequencing

The total bacterial DNA of each sample collected at the end of the 35-days treatment was extracted using the PowerSoil DNA Isolation Kit (MoBio Laboratories Inc, Solana Beach, CA, USA) according to the manufacturer's protocol. The quality and concentration of the extracted DNA were measured using a NanoDrop ND-1000UV-Vis spectrophotometer (NanoDrop Technologies, USA). The V_3–V_4 region of the bacterial 16S rRNA gene was amplified by polymerase chain reaction (PCR) using the primer pair 338F (5′-ACTCCT ACGGGAGGCAGCAG-3′) and 806R (5′-GGA CTACHVGGGTWTCTAAT-3′) with indexed adapters under the following thermal cycling program: 95 °C for 5 min, followed by 25 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s, and a final extension at 72 °C for 3 min. The amplicons were subjected to 2% agarose gel electrophoresis for detection, and samples with a bright main band of approximately 450 bp were selected and mixed at equidensity ratios. The mixture of PCR products was purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, USA) following the manufacturer's instructions. Sequencing libraries were validated using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) and quantified with a Qubit 2.0 Fluorometer (Thermo Fisher). Subsequently, paired-end sequencing was conducted using an Illumina HiSeq2500 platform (Illumina, Inc., San Diego, CA, USA) at Biomarker Bioinformatics Technology, Co., Ltd. (Beijing, China) according to standard protocols.

Bioinformatics and Statistical Analyses

The overlapping regions between the paired-end reads were merged using FLASH v1.2.7, and raw reads were quality-filtered under specific filtering conditions to obtain high-quality clean tags on the basis of the QIIME (V1.8) quality-control process [30]. Sequences that were less than 300 bp in length or that contained homopolymers longer than 8 bp were removed. Chimera sequences were detected by comparing tags with the reference database (RDPGold database) using the UCHIME algorithm and then removed. The effective sequences were then used in the final analysis.

Operational taxonomic units (OTUs) were clustered with a 97% similarity cut-off using the clustering program UPARSE [31]. The normalization process was then performed and chimeric sequences were identified and removed through UCHIME [32]. The OTUs were taxonomically classified to different levels (phylum, class, order, family, genus, and species) by means of the Ribosomal Database Program (RDP) classifier. Alpha-diversity indices (i.e., ACE, Chao1, Shannon, and Simpson) were calculated using QIIME from rarefied samples to determine the richness and diversity of the bacterial community. Linear discriminant analysis (LDA) effect size (LEfSe) analysis was performed to determine the ranking of significantly abundant modules in the different treatment groups [33]. A size-effect threshold of 4.0 on the logarithmic LDA score was used for discriminating functional biomarkers.

Alpha-diversity indices and the relative abundances of the top 10 phyla and genera are presented as the means ± SD, and were compared between groups using the independent-sample *t* test (for normally distributed data) or Mann–Whitney U-test (for non-normally distributed data). All statistical analyses were conducted with R version 3.1 software. The results of all statistical tests were regarded significant with a P-value < 0.05. For all statistical analyses, the dataset calculated for a 97% identify (species level) was used.

Functional profiles were predicted from the obtained 16S rRNA sequencing data through Tax4Fun [34]. Genes involved in transportation and those encoding key enzymes in the degradation process were identified in the resulting profiles according to their Kyoto Encyclopedia of Genes and Genomes (KEGG) orthologs.

Results

Biodegradation Efficiency of Different Treatments

As shown in Fig. 1, 81.9% of the TPH content was degraded in the samples treated by BMR after 35 days, which was significantly higher than the degradation achieved with the other treatments under the same conditions. The removal efficiency of the main components within 35 days are shown in Table 1, demonstrating no significant difference in the highest residual PAHs volume or average degradation rate, whereas the surplus of alkanes was the highest in BR and the lowest in BMR, and the average degradation rates were similar for groups NA, BR, BM, which were all lower than that of BMR. Therefore, the BMR treatment exhibited a superior degradation efficiency compared with the other treatments, and this phenomenon might be caused by the diversity of

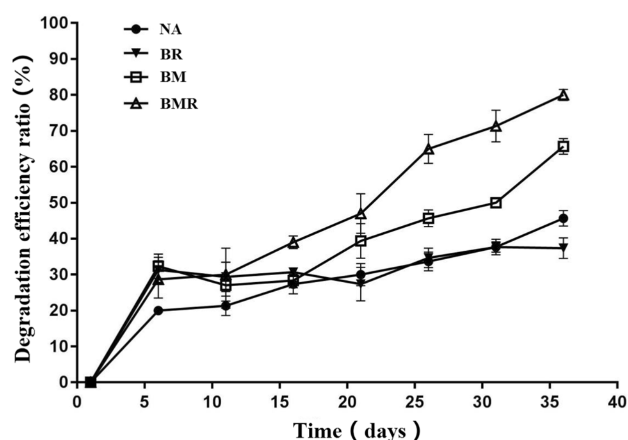

Fig. 1 Amount of TPH degradation in the different treatments during 35 days of incubation. (*NA* natural attenuation, *BR* bioremediation with rhamnolipids only, *BM* bioremediation with bacterial consortia, *BMR* bioremediation by means of bacterial consortia supplemented with rhamnolipids)

Table 1 Removal efficiency of total petroleum hydrocarbons and predominant components in the different treatments

Treatments	NA	BR	BM	BMR
Removal efficiency ratio (%)				
TPH	42.7 ± 1.1	39 ± 2.3	63.5 ± 1.8	81.9 ± 1.6
PAHs	40.1 ± 0.1	41.4 ± 1.3	53.2 ± 2	49.4 ± 0.3
Alkane	60.2 ± 0.5	58.3 ± 0.7	67.1 ± 0.3	86.4 ± 0.6
Removal rate (mg kg^{-1} d^{-1})				
TPH	437.7 ± 8	395.1 ± 7.5	645.2 ± 8.7	876.3 ± 9.5
PAHs	54.9 ± 1.8	57.1 ± 2.4	71.2 ± 3	67.7 ± 2.4
Alkane	420.7 ± 8.6	408.1 ± 5.8	469.6 ± 5.8	604.7 ± 5.5

NA natural attenuation, *BR* bioremediation with rhamnolipids, *BM* bioremediation with bacterial consortia, *BMR* bioremediation with bacterial consortia supplemented with rhamnolipids, *TPH* total petroleum hydrocarbons, *PAHs* polycyclic aromatic hydrocarbons

bacteria in the community that used crude oil as a carbon source and had the potential to remediate petroleum pollution in soil [29].

Bacterial Diversity and Richness

After quality filtering, denoising, and removal of potential chimeras and non-bacterial sequences, a number of 238,875 high-quality bacterial V_3–V_4 Illumina sequences, ranging from 57,460 to 64,365, were obtained for further analysis. Species richness and diversity indices, along with the number of OTUs in the different treatments are shown in Table 2. A total of 1878 OTUs were gained at a sequence-similarity level of 97%. The rarefaction curves showed that the quantity of OTUs detected increased with increasing sequencing depth, and the ends of the rarefaction curves tapered off with increasing numbers of sequences per sample, as is commonly observed with sequencing data (Fig. 2).

Table 2 Number of sequences, richness, and diversity indices of petroleum-polluted soils remediated in the different treatments

Samples	NA	BR	BM	BMR
Number of sequences	64,365	57,460	58,512	58,538
Observed OTUs	428	456	488	506
Number of Phyla	15	14	14	14
Number of Class	26	23	24	23
Number of Order	63	57	59	56
Number of Family	121	127	130	132
Number of Genera	170	173	180	185
Shannon index	3.62	3.71	3.95	4.27
Simpson index	0.72	0.77	0.88	0.78
Chao 1 estimator	328	331	373	419
Ace estimator	350	345	399	403

NA natural attenuation, *BR* bioremediation with rhamnolipids, *BM* bioremediation with bacterial consortia, *BMR* bioremediation with bacterial consortia supplemented with rhamnolipids, *OTUs* operational taxonomic units

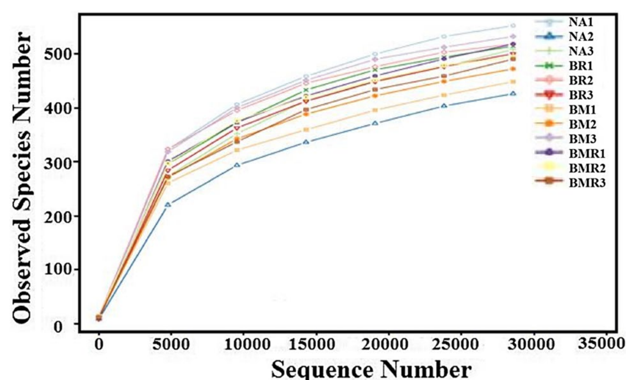

Fig. 2 Rarefaction analysis of the V3/V4 sequencing reads of the 16S rRNA gene from different treated soil samples at a 97% sequences similarity cut-off value. The x-axis shows the number of valid sequences per sample and the y-axis shows the observed species (OTUs)

The alpha-diversity indices (Ace, Chao1, Simpson, and Shannon) were used as indicators of soil bacterial richness and community diversity (Fig. 3). The rarified Ace, Chao1, and Shannon diversity indices showed remarkable differences between the BMR sample and the other three samples ($P < 0.05$), whereas there were no significant differences in the Simpson index between the BMR group and the others, demonstrating higher bacterial richness and diversity from the combination of the bacterial consortia and rhamnolipids. Comparisons among the other treatments showed similarity in the indices overall except for a higher Simpson index in the BM sample.

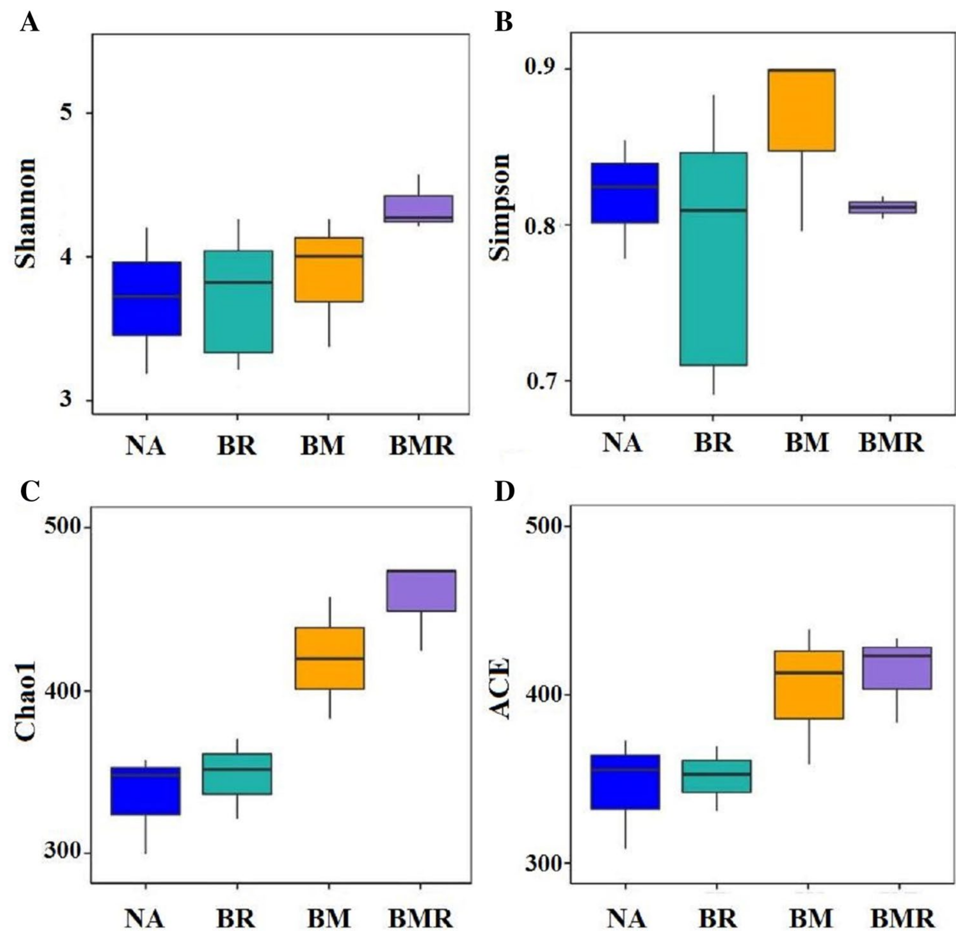

Fig. 3 Boxplot of Alpha-diversity indices Alpha-diversity indexes are composite indexes reflecting abundance and consistency. **a** Shannon and **b** Simpson indices reflect the diversity of OTU in samples, **c** Chao1 and **d** Ace estimators reflect the OTU abundance in samples

Bacterial Community Composition

More than 13 prokaryotic phyla were identified in the four treated samples accounting for 98% of the total community (Fig. 4). Actinobacteria and Proteobacteria were the core phyla in all samples, with a relative abundance ranging from 63 to 89% of the total number of phyla. For the NA, BR, and BM samples, Actinobacteria was the most dominant phylum, accounting for 47–56% of the total number of phyla, followed by Proteobacteria ranging from 16 to 33% of the total. However, in the BMR sample, Proteobacteria (52%) known as hydrocarbon degraders was the most abundant phylum [4]. The other phyla were represented at low levels in the individual samples.

More than 170 genera were identified in all samples, with significant differences in the relative abundances of the top 20 genera among the groups (Fig. 5 and Table 2). For the NA sample, *Gordonia* (25%) and *Nocardioides* (15%) were the most abundant genera. In the BR and BM samples, *Gordonia* was also the most abundant (40–42.3%), followed by *Pseudomonas* (7.3–8.1%) and *Nocardioides* (1.7–2%). Other predominant genera were well represented based on the average abundance. The BMR sample showed a unique profile, although *Gordonia* (23%) was also the most abundant genus, this was followed by *Pseudomonas* (7.8%) and *Pseudoxanthomonas* (7.3%). In addition, some genera accounting for a relatively small proportion of the community composition were also uniquely found in the BMR sample, including *Mycobacterium* (5.6%), *Cavicella* (5.6%), *Rhizobiaceae* (4.6%), and *Acinetobacter* (3.9%), which have been associated with the metabolism of petroleum hydrocarbons.

Differences in Bacterial Composition between BM and BMR Treatments

The taxa identified with LEfSe analysis can potentially explain the statistically significant and biologically consistent differences among groups more directly than comparisons of relative abundances, representing active biomarkers that can partially reflect their biological behaviors [33]. Therefore, to evaluate the effects of rhamnolipids on bacterial community dynamics during the bioremediation of petroleum-contaminated soils, LEfSe analysis was performed to reveal the differences in the ranking of significantly abundant taxa between the BM and BMR samples. Consistent with the patterns described above, the cladogram

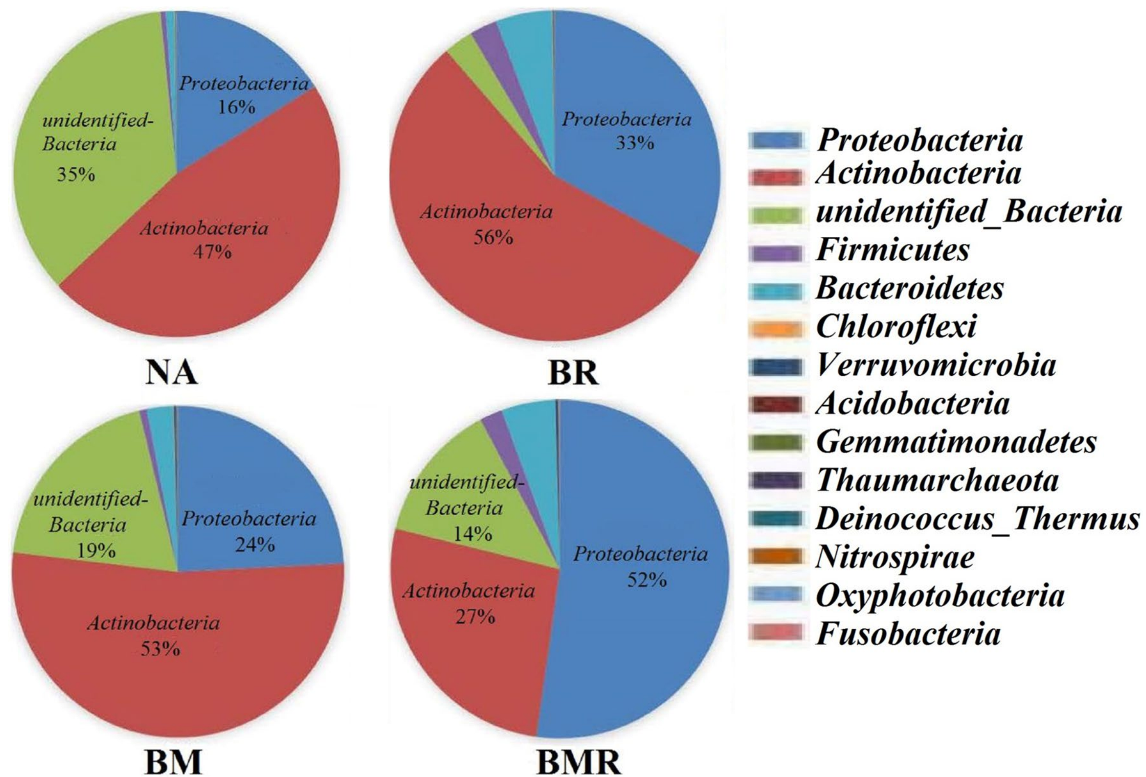

Fig. 4 Relative abundance of bacterial 16S rRNA gene sequences from different treated soil samples at phyla level. (*NA*: natural attenuation, *BR* bioremediation with rhamnolipids only, *BM* bioremediation with bacterial consortia, *BMR* bioremediation by means of bacterial consortia supplemented with rhamnolipids)

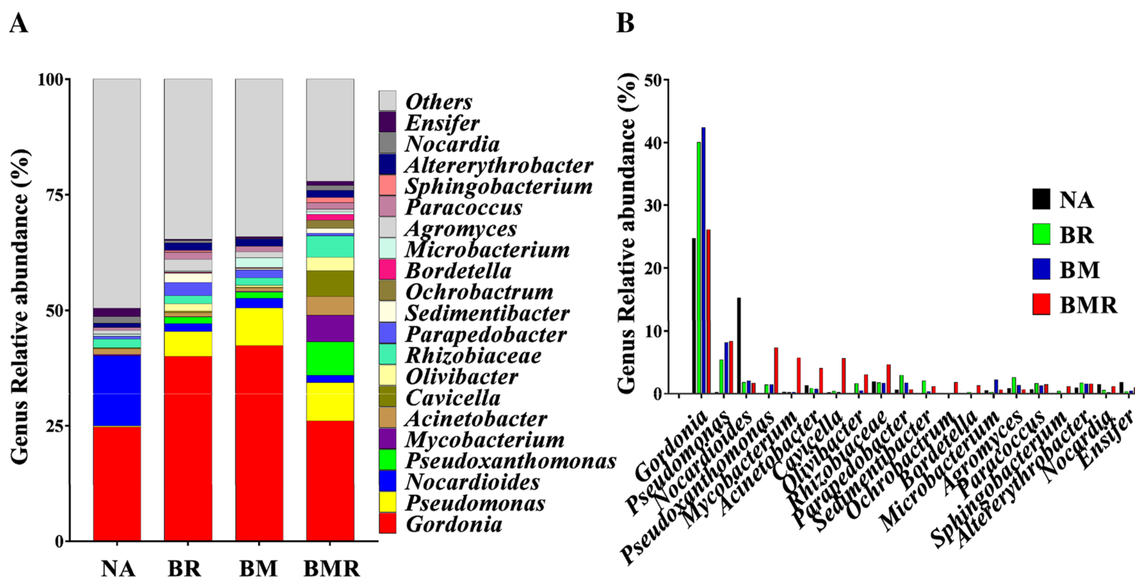

Fig. 5 Histogram of relative abundance of bacterial 16S rRNA gene sequences from different treatment at genus level (**a**) and relative abundance of the top 20 genera (**b**), other species was combined as “Others”

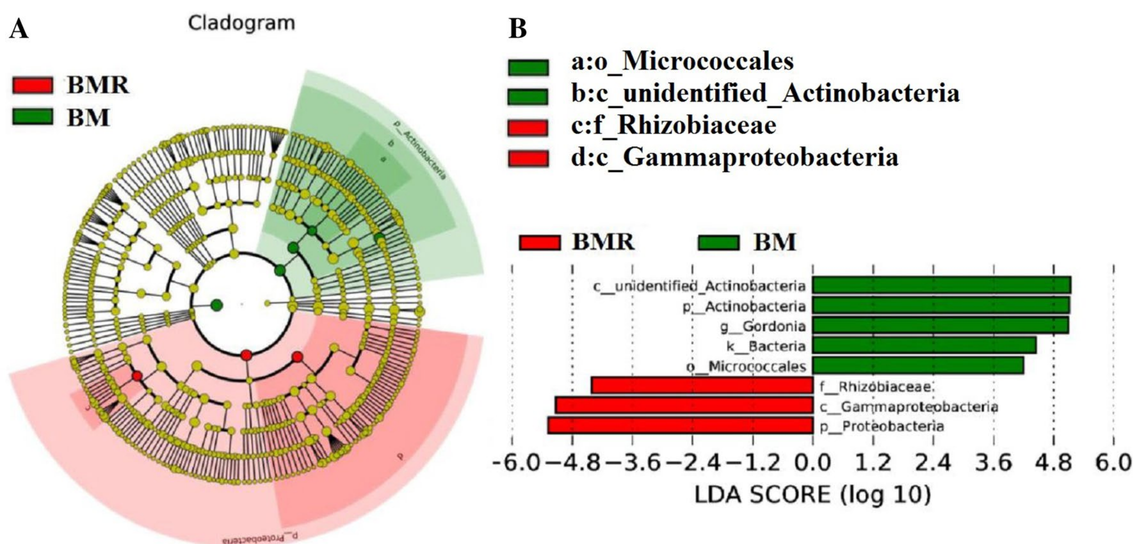

Fig. 6 LEfSe identified the most differentially abundant taxa between bioremediation supplemented with/without the rhamnolipids into bacterial consortia (BMR and BM). Taxa enriched in BM with a positive LDA score (*green*), and taxa enriched in BMR have a negative score (*red*). **a** Only taxa meeting an LDA significance threshold of 4 were shown, **b** Taxonomic cladogram obtained from LEfSe analysis of 16S rRNA sequences (relative abundance > 0.5% in at least one sample). *Small circles* and *shading* with *different colors* in the diagram represent abundance of those taxa in the respective group. *Yellow circles* represent non-significant differences in abundance between BM and BMR of those particular taxa. The *brightness* of each *dot* is proportional to its effect size (Color figure online)

(Fig. 6a) demonstrated differences in Actinobacteria and Proteobacteria between the BM and BMR groups. The plot of LDA scores from the LEfSe analysis (Fig. 6b) showed that these two phyla and the genus *Rhizobium* largely contributed to the significant differences in bacterial communities due to rhamnolipid amended or unamended.

Prediction of Functional Genes Involved in the Degradation of Hydrocarbons

We further hypothesized that bacterial functioning follows the same dynamics as bacterial community structure and diversity. To verify this hypothesis, we focused on the putative metabolic pathways involved in the degradation of hydrocarbons and compared the relative abundances of key enzyme-encoding genes in these samples (Fig. 7). The key enzymes associated with the initial oxidation of aromatic hydrocarbons (benzene dioxygenase, xylene dioxygenase, alkylbenzene dioxygenase, and naphthalene dioxygenase) showed scarcely any difference among the four sample groups, whereas the genes encoding aromatic dehydrogenase and catechol dioxygenase were slightly more abundant in the BMR samples compared to the others. Moreover, the enzymes involved in the metabolism of alkanes, such as cyclohexanone monooxygenase, alkane monooxygenase, and alcohol dehydrogenase, as well as those related to the metabolism of fatty acids, were detected at clearly higher levels in the functional profile of the BMR sample than the other samples. This might be related to the higher abundance of Proteobacteria in the BMR sample, as

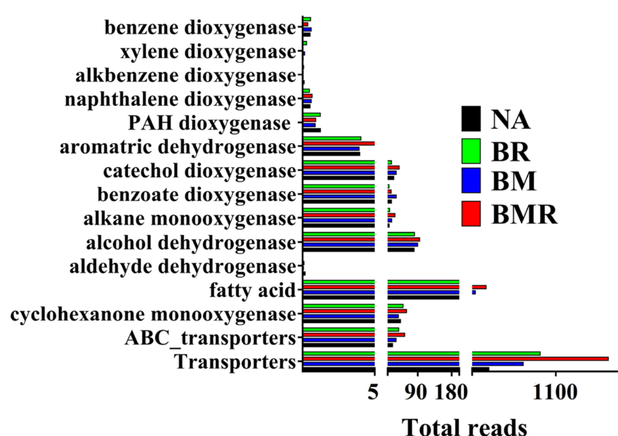

Fig. 7 Relative abundances of key Enzymes involved in hydrocarbon degradation and transportation in the different treated samples

this group is known to display catabolic activities towards both aliphatic and aromatic hydrocarbons [4]. Moreover, transporters (including ABC transporters) involved in nutrient uptake and metabolite release were also more abundant in the BMR samples.

Discussion

Bioremediation of petroleum hydrocarbons based on naturally-occurring microbial degradation capabilities is an effective and attractive tool for clean-up of polluted

environments. Bacteria are the first respondents to oil pollution, thus the bacterial species, density, and vitality are associated with hydrocarbon degradation ratio found in soils [4, 35]. However, these bacteria are not fully adapted to the contaminated soils including carbon and nitrogen source, types and bioavailability of hydrocarbons, and competition, synergism among bacteria, leading to low degradation efficiency therein. The presence of rhamnolipids had significant impacts on the removal of petroleum hydrocarbons adsorbed in soils, which was attributed to the elevated bioavailability and solubilization of particular compounds, significant attenuation of hydrophobic interactions, the increase of cell affinity to hydrocarbons, and stimulation of indigenous hydrocarbon-degrading bacteria with crude oil as a carbon source [4, 13–15]. Besides, the bacterial consortia supplemented with rhamnolipids exhibited a higher diversity and richer bacterial species content compared with the other treatments, that is, the indigenous bacterial community was enriched, which made the community more adaptable to the soils [15, 25, 36]. Consistently, the gene copies encoding monooxygenase and dioxygenase were also stimulated by the presence of rhamnolipids supplemented with bacterial consortia, which may exhibit broad, overlapping substrate preferences. All these observations clearly highlighted the synergy of rhamnolipid supplementation with bacterial consortia that degrade crude oil and produce bioemulsifier.

Members of Actinobacteria and Proteobacteria phyla are well known for displaying a broadened catabolic profile. With respect to the data presented herein, Actinobacteria and Proteobacteria were the main dominant phyla in all treatments whereas Proteobacteria was more dominant in the BMR than the other samples, since the inoculum *Pseudomonas* could survive, grow quickly and become the predominant in the early degradation of hydrocarbons owing to diversified metabolic possibilities [4, 25]. Other efficient hydrocarbon degraders, *Pseudoxanthomonas*, *Mycobacterium*, *Cavicella, Rhizobiaceae,* and *Acinetobacter* referring to metabolism of petroleum compounds respectively in genus level, were more predominant in BMR than in the other samples [4, 25, 37]. LEfSe analysis also showed that Actinobacteria and Proteobacteria could serve as biomarkers for soil with or without rhamnolipids. These bacteria are capable of biotransforming broad ranges of compounds under various environmental conditions (pH, salinity, temperature), which makes them advantageous candidates for clean-up of sites contaminated by crude oil [4, 38, 39]. Supplementing the soils with the necessary nutrients may stimulate the bacterial growth and activity, and enhance the overall biodegradation results of the pollutants. Apart from sterile solution containing $NaNO_3$ and KH_2PO_4 at an N/P ratio of 10, we proposed that the intermediates might be used as nutrient sources to supply the necessary nutrients for stimulating the bacterial activity, leading to a change in bacterial community composition.

Furthermore, it is plausible that rhamnolipids may be co-degraded with crude oil, or produced by the stimulated indigenous bacteria or inoculation. Under such conditions, whether the biodegradability or productivity of rhamnolipids is beneficial for the release of hydrocarbons from the micellar cores into aqueous phase, and the growth of indigenous microorganisms in aged contaminated soils [14, 15]. The promoting effect might be directly due to rhamnolipids, or the greater levels of dissolved organic matter released by the biosurfactants, serving as carbon sources for additional bacterial growth. Besides, rhamnolipids have no toxic effect on the growth of bacteria during the hydrocarbons biodegradation [24, 25]. The ability of the inoculation to survive in petroleum-contaminated soil matrix, to degrade both PAHs and *n*-alkanes and produce biosurfactants are all desirable characteristics for successful bioremediation. Therefore, the coexistence of multidegradative capacities in BMR is responsible for concomitant metabolic bioconversion of structurally-diverse hydrocarbons. This synergistic effect may provide useful information for the design of future bioremediation strategies.

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Compliance with Ethical Standards

Conflict of interest The authors have no conflicts of interest to declare.

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