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Research Article
Bacterial Diversity Change in Oil-contaminated Soils in Jianghan Oilfield via a High-throughput Sequencing Technique

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Abstract

Background and Objective: Oil pollution has become a severe problem in soil ecological in Jianghan Oilfield. The soil bacteria community plays a huge role in maintaining soil ecosystem balance and soil biological activity. The main objective of present study was to examine the change bacterial diversity in oil contaminated soil using a high-throughput sequencing technique. **Materials and Methods:** The Illumina MiSeq high-throughput sequencing technique was used to study the abundance, diversity and structural change in the oil-contaminated soil in Jianghan Oilfield. **Results:** The results showed that the abundance and diversity of the soil bacterial community significantly declined under oil pollution. Some dominant species (i.e., Proteobacteria and Actinobacteria) existed in the petroleum-contaminated soils and most were oil-associated and hydrocarbon degrading bacteria. **Conclusion:** On the basis of this study, the construction of the microbial flora of petroleum degradation is the focus of future research which means a more efficient degradation of petroleum in Jianghan Oilfield.

Key words: Bacterial diversity, oil-contaminated soil, Jianghan oilfield, soil bacteria, hydrocarbon degrading bacteria, MiSeq high-throughput sequencing technique

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

As an important integrated oil base in the southern part of China, Jianghan Oilfield has 24 oil and gas fields with an oil-bearing area of 139.6 km^2 and a cumulative production of crude oil of 21.1873 million t. In the development of the oil industry in the region, there have been some inappropriate operation methods in drilling and transportation, which resulted in oil seeping and soil pollution. Due to the openness of the soil system, oil pollutants evaporated into the atmosphere and entered the water system through runoff and penetration. These pollutants then are taken in by plants, animals and humans through the atmosphere, soil and water environment, causing persistent harm to human health[1,2]. Oil pollution has become a severe problem in soil ecological and environmental protection in the region.

As an important part of soil biological activity, microbes play an important role in the decomposition and transformation of soil organic matter and are an important index to assess soil quality[3]. Soil microbe is the general name for the bacteria, fungi and actinomycetes in the soil, of which the most abundant are bacteria, accounting for 70-90% of the total number of microbes[4]. Studies show that oil pollution can change the composition and diversity of soil bacteria and this change of soil properties will greatly constrain the biodegradation rate of petroleum and the bioremediation of petroleum contaminated sites[5-8]. The methods used in previous studies are mainly the traditional microbial culture method[9], Sanger sequencing[10,11]. Without enough information, it is difficult to comprehensively understand the soil bacterial community structural characteristics and diversity change pattern. Based on this situation, this study aimed to study the diversity and composition change of the oil-contaminated soil in Jianghan Oilfield in order to provide a theoretical basis for the ecological restoration of Jianghan Oilfield using high-throughput sequencing technique.

MATERIALS AND METHODS

Experimental site: The tested soil was collected from Jingzhou Oil Production Zone of Jianghan Oilfield on Huayuan-Xinchang Road in Jingzhou district, jingzhou city, Hubei province (30°23'N and 112°9'E). Jingzhou Oilfield has a subtropical monsoon climate with abundant light, warmth and a long frost-free period. Jingzhou city has an annual total solar radiation of 104-110 kcal cm^{-2} with 1800-2000 annual sunshine hours and an annual average temperature of 15.9-16.6 with an annual accumulated temperature of 5000-5350 (above 10). It has 242-263 frost-free days and 1100-1300 mm of rainfall. Therefore, the city provides good climatic resources for crops.

Soil sample collection: Oil contaminated soil samples were collected on December 11, 2017. The soil was black or dark brown and had a severe oily odor as it was polluted when the oil pipeline in Jingzhou Oil Production Zone of Jianghan Oilfield ruptured on November 20, 2017 and oil spilled into rice farmland (a pollution area of about 350 m^2). Four oil polluted soil spots were selected, topsoil samples (0-20 cm) were collected and the soil labeled as petroleum contaminated soil (PS). Another four spots were randomly selected from the rice field without oil pollution (within a 50 m radius) and topsoil samples (0-20 cm) were collected as control non-petroleum contaminated soil (NPS). The soil samples were placed in a refrigerator and brought back to the lab. Then, after sifting part of the soil with a 2 mm fine sieve, the soil was placed in a refrigerator at -20 for DNA extraction and the other part for petroleum content examination.

Determination of petroleum content in soil samples: The oil content in the soil samples was examined by the gravimetric method. Then Soxhlet extraction was carried out on 10 g of oil-contaminated soil using 20 ML of n-hexane, 20 ML of dichloromethane and 20 ML of trichloromethane successively. The extracted solutions were combined, the solvent was removed in a 100 ML round bottom flask through distillation and the product was placed in an oven to obtain a constant weight. Then it was removed and placed in a dryer. It was weighed after cooling for 30 min.

DNA extraction and PCR amplification: The total genomic DNA was extracted from the soil samples with the DNA purification OMEGA Soil DNA kit and the genome was incubated at -20 . It was melted before PCR amplification. Three melted samples were mixed for PCR amplification. The PCR product was detected by 2% agarose gel electrophoresis. The solution was mixed at the same concentration. Finally, the PCR product was reclaimed with the AxyPrepDNA gel recovery kit (AXYGEN). According to the designated sequencing region, the V3 and V4 regions of the sample were amplified by the specific primers with a barcode. General primers were F338 (ACTCCTACGGGAGGCAGCAG) and R806 (GGACTACHVGGG TWTCTAATPCR). The PCR was carried out with TransGen AP221-02: TransStart Fastpfu DNA Polymerase. The PCR instrument was ABI GeneAmp® 9700. The PCR amplification reaction procedures were 3 min of denaturation at 95, amplification for 30 cycles (30 sec at 95, 30 sec at 55 and 45 sec at 72) and 10 min at 72 and at 10°C until halted.

MiSeq library construction and sequence testing: The PCR products were examined by the QuantiFluorTM-ST Blue Fluorescence Quantitative System (Promega) and the products were diluted. A library was constructed with the TruSeqTM DNA Sample Prep Kit. The sequence was tested with HiSeq Reagent Kit v2. DNA sequencing, splicing, quality control and bioinformatics advanced analysis were performed with the help of Shanghai Major Biomedical Technology Co., Ltd.

Biological analysis: With the Qiime platform (http://qiime.org/scripts/assign_taxonomy. html), the sequence was grouped into multiple operational taxonomic units (OTU) based on the similarity of the sequence. Then the OTUs in each sample and the number of sequences in each out were checked. By searching for the closest genetic species, the taxonomic information of each one was obtained. The expected dilution curve with OTUs at 97% similarity was generated and the diversity analysis was made using the mothur calculation diversity index. Data processing was done and bacterial community distribution, principal component analysis and cluster analysis were obtained using Excel, PCoA and correlation clustering, respectively.

RESULTS

Petroleum contents in the soil sample: The results showed that the concentrations of pollutants in PS1 to PS4 were 1.06×10^5, 1.41×10^5, 1.32×10^5 and 1.46×10^5, which were 212-253 times the critical value of soil petroleum hydrocarbon (500 mg kg^{-1}) and were heavily polluted.

Soil samples sequencing results: The high-throughput sequencing showed that there were 1,578 OTUs in the eight soil samples. The samples were divided into the non-petroleum contaminated sample (NPS) group and the petroleum contaminated soil (PS) group. The two sample groups shared a total of 924 OTUs (Fig. 1), which indicated that petroleum contamination leads to a significant reduction of OTUs.

Changes in soil bacterial community abundance and diversity: The diversity of microbes in the environment can reflect the abundance and diversity of species in the bacterial community, which can be obtained with the Alpha diversity analysis. The abundance index, Chao1/ACE, the diversity index, Shannon diversity index and Simpson diversity index of each soil sample were shown in Table 1. The higher the Shannon value, the higher the diversity of the community and the smaller the Simpson index, indicating that the community diversity was high. The richness and diversity of bacterial communities in clean soils were significantly higher than those in petroleum-contaminated soils. The Good's coverage of the eight samples was above 99%, indicating that the sampling depth of all samples was very reasonable and the sequencing results represented the true situation of the bacterial distribution in the samples.

Changes in bacterial community composition: The bacteria contained in the eight soil samples belong to 25 phyla. The NPS samples contained rich soil bacteria categories such as Proteobacteria, Actinobacteria, Acidobacteria, Chloroflexi, Gemmatimonadetes, Bacteroidetes and Nitrospirae, among which Proteobacteria, Acidobacteria and Chloroflexi were relatively more numerous than other categories of soil bacteria. After the soil was polluted by oil, the relative

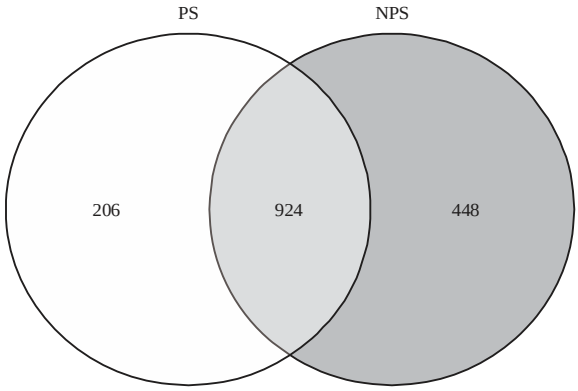

Fig. 1: Quantity of operational taxonomic units (OTUs) in the non-oil-polluted (NPS) and oil-polluted (PS) soils

Table 1: Alpha-diversity values of non-oil-polluted (NPS) and oil-polluted (PS) soil samples

Sample/estima	ACE	Chao	Coverage	Shannon	Simpson
NPS_1	1298.40539	1324.722222	0.994246	6.107695	0.004593
NPS_2	1304.530711	1323.5	0.992523	6.252519	0.00376
NPS_3	1266.728308	1268.114504	0.994082	6.129753	0.004548
NPS_4	1294.738268	1299.985294	0.992953	6.203483	0.004005
PS_1	803.783389	821.120482	0.992456	4.19955	0.050966
PS_2	956.765089	975.009709	0.990995	4.353774	0.045274
PS_3	937.688004	997.073171	0.990563	4.497473	0.039168
PS_4	1130.53489	1161.545455	0.990603	4.722448	0.031329

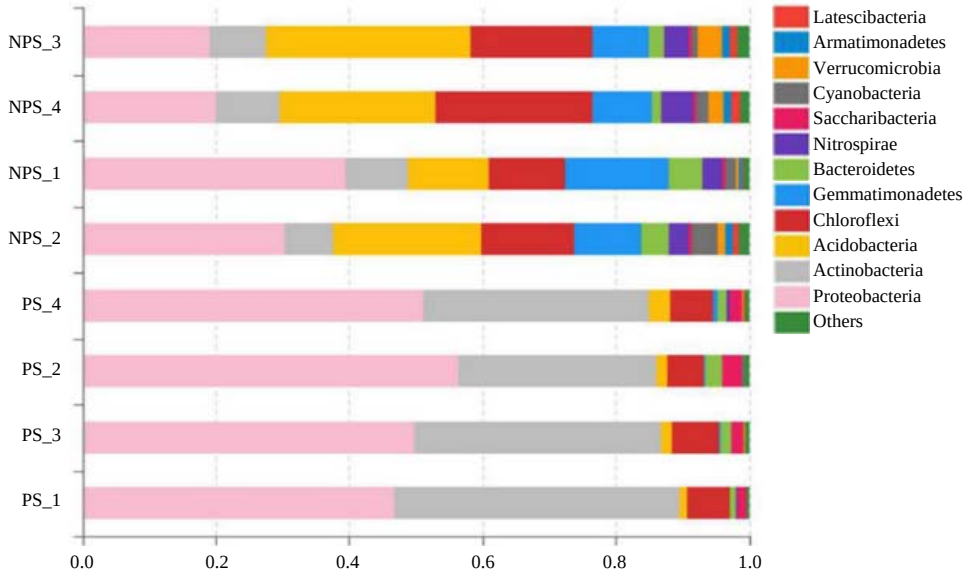

Fig. 2: Changes in relative abundance of bacteria in non-oil-polluted and oil-polluted soils

abundance of the main bacterial groups in the soil bacterial community significantly changed (Fig. 2). Proteobacteria and Actinobacteria had an absolute advantage in the bacterial community and the relative abundance of Acidobacteria and Chloroflexi declined while there were hardly any Gemmatimonadetes and Nitrospirae. Under the influence of oil pollution, the abundance of soil bacteria decreased and the species of bacteria were reduced.

Changes in soil bacteria on genus level: The changes of bacterial diversity and abundance of soil samples are shown in the Heatmap diagram (Fig. 3). The horizontal axis is the name of the soil samples and the vertical axis shows the species of each genus. The different scales of color indicate the abundance of the bacteria in the sample. The bacterial composition of the clean soil and the petroleum-polluted soil was quite different and it can be seen from the uniformity of the color distribution that the bacteria of the clean soil were more abundant and the abundance was well-distributed. After the soil was polluted by oil, the soil bacterial species and abundance were greatly changed as the abundance of bacteria in the soil sharply decreased.

Relationship between soil bacterial communities: A PCA analysis of the soil bacterial community of each soil sample sequence was done by R language (Fig. 4). The more similar the composition of the sample diversity, the closer the sample points were on the PCA figure and vice versa. The results showed that the bacterial composition of the four clean soil samples was highly consistent and the bacterial community of petroleum-contaminated soil was relatively consistent. The former tended to focus on the upper left quadrant and the latter tended to focus on the upper right quadrant. There was a significant difference between the two treatments.

DISCUSSION

Bacteria are the main microbes in the transformation and energy flow of the soil and play an important role in maintaining and restoring the balance of ecosystems. The diversity of the bacterial community structure in the soil is one of the important indices used to evaluate the environmental quality of the soil[12]. The results of this study showed that due to severe oil pollution, the abundance and diversity index of soil bacteria significantly decreased and the relative abundance of major bacterial populations greatly changed. The relative abundance of Proteobacteria and Actinobacteria sharply increased in the bacterial community, but Acidobacteria, Chloroflexi, Gemmatimonadetes, Bacteroidetes and Nitrospirae markedly declined.

The present study showed a negative effect of oil-contaminants on some bacteria. In general, some poorly tolerant bacteria cannot adapt to the oil-contaminated environment and may be decreased and disappeared. Meanwhile, the contents of Acidobacteria and Chloroflex were declined by 20 and 11%, respectively (Fig. 2) and there were hardly any Gemmatimonadetes and Nitrospirae in the PS samples. On the contrary, petroleum contaminants may have a positive impact on the growth and reproduction of other bacteria. For example, Proteobacteria accounted for 51.01%

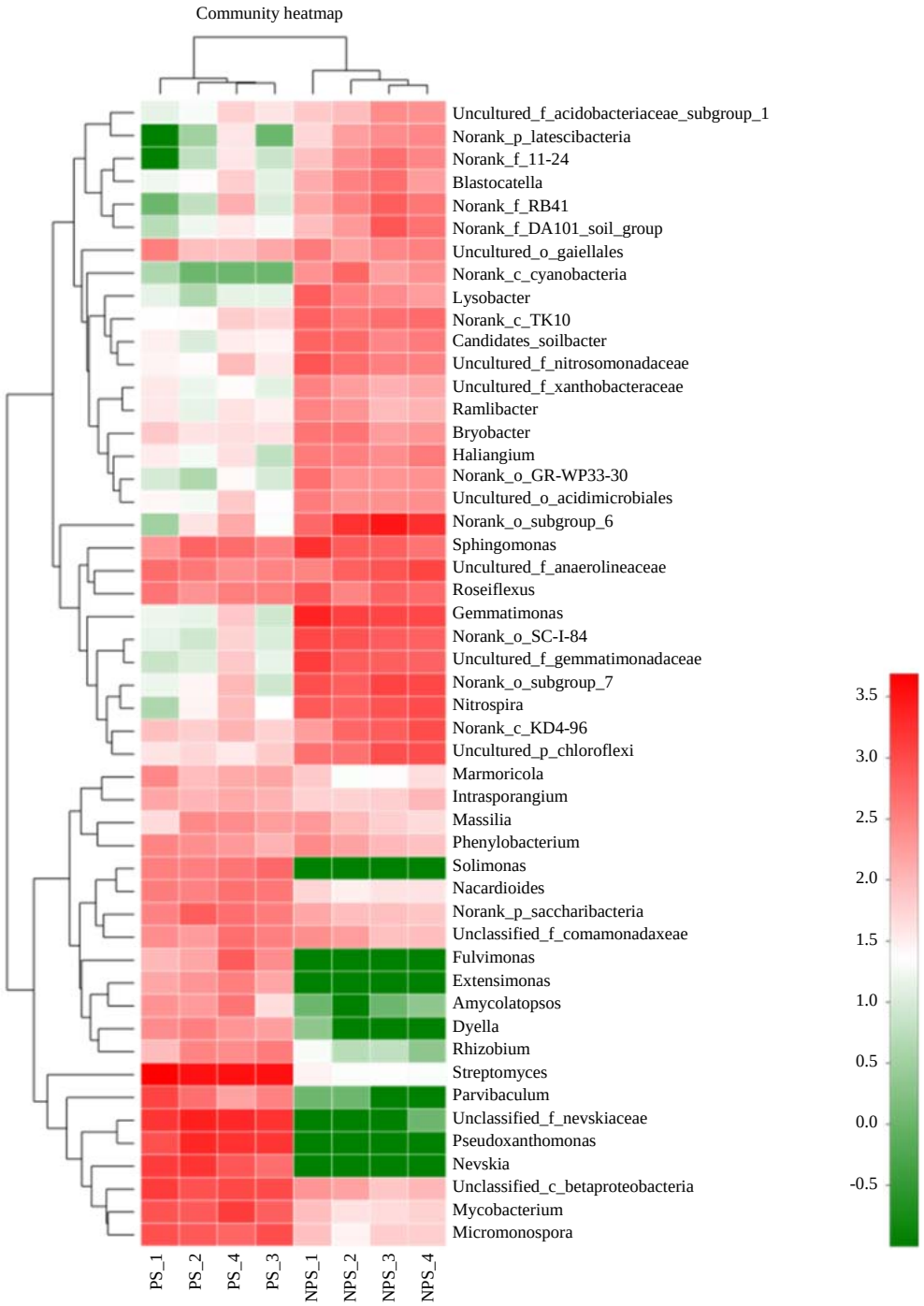

Fig. 3: Heatmap showing bacterial diversity and relative abundance of soil samples at the genus level

of the total bacteria and Actinobacteria accounted for 35.95% in the PS samples, in current study. The contents of Proteobacteria and Actinobacteria were 23 and 27%, respectively higher than those in clean soil samples. Numerous studies have shown that Proteobacteria is a dominant group degrading polycyclic aromatic hydrocarbons[13-14]. Pseudoxanthomonas and Nevskia have a strong degradation effect on petroleum hydrocarbons[15]. Actinobacteria are widely found in soil and water environments polluted by oil, especially in an environment of polycyclic aromatic hydrocarbons[16]. Mycobacteria can effectively degrade the tetracyclic and pentacyclic aromatic hydrocarbons[17]; Streptomyces can efficiently degrade most long-chain n-alkanes in C12~C34, especially C15~C21 and

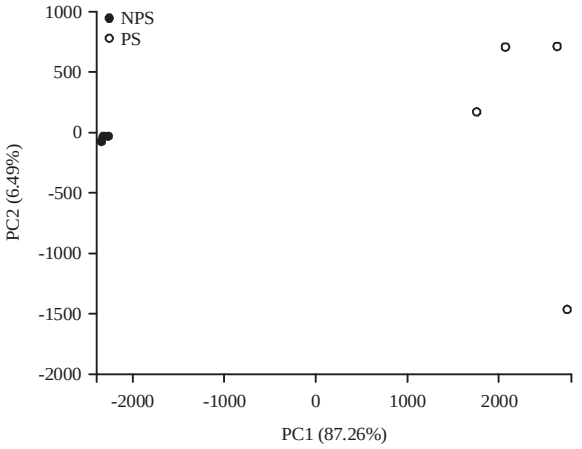

Fig. 4: PCA analysis of the soil bacterial community

C26~C29. They also have a strong degradation ability on alkyl benzene, naphthalene, phenanthrene and methyl phenanthrene[18]. For instance, petroleum hydrocarbons can be degraded to provide a carbon source and energy for degrading bacteria. Therefore, with the passage of time, as the main force in the degradation of oil, degrading bacteria gradually become the dominant bacteria in the region. Some dominant species (i.e. Proteobacteria and Actinobacteria) are the dominant bacteria in the oil-polluted soil environment.

CONCLUSION

The polluted soil in Jianghan Oilfield boasts good bioremediation capacity. In this study, the dominant bacteria (i.e., Proteobacteria and Actinobacteria) in Jianghan Oilfield area were mostly oil-related bacteria and hydrocarbon-degrading bacteria. The study proved that after soil is contaminated by oil, the hydrocarbon-degrading bacteria become the dominant bacteria in the soil. These dominant bacteria are most likely to be the critical flora of oil remediation in this habitat. On the basis of this study, the construction of the microbial flora of petroleum degradation is the focus of future research which means a more efficient degradation of petroleum in Jianghan Oilfield.

SIGNIFICANCE STATEMENT

This study evaluated the differences in bacteria diversity between oil-polluted and non-oil-polluted soils through the Illumina MiSeq high-throughput sequencing technique. These results found the significant decrease of the abundance and diversity in the soil bacterial community after soils were polluted by oils. It was firstly reported that some dominant species (i.e., Proteobacteria and Actinobacteria) existed in the petroleum-contaminated soils and most were oil-associated and hydrocarbon degrading bacteria. Such results can provide new methods in repairing oil-polluted soils by these potential degrading bacteria.

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