Soil Biology & Biochemistry 98 (2016) 64–73

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

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

Bacterial communities in oil contaminated soils: Biogeography and co-occurrence patterns

Shuo Jiao, Zhenshan Liu, Yanbing Lin, Jun Yang, Weimin Chen**, Gehong Wei*

State Key Laboratory of Crop Stress Biology in Arid Areas, College of Life Sciences, Northwest A&F University, Yangling, Shaanxi 712100, PR China

ARTICLE INFO

Article history:
Received 20 January 2016
Received in revised form
29 March 2016
Accepted 3 April 2016

Keywords:
Biogeography
Oil contamination
Co-occurrence patterns
Biodiversity

ABSTRACT

To improve the knowledge about the biogeographic patterns of bacteria in soils contaminated with crude oil, we studied the effects of local geochemical properties and geographic distance on bacterial community structure in oil contaminated soil in five oil refineries (46–360 km apart). The microbial structure was significantly affected by soil environmental factors such as pH, total petroleum hydrocarbons (TPH), total nitrogen, and cadmium level. Microbial alpha-diversity was positively correlated with pH but negatively correlated with TPH. Among sampling sites, the community dissimilarities increased with spatial distance. Variation of bacterial community was mostly attributed to simultaneous effects of spatial distance and environmental factors, and purely spatial distance contributed more to the variation. Microbial generalist OTUs were broadly distributed and dominant in contaminated soils. Their populations were in low proportion (15.75%), but they had high relative abundance (65.05%), and some were associated with TPH-degradation. Network analysis indicated that microbial communities had non-random co-occurrence patterns. Keystone taxa were *Rubrivivax*, *Nitrospira*, *Methylotenera*, *Methyloversatilis* and *Acidaminobacter*. Microbial taxa from the same module had strong ecological linkages and were involved in biological electron-transfer, C and N-cycles, and organic contaminant degradation. Our results indicate that the same microbial groups with TPH-degradation ability can be assembled from indigenous microorganisms in separate regions through long term exposure to contamination.

1. Introduction

Microbial biogeography, the study of microorganism distribution patterns across space and time, has received increasing attention since the development of molecular tools (Fenchel, 2003; Martiny et al., 2006; Hanson et al., 2012). One viewpoint of microbial biogeography is that “everything is everywhere; the environment selects” (Baas-Becking, 1934). Environmental factors were initially thought to be the dominant factors affecting variation among microbial communities. Many studies have demonstrated that environmental factors such as soil pH, nutrients, soil texture, and climatic conditions can significantly affect microbial community distribution (Fierer and Jackson, 2006; Bissett et al., 2010). However, some studies indicate that the non-random distributions of microorganisms are not only affected by environmental factors but can also be significantly correlated to geographic distance (Xiong et al., 2014; Liang et al., 2015; Wang et al., 2015). Spatial distance can limit the dispersal of microorganisms and contribute to accelerated speciation (Diniz-Filho and Telles, 2000).

Crude oil contamination is a global problem that causes great damage to natural environments and animal health. Contamination frequently occurs during oil exploration, transportation, storage, usage, or accidental spills (Liang et al., 2015). Removal of crude oil from soil is a difficult and long-term process (Peterson et al., 2003). Microbial remediation is an effective option for environment cleanup due to its low cost and limited impact on other aspects of the environment (Liebeg and Cutright, 1999). Soil is a huge reservoir for diverse microbial populations. Many microorganisms adapted to harmful environments are naturally abundant and able to degrade organic pollutants. Large numbers of functional genes contributing to degradation of alkane fractions have been detected in oil polluted soil (Fierer and Jackson, 2006). Microorganisms therefore can play important roles in bioremediation of oil damaged ecosystems. Determining the structure of microbial communities in oil contaminated soils could provide valuable clues

* Corresponding author.
** Corresponding author.
E-mail addresses: chenwm029@nwsuaf.edu.cn (W. Chen), weigehong@nwsuaf.edu.cn (G. Wei).

http://dx.doi.org/10.1016/j.soilbio.2016.04.005
0038-0717/

for ecosystem restoration and environmental management.

Microorganisms within a specific ecological niche form complex interaction webs (Faust and Raes, 2012). Co-occurrence network analysis may provide insight into the structure of complex microbial communities and the interactions between microorganisms (Barberán et al., 2012). Using the high-throughput sequencing technology, broad and deep surveys of microbial communities have been made in diverse environments including marine water (Beman et al., 2011), soil (Barberán et al., 2012) and activated sludge (Ju et al., 2014). Distinct environments are affected by abiotic factors and predict specific habitat limits which can support coexistence of species within these communities (Stegen et al., 2012). However, the co-occurrence patterns of species in oil contaminated soils are still unclear.

In this study, we conducted a metagenomic analysis of bacterial communities from oil contaminated soils via high-throughput sequencing of the 16S rRNA gene. The results were analyzed for correlations with environmental factors and spatial distances. Samples were obtained from petroleum contaminated surface soils around five oil refineries in the Shaanxi Province of China. Our main goal was to investigate the biogeographic pattern and biodiversity of soil bacterial communities in fields with a long history of oil contamination, especially to study (i) the bacterial diversity in oil-contaminated fields; (ii) the relationships among the bacterial community and soil environmental factors; (iii) the effects of environmental factors and geographic distance on bacterial community; (iv) the co-occurrence patterns among bacterial taxa responding to oil contamination.

2. Materials and methods

2.1. Sample collection

A total of 38 soil samples were collected around five oil refineries in Shaanxi Province of China in October 2013, including Yulin (JB, 9 samples), Yanchang (YC, 4 samples), Yanchuan (YP, 9 samples), Luochuan (JKH, 7 samples) and Xianyang (XY, 9 samples). Each soil sample was a mixture of five soil cores (0–15 cm) from an area of 500 × 500 m^2 around an oil refinery. Soil samples were transported to the laboratory in sterile plastic bags on dry ice. A subset of the soil was air-dried for analysis of edaphic properties. Aliquots of soil samples were stored at −80 °C for microbial analyses. Total petroleum hydrocarbon (TPH) concentrations in the soil samples were measured using the Ultrasonic-Soxhlet extraction gravimetric method (Huesemann, 1995). Physical and chemical parameters of the soils, including pH, total nitrogen (TN), available nitrogen (AN), total phosphorus (TP), available phosphorus (AP), available potassium (AK) and the heavy metals cadmium (Cd), chromium (Cr) and lead (Pb), were measured using standard soil testing procedures (Bao, 2000).

2.2. DNA extraction and purification

Community DNA was extracted from 0.5 g of soil samples using the MP FastDNA®SPIN Kit for soil (MP Biochemicals, Solon, OH, USA) according to the manufacturer protocol. The V4-V5 hypervariable regions of the 16S rRNA gene was amplified using primers 515F (5′-GTG CCA GCM GCC GCG GTA A-3′) and 907R (5′-CCG TCA ATT CCT TTG AGT TT-3′) with the forward primer modified to contain a unique 6 nt barcode at the 5′ end. All PCR reactions were performed with 30 μl system with 15 μL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs), 0.2 μM of forward and reverse primers and about 10 ng template DNA under the following thermal cycling conditions: initial denaturation at 98 °C for 1 min, followed by 30 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s, and extension at 72 °C for 60 s, and an extension step at 72 °C for 5 min after cycling was complete. All samples were amplified in triplicate, and no-template controls were included in all steps of the process. Triplicate PCR amplicons were pooled together and then mixed with the same volume of 1 × loading buffer (containing SYB green). They were detected by electrophoresis in a 2% (w/v) agarose gel. PCR products with bright band between 400 and 450bp were mixed in equal density ratios and purified with GeneJET Gel Extraction Kit (Thermo Scientific). The purified PCR amplicons were sequenced using the Illumina Miseq (300-bp paired-end reads) platform at Novogene Bioinformatics Technology Co., Ltd. (Beijing, China).

2.3. Sequence analysis of the 16S rRNA amplicons

Paired-end reads were merged using FLASH (V1.2.7, http://ccb.jhu.edu/software/FLASH/), and quality filtering of reads was performed according to the literature (Caporaso et al., 2011). The acquired sequences were chimera detected and removed using USEARCH software based on the UCHIME algorithm (Edgar et al., 2011). The sequences were then assigned to each sample with unique barcodes. Sequence analysis was performed by the UPARSE software package using the UPARSE-OTU and UPARSE-OTUref algorithms. Sequences with ≥97% similarity were assigned to the same OTUs. The low abundance OTUs were eliminated from the OTU table if they did not have a total of at least 2 counts across all the samples in the experiment. The representative sequences for each OTU were assigned to taxonomic groups using the RDP classifier (Caporaso et al., 2011).

2.4. Data analyses

Alpha diversity and beta diversity based on Bray–Curtis distance were calculated using 36,920 reads per sample (minimum number of sequences required to normalize the differences in sequencing depth) using QIIME (http://qiime.org/index.html), with multiple indices (observed species, Pielou's evenness, Shannon–Wiener index, and phylogenetic diversity) and the Bray–Curtis distance between samples.

Maps of sampling sites were made using GenGIS II (Parks et al., 2013). The relationships between geographical coordinates and sample ordination on taxonomic (Bray–Curtis) or environmental dissimilarity (Euclid distance based on environmental variables) were determined. The Mantel test was used to test the correlation between taxonomic and environmental dissimilarity.

Principal component analysis (PCA) was calculated using soil environment parameters as variables and alpha diversity indices with significance < 0.05 were fitted as factors onto the ordination. Constrained analysis of principal coordinates (CAP) based on Bray–Curtis distance was performed to investigate the relationship between bacterial community composition and environmental factors. A BEST was used to rank the importance of environmental features in influencing beta-diversity community comparisons, with feature significance confirmed by permutational MANOVA. Partial least squares regression (PLSR) analysis was used to model associations between normalized mean values for significant environmental conditions (X variables) and taxonomic features (Y variables).

The rate of distance-decay of the bacterial communities was calculated as the slope of a linear least-squares regression on the relationship between the geographic distance and the bacterial similarity based on 1 − dissimilarity of the Bray–Curtis metric. Spatial variables were derived from geographic coordinates using both linear trend and the principle coordinates of neighbor matrices (PCNM) procedure (Griffith and Peres-Neto, 2006), that