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Effects of salinization and crude oil contamination on soil bacterial community structure in the Yellow River Delta region, China

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ABSTRACT

Soil salinization is a predominant environmental character in oil fields, especially in coastal regions. However, information about the coupling effect of crude oil contamination and salinization on soil biological characteristics is lacking. Therefore, the objective of this study was to examine soil bacterial community changes in response to different gradients of salinity and total petroleum hydrocarbon (TPH) concentration. Fifteen soil samples collected from the Yellow River Delta region of China with different gradients of salinity and TPH concentration were used for analyzing soil physicochemical properties, microbial biomass and denaturing gradient gel electrophoresis (DGGE) profiles. The results showed that salinity negatively affected soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN), but little affected bacterial Shannon and evenness indices. TPH concentration was correlated negatively with soil MBC, positively with MBN and Shannon index, but had no effect on evenness index. Canonical correspondence analysis showed that salinity and TPH concentration were the main factors causing the shift of soil bacterial community structure. Soil salinity had a suppress effect on most bacterial populations without changing their dominance, while soil TPH influenced the bacterial diversity selectively. By extraction of main bacterial clusters from the dendrogram tree of DGGE profiles, the most active bacterial species involved in the shift of bacterial community structure were identified under the single or dual stresses of salinization and oil contamination. Actinobacteria, γ-Proteobacteria, Firmicutes, Deinococcus–Thermus and some unclassified bacteria were the dominant bacteria participating in crude oil degradation in dual stresses of salinization and oil contamination. Our results provide new insight and useful information in the screening of cultivable bacteria for bioremediation of crude oil contaminated saline.

1. Introduction

Environmental factors have great influence on soil bacterial community. For crude oil contaminated soil, soil structure and physicochemical and biological characteristics, e.g., soil organic matter content, bulk density, porosity, permeability, soil respiration and material transfer processes, can be altered by the high hydrophobicity of hydrocarbons (Liang et al., 2012). Bacterial communities tend to be dominated by the strains that can survive in hydrocarbon-rich environments and degrade the oil contaminants for growth (Zucchi et al., 2003). Saline and hypersaline environments are frequently accompanied with crude oil contamination as a result of industrial activities (Oren et al., 1992). Microbial community composition is also susceptible to soil salinization due to differential tolerance of microbial genotypes (Mandeel, 2006; Pankhurst et al., 2001). The transition zone of different environments are ideal systems for exploring the succession of microbial community as the abiotic factors strongly impact the distribution patterns of species (Herlemann et al., 2011; Campbell and Kirchman, 2013). The bacterial community structure at the phylum and subphylum levels changes predictably with gradients in salinity and other environmental factors (Campbell and Kirchman, 2013). As for crude oil contaminated saline soil, microbial degradation is the main mechanism for natural decontamination, and a better understanding of the microbial community structure in soil along a gradient of both oil-contamination and salinization and soil microbial responses to their stresses could provide clues about the functional

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microorganisms that adapt to such habitats. It is also helpful to find out the microorganisms with different metabolic functions and to monitor the process of bioremediation. However, most of the previous studies focused more on the effect of crude oil contamination or bioremediation on the microbial community (Bordenave et al., 2007; Evans et al., 2004; Paissé et al., 2008; Röling et al., 2004; Yu et al., 2011). Information about the coupling effect of crude oil contamination and salinization on soil biological characteristics is lacking. Kleinsteuber et al. (2006) investigated the diversity and dynamics of bacterial community in an exploited oil field soil with high salinity in Argentina. Their results showed that the bacterial community shifted during long-term incubation with diesel fuel at four salinities between 0 and 20% NaCl, and the most active species changed with the alteration of salinity accordingly. Wang et al. (2011a) studied the responses of archaeal communities to different petroleum hydrocarbon concentrations in saline–alkali soil in China; however, they did not consider soil salinity as an independent factor due to the minor difference among the sampled soils.

In this study, we evaluated soil bacterial community changes in response to different gradients of salinity and total petroleum hydrocarbon (TPH) concentration in the Yellow River Delta region of China. The objectives of this study were: (1) to analyze the effects of salinization and crude oil contamination on soil microbial biomass carbon (MBC), microbial biomass nitrogen (MBN) and bacterial diversity; (2) to clarify the main soil environmental factors affecting the bacterial community of oil-contaminated saline soil; (3) to analyze the succession of soil bacterial community structure along a gradient of soil salinity and TPH concentration and to find out the main bacterial populations participating in crude oil degradation in the saline soil. We attempted to clarify the bacterial roles in natural attenuation of oil contaminated saline soil and throw a new light on the screening of the cultivable bacteria.

2. Materials and methods

2.1. Site description

The sampling sites are located in Shengli Oilfield (118°07′–119°10′E, 36°55′–38°10′N) in the Yellow River Delta region which was formed by the fluvial sedimentation of the Yellow River, the second largest river in China. With the continuous massive deposition at the mouth of the river of silt from erosion of the Loess Plateau in central China, the delta is still under expansion at a rate of 2000 ha per year (Liu and Drost, 1997). The annual average temperature is 11.7–12.6 °C, and annual precipitation 530–630 mm. Due to the low and flat terrain, high groundwater table, high evaporation/precipitation rates, poor drainage conditions, and infiltration of seawater, soil salinization in this area has been severe. The dominant soils along the seashore are commonly Salic Fluvisols and Gleyic Solonchaks (Fang et al., 2005). According to a previous study (Wang et al., 2011a), the proportions of alkane, aromatic, polar N-, S-, O-containing compounds and asphaltene fractionated from the TPH of the chronically contaminated saline soil were about 35%, 18%, 25% and 22% in the Yellow River Delta region.

2.2. Soil sampling

In May 2010, five chronically oil-contaminated sites were selected according to their distances to the shoreline, with an interval of about 20 km between neighboring sites. One oil well was selected at each site. Considering the wellhead as the center, we sampled soils in cross directions. In each direction, four quadrats with a size of 1 m × 1 m were installed at 5-m intervals. Five soil cores ($\Phi = 2.5$ cm) were collected from the 0–20 cm layer of each quadrat with a shape of “W” and composited, and altogether 76 samples were obtained. The samples were sealed in plastic bags, stored in an ice box and sent to the laboratory within 6 h after sampling. Each field-moist soil sample was sieved (2 mm) and divided into two subsamples stored at 4 °C and −20 °C, respectively, until analysis.

2.3. Soil property and soil microbial biomass analyses

The soil samples used for the test of electrical conductivity (EC) and pH were air-dried. Soil EC and pH were measured in a 1:5 sample/water mixture with a DDS-307W microprocessor conductivity meter (Shanghai Lida Instrument Factory, China) at 25 °C after shaking for 30 min. The conversion relationship between the $EC_{1:5}$ and the salinity of coastal saline soil was calculated using the following formula (Liu et al., 2006):

$$EC_{1.5} = 0.3658S_t - 0.0152(r^2 = 0.988, P < 0.01)$$

where $EC_{1:5}$ is the EC of 1:5 soil–water extract in dS m^{-1}, and S_t is the soil salinity in g kg^{-1}.

According to the US Soil Salinity Laboratory (Richards, 1954), soil samples with EC above 4 mS cm^{-1} (salinity 11 g kg^{-1}, according to the $EC_{1:5}$ and salinity conversion formula above mentioned) were considered to be saline. The soil water-holding capacity was measured according to Alef and Nannipieri (1995, p. 106). Crude oil in the soil samples were extracted with *n*-hexane (EPA 3550B, 1996) and the organic extracts were analyzed in terms of TPH (EPA 8015B, 1996). The soil samples with TPH concentration above 1.6 g kg^{-1} were considered to be contaminated with crude oil (Wang et al., 2011b; Gao et al., 2013). Fifteen of the seventy-six soil samples with a broad gradient of both soil salinity and TPH were selected for further laboratory analysis (Sections 2.4 and 2.5) to reveal relationships between environmental stresses and soil microbial diversity and community structure. Soil total nitrogen (TN) concentration was analyzed by the Kjeldahl method, and soil total phosphorus (TP) was determined by the molybdenum–stibium colorimetry method with a continuous-flow analyzer (AutoAnalyzer III, Bran + Luebbe GmbH, Germany) after the samples were digested with H_2SO_4. Soil MBC was determined by the chloroform fumigation–extraction method (Vance et al., 1987) and soil MBN was determined by the ninhydrin-reactive N (N-nin) measurements described by Joergensen and Brookes (1990).

2.4. PCR-DGGE analysis

Total genomic DNA was extracted from the soil samples using the E.Z.N.A.^{TM} Soil DNA Kit (Omega Biotek, USA) according to the manufacturer’s instructions. The quantity of the extracted DNA was determined using Scientific NanoDrop 2000 spectrophotometer (Thermo, USA), then the DNA was diluted to about 15 ng μL^{-1} for further PCR amplification. The variable V3 region of 16S rRNA was amplified by PCR using a pair of universal primers, 338F 50-ACTCCTACGGGAGGCAGCAG-30 and 534 R 5′-ATTACCGCGGCTGCTGG-3′, to which a GC clamp (CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGG) was attached at the 5′-terminus (Muyzer et al., 1993). The PCR mixture consisted of 5 μL of DNA template, 2 μL of 338 F/534R (10 μM) primers each, 25 μL of Tiangen 2× Taq PCR Master Mix and 16 μL ddH_2O comprising a total volume of 50 μL (Tiangen Biotech, Beijing). A modified touch-down PCR procedure was used for cycling amplification in a Veriti^{TM} PCR thermal cycler (Applied Biosystems,