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Occurrence of *Pseudomonas aeruginosa* in Kuwait soil

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HIGHLIGHTS

- Factors resulting in the dominance of *P. aeruginosa* in different soil types in Kuwait was demonstrated.
- Dominant *P. aeruginosa* showed high rates of oil utilization and tolerance to metals.
- Unculturable *P. aeruginosa* in soil showed higher stability compared to the culturable fraction.

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ABSTRACT

Environmentally ubiquitous bacteria such as *Pseudomonas aeruginosa* evolved mechanisms to adapt and prevail under diverse conditions. In the current investigation, strains of *P. aeruginosa* demonstrating high rates of crude oil utilization and tolerance to high concentrations of heavy metals were found in both crude oil-contaminated and uncontaminated sites in Kuwait, and were dominant in the contaminated sites. The incidence of *P. aeruginosa* in tested soils implies the definitive pattern of crude oil contamination in the selection of the bacterial population in petroleum-contaminated sites in Kuwait. Surprisingly, the unculturable *P. aeruginosa* in different soil samples showed significant high similarity coefficients based on 16S-RFLP analyses, implying that the unculturable fraction of existing bacterial population in environmental samples is more stable and, hence, reliable for phylogenetic studies compared to the culturable bacteria.

1. Introduction

Crude oil is considered the main energy source worldwide. Accidental discharge of crude oil into the environment occurs frequently during petroleum production (Paine et al., 1996). Such discharges do cause damage to the ecosystems (Briggs et al., 1996), and constitute health hazards (Lipscomb et al., 1993, 1994). Bioremediation has proved to be a promising approach for the clean-up of crude oil pollution (Sohal and Srivastava, 1994; Alexander, 1999). It is a complex process that depends mainly on the composition of environmental microbial communities, their adaptive response to pollution and prevailing environmental conditions. Microbial communities within crude oil-contaminated sites are dominated by bacteria showing high rates of crude oil utilization and high adaptability to existing environmental conditions (AL-Saleh and Obuekwe, 2005; Obuekwe et al., 2009; AL-Saleh et al., 2009) which eventually influence the structure of the microbial communities rendering it less diverse (Macnaughton et al., 1999).

Previous studies based on culture-dependent techniques on Kuwait soils demonstrated the presence of culturable indigenous oil-degrading bacterial genera such as *Bacillus*, *Paenibacillus*, *Pseudomonas*, *Rhodococcus* and *Streptomyces* (AL-Saleh et al., 2009; Obuekwe et al., 2007, 2009; Radwan et al., 1995). Such isolates were shown to have high potentials for hydrocarbon bioremediation (Obuekwe and AL-Zarban, 1998; AL-Saleh and Obuekwe, 2005; Obuekwe et al., 2009). However, the applications of culture-dependent techniques can lead to inaccurate descriptions of microbial diversity and functionality (Zak et al., 1994; Torsvik et al., 1998; Ellis et al., 2003; Neufeld and Mohn, 2006) because unculturable microorganisms represent the larger fraction of microbial communities (Islam et al., 1993; Janssen, 2006). Any apparent microbial activity determined in environmental samples should be attributed to the actual communities present, including the unculturable microorganisms (Colwell and Grimes, 2000). Additionally, inaccurate description of crude oil-degrading microbial communities and their biodegradative potentials deduced from the application of culture-dependent techniques could result

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in imprecise and prolonged management of contaminated sites (Hawumba et al., 2010). The application of molecular methods is expected to solve the problems of inadequate characterization of isolated bacteria owing to the inability of culture-dependent techniques to differentiate among closely related species that metabolize a similar range of substrates (Stackebrandt and Ebers, 2006).

Strains of *Pseudomonas aeruginosa* capable of growing on crude oil have frequently been isolated from various Kuwait environments (Obuekwe et al., 2008; AL-Saleh et al., 2009) which suggested that the members of the genus must be playing a major role in the bioremediation of crude oil contamination in Kuwait environment. Therefore, the objective of the current study was to assess the diversity of the dominant members of crude oil-degrading bacterial community, in particular *P. aeruginosa* in crude oil-contaminated and uncontaminated sites in south of Kuwait using traditional and molecular approaches.

2. Materials and methods

2.1. Sampling site

Samples were collected from the Burgan oil field, located in the desert of south-eastern Kuwait (Fig. 1). The field has been producing petroleum for over sixty years resulting in an area extensively polluted from oil released from petroleum production activities, while patches of apparently unpolluted sites were scattered over the field. Having been subjected to crude oil pollution for more than sixty years and under constant threat of pollution from production activities, the area was considered an appropriate site to investigate the effects of crude oil pollution on microbial community.

2.2. Soil sampling

Soil samples were collected from the upper 10 cm of each of ten distantly-located locations (approximately 500 m apart). Collected soils were sandy loam characterized by brownish-black coloration and oily feel to the touch, due apparently to crude oil contamination. Similarly, ten samples were also collected from other locations in the visibly uncontaminated sites in the same general area. All soil samples were collected in sterile 1 L screw-capped bottles, kept in ice and transported immediately to the laboratory for immediate analyses, or stored at −20 °C in the case of samples which would be used for molecular analyses. Chemical and microbiological analyses of all samples were completed within 48 h after collection. Oxygen contents of samples were analysed on site. In the current study, the terms contaminated and uncontaminated soil samples were used to indicate crude oil-contaminated and crude oil-uncontaminated soil samples.

2.3. Characterizations of soil samples

Values of electrical conductivity, dissolved oxygen (DO), salinity and pH of soil samples were determined using Water Quality Checker (Horiba U-10, Horiba Instruments Limited, Northampton, UK) following standard methods (Alef and Nannipieri, 1995). For this purpose, the probe was calibrated with the appropriate standard solutions using the auto one-point calibration method following the manufacturer's instructions. The calibrated probe was inserted into locations with high water content and pebble-free soils. Measurements were taken after stabilization of the quality checker reading. The method of Forster (1995) was adopted for the determination of total organic carbon (TOC). In brief, TOC was determined in a TOC analyser (Shimatzu, TOC 5000A with SSM 5000A module, Shimadzu Scientific Instruments, Columbia, USA) according to the NF ISO 10694 standard. TOC values were obtained by subtracting inorganic carbon (IC) from total carbon (TC). The concentrations of metals present in soil samples were determined following the method of Rowell (1994). Briefly, soil samples were acid-digested (130 mL conc. HCl and 120 mL water, of this solution 150 mL was added to 50 mL HNO_3 and mixed) with heating in a reflux tube. The concentration of metals in the filtered, digested material was determined using flame atomic absorption spectroscopy and graphite furnace atomic absorption spectrometry (Varian AA-1475, California, USA), atomizing the metals with acetylene-air (1:1) flame.

2.4. Isolation and enumeration of bacteria in soils

The culturable fractions of the crude oil-degrading bacteria (CODB) in soil samples were enumerated using minimal agar following standard serial dilution method (AL-Saleh and Obuekwe, 2005). Crude oil was supplied in the vapor phase, and plates were incubated inverted at 30 °C for up to 21 d. Grown colonies were counted, expressed as colony-forming unit (cfu) g^{-1}soil, picked

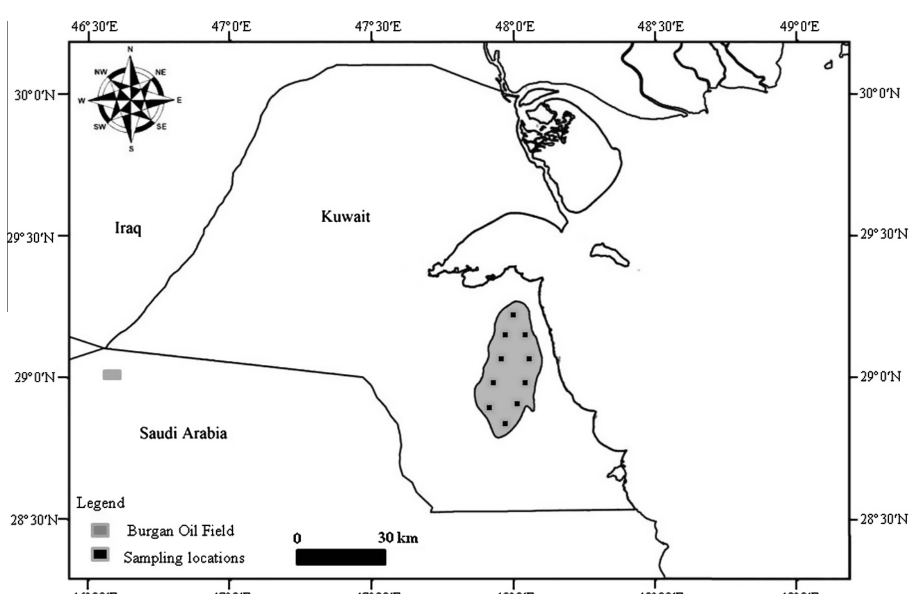

Fig. 1. Map of Kuwait showing site of Burgan oil field and sampling locations. The map is adapted from Saif ud din et al. (2008).