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Isolation and characterization of crude oil degrading bacteria from the Persian Gulf (Khorramshahr provenance)

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ABSTRACT

Fifteen crude oil degrading bacteria were isolated from oil contaminated sites in the Persian Gulf at Khorramshahr provenance. These bacteria were screened with two important factors such as growth rate on crude oil and hydrocarbon biodegradation, and then three strains were selected from 15 isolated strains for further study. One strain (PG-Z) that show the best crude oil biodegradation was selected between all isolates. Nucleotides sequencing of the gene encoding for 16S rRNA show that strain PG-Z belong to *Corynebacterium variabile* genus. This strain was efficient in degrading of crude oil. This strain was capable to degraded 82% of crude-oil after one week incubation in ONR7a medium. The PG-Z strain had high emulsification activity and biosurfactant production between all isolates. GC–MS analysis shows that *C. variabile* strain PG-Z can degrade different alkanes in crude oil.

1. Introduction

Petroleum hydrocarbons are the most common environmental pollutants in the world and oil spills pose a great hazard to terrestrial and marine ecosystems. Oil pollution may arise either accidentally or operationally whenever oil is produced, transported, stored and processed or used at sea or on land. Oil spills are a major menace to the environment as they severely damage the surrounding ecosystems (Head et al., 2006; Emtiazi et al., 2009).

Persian Gulf is a marine environment that was polluted with crude oil during the 1991 Gulf war. The pollution impact of this episode has been evaluated in several studies, all indicated that crude oil accumulated and remained for long time in coastal area (Hassanshahian et al., 2010; Tebyanian et al., 2013). The oil pollution problem is particularly acute in an oil producing area such as the Persian Gulf, where about 60% of the marine transported oil in the world is carried out (Radwan et al., 2005; Ghanavati et al., 2008).

Biodegradation by natural populations of microorganisms is the basic and the most reliable mechanism by which thousands of xenobiotic pollutants, including crude oil, are eliminated from the environment (Cappello et al., 2007). The effects of environmental conditions on the microbial degradation of hydrocarbons and the effects of hydrocarbon contamination on microbial communities are areas of great interest (Rahman et al., 2004; Cappello et al., 2012). Bioremediation is the strategy to utilize biological activities as much as possible for quick elimination of environmental pollutants. Growth stimulation of indigenous microorganisms, biostimulation, along with inoculation of foreign oil-degrading bacteria is a promising means of accelerating detoxifying and degrading activities at a polluted site with minimum impact on the ecological systems (Cappello et al., 2006).

Growth of microorganisms on hydrocarbons is a particular problem, because hydrocarbons are immiscible in water. Many bacteria are capable to emulsifying hydrocarbons in solution by producing surface active agents such as biosurfactants which increases the adhesion of cells to the substrate. Biosurfactants reduce surface tension by accumulating at the interface of immiscible fluids, increasing the surface area of insoluble compounds which leads to increased bioavailability and subsequent biodegradation of the hydrocarbons (Batista et al., 2006).

Alkanes are the major component of crude oil (Van Beilen et al., 2003). Some marine bacteria have ability to biodegradation of alkanes and these bacteria are important for marine environment (Kohno et al., 2002).

The aims of the present study is to study some bacterial strains isolated from oil polluted sites in Persian Gulf (Khorramshahr provenance) regarding their capability to producing biosurfactant and biodegradation of crude oil.

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2. Materials and methods

2.1. Sampling

For isolation of crude oil degrading bacteria sediments and seawater samples were collected from fifty stations in the Khorramshahr and Asaloyiah at Persian Gulf (37°30′N; 49°15′E). Fig. 1 shows the map of sampling sites.

Sediments samples (500 g) were taken from 1 to 12 cm below the surface of coastal using a sterile knife. Seawater samples (1 l) were collected from a depth of 15 cm in sterile 100 ml bottles and transported on ice to the laboratory for isolation.

2.2. Isolation and selection of crude-oil degrading bacteria

The ONR7a medium with 1% (v/v) of crude-oil (Iranian light crude oil) as sole carbon source and energy used for isolation of crude oil degrading bacteria. ONR7a contained (per liter of distilled water) 22.79 g of NaCl, 11.18 g of $MgCl_2{\cdot}6H_2O$, 3.98 g of Na_2SO_4, 1.46 g of $CaC1_2{\cdot}2H_2O$, 1.3 g of TAPS0 {3-[N tris(hydroxymethyl) methylamino]-2-hydroxypropanesulfonica cid}, 0.72 g of KCl, 0.27 g of NH_4Cl, 89 mg of $Na_2HP0_4{\cdot}7H_20$, 83 mg of NaBr, 31 mg of $NaHCO_3$, 27 mg of H_3BO_3, 24 mg of $SrCl_2{\cdot}6H_20$, 2.6 mg of NaF, and 2 mg of $FeCl_2{\cdot}4H_20$. For solid media, Bacto Agar (Difco) (15 g/l) was added to the solution (Dyksterhouse et al., 1995).

Portion of sediments (10 g) or condensed seawater (10 ml) were added to Erlenmeyer flasks containing 100 ml of medium and the flasks were incubated for 10 days at 30 °C on rotary shaker (180 rpm, INFORS AG). Then 5 ml aliquots were remove to fresh medium. After a series of four further subcultures, inoculums from the flask were streaked out and phenotypically different colonies purified on ONR7a agar medium. Phenotypically different colonies obtained from the plates were transferred to fresh medium with and without crude-oil to eliminate autotrophs and agar-utilizing bacteria. The procedure was repeated and isolates only exhibiting pronounced growth on crude-oil were stored in stock media with glycerol at −20 °C for further characterization (Chaillan et al., 2004; Hasanshahian and Emtiazi, 2008).

2.3. Identification of isolates

2.3.1. Biochemical characterization

To identify and characterize the bacteria isolates, biochemical tests such as Gram staining, oxidation/fermentation, production of acid from carbohydrates, hydrolysis of gelatin and citrate carried out according to the Bergey's manual is for identification (taxonomy) (Holt et al., 1998).

2.3.2. Molecular identification

Analysis of 16S rRNA was performed to the taxonomic characterization of isolated strains. Total DNA extraction of bacterial strains was performed with the CTAB method (Winnepenninckx et al., 1993). The bacterial 16S rRNA loci were amplified using the forward domain specific bacteria primer, Bac27_F (5′-AGAGTTTGATCCTGGCTCAG-3′) and universal reverse primer Uni_1492R (5′-TACGYTACCTTGTTACGACTT-3′). The amplification reaction was performed in a total volume of 25 μl consisting, 2 mM $MgCl_2$ (1 μl), 10X PCR reaction buffer (200 mM Tris; 500 mM KCl) (2.5 μl), 2 mM each dNTP (2 μl), 0.15 mM each primer (1 μl), 1U (0.5 μl) taq DNA polymerase (Qiagen, Hilden, Germany) and 2 μl of template DNA (50 p). The distill water was added for remaining of reaction (15 μl).

Amplification for 35 cycles was performed in a thermacycler GeneAmp 5700 (PE Applied Biosystem, Foster City, CA, USA). The temperature profile for PCR was kept, 94 °C for 5 min, 94 °C for 1 min, 54 °C for 1 min, 72 °C for 1 min, 30 cycles; then 72 °C for 10 min and finally storage at 4 °C (Troussellier et al., 2005). The 16S rRNA amplified was sequenced with a Big Dye terminator V3.1 cycle sequencing kit on an automated capillary sequencer (model 3100 Avant Genetic Analyzer, Applied Biosystems).

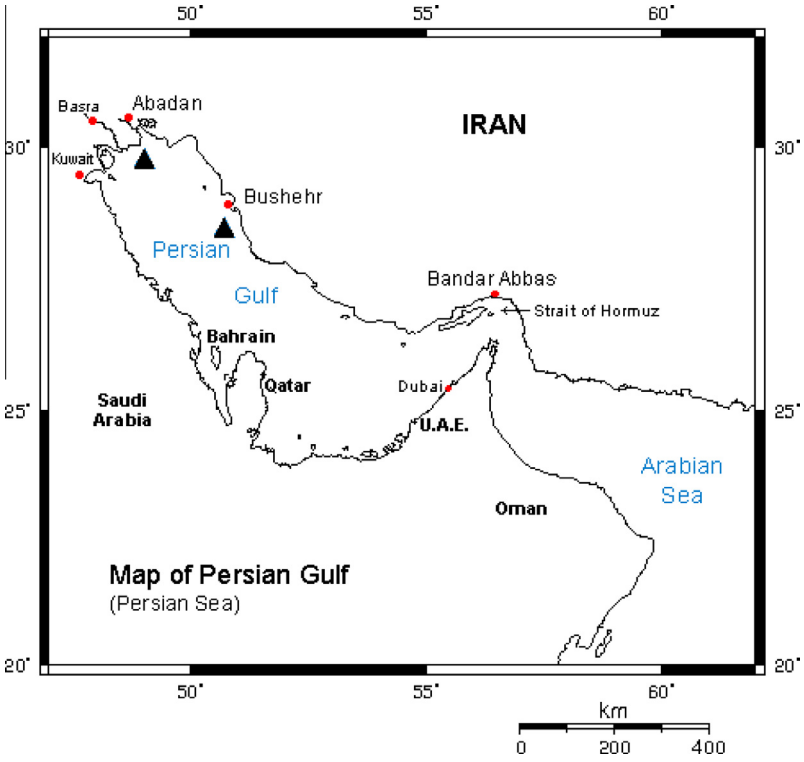

Fig. 1. Maps showing the location of sediment and seawater samples collected in the Persian Gulf (sample location showed as symbol ▲).

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