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Enrichment and isolation of crude oil degrading bacteria from some mussels collected from the Persian Gulf

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ABSTRACT

To date, little is known about existing relationships between mussels and bacteria in hydrocarbon-contaminated marine environments. The aim of this study is to find crude oil degrading bacteria in some mussels at the Persian Gulf. Twenty eight crude oil degrading bacteria were isolated from three mussels species collected from oil contaminated area at Persian Gulf. According to high growth and degradation of crude oil four strains were selected between 28 isolated strains for more study. Determination the nucleotide sequence of the gene encoding for 16S rRNA show that these isolated strains belong to: *Shewanella algae isolate* BHA1, *Micrococcus luteus isolate* BHA7, *Pseudoalteromonas sp. isolate* BHA8 and *Shewanella haliotis isolate* BHA35. The residual crude oil in culture medium was analysis by Gas Chromatography (GC). The results confirmed that these strains can degrade: 47.24%, 66.08%, 27.13% and 69.17% of crude oil respectively. These strains had high emulsification activity and biosurfactant production. Also, the effects of some factors on crude oil degradation by isolated strains were studied. The results show that the optimum concentration of crude oil was 2.5% and the best degradation take place at 12% of salinity. This research is the first reports on characterization of crude oil degrading bacteria from mussels at Persian Gulf and by using of these bacteria in the field the effect of oil pollution can be reduce on this marine environment.

1. Introduction

Many techniques are utilized to mitigate or cleanse petroleum pollution in the environment. During exploration, production, refining, transport and storage of petroleum and petroleum products, some accidental spill may be released into the sea waters (Dave and Ghaly, 2011; Ghanavati et al., 2008). Crude oil is toxic, mutagenic and carcinogenic this pollutant concedes a serious damage to marine life (Todd et al., 2010). Bioremediation is the best method for elimination of oil spills. Bioremediation of oil contamination compared with physicochemical treatment is more effective and lower cost price (Saxena et al., 2013). Bacteria are important for the biodegradation of petroleum hydrocarbons and many hydrocarbon-degrading bacteria have been isolated from different environments (Kaczorek et al., 2008; Udeh et al., 2013; Hassanshahian et al., 2012a; Radwan et al., 2002; Khan et al., 2006). Biodegradation rates can be controlled by concentration and composition of hydrocarbons, nutrients, oxygen, salt concentrations, moisture and temperature (Sathishkumar et al., 2008; Hassanshahian et al., 2010). Also, biosurfactants increase the solubility of hydrocarbons to bacteria and enhanced degradation of these toxic compounds (Hassanshahian et al., 2012a). Marine organisms can take up contaminants from bottom sediments, suspended particulate material and food sources. Filter-feeding bivalves such as mussels, oysters, and clams are very important components in the process of bioremediation of the marine environment (Stabili et al., 2005). They are capable to filter and accumulate a large number of fine particulate matters, including phytoplankton, zooplankton, microorganism and other particulate organic debris. Furthermore, the organic components in the suspended matters can be trapped and applied by filter-feeding bivalves (Manganaro et al., 2009; Zhou et al., 2014; Hassanshahian et al., 2013). Little work has been done on the distribution and physiology of autochthonous hydrocarbon-degrading microbes inhabiting marine organisms (Cappello et al., 2012a; Hassanshahian et al., 2012b). Some researchers confirmed that crude oil degrading bacteria were existing in marine organisms. For example, Radwan et al. (2005) establish that some crude oil degrading bacteria such as *Pseudomonas*, *Bacillus* and *Acinetobacter* were associated with cyanobacterial mat at Arabian Gulf (Radwan et al., 2005; Radwan et al., 2002). Also, Al-Mailem et al. (2010) study crude oil degrading bacteria that symbiosis with filamentous cyanobacteria and their results show that some bacteria such as *Halomonas aquamarina*, *Marinobacter hydrocarbonoclasticus*, *Marinobacter sp.*; *Dietzia maris and Alcanivorax* can degrade crude oil and present in cyanobacteria (Al-Mailem et al., 2010; Sorkhoh et al., 1990; Al-Awadhi et al., 2003).

In all research that studied the relationship between bacteria and marine animals, it is confirmed that the density of bacteria in the marine animal samples were higher compared to surrounding water. For

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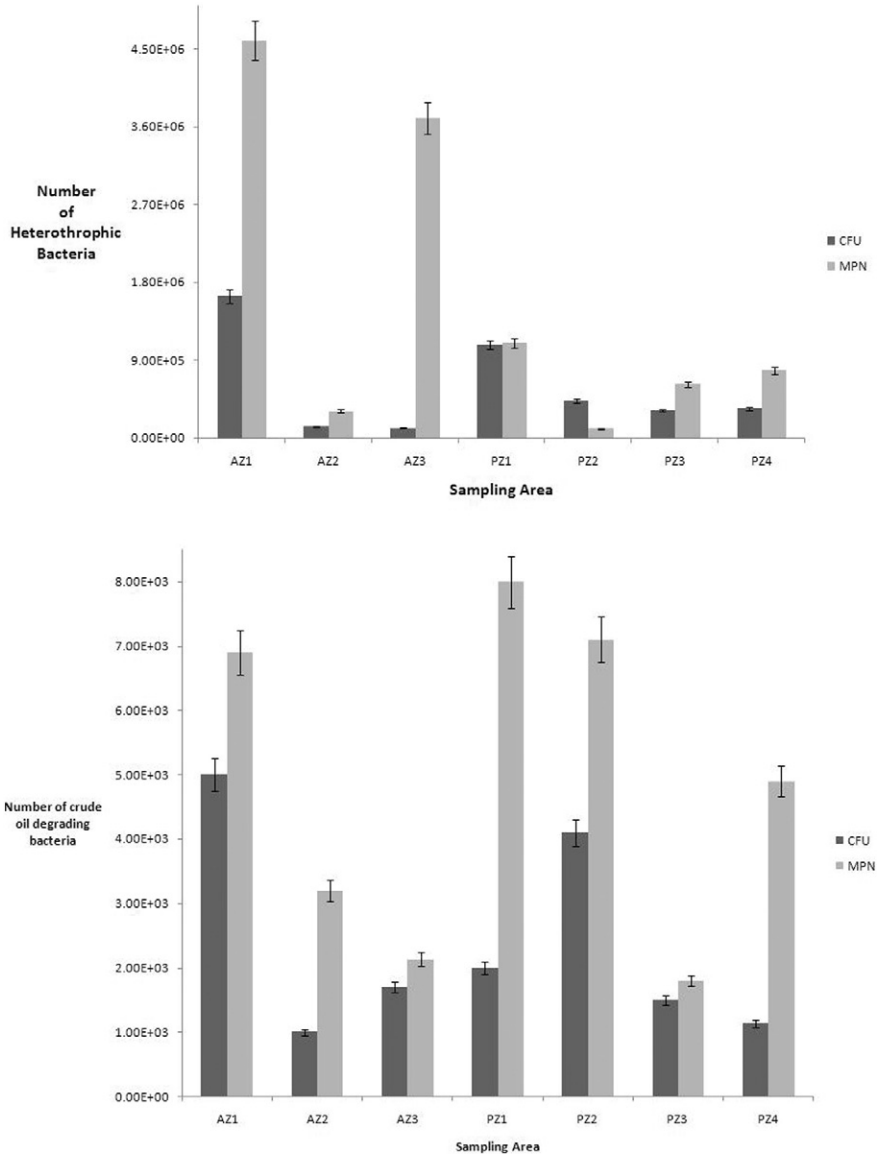

Fig. 1. Enumeration of heterotrophic and hydrocarbon-degrading bacteria by CFU and MPN methods. All data present in this figure are the average of three replicates of enumeration in the same dilution.

example, mussels can filter the seawater and concentrate the marine bacteria that cause reducing the bacterial concentration in the seawater (Cavallo et al., 2009).

To date, little is known about existing relationships between mussels and bacteria in hydrocarbon-contaminated marine environments. The aim of this study is to find crude oil degrading bacteria in some mussels at the Persian Gulf. Also identification of these strains and the degradation capacity of these isolates is another purpose of this research.

2. Materials and methods

2.1. Sampling

Mussels, seawater and sediment samples were collected from two oil contaminated sites at Persian Gulf. These two stations were located in the Qeshm island (AZ: Zakeri Harbor; 36°15, N; 34°15, E) and (PZ: Park Ziton 37°30, N; 49°15, E). Mussels were collected from depth range 5–15 m. Also, samples of seawater from above the mussels' bed in a sterile bottle, and samples of sediment from below were collected. The mussels were identified according to the standard key (Huber, 2010). The results of identification confirmed that these three mussels related to: *Crassostrea gigas*, *Chama asperella* and *Barbatia tenella* genus. Collected samples were transported on ice to the laboratory. Mussels shell was removed by sterile knife and gill and other tissue of mussel samples was washed with sterile seawater. In fact, the tissue was homogenized in buffer. Finally, macerate of mussels was used for subsequent studies.

2.2. Isolation and selection of crude-oil degrading bacteria

Crude oil degrading bacteria were isolated in ONR7a medium supplemented with 1% (v/v) of crude oil (Iranian light crude oil) as sole carbon source and energy. ONR7a contained (per liter of distilled water) 40 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 hydroxypropanesulfonic acid}, 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, Bacterial Agar (15 g/l) was added to the solution (Hasanshahian and Emtiazi, 2008).

Tissue macerate of mussel (5 ml), condensed seawater (5 ml) and portion of sediments (10 g) were added to Erlenmeyer flasks containing