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Biosurfactant-assisted bioremediation of crude oil by indigenous bacteria isolated from Taean beach sediment

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ABSTRACT

Crude oil and its derivatives are considered as one group of the most pervasive environmental pollutants in marine environments. Bioremediation using oil-degrading bacteria has emerged as a promising green cleanup alternative in more recent years. The employment of biosurfactant-producing and hydrocarbon-utilizing indigenous bacteria enhances the effectiveness of bioremediation by making hydrocarbons bioavailable for degradation. In this study, the best candidates of biosurfactant-producing indigenous bacteria were selected by screening of biochemical tests. The selected bacteria include *Bacillus algicola* (003-Phe1), *Rhodococcus soli* (102-Na5), *Isoptericola chiayiensis* (103-Na4), and *Pseudoalteromonas agarivorans* (SDRB-Py1). In general, these isolated species caused low surface tension values (33.9 −41.3 mN m^{-1}), high oil spreading (1.2−2.4 cm), and hydrocarbon emulsification (up to 65%) warranting active degradation of hydrocarbons. FT-IR and LC-MS analyses indicated that the monorhamnolipid (Rha-$C_{16:1}$) and dirhamnolipid (Rha-Rha-C_6-$C_{6:1}$) were commonly produced by the bacteria as potent biosurfactants. The residual crude oil after the biodegradation test was quantitated using GC-MS analysis. The bacteria utilized crude oil as their sole carbon source while the amount of residual crude oil significantly decreased. In addition the cell-free broth containing biosurfactants produced by bacterial strains significantly desorbed crude oil in oil-polluted marine sediment. The selected bacteria might hold additional capacity in crude oil degradation. Biosurfactant-producing indigenous bacteria therefore degrade crude oil hydrocarbon compounds, produce biosurfactants that can increase the emulsification of crude oil and are thus more conducive to the degradation of crude oil.

1. Introduction

Crude oil and its derivatives are of significant concerns among the numerous environmental pollutants that adversely impact on marine ecosystems (Okoh and Trejo-Hernandez, 2006). Oil seeps and spills in the oil and gas industry often lead to both immediate and long-term environmental damage. When crude oil enters the marine environment, it travels into the marine food web and persists in the marine environment for many years, exerting detrimental effects on biological systems (Martínez-Palou et al., 2013). These detrimental hydrocarbon pollutants therefore render the development of remediation technology for cleaning oil contaminated sites essential.

Many studies have documented that bioremediation using oil-degrading bacteria has emerged as a promising green cleanup alternative in marine environments (Nikolopoulou and Kalogerakis, 2008; Nikolopoulou et al., 2013a; Nikolopoulou et al., 2013b). Microbial remediation would be one of the best practices in cleaning oil contaminated environments because it is more efficient, eco-friendly, and cost effective methods than other typical mechanical or chemical methods (Ismail et al., 2013). Thus, developing a suitable method that can be adopted to accelerate the bioremediation of oil contaminated sites remain highly promising, such as identifying biosurfactant (BS)-producing hydrocarbon-degrading microorganisms.

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BSs are chemically active surface compounds synthesized by specific groups of microbes that utilize substrates such as simple sugars, oils, and hydrocarbons in contaminated environments. They reduce surface and interface tension between liquid and solid substances that increase the bioavailability of organic pollutants, including crude oil components, and thus enhance biodegradation (Das and Mukherjee, 2007; Whang et al., 2008; Randhawa and Rahman, 2014). BSs are amphiphilic compounds with both polar and non-polar moieties and comprise different structures, including glycolipids, phospholipids, lipopeptides, fatty acids, and polysaccharide-protein complexes (Cameotra and Makkar, 2010). Glycolipids (rhamnolipids, sophorolipids, and trehalose lipids) are low-molecular-weight BSs that exhibit a wide range of applicability due to their structural variations. These stable but readily biodegradable BSs are amphiphilic in nature; their alkyl chains are linked to sugar molecules, resulting in hydrophilic and hydrophobic regions (Costa et al., 2010).

In comparison to their synthetic counterparts, viz., chemical surfactants, BSs are lesser toxic, easily biodegradable, and also active in extreme environments such as high salinity and/or temperature condition. The development of active microbial BS-producing cultures is thus important warranting the efficiency of hydrocarbon remediation (De Rienzo et al., 2016). Many recent studies reported the application of BS-producing microbes in oil-contaminated environments to remove hydrocarbons and remediate the environment (Ibrahim et al., 2013; Ferradji et al., 2014). The enhancement of crude oil degradation by BS production has been well studied in members of several bacterial genera such as *Pseudomonas*, *Bacillus*, *Acinetobacter*, *Alcaligenes*, *Alcanivorax*, *Rhodococcus*, and *Corynebacterium* (Cameotra and Makkar, 2010; Abbasian et al., 2016; Parthipan et al., 2017; Patowary et al., 2017).

Intertidal flats along the coast of Taean, Korea, have long been heavily contaminated since the Hebei Spirit Oil Spill (HSOS) on Dec. 7th, 2007. Long-term monitoring has revealed that sedimentary polycyclic aromatic hydrocarbons (PAHs) along the beach timely decreased and almost reached ambient background concentrations, but benthic ecosystems did not seem to be fully recovered yet (Hong et al., 2014; Kim et al., 2017; Yim et al., 2017). In our recent study, we demonstrated that microbial community composition, specifically the occurrence and abundance of PAH-degrading bacteria in oil-contaminated environments, resulted in the decrease of PAHs concentrations by bioremediation (Lee et al., 2018). Thus, identifying the most active BS-producing hydrocarbon-degrading bacteria in situ would aid to site-specific bioremediation for the hot spot of oil contaminated site.

The main purpose of the present study is to evaluate whether screened PAH-degrading bacteria produce BS during the degradation process by utilizing the crude oil components and to characterize the BSs that might be produced. Functional and structural analyses of the BS were performed as part of study and the amount of residual crude oil after biodegradation was quantitated in order to determine the removal efficiency of biodegradation of residual crude oil by presently isolated BS-producing indigenous bacteria from Taean coast. In addition the extent of crude oil desorption from contaminated marine sediment containing biosurfactant was measured with GC-MS profiles. The present study will aid us in understanding the role of BSs in hydrocarbon degradation and provide a new dimension in the BS-mediated bioremediation of hydrocarbon contaminants.

2. Materials and methods

2.1. Strains and cultivation

The sampling of beach sediments and isolation of cultivable bacteria from enrichment cultures were described previously (Lee et al., 2018). Representatives of selected bacteria were employed for the degradation of crude oil and PAHs in shake flasks. Seed inocula of matching optical density ($OD_{600} = 1.0$) were prepared in marine broth 2216 (MB, Difco) from all the bacteria isolates. The seed inocula (2%) were aseptically added to flasks containing 100 mL of mineral salt medium [MSM; 10.0 g of $(NH_4)_2SO_4$, 1.1 g of KCl, 30.0 g of NaCl, 0.28 mg of FeSO4, 3.4 g of KH_2PO_4, 4.4 g of K_2HPO_4, 0.7 g of $MgSO_4 \cdot 7H_2O$, 0.5 g of yeast extract, and 0.5 mL of trace salt solution per liter]. The trace salt solution included 0.29 g of $ZnSO_4$, 0.24 g of $CaCl_2$, 0.25 g of $CuSO_4$ and 0.17 g of $MnSO_4$ per liter and was filtered with a 0.22-μm pore membrane. The pH was adjusted to 7.0 with 1.0 M NaOH and 1.0 M HCl.

2.2. Screening of potential BS-producing bacteria

2.2.1. Hemolytic activity

A hemolytic assay was performed for the screening of BS-producing bacteria as described by Sharma et al. (2015). Isolated colonies were inoculated on the surface of blood agar plates, which were then incubated at 37 °C for 24–48 h. The hemolytic activity of the bacterial culture was detected by the presence of a clear halo zone around the colonies.

2.2.2. DCPIP assay

For a rapid and simple selection of hydrocarbon-oxidizing isolates, an assay based on the redox indicator 2,6-dichlorophenol indophenol (DCPIP, Sigma) was used (Hanson et al., 1993). Briefly, a bacterial suspension (1 mL, $OD_{600} = 1.0$) was added to 9 mL of sterile MSM supplemented with crude oil (10 μL) in a sterile glass test tube (in triplicate). The final concentration of DCPIP was 0.16 mg mL^{-1}. Cultures were incubated at 28 °C with agitation (180 rpm) for 24 h. The color of the medium was subsequently observed, and the sample was evaluated as positive for microbial hydrocarbon-degrading ability if colorless (degraded) and negative for microbial hydrocarbon-degrading ability if blue (not degraded).

2.2.3. CTAB assay

The cetyltrimethylammonium bromide (CTAB) agar plate method is a rapid screening method for the detection of anionic BSs (Walter et al., 2010). MSM agar was prepared with a carbon source (glucose 2%, w/v), CTAB (0.5 mg mL^{-1}), and methylene blue (0.2 mg mL^{-1}). Then, 50 μL of a bacterial culture that had been grown in MB broth for 3 days at 180 rpm and 28 °C was spot-inoculated at the center of the CTAB agar plate, and the plate was incubated at 28 °C for 24 h. A dark halo around the colonies indicated BS production. With this method, anionic BSs, such as rhamnolipids, can be detected.

2.2.4. Oil spreading assay

An oil spreading assay was performed as described by Hassanshahian (2014). First, 40 mL of distilled water was added to petri dishes, followed by 100 μL of crude oil on the surface of the water. Then, 10 μL of cell-free culture filtrates obtained by filtering a bacterial culture through 0.22-μm pore size membrane filter paper (Millipore) was added to the center of the crude oil surface. The diameter of the resulting clear zone on the oil surface was measured. A negative control was produced with distilled water (without surfactant), which resulted in no oil displacement or clear zone. SDS (1%, v/v) was used as the positive control.

2.2.5. Emulsification index (E_{24})

The emulsification activity of BSs was evaluated by a previously described method (Panjiar et al., 2015) against n-hexane, benzene, and mineral oil. An volume of tested solution was added to an equal