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Bioremediation of oil-contaminated soil using *Candida catenulata* and food waste

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Enhancement on degradation ability of petroleum hydrocarbon by the microbial strain in the composting process with food waste.

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ABSTRACT

Even though petroleum-degrading microorganisms are widely distributed in soil and water, they may not be present in sufficient numbers to achieve contaminant remediation. In such cases, it may be useful to inoculate the polluted area with highly effective petroleum-degrading microbial strains to augment the exiting ones. In order to identify a microbial strain for bioaugmentation of oil-contaminated soil, we isolated a microbial strain with high emulsification and petroleum hydrocarbon degradation efficiency of diesel fuel in culture. The efficacy of the isolated microbial strain, identified as *Candida catenulata* CM1, was further evaluated during composting of a mixture containing 23% food waste and 77% diesel-contaminated soil including 2% (w/w) diesel. After 13 days of composting, 84% of the initial petroleum hydrocarbon was degraded in composting mixes containing a powdered form of CM1 (CM1-solid), compared with 48% of removal ratio in control reactor without inoculum. This finding suggests that CM1 is a viable microbial strain for bioremediation of oil-contaminated soil with food waste through composting processes.

1. Introduction

Bioremediation of petroleum hydrocarbon-polluted soil relies on the petroleum-degradation ability of the microbial consortium resident in the soil (Braddock et al., 1997; Franzmann et al., 2002; Lee and Merlin, 1999; Marquez-Rocha et al., 2001; Venkateswaran and Harayama, 1995). Although petroleum-degrading microorganisms are widely distributed in both soil and water, they may not be present in sufficient numbers at a given polluted site. In such cases, it may be useful to inoculate the polluted area with highly effective petroleum-degrading microbial strains in a process called bioaugmentation (Supaphol et al., 2006; Yerushalmi et al., 2003).

In general, some isolated organisms can effectively degrade single pollutant in lab-conditions but when introduced into actual field conditions with multiple pollutants, they cease to function as anticipated (Singer et al., 2005; Quan et al., 2003). In addition, the introduced strains may not compete well with the indigenous microorganism in the soil to remain dominant or viable (Bouchez et al., 2000; Das and Mukherjee, 2007; Mohanty and Mukherji, 2008; Supaphol et al., 2006). Bioremediation efficiency is thus a function of the ability of the inoculated microbial degraders to remain active in the natural environment (Alexander, 1999). From this standpoint, bioaugmentation is still experimental although it has been practiced in agriculture and in wastewater treatment for years (Agathos and El Fantroussi, 2005). To test viability of bioaugmentation of contaminated soils, a series of laboratory evaluations must be conducted to particularly characterize microbial populations and microcosm to exhaustively evaluate the selected strains capacity to colonize and to degrade target soil pollutant (Sabaté et al., 2004).

There are approximately 70 genera of known oil-degrading microorganisms, including bacteria such as *Achromobacter*, *Acinetobacter*, *Actinomyces*, *Bacillus*, *Burkholderia*, *Exiguobacterium*, *Klebsiella*, *Microbacterium*, *Nocardia*, *Pseudomonas*, *Spirillum*, *Streptomyces* and *Vibrio*, and fungi or yeast such as *Allescheria*, *Aspergillus*, *Candida*, *Debayomyces*, *Mucor*, *Penicillium*, *Saccharomyces* and *Trichoderma*. Under natural conditions, these microorganisms in most areas comprise very few, compared with the total number of identified microorganisms. However, at petroleum hydrocarbon-polluted sites, these populations may grow and increase because they use petroleum hydrocarbon as a carbon source (Ahn et al., 1999; Aldrett et al., 1997; Altas, 1981; Bento et al., 2005; Chaerun et al., 2004; Das and Mukherjee, 2007; Gallego et al., 2001;

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Hua et al., 2003; Mohanty and Mukherji, 2008; Palittapongarnpim et al., 1998; Supaphol et al., 2006).

During microbial degradation of petroleum hydrocarbons, the *n*-alkane chain length is one of the most important factors because shorter-chain petroleum hydrocarbons are generally degraded more rapidly than longer-chain hydrocarbons (Mohanty and Mukherji, 2008; Seklemova et al., 2001; Jonge et al., 1997). In addition to chain length, petroleum hydrocarbon decomposition efficiency is also determined by structures of oil hydrocarbons (for example, polycyclic structures) as well as by the basic soil system characteristics including water, pH, temperature, mineral nutrients, nitrogen, phosphorus, and organic compounds. Although petroleum is a source of abundant carbon, its lack of nitrogen and phosphate makes it far less biodegradable than other biowastes such as food waste, sewage sludge and livestock manure and so on (Altas, 1981; Beaudin et al., 1999; Head and Swannell, 1999; Michel et al., 1993; Namkoong et al., 2002; Supaphol et al., 2006). In contrast, food waste is rich in carbon, organic nitrogen, phosphorous and mineral compounds required for growth of microorganisms (Joo et al., 2001, 2007; Nakasaki et al., 1992). Therefore, addition of food waste provides required nutrients for enhanced biodegradation of petroleum hydrocarbon (Joo et al., 2001; Nakasaki et al., 1992). Composting has long been a popular treatment method for food waste, and relatively recent studies have suggested that soil composting may be a useful method for bioremediation of petroleum-contaminated soil (Beaudin et al., 1996; van Gestel et al., 2003; Joo et al., 2007; Jorgensen et al., 2000; Kirchmanm and Ewnetu, 1998; Namkoong et al., 2002; Rojas-Avelizapa et al., 2007). In a recent study we elucidated the efficacy of using food waste for supplementing deficient nutrients for enhanced microbial composting during bioremediation of 1% (w/w) petroleum hydrocarbon contaminated soil (Joo et al., 2007). The study reported in this article is an extension of this previous study and challenged the effectiveness of the inoculants at a much higher 2% petroleum hydrocarbon contaminated soil. In this study, we isolated a number of hydrocarbon-degrading microbial strains from various sources and compared their effectiveness in diesel fuel-amended medium. We then inoculated the most effective microorganism into a composting reactor containing a mixture of hydrocarbon-contaminated soil and food waste to test the enhancement of this inocula petroleum-degradation ability against a control (no inoculums but under the same environment but the different oil concentration with the previous study).

2. Materials and methods

2.1. Isolation of oil-degrading microorganisms

Oil-degrading microbial strains were isolated from oil-contaminated soil samples taken from six different sites (gas stations and auto repair shops), and from the composts of food waste composting process as well as from sewers, leaf molds, and livestock manure piles. About 1 g sample was inoculated in 100 mL 2% diesel fuel-amended medium and cultivated at 120 rpm for 3 days. Three times acclimation of 1% (v/v) inoculation of samples was carried out from which 2 to 6 different colonies for each sample were isolated from the agar plates. The basic medium was a nutrient broth (3 g bacto-beef extract, 5 g bacto-peptone and 1 L water, adjusted to pH 7.0). For diesel fuel-amended medium, the nutrient broth was supplemented with 2% (w/w) diesel oil (LG Co., Ltd., Korea). For the agar plates used to examine colonies of microbial strains, NB (the nutrient broth supplemented with 1.5% agar) and GPY agar plate (10 g glucose, 5 g peptone, 5 g yeast extract, 1 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 5H_2O$, 15 g agar and 1 L water, adjust to pH 6.8) were used. The confirmation of pure culture in every liquid- and solid-cultivation was performed with colony observation on these agar plates.

The isolated microorganisms were cultivated in 2% diesel fuel-amended medium and seven microbial strains exhibiting the highest growth were selected for further examination of their oil-degrading efficacies. The most efficient microbial strain was identified using a BIOLOG KIT (Biolog Technologies Ltd., Israel) based on biological characteristics such as carbon sources and several other characteristics summarized in Table 2.

2.2. Measurement of growth and emulsification activity

To test growth in culture medium, the seven selected microorganisms were individually cultured (three replications). In our case, 1 mL inoculums of optical density of 1 was inoculated into 75 mL fresh 2% diesel fuel-amended medium, and the microbial strains were grown at 30 °C at 120 rpm for 3 days. The cell density in each culture was measured at a wavelength of 660 nm with a spectrophotometer.

For analysis of emulsification effectiveness, 1 mL inoculum of the seven selected microorganisms were individually inoculated into a 50 mL 2% diesel fuel-amended medium and incubated at 30 °C at 120 rpm for 3 days (three replications). Exactly 1 mL of each culture broth was then inoculated into a 50 mL fresh 2% diesel fuel-amended medium, and samples were cultured for 1 day. Each culture was then filtered through a polytetrafluorethylene (PTEF) filter (pore size = 0.2 μm), and 1 mL of the resulting supernatant was mixed with 5.2 mL potassium phosphate buffer (pH 7.0) containing 0.2 mL *n*-hexadecane. The mixture was shaken for 2 min and allowed to settle for 10 min. Absorbance of the suspension at 540 nm was then measured using a spectrophotometer.

2.3. Evaluation of oil-degrading ability in diesel fuel-amended medium

Each of the seven microbial strains selected were cultured as above, and 1 mL of each inoculum (optical density of 1) was inoculated into a 100 mL 2% diesel fuel-amended medium and incubated at 30 °C at 120 rpm for 3 days (three replications). Each culture was mixed with 50 mL methylene chloride and centrifuged at 10,000 rpm for 10 min. The supernatants were introduced into a separating funnel and oil component were extracted two to three times via shaking and settling (Hwang et al., 2000). Petroleum hydrocarbon contents were analyzed using a gas chromatograph with a flame ionization detector (GC/FID, HP-6890 PLUS, Hewlett–Packard, USA) and a HP-5 column (crosslinked 5% PHME siloxane, 0.32 mm × 30 m, Hewlett–Packard, USA), after filtration through a PTEF filter (pore size = 0.45 μm). Oil-degradation ratio was calculated as the concentration of removed petroleum hydrocarbon against a control test (without inoculums of microorganisms).

2.4. Evaluation of oil-degrading ability in a contaminated soil

To test the petroleum-degrading ability of the selected best-performing microbial strain (based on the previous experiments) composting was performed in soil mixed with food waste and diesel oil. The composting reactor used was cylindrical with insulated walls and a working volume of 6 L and total volume of 8 L. The lid held a temperature sensor/recorder, and could be tightly sealed to prevent leakage of the generated gas. To maintain aerobic conditions, compressed air was input at 0.5 L/min through a flow meter located near the bottom of the reactor. A multi-gas analyzer (Gas Data LMSx, Gas Data Ltd., UK) was used to measure generated CO_2 gas and consumed O_2 gas during the composting reaction.

Contaminated soil composting was conducted under three experimental conditions (see Table 1): control (no CM1, CM1 is selected the best strain of seven), CM1-liquid (augmented with CM1 cultivated in nutrient broth) and CM1-solid (augmented with powder-like CM1). The CM1-liquid was the culture liquid after CM1 was inoculated and cultivated in nutrient broth. For generation of powder-like CM1 (CM1-solid), 0.5% CM1-liquid was inoculated with 70% rice bran and 30% okara (the edible residue left after the production of bean curd), and composting process was conducted under aerobic condition to manufacture a pure CM1 composting-microbial agent for 3 days. Water content of this material was about 15% after sun-drying, and 110 g of this was added as a seed in the CM1-solid and minor in a carbon sources. Under each experimental condition, soil [77% (w/w)] and food waste [23% (w/w)] were combined on a wet basis, mixed to homogeneity with 2% diesel oil, and equally fed to three reactors. A 110 g of CM1-liquid and CM1-solid, culture broth and microbial agent were, respectively, added to two of the three reactors, while the third reactor was used as the control. The soil used was an organic-poor (4.5% volatile solids) sandy soil with a water content of 13% (Joo et al., 2007). The food waste consisted of 32% grain, 51% vegetable, 15% meat and fish, and 2% other ingredients with the following overall characteristics: 5.1% nitrogen, 1.1% phosphorus, 1% potassium, and a C/N ratio of 11. Oil-degradation ratio was calculated as the amount of the removed petroleum hydrocarbon against the initial concentration of the hydrocarbon in soil sample.

Table 1
Mixing ratios during various remediation experiments

	Mixing ratios
Control	Soil 5 kg (77%) + food waste 1.5 kg (23%) + diesel oil 200 ml (2% w/w)
CM1-solid	Control + *C. catenulata* CM1 microbial agent 110 g (powder form)
CM1-liquid	Control + *C. catenulata* CM1 culture broth 100 ml (110 g)