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Crude oil depletion by bacterial strains isolated from a petroleum hydrocarbon impacted solid waste management site in California

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ABSTRACT

This research is part of a multidisciplinary research program to develop a bioremediation protocol for a solid waste management (SWM) site in Northern California - a site which is heavily contaminated with petroleum hydrocarbons. In this initial study, 30 bacterial strains were isolated and evaluated for their efficiencies to deplete crude oil. The 3 most efficient bacterial isolates for crude oil depletion were designated as S1BD1, OPKDS2, and OSDS1; they were identified as *Serratia proteamaculans*, *Alcaligenes* sp. and *Rhodococcus erythropolis*, respectively, based on partial 16S rRNA gene sequences. Determination of crude oil depletion efficiency by gas chromatography–mass spectrometry (GC-MS) revealed that *Serratia proteamaculans* S1BD1 was the most efficient (68.0 ± 1.78%), followed by *Alcaligenes* sp. OPKDS2 (63.7 ± 3.28%), and *Rhodococcus erythropolis* OSDS1 (54.9 ± 5.07%). *S. proteamaculans* S1BD1 was able to deplete a wide spectrum of carbon compounds within the individual components of crude oil. *Alcaligenes* sp. OPKDS2 was the most efficient at depleting BTEX (91.2 ± 1.90%), and *R. erythropolis* OSDS1 exhibited a substrate preference of n-alkanes. All three strains exhibited unusually high crude oil depletion efficiencies and tolerated a wide range of salinity and pH levels, which makes them excellent candidates for bioaugmentation of the SWM site.

1. Introduction

Crude oil is an important source of energy and raw materials. However, its accidental release to soils, ground or surface water poses a significant environmental threat to human health (Xia et al., 2014; Zhang et al., 2012). The Deepwater Horizon oil spill in the Gulf of Mexico in 2010 was one of the largest accidental spills, which resulted in the release of 636 million liters of crude oil into sea water (Camilli et al., 2010). This spill impacted 1773 km of coastline (Michel et al., 2013) and resulted in 11 deaths. The oil spill continues to pose a significant threat to the marine environments of the Gulf of Mexico and may take years or even decades to recover (Xia et al., 2014).

Several techniques exist to remove petroleum compounds from the environment, including chemical and physical treatments (Riser-Roberts, 1998). However, these methods are energy-intensive, expensive, and often only partially effective, and some may even create additional uncontrolled hazardous waste (Riser-Roberts, 1998). Bioremediation using bacteria, fungi or yeast is a far less expensive and more eco-friendly approach to removing petroleum hydrocarbons (Harayama et al., 2004). Several species of bacteria have been confirmed that can utilize or degrade hydrocarbons. *Mycobacterium* strains have been shown to degrade 100% of a PAH mix, which included phenanthrene (Phe), fluoranthene (Fla) and pyrene (Pyr), in 14 days (Guo et al., 2010). *Acinetobacter* sp. was reported to be able to utilize n-alkanes of chain length C10–C40 as a sole carbon source (Throne-Holst et al., 2007).

Bioaugmentation of oil-contaminated sites has been carried out using bacterial strains: Mishra et al. (2001) reported that 90% of total petroleum hydrocarbons (TPHs) were removed through bioaugmentation of a field site in one year (compared to only 14% removal in the control). Developing a bioremediation program for a given site depends on the specific characteristics of the site, i.e., the type and variability of contaminants, the soil and climatic

Abbreviation: SWM, Solid waste management.

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environments, as well as the regulatory restrictions placed by local or federal government agencies. In the present study the strategy was to isolate highly efficient bacterial strains for petroleum degradation that could be utilized for bioaugmentation. Certain microorganisms are capable of mineralization and utilization of petroleum hydrocarbons as carbon and energy sources (Montagnolli et al., 2015). However, the rate and extent of depletion of petroleum hydrocarbons depends on the microorganisms' metabolic capabilities. Indigenous microbes associated with the extreme conditions of the site are more adapted to the prevailing conditions and are thus most suitable for use. Such indigenous microbes may be present at low abundances (Sayara et al., 2009) so that isolating the most efficient strains from the contaminated site and then utilizing these for bioaugmentation may be considered as the best strategy for enhancing biodegradation.

In the present study we were fortunate to have access to a solid waste management (SWM) site in Northern California that was contaminated over a period of 30 years (1950–1980) with petroleum hydrocarbons, heavy metals and other contaminants of concern. The overall goal of the present research was to isolate and identify crude oil-degrading bacterial strains present in the soil of this specific SWM site – strains that might eventually prove useful for bioaugmentation. The specific objectives were to: 1) isolate and identify bacterial strains native to the site, 2) characterize their ability to deplete petroleum hydrocarbons, and 3) to determine their tolerance to site levels of salinity and pH.

2. Materials and methods

2.1. Description of the site and sampling

The 29-ha solid waste management site is located in Contra Costa County, California. This site is contaminated with petroleum hydrocarbons (~15,000 mg/kg, especially those with a carbon number under 20 (Laboratories, 2013)), heavy metals, and carbon black (fly ash residue from coal burning industry, particle size <10 μm). Certain areas of the site also contain polycyclic aromatic hydrocarbons (PAHs), especially benzo(ghi)perylene, and pyrene (CH2MHill, 2010). The site is currently covered with a water cap to prevent the release of offensive odors to neighboring public areas. The water and soil are moderately saline and some areas exhibit high or low pH values (Table S1).

Impacted soil samples were collected from different parts of the site, including 1) unplanted soil, 2) saltgrass (*Distichlis spicata*) rhizosphere soil, 3) pickleweed (*Salicornia virginica*) rhizosphere soil and 4) sea-purslane (*Sesuvium verrucosum*) rhizosphere soil (all three plant species are native to the site). Samples were collected using disinfected shovels to dig approximately 6 inches deep, brought back to lab in sterilized containers and stored at 4 °C; the soils collectively weighed ~1200 g.

2.2. Culture media and carbon sources

Soil samples were used as the bacterial source for the enrichment culture in the initial screening step. The enrichment culture used a medium composed of sterilized mineral salts medium (MSM) plus 1% (v/v) of a sole carbon source. The MSM contained g/L 2.42 KH_2PO_4, 5.60 K_2HPO_4, 2.00 $(NH_4)_2SO_4$, 0.30 $MgSO_4 \cdot 7H_2O$, 0.04 $CaCl_2 \cdot 2H_2O$, 4.5×10^{-3} $MnSO_4 \cdot 7H_2O$, 0.1×10^{-3} $CuSO_4 \cdot 5H_2O$ and 0.1×10^{-3} $FeSO_4 \cdot 7H_2O$, the pH was adjusted to 7.0 ± 0.2 before autoclaving (Bury and Miller, 1993). Three kinds of carbon sources (crude oil, a gasoline/diesel mix (v/v = 1) and pyrene) were used as the sole carbon sources for enriching bacterial strains potentially useful for petroleum hydrocarbons depletion. Crude oil was collected from a "Cardinal Field" and stored at room temperature. Gasoline was purchased from Chevron Corporation (USA), diesel was purchased from ConocoPhilips (USA), and pyrene was purchased from Spectrum Chemical (USA).

2.3. Enrichment and isolation of petroleum hydrocarbons degrading bacterial strains

Crude oil, a gasoline/diesel mix (v/v = 1) and pyrene were individually used as the sole carbon source in enrichment culture process. These compounds were selected to represent a wide range of chemical compositions and amount of carbon chains present in petroleum hydrocarbons. The enrichment and isolation methods were modified from previous work (Kumari et al., 2012; Wongsa et al., 2004; Zhang et al., 2012). Five grams of a soil sample were added to a flask containing 200 ml MSM plus 1% (v/v) of a sole carbon source. The step was repeated for each soil sample collected and for each carbon source for a total of 12 enrichment flasks (4 soil samples x 3 carbon sources). The flasks were incubated on a shaker at 130 rpm, room temperature (approximately 25 °C) for the enrichment phase. After approximately 10 days (d) of incubation, 5 ml of inoculum was aseptically transferred from each individual flask to a flask containing fresh MSM and incubated at the same conditions for another 10 d. This was repeated two additional times. After the fourth subculture, the inoculum was serial diluted and spread on Lysogeny broth (LB, formulation per 1 L: 10 g SELECT Peptone 140; 5 g SELECT Yeast Extract; 10 g sodium chloride) agar plates (Thermo Fisher Scientific, USA) for isolation of bacterial strains. Individual colonies on LB agar plates were further isolated using the four quadrant streaking method. Then, individual strains were prepared for storage using a glycerol preservation method and stored in 1 ml cryogenic vials at minus 80 °C until further investigation. In this study, 30 strains were stored in total and were used as the stock source for further experiments.

2.4. Identification and characterization of strains

Genomic DNA was extracted from each bacterial isolate culture broth after 24 h incubation at 30 °C using DNA extraction kit (MO BIO, USA) according to the manufacturer's instructions. The 16S rRNA gene fragment was amplified with a pair of universal primers (27F): 5′ AGA GTT TGA TCM TGG CTC AG 3′ and (1525R): 5′ AAG GAG GTG WTC CAR CC 3′ using the extracted DNA as template. Polymerase chain reaction (PCR) was performed in a thermal cycler using the following conditions: pre-heating for 3 min at 95 °C followed by 30 cycles of 30 s at 95 °C; 45 s at 55 °C; 90 s at 72 °C with a final extension of 10 min at 72 °C. The size of the PCR product was confirmed with electrophoresis using a 1.5% agarose gel. The PCR products were purified by using the QIAquick PCR Purification Kit (QIAGEN, USA). The amplicons were sequenced at the University of California Berkeley DNA Sequencing Facility. The raw sequences were aligned, edited manually and BLAST searched using a BLAST search option of NCBI GenBenk (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to estimate the degree of similarity to other species.

2.5. Crude oil depletion efficiencies of individual strains

Bacterial inoculum were prepared by growing each bacterial isolate overnight at 30 °C in LB broth. The bacterial cells were harvested by centrifuging at 5000 rpm for 15 min, supernatant was discarded and obtained cells pellets were suspended in sterilized deionized water to adjust OD_{600} of 0.5. Bacterial inoculum (0.3 ml) was transferred to a 60 ml screw-capped vial (to prevent loss of volatile hydrocarbon components) containing 30 ml MSM plus 1% (v/v) crude oil; vials were incubated in a rotary shaker at 30 °C and 130 rpm. MSM plus crude oil vials without cells were used as a

control. All vials were prepared in triplicate with 93 experiment vials in total (30 strains and the control). After 15 days all vials were harvested for measuring the amount of residual crude oil.

2.6. GC-MS analyses of crude oil

The amount of specific compounds in crude oil was determined using high-performance capillary gas chromatography-mass spectrometry (GC-MS) (SHIMADZU GCMS-QP2010, Japan) operated in the selected-ion monitoring (SIM) mode. System control and data acquisition was achieved by SHIMADZU LabSolutions GCMSsolution. Residual crude oil in each vial was extracted with 2 ml chloroform and diluted 20 times before injected into the GC-MS. Gas chromatography was operated under following conditions: helium gas was used as the carrier gas at a total flow rate of 11.5 ml/min, column flow rate of 1.00 mL/min, purge flow rate of 0.5 mL/min, linear velocity of 36.1 cm/s and a pressure of 51.2 kPa. Samples were injected onto a 30 m ZB-5HT INFERNO column (Zebron, United States) with a 0.25 mm i. d. and 0.25 μm film thickness. Injection temperature was 280 °C and injection mode was split at a ratio of 10:1. One micro-liter of the crude oil/chloroform extract was injected into the equipment for each sample. The column oven temperature program was: hold for 7 min at 45 °C, then increase to 180 °C (5 °C/min), increase to 380 °C (35 °C/min), hold for 3 min, then decrease to 50 °C (60 °C/min). In selected ion monitoring (SIM) mode, about 150 target compounds of different classes including alkanes, PAHs, BTEX and cycloalkane were detected. The internal standard, 17α(H),21β(H)-Hopane, a common constituent in crude oil, was selected because it is neither synthesizable nor biodegradable during the process of crude oil biodegradation (Prince et al., 1994). The relative abundance of different compounds was calculated as the ratio of the peak area of each hydrocarbon to the peak area of 17α(H),21β(H)-Hopane in the GC chromatograph (Wang et al., 2011). In this study, total amount of residual crude oil in each sample was calculated by summarizing all the detectable peaks in SIM mode together. Percentage removal of the total C compounds is calculated as the difference between the control (MSM + crude oil − bacteria) and samples (MSM + crude oil + bacteria), called potential biodepletion efficiency.

2.7. Bacterial environmental tolerance testing

Growth of each isolate under varying concentration of NaCl ranging from 5 g/L to 100 g/L were tested with 5 mg/L to 10 mg/L increments in LB broth. The salinity gradient LB broth were inoculated with 1% (v/v) of bacterial cells while LB broth without adding salt was set as control. Similarly, LB broth with different pH levels ranging from 4 to 9 were prepared by adjusting the pH with 1N NaOH or 1N HCl at an increment of 1, whereas LB broth at pH 7 was set as the control. The salinity and pH ranges were selected based upon site characterization data (Laboratories, 2013). For all tolerance tests, after 24–48 h of incubation under 30 °C and shaking at 130 rpm, samples were measured using a spectrometer (Thermo Fisher Scientific, US) at OD_{600} to reflect bacterial concentration.

2.8. Data analysis

All experiments were conducted in triplicate. Data analysis were conducted in Excel 2010 and Prism version 6. Figures were produced by Origin 8.5.

3. Results and discussion

3.1. Isolation and identification of petroleum hydrocarbon degrading bacteria

After carrying out four enrichment cycles, the SWM soil microbes were subsequently cultured on LB plates (Fig. S1). Comparing the colonies visually by color, form and colony size indicated that the plant rhizospheres contained a greater abundance of cultivable bacteria than unplanted soils. Counts of the numbers and types of colonies confirmed that the rhizosphere soil of saltgrass, pickleweed and sea-purslane have greater bacterial abundances and diversities compared to the unplanted soil (Table 1). Other researchers have also reported increased abundances of rhizosphere bacteria (as well as bacterial communities capable of degrading petroleum hydrocarbons) in petroleum hydrocarbon contaminated soil (Kirk et al., 2005).

The bacterial colonies obtained from all of the different soil samples, were identified by 16S rRNA gene sequencing and BLAST searching for nucleotide percentage homology in NCBI GenBank (Fig. 1 and Table 2). The molecular identification results revealed that the 30 isolates belong to 13 different genera – *Brevundimonas*, *Ochrobactrum*, *Rhizobium*, *Rhodococcus*, *Achromobacter*, *Alcaligenes*, *Serratia*, *Pseudomonas* (Protobacteria), *Arthrobacter*, *Corynebacterium*, *Isoptericola*, *Microbacterium* (Actinobacteria), and *Bacillus* (Firmicutes) distributed across the three major phyla of bacteria.

A phylogenetic tree (based on maximum parsimony analysis was constructed using 16S rRNA gene sequences) resolved all the isolates into different clades with closely homologous strains clustered together; these clades were strongly supported by bootstrap values (100%) (Fig. 1). Of the 3 major phyla, Protobacteria appears to be the dominant phylum. The culture-independent microbial community analysis through 16S rRNA gene sequencing by Next Generation Sequencing (NGS) also confirmed the existence of Protobacteria as the dominant phyla among all the bacterial phyla (unpublished data) in SWM soil. A high percentage of the isolates were related to the genera, *Serratia* and *Pseudomonas*, Protobacteria that are known for their ability to degrade petroleum hydrocarbons (Wongsa et al., 2004). Radwan et al. (2005) reported that crude-oil degrading bacteria (including *Pseudomonas*, *Bacillus*, and *Acinetobacter*) were associated with cyanobacterial mats in the Persian Gulf. Similarly, a member of the genus, *Rhodococcus*, has been shown to deplete almost 90% of the total n-alkanes of petroleum products within one week (Quek et al., 2006).

Table 1
Total numbers and types of colonies observed on LB agar plates after 48 h of culturing at 30 °C. Unplanted soil had smaller numbers and types of colonies (except for pyrene) than the planted rhizosphere soil.

Sole carbon source	Unplanted soil		Saltgrass rhizosphere		Pickleweed rhizosphere		Sea-purslane rhizosphere	
	Colonies	Types	Colonies	Types	Colonies	Types	Colonies	Types
Crude oil	24	3	161	5	672	4	702	3
Gasoline & diesel mix (v/v = 1/1)	18	1	51	2	763	3	688	3
Pyrene	19	4	50	4	124	4	49	4

Note: Types on LB agar plates were differentiated by macroscopic (color, form and colony size).

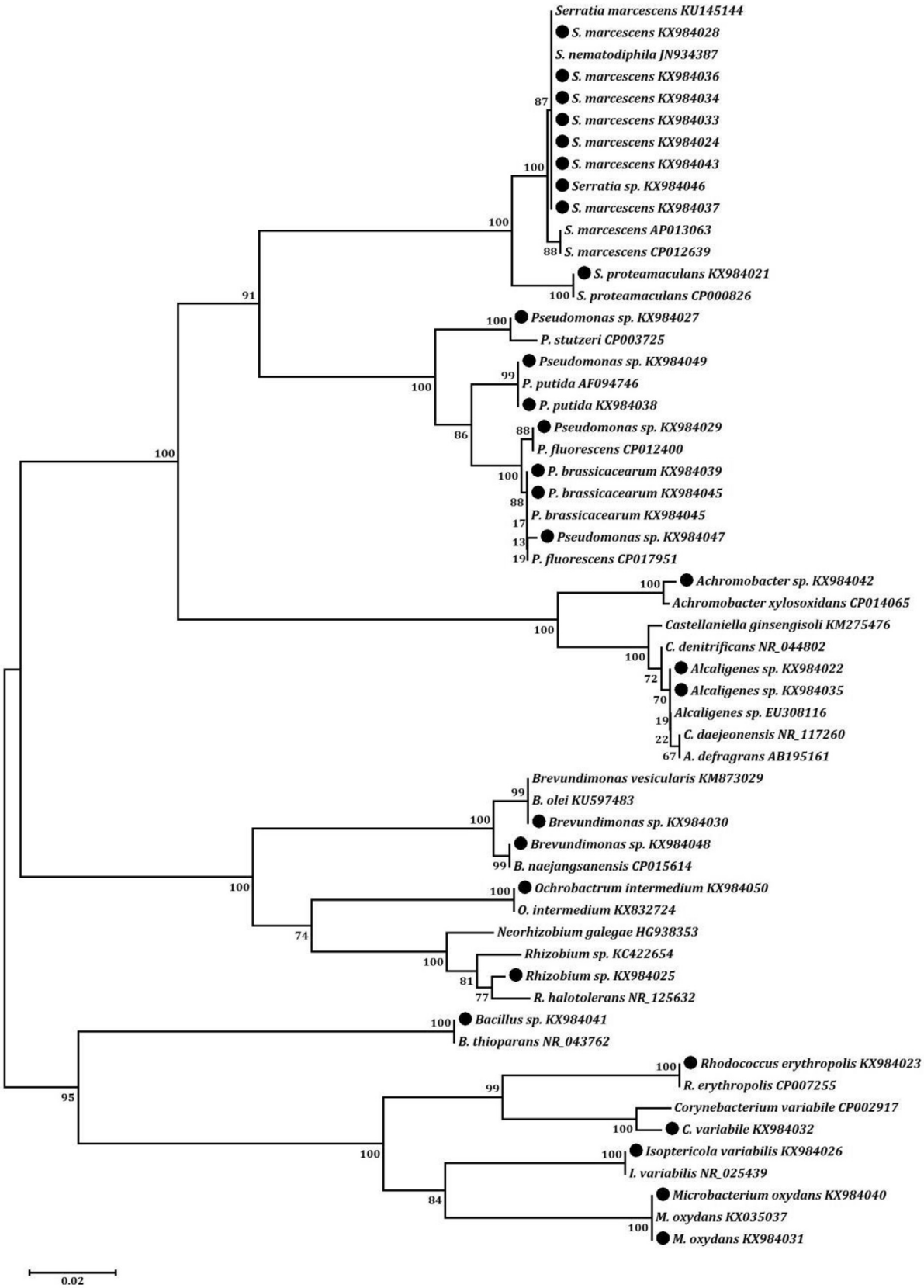

Fig. 1. Phylogenetic analysis of bacterial strains isolated from the waste management site.

Table 2
Characteristics of selected bacterial strains isolated from the solid waste management site.

Strain ID	Crude oil depletion efficiency %	Nearest BLAST relatives	Accession No.	ID (%)	Salinity (g/L NaCl)	pH
S1BD1	68.0 ± 1.78	*Serratia proteamaculans*	KX984021	99	0–60	4–9
OPKDS2	63.7 ± 3.28	*Alcaligenes* sp.	KX984022	99	0–40	5–9
OSDS1	54.9 ± 5.07	*Rhodococcus erythropolis*	KX984023	99	0–30	5–9

Identification information are based on 16S rRNA gene sequencing and BLAST. The salinity and pH ranges are the optimal range in which bacterial growth was measured for each strain. Experiments for depletion rate, salinity range and pH range were conducted in triplicate. Total amount of residual crude oil in each sample was calculated by summarizing all the detectable peaks in SIM mode together. Percentage removal of the total C compounds is calculated as the difference between the control (MSM + crude oil − bacteria) and samples (MSM + crude oil + bacteria), called potential biodegradation efficiency.

3.2. Crude oil depletion efficiencies of isolated strains

3.2.1. Chemical composition of crude oil

GC-MS analysis of the crude oil samples revealed the following components: ~40% alkane, ~25% cycloalkane and ~26% BTEX, with PAHs and hopane being minor components (together constituting ~9% of the crude oil components). Among the alkanes, the most abundant compounds identified were nonane, undecane, decane, dodecane and octane. Most of the cycloalkane were in the form of methylcyclohexane, while among the BTEX constituents, toluene and xylene were dominant. The dominant form of PAH was 1,3,3-trimethyl-1-phenylindan, followed by naphthalene and ethyl-naphthalene. The GC-MS analysis confirmed that the crude oil sample represents an appropriate surrogate for the petroleum hydrocarbon compounds found at the SWM site. The crude oil contains almost all the different components found at the site, including alkanes, cycloalkanes and PAHs (Laboratories, 2013). Furthermore, most of the detectable compounds in the crude oil are those with a carbon number less than 20, which was also true for petroleum hydrocarbons from the SWM site (Laboratories, 2013).

3.2.2. Efficiencies of the isolated strains for the depletion of crude oil

In order to identify potential strains that might be useful for the bioaugmentation of the SWM site, crude oil depletion efficiencies of the 30 isolated bacterial strains were determined. Of these strains, *Serratia proteamaculans* S1BD1 exhibited the highest depletion efficiency, i.e., 68.0 ± 1.78%. *Alcaligenes* sp. OPKDS2 and *Rhodococcus erythropolis* OSDS1 also exhibited high depletion efficiencies, i.e., 63.7 ± 3.28%, and 54.9 ± 5.07%, respectively (Table 2). With respect to *S. proteamaculans* S1BD1, the depletion efficiency of 68% was greater than other reported values (Table 3). Although Wongsa et al. (2004) reported a value of 67% degradation efficiency for other strains of the genus *Serratia*, this was achieved over a much longer period (30 days), versus 15 days in the present study. With respect to *Alcaligenes* sp., Ijah and Antai (2003) reported that *Alcaligenes* sp. isolated from soil was capable of degrading 34.4% of crude oil in 16 days (compared to ~64% in our study). The depletion efficiency of *R. erythropolis* in the present work is similar to values reported for several other strains of *Rhodococcus* sp. (Kumari et al., 2012; Liu and Liu, 2011; Sharma and Pant, 2000).

The results of the present work suggest that two of the 30 bacterial strains isolated from the SWM site appear to release biosurfactants. Biosurfactants have been shown to play a vital role in the depletion of petroleum hydrocarbons (Cameotra and Bollag, 2003; Lai et al., 2009; Whang et al., 2008). Hydrophobicity resulted in the physical separation of the crude oil at the air-MSM interface (e.g., in the controls, or in vials inoculated with *S. proteamaculans* S1BD1, Fig. S3). However, vials inoculated with *Alcaligenes* sp. OPKDS2 and *R. erythropolis* OSDS1 differed in that there was a clear dispersion and emulsification of the crude oil, suggesting that these

Table 3
Crude oil depletion efficiencies of our top three strains (*) compared with published values of other reported strains.

	Strains	Depletion efficiencies	Descriptions	References
1*	*Serratia proteamaculans* S1BD1	68.0%	*Serratia proteamaculans* S1BD1 isolated from the waste management site depleted 68.0 ± 1.78% crude oil after 15 days of inoculation in MSM plus 1% (v/v) crude oil under shaking at 130 rpm.	Present study
2*	*Alcaligenes* sp. OPKDS2	63.7%	*Alcaligenes* sp. OPKDS2 isolated from the waste management site depleted 63.7 ± 3.28% crude oil after 15 days of inoculation in MSM plus 1% (v/v) crude oil under shaking at 130 rpm.	Present study
3*	*Rhodococcus erythropolis* OSDS1	54.9%	*Rhodococcus erythropolis* OSDS1 isolated from the waste management site depleted 54.9 ± 5.07% crude oil after 15 days of inoculation in MSM plus 1% (v/v) crude oil under shaking at 130 rpm.	Present study
4	*Serratia marcescens* HokM	67.0%	*Serratia marcescens* HokM isolated from the hot spring water, slightly contaminated with crude oil. The cells were grown for 4 weeks in10 mL of MSM plus one of the petroleum products (e.g., gasoline, kerosene, diesel oil, or lubricating oil) at a concentration of 10 g L^{-1}.	(Wongsa et al., 2004)
5	*Serratia marcesens* ACE2	58%	*Serratia marcesens* ACE2 isolated from corrosion product. Incubated aerobically in 300 ml Bushnell Haas broth containing 1 g of diesel at 27 °C on a rotary shaker operated at 200 rpm for 30 days.	(Rajasekar et al., 2007)
6	*Alcaligenes odorans* P20	45% & 37%	*Alcaligenes odorans* P20 depleted 45% of Bombay High crude oil while 37% of Gujarat crude oil, it depleted alkanes and aromatics equally	(Lal and Khanna, 1996)
7	*Rhodococcus* sp.	50%	A *Rhodococcus* sp. isolated from a chronically oil-polluted marine site depleted up to 50% of the aliphatic fraction of Assam crude oil in 72 h in seawater supplemented with 35 mM nitrogen and 0.1 mM phosphorus.	(Sharma and Pant, 2000)
8	*Rhodococcus* sp. NJ2	49.5%	*Rhodococcus* sp. NJ2 isolated from crude oil contaminated site metabolized 49.5% crude oil after 30 days of incubation in MSM (minimal salt media) with 2% of crude oil at their optimum conditions.	(Kumari et al., 2012)
9	*Rhodococcus erythropolis* NTU-1	30%	In batch cultures with 10,000 ppmv crude oil, approximately 90% oil were removed (about 30% of biodepletion and 60% of biosorption) within 4 days by *Rhodococcus erythropolis* NTU-1, it depleted C10–C32 n-alkanes in diesel oil or crude oil.	(Liu and Liu, 2011)

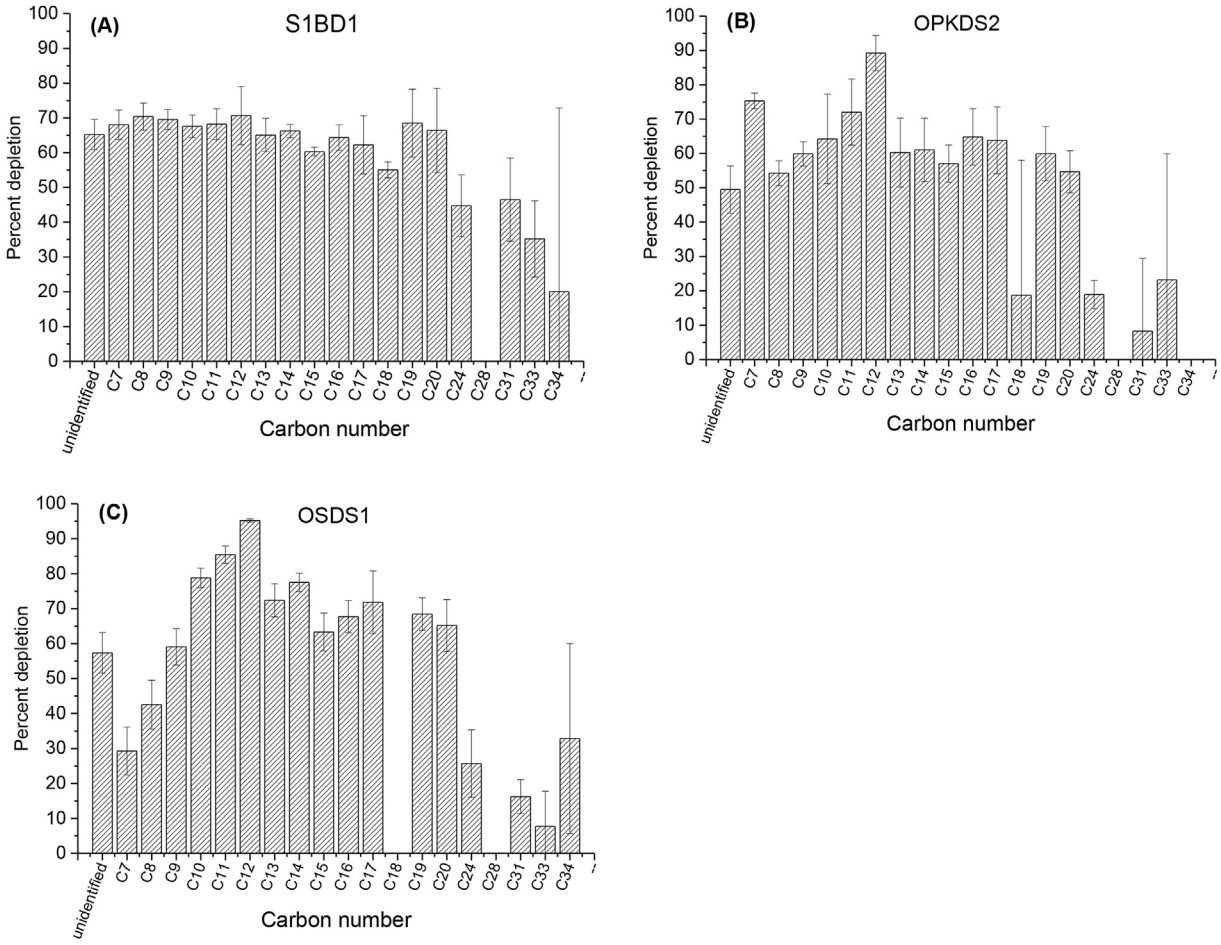

Fig. 2. Depletion rates of bacterial strains, S1BD1, OPKDS2 and OSDS1, for carbon numbers of different components found in the crude oil samples. The relative abundance of different compounds was calculated as the ratio of the peak area of each hydrocarbon to the peak area of 17α(H),21β(H)-hopane in the GC chromatograph. Percentage removal of the total C compounds is calculated as the difference between the control (MSM + crude oil − bacteria) and samples (MSM + crude oil + bacteria).

two strains are releasing biosurfactants that lowered the surface tension of the petroleum hydrocarbons. *R. erythropolis* has been shown to be an effective releaser of biosurfactants (Bicca et al., 1999; Pacheco et al., 2010; Peng et al., 2007). Although the visually observed crude oil emulsification associated with the *Alcaligenes* sp. OPKDS2, and with the *R. erythropolis* OSDS1, suggest that these two strains may both be involved in biosurfactant production, it should be pointed out that biosurfactants were not directly measured in the present work.

3.2.3. Crude oil depletion characteristics of selected strains

The three strains with the highest efficiencies of crude oil depletion, *S. proteamaculans* S1BD1, *Alcaligenes* sp. OPKDS2, and *R. erythropolis* OSDS1, were selected for further analysis. Specifically, we analyzed the depletion of individual components, i.e., those with different carbon numbers (ranging from C7 to C34), and belonging to five different hydrocarbon classes, i.e., n-alkane, alkane, cycloalkane, BTEX, and PAHs (n-alkane represents an acyclic saturated hydrocarbon without a branch, alkane - an acyclic saturated hydrocarbon with branches, and cycloalkane - a monocyclic saturated hydrocarbon (Wang and Fingas, 2003). *S. proteamaculans* S1BD1, the most efficient strain of crude oil depletion, was able to deplete a wide spectrum of petroleum hydrocarbons of different structure (Fig. 2A). With respect to components of the different classes, n-alkane, PAH, BTEX, cycloalkane, alkane (Fig. 3), the best depletion rates of PAH, cycloalkane and alkane were attained by *S. proteamaculans* S1BD1, i.e., 61.6 ± 1.72%, 69.1 ± 3.19% and 67.1 ± 3.43%, respectively.

Alcaligenes sp. OPKDS2 efficiently utilized C7 (75.3 ± 2.29%) and C12 (89.3 ± 5.12%) (Fig. 2B). *Alcaligenes* sp. OPKDS2, which depleted >90% of BTEX, attained the highest depletion rate of BTEX (91.2 ± 1.90%) of all three isolates (Fig. 3). *Alcaligenes* sp., which have been reported as potential degraders of petroleum hydrocarbons in other studies (Ijah and Antai, 2003; Li et al., 2006), are usually found as a member of degrading consortia (Adebusoye et al., 2007).

R. erythropolis OSDS1 attained the highest depletion rates of n-alkane (78.7 ± 2.49%), as well as C10 to C17 components (63.3 ± 5.43% to 95.22 ± 0.45%) (Fig. 2C). Members of *Rhodococci* have often been reported as efficient degraders for a broad range of n-alkanes. The capability of alkane degradation is often attributed to their ability of biosurfactants production (Ganesh and Lin, 2009). Margesin et al. (2013) also reported most efficient n-alkane degraders belonged to genus *Rhodococcus* that were able to deplete C12–C22, with the best efficiency for C12–C18.

In some instances, GC-MS revealed that the chromatographic peaks associated with certain individual compounds completely disappeared in samples inoculated with bacterial cells (Fig. S4). Such complete removal of the individual carbon mass components may have been due to: 1) complete metabolism to CO_2 and water, and/or 2) transformation of the compounds to smaller chain C compounds, or, compounds that have been modified with an increased bioavailability (Das and Chandran, 2010).

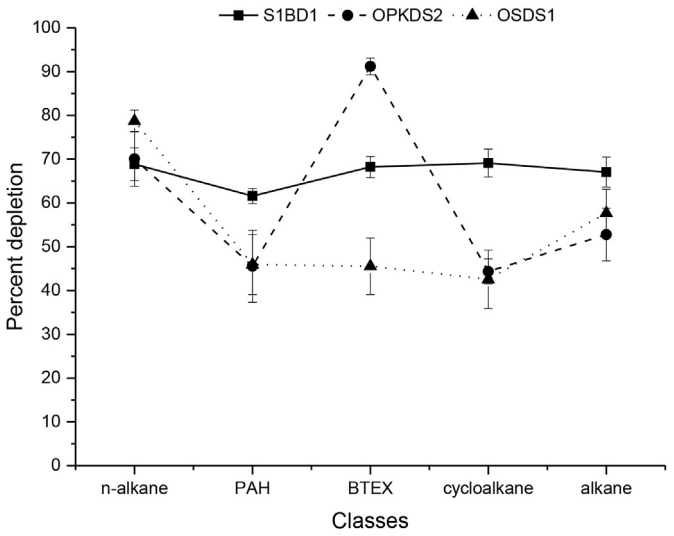

Fig. 3. Percent depletion (±SD) of five different classes of petroleum hydrocarbons (present in the crude oil samples) for each of the bacterial strains, S1BD1, OPKDS2 and OSDS1.

3.3. Salinity and pH tolerance of selected strains

To obtain maximum depletion efficiencies at the impacted site, strains used for bioaugmentation should have physiological characteristics that are appropriate for the particular environment. Since the SWM site is moderately saline (up to 10 dS/m), and is characterized by a broad range of pH (4.8–8.3), bacterial strains were tested for their growth at different salinity and pH levels (Table 2). Three of the selected high efficiency strains, *Serratia proteamaculans* S1BD1, *Alcaligenes* sp. OPKDS2, *Rhodococcus erythropolis* OSDS1 tolerated a wide range of salinity and pH levels; *S proteamaculans* S1BD1 tolerated up to 70 g/L NaCl and the entire pH range of 4–9 (Table 2).

4. Conclusion

Three bacterial strains with high efficiencies for crude oil depletion were isolated and identified. The isolate, *Serratia proteamaculans* S1BD1 was the most efficient and was potentially able to utilize a wide spectrum of petroleum hydrocarbons of different structure. *Alcaligenes* sp. OPKDS2 exhibited an impressive ability to deplete BTEX (more than 90% of present BTEX was removed in 15d). The third most efficient strain, *Rhodococcus erythropolis* OSDS1, emulsified the crude oil and exhibited a substrate preference for the depletion of C10 to C17 and n-alkanes.

Since the three selected high efficiency strains, *S. proteamaculans* S1BD1, *Alcaligenes* sp. OPKDS2, *R. erythropolis* OSDS1, tolerated a wide range of salinity and pH levels, it is proposed that they could potentially be used for the bioaugmentation of the SWM waste management site (and possibly other sites with similar physical and chemical characteristics).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ibiod.2017.06.003.

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