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EDTA addition enhances bacterial respiration activities and hydrocarbon degradation in bioaugmented and non-bioaugmented oil-contaminated desert soils

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HIGHLIGHTS

- EDTA addition enhances bacterial respiration rates and oil biodegradation.
- EDTA enhances oil degradation by increasing the bioavailability of hydrocarbons.
- Desert soils can be efficiently bioremediated using EDTA and exogenous bacteria.
- Some bacteria can grow on EDTA as a carbon source.
- *Alcanivorax* sp. and *Parvibaculum* sp. can be ideally used in bioaugmentation.

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ABSTRACT

The low number and activity of hydrocarbon-degrading bacteria and the low solubility and availability of hydrocarbons hamper bioremediation of oil-contaminated soils in arid deserts, thus bioremediation treatments that circumvent these limitations are required. We tested the effect of Ethylenediaminetetraacetic acid (EDTA) addition, at different concentrations (i.e. 0.1, 1 and 10 mM), on bacterial respiration and biodegradation of Arabian light oil in bioaugmented (i.e. with the addition of exogenous alkane-degrading consortium) and non-bioaugmented oil-contaminated desert soils. Post-treatment shifts in the soils' bacterial community structure were monitored using MiSeq sequencing. Bacterial respiration, indicated by the amount of evolved CO_2, was highest at 10 mM EDTA in bioaugmented and non-bioaugmented soils, reaching an amount of 2.2 ± 0.08 and 1.6 ± 0.02 mg-CO_2 g^{-1} after 14 days of incubation, respectively. GC–MS revealed that 91.5% of the C_{14}–C_{30} alkanes were degraded after 42 days when 10 mM EDTA and the bacterial consortium were added together. MiSeq sequencing showed that 78–91% of retrieved sequences in the original soil belonged to *Deinococci*, *Alphaproteobacteria*, *Gammaproteobacteia* and *Bacilli*. The same bacterial classes were detected in the 10 mM EDTA-treated soils, however with slight differences in their relative abundances. In the bioaugmented soils, only *Alcanivorax* sp. MH3 and *Parvibaculum* sp. MH21 from the exogenous bacterial consortium could survive until the end of the experiment. We conclude that the addition of EDTA at appropriate concentrations could facilitate biodegradation processes by increasing hydrocarbon availability to microbes. The addition of exogenous oil-degrading bacteria along with EDTA could serve as an ideal solution for the decontamination of oil-contaminated desert soils.

1. Introduction

Human activities rely on the use of crude oil which potentially leads to environmental contamination (Macaulay and Rees, 2014). Many attempts have been made to remove oil pollutants from the environment; however, the success of these remediation processes, in many cases, is hindered by the existence of a low number of oil-

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degrading microorganisms and the low solubility and availability of hydrocarbons (Whitehouse, 1984; Jain et al., 1992; Northcott and Jones, 2000; Millioli et al., 2009). To circumvent these problems, several bioremediation approaches have been designed that involved the addition of exogenous oil-degrading bacteria (known as bioaugmentation) or the addition of chemicals such as surfactants to emulsify oil and increase its bioavailability (Volkering et al., 1995; Li and Chen, 2009; Tyagi et al., 2011; Chen et al., 2015). The oil-degrading bacteria will then utilize hydrocarbons either by directly uptaking the dissolved fraction from the aqueous medium or by adhesion to the small-emulsified oil droplets (Beal and Betts, 2000; Hua et al., 2007; Mishra and Singh, 2012; Hua and Wang, 2013; Abdel-Megeed et al., 2014). So far, most bioremediation studies have been performed either by the addition of exogenous bacteria or chemicals to change the physical properties of oil but never both together. The major concern in this case is that the added chemicals would have a negative effect on growth of the introduced exogenous oil-degrading bacteria.

Chelating agents, such as ethylenediaminetetraacetic acid (EDTA), have been previously used in oil fields in a mixture with other chemicals to increase oil production, to make drilling more efficient and to inhibit scale formation (Cikes et al., 1990; Crabtree et al., 1999). Also, EDTA has been reported to have the ability to remove adsorbed hydrocarbons from soil particles, thus rendering them available to hydrocarbon-degrading bacteria (Hua et al., 2007; Han et al., 2009; Baziar et al., 2013). In spite of that, the use of EDTA in bioremediation has been very limited, which could be attributed to its adverse effect on microorganisms. For instance, EDTA was found to increase dispersal of some bacterial biofilms (Banin et al., 2006; Ramage et al., 2007; Robertson et al., 2012; Meng et al., 2013; Saadat et al., 2013) and to strongly suppress the catalytic properties of microbes (Meng et al., 2013). The strong chelating binding affinity of EDTA to different metal ions such as Ca^{2+}, Mg^{2+} and Fe^{3+} destabilizes the microbial extrapolymeric substances (EPS) matrix and causes cells to separate from biofilms. The divalent chelating capacity of EDTA causes lipopolysaccharides to separate from the outer membrane of microbial cells, thus increasing the membrane permeability and subsequently cell death (Gray and Wilkinson, 1965; Banin et al., 2006; Yakandawala et al., 2007; Saadat et al., 2013). For these reasons, EDTA has been used in food and therapeutic industry to eradicate microbes and their biofilm formation (Ramage et al., 2007; Juda et al., 2008; Chauhan et al., 2012; Shaikh and Musaddiq, 2012).

In this study, we tested the role of EDTA in stimulating bacterial respiration and oil biodegradation in an oil-polluted desert soil subjected to a bioaugmentation treatment. Our hypothesis is that EDTA will increase the bioavailability of hydrocarbons to the oil-utilizing bacteria, hence facilitating the degradation process. Moreover, the effect of EDTA on the fate of the introduced oil-degrading bacterial consortium as well as on the soil's bacterial community composition was investigated.

2. Material and methods

2.1. Sample collection and characterization

Oil contaminated soils were collected from an area close to an oil production facility in Mina Al-Fahal, a coastal area in Muscat, Oman (23° 37′ 49.92″N; 58° 31′ 23.84″E). Soils from this area have been previously investigated for their physical and chemical properties as well as for their response to different bioremediation treatments including the addition of inorganic nutrients and exogenous microorganisms (Abed et al., 2014a). Soils were collected in sterile plastic boxes and brought back to the laboratory, where all incubations were performed.

2.2. Effect of EDTA on respiration activities and oil degradation

Five alkane-degrading bacterial strains (i.e. *Alcanivorax* sp. MH3, *Parvibaculum* MH21, *Azospirillum* sp. AH2, *Marinobacter* sp. AH3 and *Marinobacter* sp. AH6) were cultivated in a minimal salt medium (see below) at 30 °C using acetate as a carbon source. Detailed biochemical, physiological and phylogenetic characterization of the strains can be found in Abed et al. (2014a,b). In brief, the strains were Gram negative, rod in shape and were phylogenetically affiliated to the classes *Alphaproteobacteria* (i.e. *Parvibaculum* MH21, *Azospirillum* sp. AH2) and *Gammaproteobacteria* (i.e. *Alcanivorax* sp. MH3, *Marinobacter* sp. AH3 and *Marinobacter* sp. AH6). They grew well at 2–7% salinity and between 20 and 60 °C. All strains exhibited a better growth on long chain than on short chain alkanes. The selection of these strains was based on their utilization of different alkanes and representation of different bacterial genera. The cells were concentrated by centrifugation, washed with sterile water and then re-suspended in carbon- and nutrient-free (i.e. without KH_2PO_4 and NH_4Cl) minimal salt medium. The medium contained $MgCl_2.6{\cdot}H_2O$ (5.6 gl^{-1}), $MgSO_4.7{\cdot}H_2O$ (6.8 gl^{-1}), $CaCl_2.2{\cdot}H_2O$ (1.47 gl^{-1}), KCl (0.66 gl^{-1}), KBr (0.09 gl^{-1}) and was supplemented with trace elements mixture (Widdel and Bak, 1992) and vitamins (Heijthuijsen and Hansen, 1986). A bacterial consortium was prepared by mixing equal volumes of each bacterial culture ($OD_{600} = 0.2$) and 1 ml of this mixture was added to 19 ml medium for each treatment (see below).

The experiment was performed in glass bottles (volume 165 ml). Each bottle received 10 g of the oil-polluted soil mixed with 20 ml of carbon- and nutrient-free minimal salt medium. Soils with and without the addition of exogenous bacterial consortium were incubated in the presence of different concentrations (i.e. 0.1, 1 and 10 mM) of EDTA (Sigma Aldrich, USA) in the medium. The same concentrations of EDTA were also added to the bacterial consortium alone without soil. Incubations (i.e. bioaugmented soil, non-bioaugmented soil and the bacterial consortium without soil) in the absence of EDTA served as controls. All treatments and controls were maintained in triplicates. The bottles were sealed with thick, black rubber stoppers to ensure no gas leakage and incubated at 30 °C for 42 days.

Respiration activities were measured in all treatments by following evolved CO_2 at different time intervals using gas chromatography (GC). From the headspace, 250 μl of gas was withdrawn using a gas-tight syringe and then manually injected into the GC (GC, Agilent model 6890N). The GC used helium gas as a carrier gas at a flow rate of 4 ml min^{-1} in the 30 m × 250 μm capillary column (HP-PLOT Q). The equipped thermal conductivity detector was maintained at 200 °C while the injector temperature was 210 °C. The oven temperature was programmed from 50 °C to 80 °C with a final hold time 3 min at a rate of 20 °C min^{-1}. Actual oil mineralization rates were calculated from the evolved CO_2, assuming that oil was the only carbon source available for microbes. These rates were estimated by comparing the experimentally quantified CO_2 with the theoretical amount of CO_2 that would be produced by complete oxidation of the present oil (Abed et al., 2015).

Alkane degradation at the end of all treatments was evaluated using gas chromatography-mass spectrometry (GC–MS). One gram of each soil sample was extracted 2–3 times in 5 ml dichloromethane (DCM, Sigma–Aldrich, Germany) and then sonicated for 25 min at 10 °C. The extracted supernatant was mixed with sodium sulfate and was filtered with non-absorbent cotton to remove solid particles. The filtrate was then evaporated using a rotary evaporator. The dry extract was re-dissolved into DCM and passed through silica gel to remove any solid particles prior to injection. Individual alkanes were quantified after injecting the extract into

GC–MS (Perkin Elmer Clarus 600 GC/MS). The Perkin Elmer Clarus 600C MS was coupled with Rtx®-5MS capillary column (30 m × 0.25 mm I.D. × 0.25 μm film thickness; maximum temperature, 350 °C). Ultra-high purity helium was used as a carrier gas at a constant flow of 1.0 ml/min. The ionizing energy was 70 eV. Electron multiplier (EM) voltage was obtained from autotune. The injection, transfer line and ion source temperatures were 290, 280 and 280 °C, respectively. The oven temperature program was hold at 80 °C for 5 min and then accelerated at a rate of 10° C/min to 280 °C (hold for 30 min). The volume of injected sample was 1 μl with a split ratio of 10:1. The standard mix solution (C_7–C_{30}) of concentrations 10, 20, 30, 40 and 50 ppm were used for confirmation and quantification purposes (Lot: LB87941, Supelco, Bellefonte, PA, USA). The alkanes were identified by comparing the spectra obtained with mass spectrum libraries (NIST 2011 v.2.3 and Wiley, 9th edition).

2.3. Illumina MiSeq 16S rRNA amplicon sequencing

At the end of the experiment, the fate of the bacterial consortium and changes in the bacterial community composition in the bioaugmented and non-bioaugmented soils with (10 mM) and without EDTA were followed using illumina MiSeq sequencing. DNA was extracted from triplicate soil samples using skim milk protocol (Volossiouk et al., 1995). Purified DNA extracts were then submitted to Molecular Research MR DNA laboratory (www.mrdnalab.com, Shallowater, TX, USA) for illumina MiSeq sequencing of the bacterial 16S rRNA genes using the primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 805R (5′-GACTACHVGGGTATCTAATCC-3′) with barcode on the forward primer (Klindworth et al., 2013). Sequence analysis was carried out using the Mothur MiSeq SOP pipeline (Schloss et al., 2009). Briefly, barcodes were removed and sequences <200 bp and sequences with ambiguous base calls were eliminated. Sequences were de-noised, operational taxonomic units (OTUs) generated and chimeras removed. OTUs were defined by clustering at 3% divergence (97% similarity). Final OTUs were taxonomically classified using BLASTn against a curated GreenGenes database (DeSantis et al., 2006). Rarefaction curves and diversity indices (OTU richness, Chao and ACE) were calculated using the Mothur software. SIMPER (similarity percentage) analysis of the sequence data was performed using PAST program to find out the bacterial genera that accounted for the community differences in the different soil samples.

2.4. Effect of EDTA on the bacterial consortium growth

Additional experiments were conducted to find out if the consortium bacteria can tolerate EDTA and if they can use it as a carbon source. To test the strains' tolerance to EDTA, individual strains of the consortium were grown on acetate as a carbon source in the presence of different concentrations (i.e. 0.1, 1 and 10 mM) of EDTA. Briefly, 50 μl of a minimal salt medium (see above) supplemented with nutrients i.e. NH_4Cl and NaH_2PO_4, vitamins and 20 mM acetate were added into 96 well plate and then inoculated with 50 μl of each strain culture. EDTA was then added to each well at the above mentioned concentrations. Biotic (bacteria without EDTA) and abiotic (EDTA without bacteria) controls were maintained, and all plates were incubated at 30 °C. The growth of bacteria was monitored by measuring cell density at 620 nm over time using a microplate photometer reader. To test the growth of the strains on EDTA as the sole carbon source, a similar experiment was carried out except that EDTA was used instead of acetate as the carbon source in the medium.

2.5. Statistical analysis

CO_2 evolution data over time and GC–MS data were statistically analyzed using two-way ANOVA. The assumption of normality of data was verified with the Shapiro–Wilk's test (Shapiro and Wilk, 1965). Turkey post-hoc test was used in order to compare individual means. In all cases, the threshold for significance was 5%.

3. Results

3.1. EDTA and bacterial respiration activities

Analysis of variance (ANOVA) demonstrated that different concentrations of EDTA, duration of the experiment and their combination had a significant ($p < 0.05$) effect on the production of CO_2 (Table 1). The respiration activity of the bacterial consortium, as indicated by the evolved CO_2 showed a slight significant increase (Turkey HSD, $p < 0.05$) with the addition of EDTA in comparison with the EDTA-free control (Fig. 1). This increase occurred in the first 8 days of incubation, after which the amount of evolved CO_2 remained unchanged for the remaining period of incubation (i.e. 42 days). In the non-bioaugmented contaminated soil, the addition of EDTA at the concentrations of 0.1 and 1 mM resulted in a slight increase in the evolved CO_2 at the end of the experiment from 0.5 ± 0.07 to 1.0 ± 0.07 and to 0.7 ± 0.06 mg CO_2 g^{-1} soil, respectively. The most significant (Turkey HSD, $p < 0.05$) increase in the evolved CO_2 was detectable after the addition of 10 mM EDTA, reaching a maximum amount of 2.2 ± 0.08 mg-CO^2 g^{-1} after 14 days. This amount did not significantly ($p > 0.05$) change in the period between 14 and 42 days of incubation. The addition of EDTA to the bioaugmented soil (i.e. soil + bacterial consortium) had a comparable effect on respiration activities as in the non-bioaugmented soil (Fig. 1). The highest CO_2 evolution was 1.6 ± 0.4 mg CO_2 g^{-1} in the presence of 10 mM of EDTA after 14 days.

3.2. EDTA and oil biodegradation

Oil degradation was assessed either by calculating the complete hydrocarbon mineralization to CO_2 from the evolved CO_2, assuming that oil was the major available carbon source, or by GC–MS analysis of partially and completely degraded hydrocarbons. The highest oil mineralization rate was detected in the case of the addition of 10 mM EDTA in all incubations. Oil mineralization extent in the bioaugmented soil after the addition of 10 mM EDTA reached around 1.7 ± 0.1% of the initial oil present, which corresponds to ca. 0.8 ± 0.1 mg of hydrocarbons g^{-1} soil (Table 2).

GC–MS analysis of the residual oil at the end of the experiment revealed that mostly C_{14}–C_{30} alkanes were degraded (Fig. S1, 2). The concentrations of alkanes were significantly ($p < 0.05$) lower in the soils with 10 mM EDTA compared to the soils without EDTA (Fig. 2). While the addition of EDTA to the soil resulted in the degradation of 62% of the alkanes (i.e. 0.8 ± 0.5 mg g^{-1} soil), the addition of the consortium resulted in the degradation of 68.7% (i.e. 0.9 ± 0.4 mg g^{-1} soil). The highest degradation was observed when

Table 1
The effect of different concentrations of EDTA on CO_2 production determined by ANOVA. An asterisk (*) denotes significant *p*-values at $\alpha = 0.05$.

Factor	Mean square	F	*p*-value
Concentrations	27.889	366.451	>0.0001*
Time	22.778	299.288	>0.0001*
Concentrations × time	1.005	13.21	>0.0001*
Residuals	0.107		

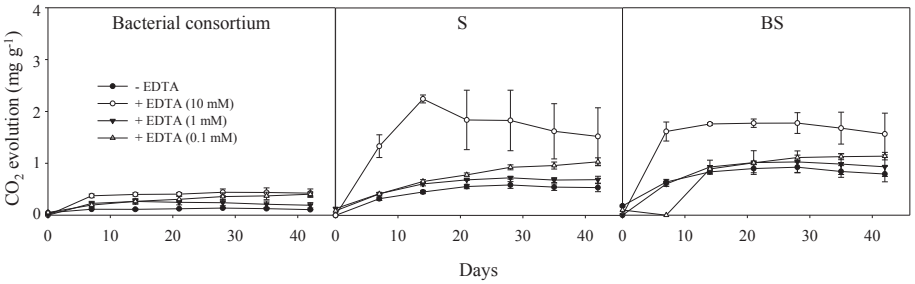

Fig. 1. The effect of different concentrations of EDTA (+EDTA) on CO_2 evolution rates of the non-bioaugmented oil-contaminated soil (S), the bioaugmented soil with the bacterial consortium (BS) and the bacterial consortium alone. Samples without EDTA were used as controls (-EDTA). All treatments were maintained in triplicates.

Table 2
Accumulative evolved CO_2, oil mineralization as calculated from evolved CO_2, assuming that oil was the only carbon source, and amount of alkane degradation as revealed by GC–MS analysis of the bioaugmented (BS) and the non-bioaugmented (S) soils in the presence of 10 mM EDTA.

Treatment	Total evolved CO_2 after 63 days	Oil mineralization calculated from evolved CO_2		Amount of C_{14}–C_{30} biodegraded by GC–MS	
	mg CO_2 g^{-1} soil	%	mg g^{-1}	%	mg g^{-1}
BS	0.8 ± 0.2	1.0 ± 0.2	0.5 ± 0.1	68.7	0.9 ± 0.4
S + EDTA	1.1 ± 0.1	1.5 ± 0.2	0.7 ± 0.1	62.0	0.8 ± 0.5
BS + EDTA	1.3 ± 0.09	1.7 ± 0.1	0.8 ± 0.1	91.5	1.2 ± 0.7

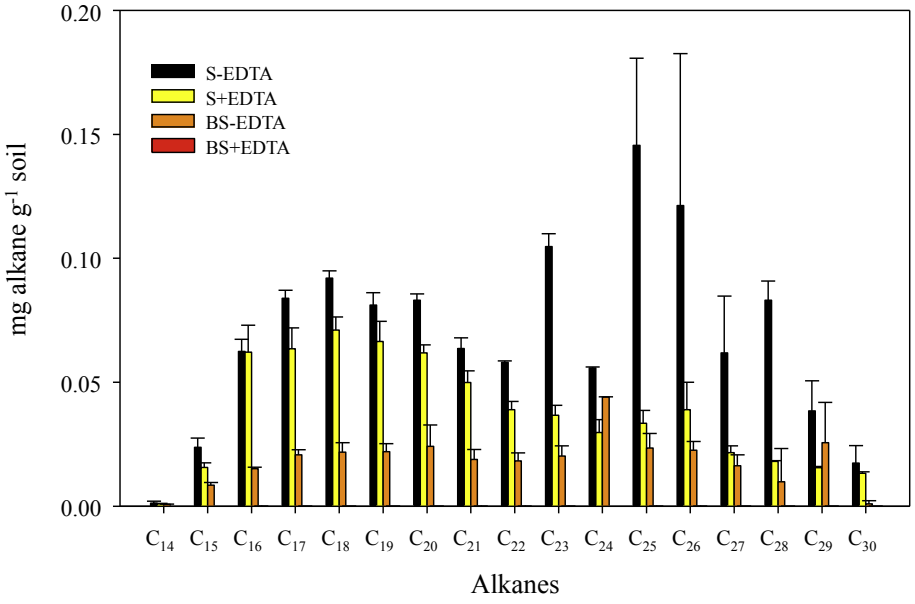

Fig. 2. Degradation of individual alkanes (C_{14}–C_{30}) at the end of the bioaugmentation experiments in bioaugmented (BS) and non-bioaugmented (S) soils with (+) and without (−) EDTA. Triplicate samples from each soil were analyzed.

both the bacterial consortium and EDTA were added. This degradation reached 91.5% of the C_{14}–C_{30} alkanes, corresponding to 1.2 ± 0.7 mg g^{-1} soil (Table 2).

3.3. EDTA and soil bacterial community

A total of 1,348,936 16S rRNA sequences, with 922,075 unique sequences, were obtained by MiSeq sequencing at the end of the experiment (Table 3). Cluster analysis showed clear variations among the triplicates of each treatment and all soils shared more than 49% of their OTUs (Fig. 3). The bacterial community structure of the original contaminated soil was dominated by the bacterial classes *Deinococci*, *Alphaproteobacteria*, *Gammaproteobacteia* and *Bacilli* 78–91% of total sequences (Fig. 3). Sequences belonging to the Candidate division tm7 group made up to 17% of total sequences only in one of the replicates (i.e. S–B). *Truepera* and *Sphingomonas* were the two major genera in the triplicate samples of this soil, accounting for 46–67% and 11–21% of the total sequences, respectively (Fig. 4). The genera *Gemmatimonas*, *Aneurinibacillus* and *Nitrococcus* were less dominant. Addition of 10 mM of EDTA to the contaminated soil did not cause a dramatic change in the bacterial community composition (Fig. 4). Only the classes *Gammaproteobacteria* and *Actinobacteria* showed an increase in their relative abundance. While *Gammaproteobacteria* increased from 4.5–5.5% to 5–14% of total sequences, *Actinobacteria* increased from 1–2.6% to 3–13%, respectively (Fig. 3). At the genera

Table 3
MiSeq pyrosequencing and diversity indices of the bioaugmented (BS) and the non-bioaugemnted (S) soils at the end of the EDTA treatments.

Treatment	Replicates	No. of sequences	No. of $OTU_{0.03}$[a]	Chao1	ACE
S	A	98402	526	890	919
	B	146687	590	1039	1051
	C	120261	662	1088	1121
S + EDTA	A	40849	602	1097	1088
	B	121988	916	1395	1406
	C	50124	576	1026	1073
BS	A	152882	776	1279	1330
	B	132855	627	1059	1109
	C	96543	517	905	922
BS + EDTA	A	97085	644	1088	1110
	B	74890	654	1138	1191
	C	119322	708	1178	1254

[a] Operational taxonomic unit at 3% sequence dissimilarity based on equal subsets of sequences for all samples.

level, similar bacterial genera were detected in the soils with and without EDTA with the exception of the genera *Rubrobacter* and *Gilvimainus*, which were only detected in the presence of EDTA (Fig. 4). In the soils supplemented with the consortium alone (no EDTA), the same bacterial classes and genera were detected, but with an increase in the relative abundance of *Gammaproteobacteria* (5–26% of total sequences). Out of the five members of the consortium, only *Alcanivorax* sp. MH3 and *Parvibaculum* sp. MH21 were detected. In comparison with the original soil, the relative abundance of candidate division tm7, *Nitrosococcus* and *Gemmatimonas* slightly decreased, while the abundance of *Salinimicrobium*, and *Aquicella* slightly increased. This increase or decrease was at least observed in one of the replicate samples. The most profound changes in the bacterial community structure were detectable in the soils amended with EDTA and the bacterial consortium. While the relative abundance of *Deinococci* decreased to 27–45% of total sequences, the relative abundance of *Bacilli* and *Flavobacteria* increased to 3–10% and to 2–13% of total sequences, respectively. In this treatment, the bacterial consortium member *Alcanivorax* sp. MH3 was still detectable at the end of the experiment, making up 7.5–15% of total sequences. Sequences belonging to Candidate division tm7 and *Cloacibacterium*, increased in relative abundance in this soil. SIMPER analysis revealed that the genera *Alcanivorax*, *Truepera* and *Sphingomonas* were responsible for the dissimilarities between the bacterial communities (Table S1). The contribution of other genera did not exceed 4%.

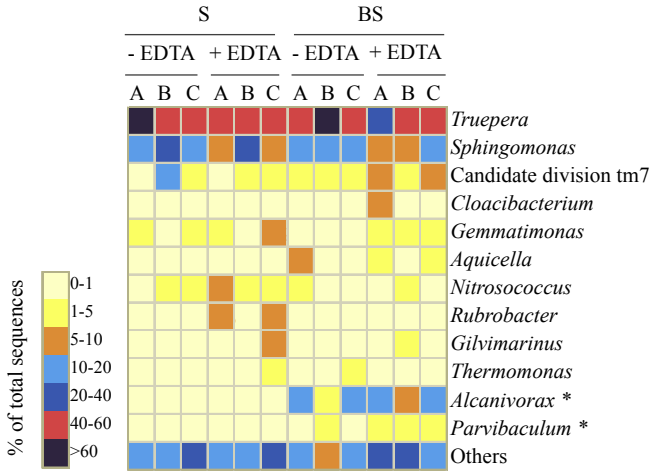

Fig. 4. A heatmap showing the relative abundance (%) of the most dominant bacterial genera detected in the bioaugmented (BS) and non-bioaugmented (S) soils with (+EDTA) or without (-EDTA) the addition of 10 mM EDTA after 42 days. Asterisks indicate bacteria that are members of the introduced consortium.

3.4. Effect of EDTA on growth of the consortium bacteria

All consortium bacteria were able to grow on acetate in the presence of 0.1, 1 and 10 mM EDTA, except *Azospirillum* AH2 (Table S2). This strain did not grow at 10 mM EDTA, although it grew at 0.1 and 1 mM concentrations. On the other hand, when the medium was deprived of acetate and only EDTA was added, four strains out of five (i.e. except *Azospirillum* AH2) grew at 0.1 mM, only two (i.e. *Alcanivorax* sp. MH3 and *Parvibaculum* sp. MH21) grew at 1 mM and none grew at 10 mM (Table S2).

4. Discussion

Our results demonstrated a positive correlation between the concentration of EDTA and the stimulation of bacterial respiration activities and oil biodegradation rates. This relation was consistently observed in the bioaugmented and non-bioaugmented soils and even in the bacterial consortium without soil. The increase in respiration activities with increasing EDTA concentration points out to the ability of the consortium members and possibly some bacteria in the soil to survive the previously reported lethal effects

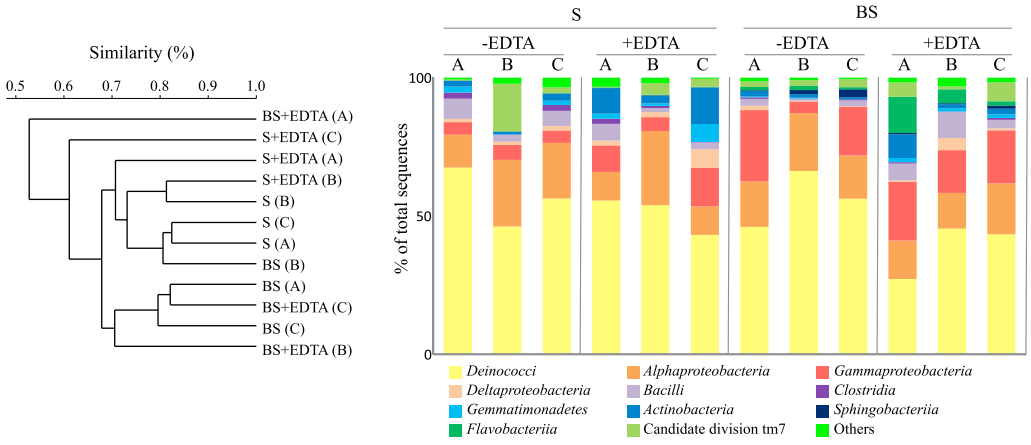

Fig. 3. The impact of 10 mM EDTA and addition of the bacterial consortium on the diversity of soil microbial communities analyzed after 42 days of treatment. **A.** Cluster analysis showing similarities among studied microbial communities based on OTUs and **B.** the relative abundance (%) of major classes of bacteria present in all treatments. The bioaugmented (BS) and non-bioaugmented (S) soils were incubated with (+EDTA) and without the addition of EDTA (-EDTA).

of high concentrations of EDTA (Gray and Wilkinson, 1965; Banin et al., 2006; Yakandawala et al., 2007; Saadat et al., 2013). This assumption was supported by our growth experiments, which demonstrated the ability of four out of five strains of the consortium to grow on acetate in the presence of as high as 10 mM EDTA. Indeed, the consortium strains were even able to utilize EDTA as a carbon source at 0.1 mM but not at 10 mM concentration. Previous research has demonstrated the ability of individual bacterial strains to catabolize EDTA, through intracellular oxidation (Nörtemann, 1999), as a sole carbon source (Tiedje, 1975; Oviedo and Rodríguez, 2003). For instance, the strains *Agrobacterium* sp. strain ATCC 55002 (Lauff et al., 1990; Oviedo and Rodríguez, 2003), *Chelativorans oligotrophicus* (Kaparullina et al., 2012), *Aminobacter aminovorans* (Weilenmann et al., 2004), *Burkholderia cepacia* (Chen et al., 2005) and *Stenotrophomonas chelatiphaga* (Kaparullina et al., 2009) were shown to degrade up to 35 mM of EDTA metal complexes. A strain of the genus *Agobacterium* could even use EDTA as the sole source of nitrogen (Nörtemann, 1999). OTUs related to *Agrobacterium*, *Burkholderia* and *Stenotrophomonas* were detected in our soils using MiSeq, suggesting a possibility that some of the soil's bacteria could grow on the added EDTA.

The addition of EDTA at 10 mM concentration exhibited the most profound stimulatory effect on bacterial respiration activities. The amounts of evolved CO_2 at this concentration in the soils with and without the bacterial consortium were comparable, although more CO_2 was expected to evolve after the addition of the consortium. This could be attributed to the variable effect of EDTA on microorganisms, promoting the growth and activity of some bacteria but inhibiting others, thus resulting in the same net CO_2 production. Indeed, our MiSeq data showed that out of the five consortium strains, only *Alcanivorax* sp. MH3 and *Parvibaculum* sp. MH21 survived until the end of the experiment. Moreover, the growth of all consortium strains was inhibited at 10 mM EDTA when no other carbon source was available. This means that EDTA at this concentration could inhibit, has no effect or promote the growth of different bacteria in the contaminated soils.

Although the evolved CO_2 from soils with and without the bacterial consortium suggested a minimal role of the consortium in enhancing oil degradation, GC–MS data showed the opposite. This could be due to the fact that CO_2-based method indirectly measures biodegradation by following the evolved CO_2 from completely mineralized hydrocarbons as well as other available carbon sources, such as EDTA. Our growth experiments demonstrated that EDTA could indeed serve as a carbon source for some microorganisms. Hence, in our experiments, GC–MS analysis is more reliable for the estimation of hydrocarbon degradation. GC–MS analysis demonstrated that the addition of either EDTA or the bacterial consortium resulted in the degradation of 62% and 68.7% of C_{14}–C_{30} alkanes, respectively whereas the combination of both resulted in the degradation of 91.5% of the alkanes. This indicates that both biostimulation and bioaugmentation approaches can be efficiently applied for the cleanup of desert soils. The increase in the relative abundance of sequences related to the consortium strains *Alcanivorax* sp. MH3 and *Parvibaculum* sp. MH21 and their survival until the end of the treatments suggest an active role of these strains in the observed alkane degradation. This highlights the importance of *Alcanivorax* and *Parvibaculum* spp. in bioaugmentation of oil-polluted soils. These species have been previously reported in oil-impacted ecosystems (Schneiker et al., 2006; Lai et al., 2011; Looper et al., 2013), and could degrade several aliphatic and aromatic compounds (Sipilä et al., 2008; Wang and Shao, 2013). Several studies have demonstrated the efficient use of *Alcanivorax* spp. individually or in a consortium in the bioaugmentation of contaminated soils (McKew et al., 2007; Gertler et al., 2009; Hassanshahian et al., 2012).

The addition of EDTA stimulated alkane degradation by increasing their bioavailability. Upon oil pollution, hydrocarbons bind to soil surfaces through weak non-covalent bonds, such as Van der Waals and hydrogen bonding, besides cation and water bridging (Yong et al., 1994). Metal ions function as cross-linking agents in soils by binding to multiple carboxyl or phenolate groups from different strands of the humic macromolecules, hence increasing the binding between oil and soil surfaces (Yong et al., 1994). It was previously reported that the addition of chelating agents such as EDTA facilitates the desorption of organic contaminants from soil (Yang et al., 2001). The mechanism underlying this effect was attributed to the involvement of EDTA in the removal of metal ions, hence changing the surface properties of soils. This will consequently result in the release of hydrocarbons, which are bound to the soil via the metal ions, into the aqueous phase. The diffusion of hydrocarbons into solution makes them more available to hydrocarbon utilizing bacteria. Previous studies have demonstrated the efficient use of EDTA in facilitating oil bioavailability and degradation (Hua et al., 2007; Han et al., 2009; Baziar et al., 2013). For instance, the addition of 0.005 M and 0.01 M EDTA was shown to remove 35% and 38% of the bound organic contaminants from soils, respectively (Han et al., 2009). The addition of EDTA was also shown to increase the desorption and diffusion of polycyclic aromatic hydrocarbons (PAH) into the aqueous phase (Yang et al., 2001).

MiSeq sequencing revealed the dominance of the bacterial class *Deinococci* in all soils with or without EDTA. This phylum is well-known for its resilience in environments that experience extreme toxicity and radiation and it includes members that are UV and radiation-resistant (Ellis et al., 2003; Suzuki and Banfield, 2004; Chanal et al., 2006; Zhang et al., 2007). UV-resistant bacteria of the actinobacterial genus *Rubrobacter* (Kim and Crowley, 2007) have also been detected in the EDTA-amended soils. The occurrence of the radiation-resistant *Deinococci* and *Rubrobacter* in our samples is consistent with the prevailing harsh environmental conditions of the arid desert, where they originate. Sequences belonging to *Deinococci* have been previously encountered in oil-polluted soils (Liang et al., 2011; Abed et al., 2014b). Although members of this group are not regarded as well-known oil-degraders, some strains were reported to grow, or have the potential to grow, on hydrocarbons (Bisht et al., 2010; Dalmaso et al., 2010). For instance, *Trupera radiovictrix*, which constituted 46–67% of total sequences in our soils, possesses all genes for naphthalene degradation (KEGG database, Kanehisa and Goto, 2000). Species of *Truepera* contributed to the degradation of naphthenic acids in oil-polluted waters (McKenzie et al., 2014). *Deinococcus radiodurans*, which was also detected in our soils but at a very low abundance, was genetically engineered for the biodegradation of ionic mercury and toluene in radioactive mixed waste environments (Brim et al., 2000). This highlights the importance of UV-resistance bacteria in the bioremediation of oil-polluted sites that are simultaneously exposed to high levels of radiations, such as arid deserts.

Sequences belonging to *Sphingomonas*, Candidate division tm7 and *Gemmatimonas* were abundantly encountered in all soils. The detection of $\leq$17% of total sequences belonging to the Candidate division tm7 indicates the presence of yet uncultured novel species in these soils. The genus *Sphingomonas*, which constituted 11–21% of total sequences, has been frequently detected in oil-contaminated sites (Leys et al., 2004). *Sphingomonas* spp. are known to play an important role in bioremediation and biodegradation of organic pollutants. For instance, *Sphingomonas formosensis* could utilize polycyclic aromatic hydrocarbons such as naphthalene, phenanthrene and pyrene (Lin et al., 2012). *Sphingomonas paucimobilis* could degrade numerous aromatic hydrocarbons (Story et al., 2004; Ichikawa et al., 2010). Bacteria belonging to

the genes *Gemmatimonas* are widespread in nature, particularly soil habitats, and have a special cell wall structure (Zhang et al., 2009). This cell wall makes them highly resistant to heavy metals and oil pollution (Zhang et al., 2009), which could explain their enrichment after addition of EDTA in our experiments. The increase in the relative abundance of *Bacilli* and *Flavobacteria* in the soils where both EDTA and the bacterial consortium were added, suggests a role of these bacteria in oil degradation, particularly since the highest alkane degradation was observed in this treatment. Indeed, *Bacilli* is known to contain hydrocarbon-degrading species that also possess the ability to produce biosurfactants (Morán et al., 2000; Christova et al., 2004; Das and Mukherjee, 2007). *Bacilli* was detected in oil-polluted soils and even in crude oils, suggesting their high tolerance to oil contamination. Several studies demonstrated the ability of isolates of *Bacillus* and *Paenibacillus*, which were detected in our soils, to degrade crude oil and naphthalene, respectively (Daane et al., 2002; Prince et al., 2010). On the other hand, the detection of *Cloacibacterium* genus of the class *Falvobacteria* in our soils is interesting since this genus has been reported for contaminated freshwaters but not soils (Allen et al., 2006; Cao et al., 2010). No studies have described the ability of bacteria within this group to degrade hydrocarbons. Hence, the presence of *Cloacibacterium* spp. in soils and their possible role in oil degradation deserves further investigations.

5. Conclusion

Our study demonstrated that EDTA at 10 mM can be used in bioremediation of oil-polluted desert soils, which are otherwise difficult to decontaminate due to the excessive temperature and radiation that renders hydrocarbons inaccessible to microorganisms. EDTA increases the dissolution of hydrocarbons, hence increasing their bioavailability and degradation. The bioaugmentation of the contaminated desert soils with hydrocarbon-degrading bacterial consortium could further increase the degradation efficiency. *Alcanivorax* sp. MH3 and *Parvibaculum* sp. 21 were the most competent members of the bacterial consortium and could be used in future bioaugmentation treatments. The combination of bioaugmentation and addition of EDTA can serve in the future as an efficient approach for the cleanup of contaminated desert soils.

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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.chemosphere.2015.12.114.

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