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Original Article

Effective bioremediation of a petroleum-polluted saline soil by a surfactant-producing *Pseudomonas aeruginosa* consortium

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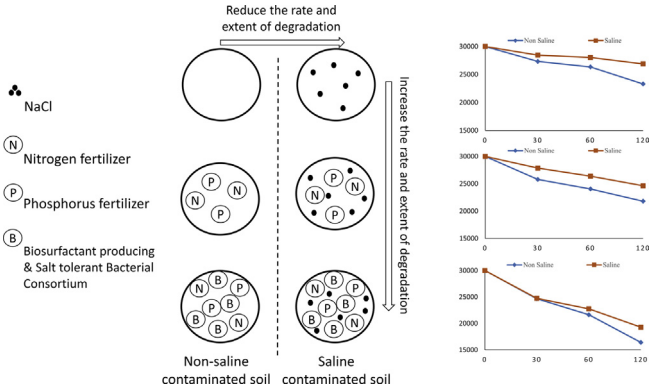
GRAPHICAL ABSTRACT



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ABSTRACT

Bacteria able to produce biosurfactants can use petroleum-based hydrocarbons as a carbon source. Herein, four biosurfactant-producing *Pseudomonas aeruginosa* strains, isolated from oil-contaminated saline soil, were combined to form a bacterial consortium. The inoculation of the consortium to contaminated soil alleviated the adverse effects of salinity on biodegradation and increased the rate of degradation of petroleum hydrocarbon approximately 30% compared to the rate achieved in non-treated soil. In saline condition, treatment of polluted soil with the consortium led to a significant boost in the activity of dehydrogenase (approximately 2-fold). A lettuce seedling bioassay showed that, following the treatment, the soil's level of phytotoxicity was reduced up to 30% compared to non-treated soil. Treatment with an appropriate bacterial consortium can represent an effective means of reducing the adverse effects of salinity on the microbial degradation of petroleum and thus provides enhancement in the efficiency of microbial remediation of oil-contaminated saline soils.

Introduction

The dependence of the modern economy on petroleum remains high, bringing along with it the risk of environmental contamination during the extraction, transport and storage of crude oil and derived products [1]. The estimated annual volume of crude oil

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spillage ranges from 0.2 to 2.0 million tons in metric units [2]. The crude oil is a complex mixture of alkanes, aromatic hydrocarbons and nitrogen-, oxygen- and sulfur-containing compounds [3], imposing adverse effects on human, animal and plant life [4]. Remediation of spills requires a range of effective, environmentally benign technologies to be devised. The contribution of microbes in this context is receiving particular attention [5]. The effectiveness of applying a bioremediation strategy to petroleum-hydrocarbons polluted soils depends on the biotic and abiotic elements which are impressive on growth and activity of degrading microorganisms [6]. A major constraint to the biodegradation process in soil is the lack of bioavailability or mass transfer limitation of the polluting entities, which restricts the access of the microbes to petroleum pollutant components, thereby decreasing the rate of contaminants biodegradation [7]. Some bacteria and fungi are capable of producing and excreting amphipathic molecules referred to as biosurfactants, which act to pseudosolubilize hydrocarbons, allowing them to be more effectively desorbed from the soil matrix. Such microbes have considerable potential as bioremediation agents of crude oil contaminated soils [8–10]. Many of these contaminated soils also suffer from salinization as a consequence of industrial activity [11]. Soil salinity suppresses the growth of most microbes, reducing their value as degraders of petroleum pollution [12]. In such environments, therefore, it is necessary that the potential bioremediation agents are also high salinity tolerant. Bacterial communities (“consortia”) are typically more flexible than any single species, so can be expected to be capable of degrading a wider range of pollutants [13].

As yet, little attention has been paid to assessing the bioremediation potential of salt-enriched soils contaminated with crude oil. The objectives of the current study were to determine the effect of salinity on soil microbial activity and hydrocarbons bioremediation process, and to evaluate degradation efficiency of biosurfactant-producing bacterial consortium in oil-contaminated saline soil. This study also attempted to estimate the correlation between dehydrogenase activity (DHA) and most probable number (MPN) of hydrocarbon-degrading bacteria, to confirm the utility of this indicator in the monitoring of bioaugmentation process.

Material and methods

Soil samples

Non-contaminated, non-saline soil was collected from the soil surface layer (0–30 cm) at a clean site (Lat: 35° 45′ 16″; Long: 50° 57′ 56″). After air drying, the soil was passed through a 2 mm sieve to allow the measurement of a set of standard soil characteristics (pH, electrical conductivity, cation exchange capacity and organic carbon content) (Table 1). The soil was then mixed with varying amounts of crude oil and NaCl. The chosen levels of crude oil were 10 and 30 g/kg, and the NaCl concentration was set to either 0, 150 or 300 mM. Salinity and contamination levels were chosen based on reports of literatures from contaminated sites of Iran [14–16].

Table 1
Physical and chemical characteristics of the experimental soil.

Soil property	Value
Sand	53%
Silt	25%
Clay	22%
pH	7.33
EC (Electrical conductivity)	1.48 dS m^{-1}
CEC (Cation exchangeable capacity)	14.3 meq 100 g^{-1} dry soil
Organic carbon	0.55%
Organic matter	0.95%
Sodium	71 mg kg^{-1} dry soil
Potassium	204 mg kg^{-1} dry soil

Bacterial consortium

The bacterial consortium tested comprised four bacterial strains isolated from two saline petroleum-contaminated soils, based on the modified mineral salt medium (MSM) described by Zhang et al. [17]. The strains were identified and differentiated from one another via 16S rRNA sequencing using the pair of universal primers 27F (5′AGA GTT TGA TCC TGG CTCAG3′) and 1429R (5′TAC GGY TAC CTT GTT ACG ACTT3′). An evaluation was conducted of each strain's capacity to produce biosurfactant and to degrade crude oil by culturing them in a saline medium which essentially as previously described by our group [14]. To construct the consortium, the strains were first cultured separately in aerobic tryptic soy broth at 30 °C for 24 h. The cells were harvested by centrifugation (10,000*g* for 10 min) and resuspended in sterile 0.9% NaCl. The concentration of the subsequent suspensions was inferred by turbidimetry at 630 nm. Finally, the strains were resuspended together in a 1:1:1:1 ratio [18].

Bioaugmentation and biostimulation experiments

A 120-day pot experiment was conducted in a greenhouse where the temperature varied from 20 to 30 °C. Each pot was filled with 3 kg of sieved (4 mm), salinized (0, 150 or 300 mM NaCl) and crude oil-contaminated (10 or 30 g/kg) soil. The soil moisture was maintained at about 70% water holding capacity throughout. Plastic saucers were used to prevent water draining from the pots and to maintain salinity in considered levels. The pots were fully randomized in triplicates, and three treatments (T1 through T3) were imposed: T1 – no additives; T2 – the C:N:P ratio was adjusted to 100:15:3 to promote the growth of native microbes (“biostimulation”); and T3 – 10^6 CFU/g soil of the consortium was added to the T2 treatment (“bioaugmentation” + “biostimulation”). Treatment combinations were run in 18 groups (non-saline soils dosed with 10 and 30 g/kg crude oil imposed with T1, T2, and T3 treatments, salinized soils by 150 mM NaCl and dosed with 10 and 30 g/kg crude oil imposed with T1, T2, and T3 treatments, salinized soils by 300 mM NaCl and dosed with 10 and 30 g/kg crude oil imposed with T1, T2, and T3 treatments). During experiment the composite soil samples were taken from each pot (54 samples) after 30, 60, and 120 days, and was stored at 4 °C for subsequent analysis.

Total petroleum hydrocarbons (TPHs) measurement

The TPHs content of each sample was determined by ultrasonic treatment of soil extracted in a 1:1 (*v*/*v*) mixture of hexane and acetone (extraction method EPA 3550b). Each 2 g sample of soil was first mixed with 1 g anhydrous Na_2SO_4 and then extracted at 20 °C in 15 mL of the solvent with the aid of an ultrasound device delivering 250 W (Branson M8800). The resulting suspension was centrifuged (10,000*g*, 5 min) to remove soil particles. The procedure was repeated and the two extracts combined. The solvent was evaporated using a concentrator (Eppendorf vacufuge plus), and the residual TPH amount was determined gravimetrically [19].

Biological indicators

The abundance of hydrocarbon-degrading microbes in the soil was estimated using the “most probable number” (MPN) protocol, carried out in a 96 well microtiter plate. The growth medium was MSM medium supplemented with various amounts of crude oil. A series of tenfold serial dilution was performed from a suspension of 1 g of soil in 10 mL MSM, and each plate was inoculated with

10^{-4}–10^{-8} serial dilutions. A 5 μL aliquot of filtered 50 mg/L resazurin was added to each well, the plate was sealed with Parafilm and then held at 30 °C for one week. Wells which had changed in color from blue to pink were deemed to be positive and the MPN of hydrocarbon-degrading microbes per g of soil was calculated [20].

Dehydrogenase activity (DHA) was measured using the triphenyl tetrazolium chloride reduction method [21]. Briefly, 2 g samples of soil were mixed with 2 mL 4% (*w*/*v*) triphenyl tetrazolium chloride and incubated at 30 °C for 24 h in the dark. The resulting triphenyl formazan generated was acetone-extracted and quantified colorimetrically (absorbance wavelength 485 nm). DHA was expressed as in the form triphenyl formazan per g soil per h.

Soil phytotoxicity was evaluated using a lettuce seed germination/root elongation test. Lots of 20 seeds were sown in 50 g air dried soil, which was then brought to 75% water holding capacity. After holding in the dark at 25 °C for 120 h, the number of germinated seeds was counted and the seedling root length measured. A root elongation inhibition index was then calculated [22].

Statistical analysis

The experiments were all run in triplicate and the data were subjected to a standard analysis of variance. Means were compared using Duncan's multiple range test ($P < 0.05$). Statistical calculations were made using SPSS v17.0 software (SPSS Inc., Chicago, IL, USA). Data are the mean ± S.E. Also the measured factors which reported as percentage was analyzed by the non-parametric Kruskal-Wallis test and values expressed as median ± range.

Results and discussion

Characterization of the bacterial consortium

On the basis of their 16S rDNA sequence, all four bacterial strains isolated were determined to be *Pseudomonas aeruginosa* (similarity over 99%). A phylogenetic analysis of the four sequences is given in Fig. 1S (Supplementary material). The oil spreading and emulsification assay indicated that each bacterial strain had the ability to produce biosurfactant, varying in quantity from 2.08 to 3.72 g/L. Based on a gravimetric analysis, their efficiency to degrade crude oil in saline mineral broth varied from 33 to 39.2% (Table 2). Details related to results of isolation and characterization of biosurfactant-producing and oil-degrading bacteria are reported in our previous work [14]. A gas chromatography-flame ionization based analysis of the crude oil degradation by each strain is given in Fig. 2S (Supplementary material). The GC analysis showed that the isolated bacterial strains present different patterns of hydrocarbon chain degradation. The T4 strain exhibited a similar ability to degrade short and long chain hydrocarbon chains while E1 strain was more efficient towards long chain hydrocarbons. Differential ability to degrade hydrocarbon compounds by bacterial strains has been documented [17,23].

The biodegradation of crude oil

The residual crude oil concentration following the various soil treatments is summarized in Table 3. According to the gravimetric analysis, degradation efficiency at each salinity level of T3 was significantly ($P < 0.05$) greater than those measured in both T2 and T1 samples. The inoculation of the consortium after 120 days in soil dosed with 10 g/kg crude oil at 0, 150, and 300 mM NaCl led to degradation of crude oil in the amount of 49.5, 47.0, and 42.3% respectively; the equivalent proportions when the initial crude oil load was 30 g/kg were 45.2, 39.9, and 35.7%. Biodegradation kinetics were modeled by the expression $\ln(C/C_0) = -kt$ or $C = C_0e^{-kt}$, where C represents the hydrocarbon concentration (mg kg^{-1}), C_0 the initial concentration of crude oil (mg kg^{-1}), t the number of days elapsed and k the rate constant (day^{-1}). Under non-saline conditions, k varied from 0.0029 to 0.0054 between the three treatments under the lower initial crude oil load, and from 0.002 to 0.0049 at the higher one (Table 4). Salinity inhibited the degradation process in both T1 and T2, but not in T3; in the former treatment, the effect of adding NaCl increased k from 0.0029 to 0.0014 in the soil dosed with 10 g/kg crude oil, and from 0.002 to 0.0009 in the one dosed with 30 g/kg. The presence of the bacterial consortium, however, reduced the decrease of k by salinity, resulting in the absence of any significant differences in removal efficiency between the three salinity levels ($P < 0.05$). Finally, the inoculation

Table 2
Biochemical performance of the individual components of the bacterial consortium. Values expressed as mean/median ± SE/range ($n = 3$).

Isolates	Oil spreading (mm)	Emulsification index (%)	Glycolipid production (g L^{-1})	Oil degradation (%)	16S rDNA identification
T4	3 ± 0.28	22.2 ± 2.1	2.08 ± 0.09	39.2 ± 2.8	*P. aeruginosa* (MF 289987)
T27	2.85 ± 0.15	33.5 ± 5.5	3.72 ± 0.11	33.3 ± 1.2	*P. aeruginosa* (MF 289986)
T30	2.4 ± 0.51	38 ± 2.9	2.12 ± 0.28	38.4 ± 1.5	*P. aeruginosa* (MF 289985)
E1	1.85 ± 0.2	24.5 ± 3	2.2 ± 0.28	33 ± 3.9	*P. aeruginosa* (MF 289988)

Table 3
Residual crude oil content during bioremediation process in the various treatments. The initial crude oil concentration was 10 g/kg (left), 30 g/kg (right). Values expressed as mean ± S.E. ($n = 3$).

Sampling time (day)	Salinity (mM NaCl)	10 (g kg^{-1})			30 (g kg^{-1})		
		Natural attenuation	Biostimulation	Bioaugmentation	Natural attenuation	Biostimulation	Bioaugmentation
30	0	9.51 ± 0.06^{no}	8.59 ± 0.09^{jk}	7.53 ± 0.094^{f}	27.33 ± 0.223^{lmn}	25.78 ± 0.369^{ij}	24.67 ± 0.192^{gh}
	150	9.73 ± 0.08^{op}	9.18 ± 0.08^{lm}	7.83 ± 0.146^{g}	28.52 ± 0.123^{p}	27.54 ± 0.139^{mno}	25.54 ± 0.364^{hij}
	300	9.92 ± 0.05^{p}	8.93 ± 0.072^{l}	8.21 ± 0.101^{hi}	28.45 ± 0.104^{op}	27.85 ± 0.229^{nop}	24.74 ± 0.487^{gh}
60	0	8.66 ± 0.107^{k}	7.95 ± 0.112^{gh}	6.63 ± 0.098^{d}	26.34 ± 0.286^{jk}	24.04 ± 0.497^{fg}	21.64 ± 0.117^{d}
	150	9.13 ± 0.07^{lm}	8.65 ± 0.088^{k}	6.78 ± 0.125^{d}	$27.63 \pm 0.135^{m-p}$	26.87 ± 0.319^{klm}	21.75 ± 0.185^{d}
	300	9.32 ± 0.08^{mn}	8.42 ± 0.092^{ijk}	7.18 ± 0.132^{e}	28.03 ± 0.2^{nop}	26.38 ± 0.178^{jkl}	22.75 ± 0.203^{e}
120	0	7.15 ± 0.245^{e}	6.72 ± 0.092^{d}	5.04 ± 0.143^{a}	23.30 ± 0.677^{ef}	21.80 ± 0.601^{d}	16.41 ± 0.73^{a}
	150	8.32 ± 0.105^{ij}	7.23 ± 0.056^{e}	5.32 ± 0.196^{ab}	26.75 ± 0.318^{klm}	25.23 ± 0.389^{hi}	18.02 ± 0.32^{b}
	300	8.54 ± 0.118^{jk}	7.16 ± 0.036^{e}	5.76 ± 0.163^{c}	26.89 ± 0.359^{klm}	24.62 ± 0.212^{gh}	19.28 ± 0.397^{c}

Similar lower case letters indicate that data are not significantly different from each other according to Duncan's multiple range test ($P = 0.05$).

Table 4
Rate constant for hydrocarbon biodegradation (k) in soils subjected to various treatments.

Treatments	k (day^{-1})					
	10 (g kg^{-1})			30 (g kg^{-1})		
	0 mM	150 mM	300 mM	0 mM	150 mM	300 mM
Natural attenuation	0.0029	0.0016	0.0014	0.002	0.0009	0.0009
Biostimulation	0.0032	0.0027	0.0027	0.0025	0.0013	0.0016
Bioaugmentation	0.0054	0.0051	0.0045	0.0049	0.0042	0.0035

of saline soils by the consortium boosted the removal of crude oil by 31% in the less heavily polluted soil and by 29% in the more heavily polluted one.

According to Darvishi et al. [24], both microbial growth and the rate of oil degradation are negatively impacted by increasing the level of soil salinity , while Qin et al. [25] have suggested that inoculation with a consortium can effectively enhance the biodegradation of petroleum-based hydrocarbons in a saline-alkaline soil. The presence of salinity is known to compromise the metabolic activity of many microbes, thereby compromising their ability to biodegrade oil [11]. In particular, salinity has an adverse effect on the activity of some key enzymes involved in the hydrocarbon

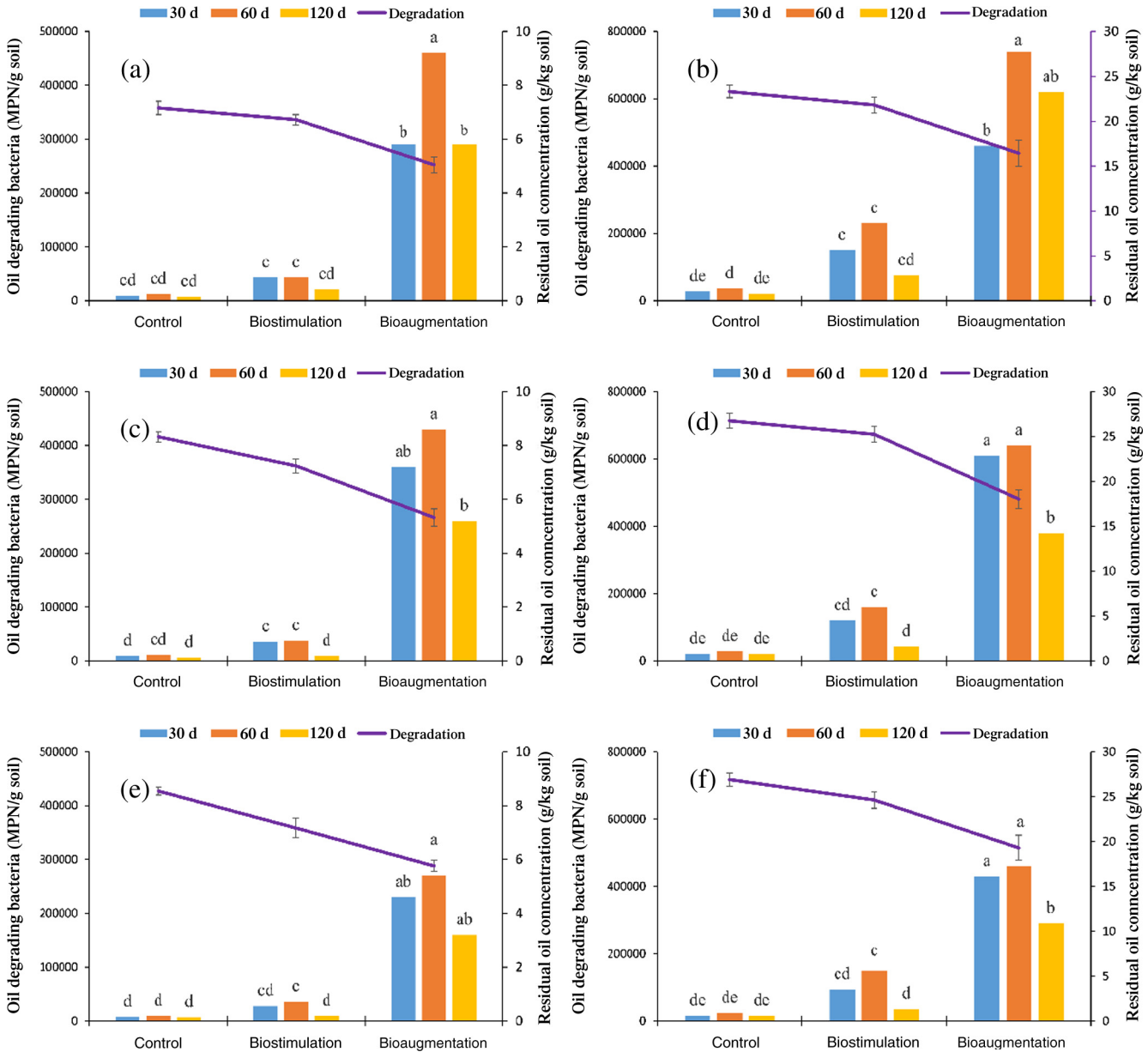

Fig. 1. Most probable number of oil-degrading bacteria (MPN) during the bioremediation process and the residual oil concentration (g kg^{-1} soil) from an initial crude oil concentration of (a, c, e) 10 g/kg, (b, d, f) 30 g/kg, in the presence of (a, b) 0 mM NaCl, (c, d) 150 mM NaCl, (e, f) 300 mM NaCl. Values expressed as mean ± S.E. (n = 3). Bars sharing the similar lower case letters indicate that data are not significantly different from each other according to Duncan's multiple range test (P = 0.05).

Table 5
Dehydrogenase activity (DHA) based on triphenyl formazan (TPF) reduction, during bioremediation process in the various treatments. The initial crude oil concentration was (left) 10 g/kg, (right) 30 g/kg. Values expressed as mean ± S.E. (n = 3).

Sampling time (day)	Salinity (mM NaCl)	DHA (μg TPF g^{-1} h^{-1})					
		10 (g kg^{-1})			30 (g kg^{-1})		
		Natural attenuation	Biostimulation	Bioaugmentation	Natural attenuation	Biostimulation	Bioaugmentation
30	0	1.33 ± 0.18^{g}	3.47 ± 0.53^{de}	5.81 ± 0.43^{ab}	4.98 ± 0.51^{fgh}	7.66 ± 0.25^{bcd}	10.45 ± 0.89^{ab}
	150	2.23 ± 0.69^{ef}	3.24 ± 0.95^{de}	5.05 ± 0.44^{bc}	3.86 ± 0.69^{ghi}	$5.00 \pm 0.34^{e-h}$	8.74 ± 0.88^{bc}
	300	1.76 ± 0.34^{fg}	2.44 ± 0.81^{ef}	3.84 ± 0.12^{d}	2.88 ± 0.33^{ij}	4.54 ± 0.54^{fgh}	7.29 ± 0.58^{cde}
60	0	3.51 ± 0.16^{de}	6.66 ± 0.80^{a}	7.04 ± 0.38^{a}	5.22 ± 0.17^{fgh}	7.86 ± 0.90^{bc}	11.08 ± 0.48^{a}
	150	3.04 ± 0.24^{def}	4.29 ± 0.31^{cd}	6.88 ± 0.11^{a}	4.73 ± 0.38^{fgh}	6.59 ± 0.22^{cde}	10.38 ± 0.76^{ab}
	300	2.93 ± 0.29^{def}	3.16 ± 0.54^{cde}	4.92 ± 0.23^{bc}	3.35 ± 0.32^{ij}	$6.04 \pm 0.66^{d-g}$	8.90 ± 0.96^{bc}
120	0	2.96 ± 0.35^{def}	5.22 ± 0.23^{bc}	4.27 ± 0.35^{bcd}	5.06 ± 0.58^{fgh}	6.47 ± 0.47^{cde}	8.64 ± 0.69^{bc}
	150	2.26 ± 0.23^{ef}	3.39 ± 0.12^{de}	3.89 ± 0.20^{cd}	3.37 ± 0.30^{ij}	$6.09 \pm 0.49^{d-g}$	8.09 ± 0.84^{cd}
	300	2.93 ± 0.22^{def}	3.04 ± 0.42^{de}	3.38 ± 0.18^{de}	3.41 ± 0.59^{hij}	$5.35 \pm 0.50^{d-g}$	$6.35 \pm 0.62^{d-g}$

Similar lower case letters indicate that data are not significantly different from each other according to Duncan's multiple range test (P = 0.05).

Table 6
Pearson's correlation between dehydrogenase activity (DHA) and either oil degrading bacteria (MPN) or total petroleum hydrocarbon degradation (TPH).

Treatment	Correlation coefficient (r)	
	DHA vs. MPN	DHA vs. TPH
Natural attenuation	0.83**	0.65**
Biostimulation	0.72**	0.65**
Bioaugmentation	0.9**	0.82**

** Correlation is significant at the 0.01 level of probability.

degradation process [2]. The possible mechanisms used by the bacterial consortium to preferentially utilize easily degradable components may contribute to the higher removal rate in the initial 30 days of bioremediation [26]. In principle, crude oil represents a source of bioavailable and metabolizable carbon, which should therefore stimulate microbial activity and hence accelerate the biodegradation process [11]. However, as this is not the general observation, it is clear that much of the carbon present in the oil must remain in non-available form. Since both the level of soil salinity and the extent of the pollutant load exert such a strong effect on the growth and activity of soil bacteria, in the context of bioremediation, it will be important to identify those bacterial strains which not only display a strong ability to degrade oil, but also a high level of salinity tolerance.

Biological indicators

The MPNs of the hydrocarbon-degrading bacteria in the various treatments and at the various sampling time points are shown in Fig. 1. The highest MPN reached was 7.4×10^5 per g: this was in T3 following a 60-day incubation of a non-saline soil polluted with 30 g/kg crude oil. T2 was superior to T1 in terms of the growth of hydrocarbon-degrading bacteria. In all three treatments, the number of bacteria increased significantly when the initial crude oil load was increased, while salinity had a negative effect (except in T3). The statistically significant difference in the number of bacteria present as a result of the various treatments implied that the bacterial consortium was able to compete well with the native community, especially in the presence of salinity. Under T3, by the time of the later sample time points, the population of hydrocarbon-degrading bacteria had begun to decline, possibly reflecting a fall in the concentration of hydrocarbon pollutant and/or the level of carbon bioavailability [27].

The relationship between the MPN and the rate of TPH degradation implies that the latter depends strongly on the growth and activity of the bacteria. Petroleum hydrocarbons are known to be hydrophobic and their adsorption onto the soil matrix over time further reduces their solubility in water [28]. In order to be successfully biodegraded, these compounds must first be desorbed from the soil, so that they can be released into the soil water and from thence taken up into the microbes' cells. Their rate of transfer from the adsorbed (insoluble) to the desorbed (soluble) phase is considered to be the major rate-limiting step for their biodegradation [28]. The ability of biosurfactants to increase their solubility is clearly important in this context, as has been shown by the successful enhancement of hydrocarbon degradation achieved by adding biological or chemical surfactants to the soil [29]. Here, the implication was that biosurfactants produced by the bacterial consortium acted to solubilize some of the crude oil, and hence to promote its degradation.

It has been suggested that the activity of a number of soil enzymes (dehydrogenases, lipases, ureases and catalases) can act as a sensitive indicator of soil quality, so that their measurement could be well suited to assess the impact of pollution [30]. Dehydrogenase is produced by all living organisms; soil DHA is directly related to the metabolic activity of soil microbes [31], which has been exploited to develop its use as a monitoring tool for the biodegradation efficiency of petroleum hydrocarbons in soil [25]. Here, both the T2 and T3 treatments exerted a significant positive influence on the level of soil DHA, which increased markedly when the initial crude oil load was increased from 10 to 30 g/kg; DHA reached 11 μg TPF per g per h in T3 after 60 days in the absence of salinity following the addition of the 30 g/kg crude oil load. This level was double that measured in T1 at the same time point. Salinity tended to depress DHA in all three treatments; the exception was in T3 subjected to 150 mM NaCl, where the DHA did not differ significantly from that measured in non-saline soil (Table 5). DHA rose initially in all three treatments, but later fell away. A possible explanation for this behavior is that the available fraction of petroleum hydrocarbons degrades early and relatively easily, leading to a build-up over time of less readily degraded compounds [25]. DHA was positively correlated with the TPH degradation and the MPN of oil-degrading bacteria (Table 6). In T3, the correlation coefficient between DHA and MPN ($r = 0.9$, $P \leq 0.01$) was higher than that in both T2 ($r = 0.72$, $P \leq 0.01$) and T1 ($r = 0.83$, $P \leq 0.01$), assumed to reflect the successful establishment of the bacterial consortium in the soil. Correlations between DHA and TPH degradation have been reported elsewhere in the literature [25,32], and have been interpreted as implying that microbial dehydrogenases are involved in the degradation process of crude oil [32]. The significant correlation established here between DHA and MPN confirms that DHA can be used to monitor the activity and efficiency of specific bacteria or bacterial consortia during bioaugmentation.

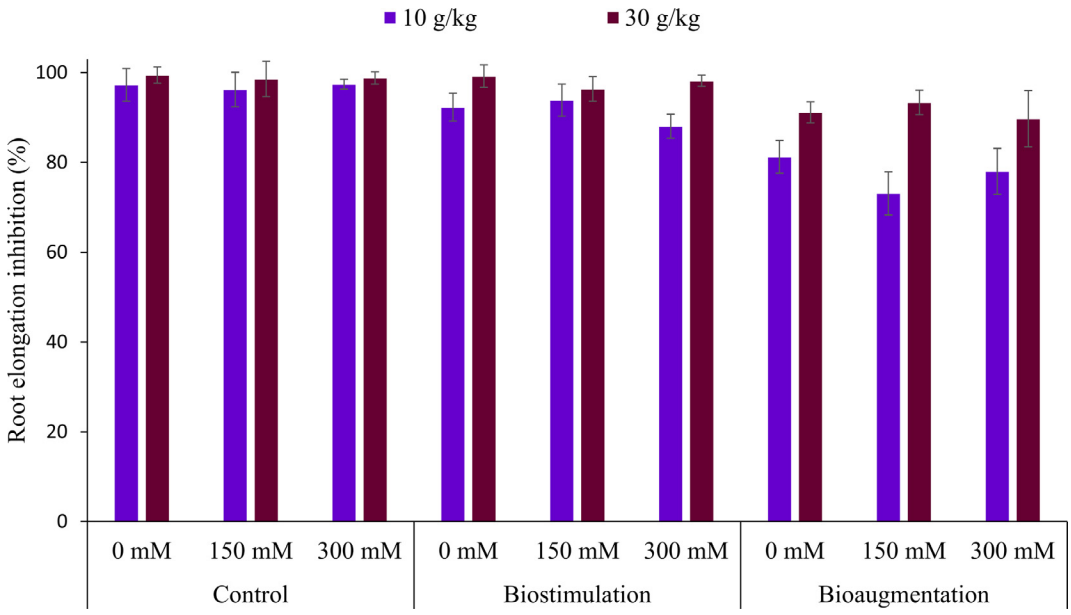

Fig. 2. The lettuce seedling root elongation inhibition test of remediated soil. Values expressed as median, bars indicate the range of three replicate.

Crude oil contains a variety of compounds associated with varying degrees of toxicity, mutagenicity and carcinogenicity. Since the major purpose of attempting the bioremediation of oil-contaminated soil is to permit further rehabilitation via phytoremediation, a mere decrease in the content of TPH may be insufficient. Rather, it is necessary to establish whether the soil treatment has been successfully enough to support plant growth [5]. Here, the residual phytotoxicity of the remediated soils was determined using a bioassay based on the germination of lettuce seed. Neither the T1 nor the T2 treatments led to any improvement, but there was a statistically significant positive effect as a result of T3 (Fig. 2), presumably as a result of the conversion by the bacterial consortium of some of the toxic compounds to those which were less or even non-toxic to lettuce seed [28].

Conclusions

The present experiments have demonstrated that biosurfactant-producing *P. aeruginosa* strains are capable of degrading crude oil, even in the presence of salinity. The inoculation of saline, contaminated soils with a consortium of four strains was able to alleviate the inhibition imposed by salinity on microbial growth and activity, thereby promoting TPH degradation. The plant-based bioassay showed that soil partially remediated in this way contained a reduced level of toxic compounds. Its correlation with the MPN of oil-degrading bacteria allows DHA to be used to monitor the activity and efficiency of bacterial consortia used for bioaugmentation. Clearly, further study will be needed to increase the effectiveness of the bioaugmentation technique as well as to investigate the potential benefit of combing bacterial consortia with other approaches.

Conflict of Interest

The authors declare that there is no conflict of interest..

Compliance with Ethics Requirements

This article does not contain any studies with human or animal subjects.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jare.2017.06.008.

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