A newly isolated strain of *Serratia* sp. from an oil spillage site of Assam shows excellent bioremediation potential

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Abstract

A hydrocarbon-degrading strain was isolated from a petroleum oil-contaminated site which was identified on the basis of 16S rDNA gene sequencing as a member of the genus *Serratia*. The isolate reduced surface tension of petroleum oil supplemented medium by 48.35% with respect to control after 7 days of treatment. Fluorescence microscopy revealed that its chemotaxis was towards hydrocarbon. The isolate degraded 87.54 and 85.48% of diesel and kerosene in liquid culture, respectively, after 28 day incubation at 37 ± 2 °C. The ex situ pilot scale bioremediation experiment in which artificially contaminated soil (10 and 20% v/w kerosene) was treated for 7 days showed a germination rate of *Vigna radiate* seeds of 52% and 72%, respectively. Interestingly, a germination rate of 31% was obtained with the heavily contaminated soil samples collected from the oil spillage site after 20 days of bioremediation treatment. The presence of ν_{CH_3} (asymmetric stretching), $\nu_{\text{C}=\text{C}}$ (stretch), and $\nu_{\text{C}-\text{C}}$ (stretch) in the crude biosurfactant produced by the isolate was revealed by FTIR analysis, and emulsification index (E_{24}) was found 60 and 56.6%, respectively, against diesel and kerosene oil. The non-cytotoxicity nature of the biosurfactant also supports its potential application in field trial.

Keywords Oil spillage · Hydrocarbon degradation · *Serratia* sp. · Biosurfactant · Pilot scale bioremediation

Introduction

Fossil oil contamination in terrestrial and water body largely hampers the ecosystem by increasing the toxicity level in the environment and is considered as a serious issue of global concern (Gordon et al. 2018; Méndez et al. 2017). Oil spillage may take place due to a number of reasons including onshore or offshore oil exploration practices, oil disposal practices, human negligence, and accidents during

transportation of oil, natural calamities or may be due to intentional release of oil during wars (Chen et al. 2018; Gong et al. 2014). Statistics carried out by the International Tanker Owners Pollution Federation Limited (ITOPF) reports two large oil spills, i.e., more than 700 ton each and four medium spills, i.e., 7–700 ton in the year 2017 itself (<http://www.itopf.com/knowledge-resources/data-statistics/statistics/>).

Physical methods such as skimming, wiping, burning, water flushing, etc. are used rarely as ancient methods for dealing with the problem but are least effective (Padaki et al. 2015). On the other hand, certain chemical surfactants are also used for the removal of oil, but are less acceptable as less than only 8% usable oil may be recovered (Hemmer et al. 2011). Therefore, microbial bioremediation is one of the most widely accepted models to fight against the problem due to its environment benign nature (Lim et al. 2016; King et al. 2015). Biosurfactants produced by petroleum degrading microorganism play an important role in emulsifying hydrocarbons to convert them into smaller, soluble, and less toxic form, so that it can be taken up by the microbes for metabolic conversion (Deepak and Jayapradha

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2015; Mahanty et al. 2006; Rosenberg and Ron 2002). A diverse group of microorganisms mostly belongs to the genus *Pseudomonas*, *Acenatobacterium*, *Bacillus*, etc. are largely explored for their potential to degrade hydrocarbon but least explored for their in situ or ex situ potential (Mukherjee and Bordoloi 2011; Sangeetha and Thangadurai 2014; Yenn et al. 2014; Guermouche et al. 2015; Patowary et al. 2018; Ramadass et al. 2018), whereas we report the potential application of *Serratia nematodiphila* for such studies for the very first time. However, petroleum-contaminated sites also serve as a potential source of novel indigenous hydrocarbon-degrading microorganisms (Chaudhary and Kim 2017; Méndez et al. 2017). Considering the above facts, a study was carried out for the exploitation of novel indigenous hydrocarbon-degrading microorganisms from a petroleum oil spillage site located in Rudrasagar area (26.11°N, 91.83°E) of Assam, Northeast India. Apart from bioremediation study by the most potential isolate in liquid culture, a set of pilot scale ex situ bioremediation experiments were also carried out with contaminated soil samples collected from the contaminated site and also with artificially contaminated soil samples. It may also be noted that the Northeast region of India is a biodiversity hotspot for flora and fauna with largely unexplored microbiota.

Materials and methods

Chemicals and reagents

Petroleum oil used in the study was procured locally from Numaligarh Refinery Limited (NRL) oil depots of Dibrugarh, Assam. Chemicals and consumables were purchased from Merck India Ltd., and all the microbial media were purchased from HiMedia India Pvt. Ltd.

Collection of petroleum-contaminated soil samples

Petroleum-contaminated soil samples ($n = 20$) were collected from an oil spillage site located in Rudrasagar area (26.11° N, 91.83° E) of Sibsagar district, Assam in sterile plastic bags and brought to the lab (Fig. 1).

Isolation and screening of hydrocarbon-degrading microorganisms

Serially diluted oil-contaminated samples were plated on BH (Bushnell and Haas) agar plates. The composition of medium was in g l^{-1} : MgSO_4 -0.2, CaCl_2 -0.02, KH_2PO_4 -1.0,

Fig. 1 a, b Location of the oil spillage site in the map (26.11° N, 91.83° E) and c, d sample collection procedure

K_2HPO_4 -1.0, NH_4NO_3 -1.0, $FeCl_3$ -0.05, agar-agar-20.0, pH-7.0 at 25 °C, and was supplemented with 200 µL kerosene and incubated at 37 ± 2 °C for 36 h. Each isolate was inoculated in 2% (v/v) kerosene supplemented BH broth followed by incubation at 37 ± 2 °C for 5 days at 135 rpm. Most potential isolate capable of degrading hydrocarbon was screened on the basis of their cell population (*cfu* count), the protein content of cell pellet, cell dry biomass, and a decrease in surface tension of hydrocarbon-supplemented medium (Silva et al. 2014; Viramontes-Ramos et al. 2010).

Agar plug assay for bacterial chemotaxis

Chemotaxis of the potential isolate towards hydrocarbon was demonstrated by agar plug assay method (Lacal et al. 2011). Briefly, 200 µL of overnight bacterial culture ($OD_{600} = 1$) was spread plated on BH agar plates, which were loaded with 20 µL kerosene in a well bored at the centre of the agar plate followed by incubation at 37 ± 2 °C for 36 h.

Fluorescence microscopy

In addition to this, fluorescence microscopy was also performed to observe the presence of hydrocarbon-degrading bacterial cells around the oil droplets using acridine orange fluorescence dye.

Molecular characterization of the most potential isolate

The isolate was identified by 16 s rDNA gene sequencing method using P3-forward (5'-AGA GTT TGA TCA TGG CTC AG-3') and P13-reverse (5'-GGT TAC CTT GTT ACG ACT T-3') primers. Phylogenetic tree of the isolate was constructed with the help of MEGA6™ tool by Neighbour-Joining method forcing *Staphylococcus aureus* ATCC29213 (GenBank AB680755.1) as out group with 1000 bootstrap value.

Cell surface trait analysis

Bacterial adhesion to hydrocarbon (BATH) was analyzed by the method described by Rosenberg and Ron (2002). Briefly, the cell pellets collected from the culture medium were washed twice and re-suspended in a buffer salt solution ($g\ L^{-1}$ composition: K_2HPO_4 -16.9, K_2HPO_4 -7.3) and diluted using the same buffer solution to an optical density $OD_{600} = 0.5$. 2 ml of the cell suspension was mixed with equal amount of diesel and kerosene in separate vials and vortex for 3 min. Oil and aqueous phases were allowed to separate for 1 h after vortex. OD of the aqueous phase was then measured at 610 nm in a spectrophotometer. From the

OD values, percentage of cells attached to diesel and kerosene oil was calculated using the following formula:

Bacterial cell adherence (%) =

$$1 - \frac{OD \text{ of the mixture of cells and oil after shaking}}{OD \text{ of the cell suspension in buffer before mixing with oil}} \times 100.$$

Determination of hydrocarbon degradation by GLC method

The biodegradation efficiency (BE) of the most potential isolate based on the decrease in the total concentration of hydrocarbons was calculated using the following expression (Ramasamy et al. 2014):

$$BE (\%) = 100 - \left[\frac{A_t}{A_c} \right] \times 100,$$

where A_t = total area of peaks of hydrocarbons in the test after degradation; A_c = total area of peaks of hydrocarbons in the control BH broth supplemented with respective hydrocarbons (i.e., diesel and kerosene separately), but devoid of inoculums was considered as control.

Extraction of biosurfactant

Biosurfactant was extracted from the cell free media by cold acetone precipitation method followed by freeze drying.

Characterisation of biosurfactant

Drop-collapse test

The drop-collapse test was performed using the method of Tugrul and Cansunar (2005).

Determination of the emulsification index

The emulsification indexes of culture samples was determined by adding 2.5 mL of diesel and kerosene in separate tubes to the same amount of bacterial culture. The mixtures were vortex for 1 min and left to stand for 24 h. The emulsification indexes for both the samples were determined in every 4th hour till 24th hour and calculated as shown in the following equation (Desai and Banat 1997):

$$\text{Emulsification Index} = \frac{\text{Height of the emulsion layer (mm)}}{\text{Total height of the solution (mm)}} \times 100.$$

Determination of the foaming index

The foaming indexes for both kerosene and diesel supplemented culture were determined by taking 2 mL of cell free

extracts in two separate tubes followed by vortex for 1 min, and the foaming index was calculated as shown in the following equation (El-Sheshtawy et al. 2015):

$$\text{Foaming Index} = \frac{\text{Height of the foam (mm)}}{\text{Total height of the solution (mm)}} \times 100.$$

Determination of critical micelle concentration (CMC) of the biosurfactant

The CMC of the biosurfactant produced by the isolate was determined by the method described by Liu et al. (2013). Aliquots of 5, 10, 25 mg/L, and so on till 50 mg/L of the extracted biosurfactant were prepared in distilled water and the surface tension was measured using a tensiometer at 25 °C where distilled water devoid of surfactant was taken as control. The CMC of the surfactant was determined by monitoring the surface tension of the emulsion when became minimum with the increasing concentration of biosurfactant above which the change in surface tension becomes independent of its concentration (Liu et al. 2013).

FTIR analysis of the biosurfactant

Dried biosurfactant produced by the isolate was analyzed using an FTIR instrument in the range of 1000–4000 cm⁻¹ with a resolution of 5 cm⁻¹.

Biochemical analysis of the crude biosurfactant

Ninhydrin, anthrone, and saponification tests were performed to confirm the presence of amino acids, carbohydrate, and lipid moieties, respectively, in the bacterial biosurfactant.

In vitro cytotoxicity assay

Cytotoxicity assay of the extracted biosurfactant was tested on primary mouse (*Mus musculus*) liver cell line (hepatocytes) by MTT assay. Absorbance was measured at 590 nm against the PBS which is taken as blank.

Pilot scale ex situ bioremediation assay by seed germination tests

The test was conducted as per the methods of Maila and Cloete (2002) with necessary modification. Briefly, contaminated soil samples (250 gm in each pot) collected from oil spillage site of Rudrasagar area were seeded with 10% (v/w) of bacterial broth (OD₆₀₀ ≥ 1) maintaining 60% relative humidity (RH) for 20 days at room temperature. Appropriate control devoid of bacterial inoculum was also maintained under similar condition. It was further experimented with

pre-sterilized non-polluted soil samples collected from the nearest point of the same oil spillage site with the following combinations: microbial treated: soil + bacterial broth + 10% (v/w) kerosene oil, augmented: soil + fresh un-inoculated BH broth + 10% (v/w) kerosene, control: soil + 10% (v/w) kerosene oil. Same experiment was repeated in the presence of 20% (v/w) kerosene oil in soil. *Vigna radiata* seeds (*n* = 30) were planted after 7 days of treatment and are allowed to sprout and grow. Overall heights of the plantlets were noted down for analysis after 7 days of sprouting. All the pots were sprinkled with 25 ml of mineral water and kept at room temperature maintaining 60% RH throughout the experiment.

Statistical analysis

All the experiments were performed in triplicate and results were presented in the form of mean ± SD. Student's *t* test was performed to check the significance of the findings using Grphpad™ online tool.

Results

Screening and identification of hydrocarbon-degrading isolates from contaminated soil

A total of 165 potential colonies with 30 different colony morphologies were obtained on screening media. The best isolate capable of degrading petroleum hydrocarbon efficiently was identified on the basis of its growth profile in terms of *cfu*, dry cell biomass, respective total protein content, and reduction in surface tension of the hydrocarbon-supplemented medium. The best isolate with strain ID KDS showed maximum *cfu* of 3.39 × 10⁷ cells/ml and dry cell biomass of 8.4 mg/ml in petroleum supplemented medium. The respective total protein content of the strain KDS was determined to be 146.26 µg/ml (Table 1).

All the isolates were tested for the reduction in surface tension, and the result showed that only 3 strains out of the 30 strains tested had the potential to reduce the surface tension of the culture liquid to less than 40 mN/m and out of which the isolated strain KDS showed the minimum surface tension of 32.54 ± 1.78 mN/m with 48.35% reduction of surface tension as compared to control, i.e., 63.42 ± 0.22 mN/m. This gives a strong indication on biosurfactant production by the strain (Viramontes-Ramos et al. 2010).

The presence of bacterial colonies around the agar well filled with kerosene clearly indicated bacterial chemotaxis towards petroleum hydrocarbon which further broadens its scope as a potential hydrocarbon degrader (Meng et al. 2017). Furthermore, both light and fluorescence microscopy revealed the presence of a large population

Table 1 Growth profile of all the isolates in hydrocarbon-supplemented medium after 7 days of incubation

Sample ID	cfu	Biomass (mg/ml)	Protein content (μg/ml)	Surface tension (mN/m)	Reduction percentage of surface tension (mN/m) (%)
A ₁	$(9.40 \pm 1.2) \times 10^6$	4.32 ± 0.42	62.45 ± 2.12	53 ± 1.34	15.87
KDS	$(3.39 \pm 1) \times 10^7$	8.4 ± 0.29	146.26 ± 2.22	32.54 ± 1.78	48.35
B ₁	$(1.02 \pm 1.3) \times 10^7$	5.9 ± 0.24	71.61 ± 1.83	48.34 ± 1.54	23.27
C ₁	$(2.64 \pm 1.2) \times 10^7$	8.2 ± 0.24	86.53 ± 1.89	35.34 ± 2.35	43.9
D ₁	$(8.90 \pm 2) \times 10^6$	4.5 ± 0.51	60.9 ± 1.32	52.1 ± 3.54	17.3
E ₁	$(9.80 \pm 1.5) \times 10^6$	4.4 ± 0.32	61.9 ± 1.36	52.8 ± 2.51	17.3
F ₁	$(1.92 \pm 0.4) \times 10^7$	7.62 ± 0.21	82.46 ± 1.98	38 ± 2.46	39.68
G ₁	$(1.01 \pm 0.6) \times 10^7$	5 ± 0.13	70.2 ± 3.23	45.2 ± 1.78	28.25
G ₂	$(1.55 \pm 0.4) \times 10^7$	6.72 ± 0.3	75.35 ± 4.32	46.7 ± 1.54	25.87
H ₁	$(1.39 \pm 0.2) \times 10^7$	5.77 ± 0.22	44.26 ± 2.34	49.2 ± 2.58	21.9
H ₂	$(1.51 \pm 0.5) \times 10^7$	6.1 ± 0.21	72.23 ± 3.32	49.32 ± 3.42	21.71
I ₁	$(1.10 \pm 0.2) \times 10^7$	4.8 ± 0.32	68.4 ± 3.45	51.3 ± 2.18	18.57
I ₂	$(1.89 \pm 0.4) \times 10^7$	7.24 ± 0.28	80.6 ± 4.54	40.8 ± 4.12	35.24
J ₁	$(9.60 \pm 1.2) \times 10^6$	4.43 ± 0.23	61 ± 3.76	52.67 ± 3.45	16.4
J ₂	$(5.60 \pm 1.4) \times 10^6$	2.98 ± 0.12	48 ± 4.56	49 ± 1.23	22.22
K ₁	$(1.04 \pm 0.3) \times 10^7$	4.78 ± 0.14	68.5 ± 3.23	49.1 ± 1.72	22.06
K ₂	$(9.80 \pm 1.4) \times 10^6$	4.4 ± 0.23	61.2 ± 3.76	53 ± 2.34	15.87
L ₁	$(9.50 \pm 1.5) \times 10^6$	4.12 ± 0.16	61.5 ± 4.56	52.2 ± 1.22	17.14
L ₂	$(7.80 \pm 1.3) \times 10^6$	3.89 ± 0.19	59.54 ± 4.32	52.45 ± 1.34	16.74
M ₁	$(1.14 \pm 0.5) \times 10^7$	4.6 ± 0.34	67.9 ± 2.32	48.8 ± 1.56	22.54
M ₂	$(1.43 \pm 0.5) \times 10^7$	5.82 ± 0.51	75.5 ± 2.12	49.2 ± 1.78	21.9
N ₁	$(8.30 \pm 1.2) \times 10^6$	4.1 ± 0.52	60 ± 4.21	52.14 ± 1.98	17.24
O ₁	$(1.34 \pm 0.4) \times 10^7$	4.9 ± 0.34	69.1 ± 4.21	49.1 ± 1.56	22.06
P ₁	$(9.20 \pm 1.4) \times 10^6$	4.1 ± 0.39	61.4 ± 2.33	51.46 ± 1.35	18.32
P ₂	$(9.80 \pm 1.1) \times 10^6$	4.3 ± 0.44	63 ± 3.51	53.4 ± 1.98	15.24
Q ₁	$(1.27 \pm 0.4) \times 10^7$	5.87 ± 0.42	70.8 ± 2.34	49.56 ± 2.35	21.33
R ₁	$(1.42 \pm 0.2) \times 10^7$	4.9 ± 0.65	68.43 ± 3.76	51.2 ± 2.21	18.73
S ₁	$(8.40 \pm 1.1) \times 10^6$	4.18 ± 0.24	61.12 ± 4.32	52.72 ± 2.22	16.32
S ₂	$(1.01 \pm 0.4) \times 10^7$	4.6 ± 0.37	69 ± 2.56	51.5 ± 2.35	18.25
T ₁	$(9.90 \pm 1.4) \times 10^6$	4.52 ± 0.53	65.5 ± 3.32	53.2 ± 2.12	15.56

Results are in mean ± SD form where, $n=3$ and $p < 0.5$

of the bacterial isolate around and inside the kerosene oil droplet during the study which is in accordance with the findings of other researcher (Ripley et al. 2002; Gong et al. 2014; Pi et al. 2015).

The most potential isolate was primarily identified as a member of the genus *Serratia* (Genbank accession no. MH507504) on the basis of 16S rDNA gene sequencing method and given a unique strain name KDS until the taxonomy is resolved. The phylogenetic analysis of the isolate showed maximum similarity with *Serratia marcescens* and *Serratia nematodiphila* on the basis

of evolutionary relatedness by forcing *Staphylococcus aureus* ATCC29213 with 1000 bootstrap value (Fig. 2).

Cell surface trait analysis and hydrocarbon degradation assay by GLC method

GLC analysis confirmed reduction of total peak area up to 3731.495 mV s in the treated sample in the presence of diesel as compared to the total peak area of control 29,938.211 mV s (Fig. 3a, b), which indicates 87.54% TPH degradation just after 28 days, whereas it shows 85.48% TPH degradation for kerosene under similar condition (Fig. 4a,

Fig. 2 The phylogenetic position of *Serratia* sp. strain KDS (Genbank accession: MH507504) with its close relatives and other members of family *Enterobacteriaceae*. The out group was *Staphylococcus aureus* ATCC2931. The nucleotide accession numbers are given in brackets. The scale bar represents 0.02 exchanges per nucleotide. The abbreviations for the culture collections are ATCC, American type culture collection; NBRC, NITE Biological Research Centre; DSM, Deutsche Sammlung von Mikroorganismen; JCM, Japan Collection of Microorganisms

b). Cell surface trait analysis of the isolate showed 36.8 and 33.2% hydrophobicity against diesel and kerosene oil, respectively.

Characterization of biosurfactant produced by the isolate

Collapse of the drop of diesel oil just in 30 s indicated successful production of biosurfactant by the isolate. The emulsification index of the biosurfactant, hence, produced against diesel oil were found 10, 16, 21, 28, 46, and 60%, respectively, after 4, 8, 12, 16, 20, and 24 h (Fig. 5). The same were found 10, 12, 18, 28, 41, and 56.6%, respectively, after every 4th hour of interval till 24th hour against kerosene oil (Fig. 5). The foaming index of the surfactant was found 15% for both of kerosene and diesel supplemented medium. The CMC of the crude biosurfactant was determined as 33 mg/L which reduced the surface tension of the emulsion to 15 mN/m (Fig. 6).

The FTIR spectra of dried crude biosurfactant showed five very prominent peaks at 2954.95, 1691.57, 1529.55, 1008.77, and 802.39 cm^{-1} which could be attributed, respectively, to ν_{CH_3} (asymmetric stretching) and $\nu_{\text{C}=\text{C}}$ (stretch) for next two peaks followed by two $\nu_{\text{C}-\text{C}}$ (stretch) for next two peaks (Fig. 7). The presence of amino acids, carbohydrate moieties, and lipids in the biosurfactant was confirmed by ninhydrin, anthrone, and saponification tests, respectively. Cytotoxicity assay of the crude biosurfactant on primary mouse liver cell line reveals noncytotoxic nature of the biosurfactant (Fig. 8).

Ex situ bioremediation study by seed germination assay

Pilot scale bioremediation study shows excellent results in terms of plant growth in the treated soil. The average heights of the plantlets in the contaminated soil sample (10% kerosene) were found to be 23 ± 1.6 cm just after 7 days of treatment with the bacterial isolate. Whereas the average heights of the plantlets in 20% kerosene-contaminated soil sample were found 15.5 ± 1.2 cm. The heights of the plantlets for the augmented soil samples with un-inoculated BH broth were found 6.5 ± 0.8 cm and 5 ± 0.8 cm, respectively (Fig. 9). Whereas, the average heights of the plantlets in contaminated soil samples collected directly from the oil spillage site shows 4.2 ± 0.7 cm with a germination rate of 31% after bioremediation. However, the controls for all the cases show no seed germination.

The germination rate was found 52% and 72% ($n=30$), respectively, in 20% and 10% (v/w) kerosene-contaminated soil after bioremediation. Whereas, 13% and 17% germination rates were obtained, respectively, in 20% and 10% (v/w) kerosene-contaminated soil augmented with un-inoculated BH broth. Interestingly 31% germination rate was seen after treating the heavily contaminated soil samples collected from oil spillage site of Rudrasagar area of Sibsagar district for 20 days.

Discussion

Salt medium supplemented with petroleum oil as sole source of carbon is most widely used as a screening medium for hydrocarbon-degrading microorganisms as it is expected that

Fig. 3 GLC chromatogram of diesel oil: **a** before degradation (control) and **b** after degradation (test) in liquid culture

only the microbes which are capable of hydrocarbon degradation will utilize petroleum oil for their growth and proliferation. Hence, an increase in cell density in such media may be used as an indicator of higher order of petroleum degradation (Das and Mukherjee 2007; Mandri and Lin 2007). This may be further validated with corresponding cell dry biomass obtained in such media with respective protein content of the cell pallet (Das and Mukherjee 2007; Mandri and Lin 2007). The diversity of biosurfactant producing microorganisms and of the biosurfactants produced is well known. Surfactants possess both hydrophilic and hydrophobic moieties in its structure which gives rise to the capability to lower the surface tension between air and water interface

(biosurfactants), and solid–liquid interface (bioemulsifiers) and also can reduce interfacial tension between two immiscible liquids (Batista et al. 2006). The biosynthesis of surfactants by microbial cells most often results in foaming, a decrease in surface tension, and emulsification of hydrophobic substrates (Marchal et al. 1998).

Some studies report cell hydrophobicity of hydrocarbon-degrading *Pseudomonas* sp. as high as 80–82% against engine oil, whereas, in this study, they could achieve only 22% hydrocarbon degradation (Table 2). In contrast, the current isolate showed 36.8 and 33.2% hydrophobicity against diesel and kerosene, but could achieve up to 87% degradation in liquid culture. It is worth mentioning that

Fig. 4 GLC chromatogram of kerosene oil: **a** before degradation (control) and **b** after degradation (test) in liquid culture

Fig. 5 Emulsification index of bacterial culture against diesel and kerosene after every 4th hour till 24th hour; inset figure shows the emulsion layer of diesel and kerosene after incubation

Fig. 6 Surface tension of the biosurfactant in water emulsion at different concentration

the emulsification index of the same bacterial biosurfactant may vary against different hydrocarbon oil (Abouseoud et al. 2008; Ferhat et al. 2011). In accordance, the current study shows a gradual increase in emulsification index values from 10% after 4 h of incubation against both diesel and kerosene oil up to 56–60% after 24th hour of incubation (Fig. 5). The formed emulsion exposed their stability at room temperature even after 5 days. This property demonstrates the applicability of this strain not only against fossil oil based contamination but also for microbial enhanced oil recovery (MEOR) studies (Aparna and Smitha 2012). Determination of CMC value of the biosurfactant is also an important parameter which gives an idea about the minimum concentration of the biosurfactant required to have a lower maximum surface tension in a solution (Liu et al. 2013). Gradual decrease in surface tension of the biosurfactant solution was observed with the increased concentration of the biosurfactant and the CMC for the biosurfactant was determined as 33 mg/L which reduced the surface tension of the emulsion to

Fig. 8 Cytotoxicity assay of the extracted biosurfactant on mouse liver cell

15 mN/m which is fairly in accordance with some of the reported values (Sousa et al. 2012; Liu et al. 2013).

In the pilot scale of bioremediation study, *Vigna radiata* showed sensitivity to kerosene, as the overall heights of the plantlets decreased with an increase in the concentration of the kerosene. Height of the plantlets of germinated seeds in soil with no bacterial broth was found significantly low ($p < 0.05$) as compared to the heights of the plantlets in treated soil with bacterial broth. Kerosene was preferred over diesel for the pilot scale bioremediation study considering the fact that it is less volatile as compared to diesel which will sustain in the soil for a longer period of time, and thus, it will not affect the efficacy of the test. The current finding shows a significant increase ($p < 0.05$) in the heights of *Vigna radiata* plantlets in artificially contaminated soil samples (10 and 20% v/v kerosene oil) after treatment with *Serratia* sp. strain KDS as compared to the heights of the plantlets in the un-inoculated BH broth-augmented soil sample (Fig. 9). Moreover, the non-cytotoxicity nature of the biosurfactant produced by the isolate on the primary mouse liver cell line clearly advocates its possible applicability for

Fig. 7 FTIR spectrum of the crude biosurfactant extracted from the isolate; inset figure shows the foaming level of the crude biosurfactant

Fig. 9 Overall comparison of heights of the plantlets from *Vigna radiata* seeds exposed for germination during pilot scale bioremediation experiment with petroleum-contaminated soil

Table 2 Comparison of some recent reports on microbial bioremediation of petroleum oil contaminants

Name of the microorganism	Petroleum substrate	Culture condition	Max. percentage of biodegradation	E_{24}	In situ/ex situ bioremediation test on contaminated soil	References
<i>Serratia</i> sp. strain KDS	Kerosene oil Diesel oil	Liquid	85.48% 87.54%	56.6% 60%	+ve	Present study
Consortium of <i>Bacillus subtilis</i> and <i>Pseudomonas aeruginosa</i>	Crude oil	Solid	76%	—	+ve	Mukherjee and Bordoloi (2011)
Undefined bacterial consortium	Diesel	Solid	—	—	+ve	Graj et al. (2013)
<i>Citrobacter sedlakii</i> , <i>Enterobacter hormeachei</i> , <i>Enterobacter cloacae</i>	Kerosene	Liquid	42–69%	—	—	Ghoreishi et al. (2017)
<i>Raoultella ornithinolytica</i> , <i>Serratia marcescens</i> , <i>Bacillus megaterium</i> , and <i>Aeromonas hydrophila</i>	Acenaphthene Fluorene	Liquid	90–99% 97–99%	—	—	Alegbeleye et al. (2017)
<i>Phormidium ambiguum</i> strain TISTR 8296	Engine oil	Liquid	20–65%	—	—	Pimda and Bunnag (2017)
<i>Klebsiella pneumoniae</i> ATCC13883	Crude oil	Liquid	66.5%	—	—	Ozyurek and Bilkay (2017)
<i>Acinetobacter calcoaceticus</i>	Diesel oil	Liquid	90%	> 50%	—	Balseiro-Romero et al. (2017)
<i>Enterobacter cloacae</i> strain C3	Diesel	Liquid	48%	55%	—	Jemil et al. (2018)
<i>Pseudomonas aeruginosa</i> SR17	Crude oil	Liquid	65–100%	—	—	Patowary et al. (2018)
<i>P. aeruginosa</i> TPHK and <i>Pseudomonas putida</i>	Engine oil	Contaminated soil	22% 18%	80–82%	+ve	Ramadass et al. (2018)

bioremediation field trial. Even though a handful of reports are available with successful ex situ microbial bioremediation study on contaminated soil with defined bacterial strains or consortium (Morelli et al. 2005; Mukherjee and Bordoloi 2011; Sangeetha and Thangadurai 2014; Patowary et al. 2018; Ramadass et al. 2018), but very limited reports show different members of the genus *Serratia*, mostly the species *marcescens* involved in hydrocarbon degradation (Mohan et al. 2007; Rajasekar et al. 2011; Wu et al. 2012; Almansoory et al. 2016; Rajasekar 2017). Herein, we report a newly isolated strain belongs to the genus *Serratia* involved in ex situ bioremediation of petroleum-contaminated soil and water.

Conclusions

Microbial bioremediation is an eco-friendly method of dealing with soil and water body contamination due to petroleum hydrocarbon. However, the efficacy of the inducted microorganisms may depend on a number of factors such as the toxicity level in the contaminated site, growth factors, environmental conditions, etc. The novel indigenous strain of *Serratia* sp. with excellent bioremediation efficacy both in liquid culture and in pilot scale ex situ experiment with no cytotoxicity due to the biosurfactant released advocates its suitability to implement for field trial. However, a little further experimental validation may be expected to release the isolate to natural environment for bioremediation operations.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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