# Bioremediation of petroleum contaminated soil through biosurfactant and *Pseudomonas sp.* SA3 amended design treatments

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A B S T R A C T

Toxicity of agricultural soil due to petroleum contamination has become a serious issue in recent times. Petrol oil exhibits toxic effects in agricultural crops due to the presence of various hazardous hydrocarbons. The degradation of petroleum hydrocarbon has been widely studied by the researchers that signify the requirement of effective treatments for the detoxification of petroleum contaminated soil and their reuse for growing crops. Hence, with this intention in the present study secondary metabolites "biosurfactant" (natural surfactant) along with the potent plant growth promoting (PGP) bacterial strain *Pseudomonas sp.* SA3 was used in the designed treatments for growing agricultural crop. The biosurfactant produced by the strain has the emulsification capacity of 43% and surface tension reduction ability to 34.5 mN/m whereas the plant growth promoting traits demonstrates 93.46 μg/mL phosphate solubilisation ability, siderophores (iron chelating compound) production upto 69.41% units and 81.41 μg/mL indole acetic acid  (IAA) production ability. Further, the results of the design treatments signifies that treatments amended with the strain SA3 and biosurfactant is effective in the management of petroleum contaminated soil indicating treatment EX 5 (1 kg soil + 1 L water + *Pseudomonas sp.* SA3 + 300 mL crude biosurfactant), as an efficient treatment in increment of phytochemical constituents and 10–15% enhancement in growth parameters as compared to negative control. Hence, the developed treatments can be efficaciously used for the management of petroleum contaminated soil for agronomy.

## 1. Introduction

Environmental pollution due to petroleum products such as gasoline, diesel, and crude oil has attracted much attention to the ecological concern (Jimoh and Lin, 2019). Release of the pollutant from petroleum and their by-products in the environment causes a consequential threat to human and animal health due to their hazardous nature (Rahman et al., 2003; Langworthy et al., 2002; Yadav et al., 2016). Petroleum products consist of harmful hydrocarbons that cause environmental pollution inducing toxic, mutagenic, carcinogenic effects in aquatic and terrestrial ecosystems. Defalcation of oxygen and water as well as to limitation in availability of nitrogen, phosphorus and iron are the main changes due to petroleum derived pollutants in soil (Nogueira et al., 2011; Das and Kumar, 2016) Hence, their remediation is necessary to focus on. Many researchers demonstrated that association of plants with hydrocarbons degrading bacteria with plant growth promoting traits (PGP traits) as an efficient technology for remediation of petroleum hydrocarbons contaminated soils (Pawlik et al., 2017). PGP traits are the secondary metabolite produced by the rhizobacteria collectively called as Plant growth promoting rhizobacteria (PGPR) (Pawlik et al., 2017). PGPR can affect positively the plant growth by various PGP traits like siderophore produc-

tion, phosphate solubilization, indole acetic acid (IAA) production, hydrogen cyanide (HCN) production, production of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, and induction of systemic resistance (Benaissa, 2019; Kumar et al., 2015, Glick, 2012). Introducing PGPR in early stages of plant growth can significantly increase the survival rate of host plants (Azri et al., 2018). These microorganisms enhance the remediation potentiality by producing biosurfactants. Biosurfactants are a surface-active secondary metabolite of amphiphilic nature produced mainly by microorganisms like bacteria, yeast and fungi (Santos et al., 2016). They enhance the remediation of petroleum hydrocarbons contaminated environments through two distinct mechanisms. The first process involves the enhancement of substrate bioavailability for microorganisms whereas the second process engages the interaction of bacterial cell surface with hydrophobic substrates by intensifying the hydrophobicity (Pacwa- Płociniczak et al., 2011, Mulligan and Gibbs, 2004). Hence, employing biosurfactant producing PGP bacteria not only improves the immunity of plant to contaminants by degrading the organic compounds, furthermore, they also have the efficiency to alleviate the stress along with the intensification of the growth and development of the plant (Cruz-Morales et al., 2016; Almansoory et al., 2019).
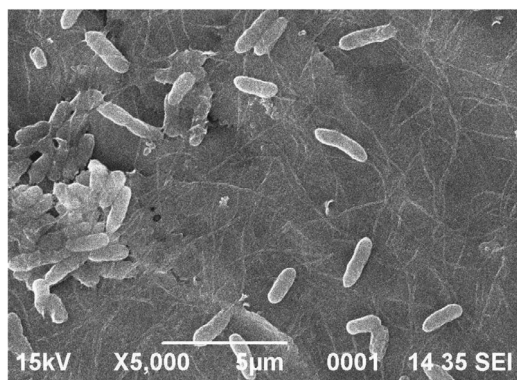
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**Fig. 1.** SEM micrograph of the strain.

Hence, with this intention in the present study, various treatments were designed employing biosurfactant and *Pseudomonas sp.* SA3 with PGP traits for the management and detoxification of petrol contaminated soil for growing crops.

## 2. Material and methods

### 2.1. Enrichment and isolation and characterization of the selected strain

Petroleum contaminated soil samples were collected from the rhizospheric zone of *Ricinus communis* of the vicinity of Charbagh railway station, Lucknow, Uttar Pradesh, India (26°55′ N latitude and 80°59′ E longitude) in a sterile polybag and stored in the laboratory. For the isolation of bacteria, 1 g of soil sample were mixed in mineral salt media (MSM)(composition g/l $KH_2PO_4$–1.0, $K_2HPO_4$–3.0, $MgSO_4$- 0.5, $FeSO_4$–0.01, $Na_2HPO_4$–5.67, $Cacl_2$–0.1, $NH_4NO_3$–0.39, $MnSO_4$–0.002, Dextrose-15) containing 3–4% diesel and petrol oil and incubated for a week on a shaker at 150 rpm. Further, the bacterial strains were isolated using serial dilution technique on MSM agar plate amended with 1% petrol oil at 37°C for 4–5 days. The colonies observed were streaked and purified in nutrient agar media (composition g/l Peptone – 5; Sodium chloride – 5; Beef extract - 1.5; Yeast extract - 1.5; Agar - 15). All the colonies selected were morphologically characterized and biochemically tested using Hi-Media HiIMViC$^{TM}$ Test Kit (product code: KB001–5KT). Further, the selected potent strain was characterized using scanning electron microscope JEOL (JSM 6490 LV). Briefly, cells grown in nutrient broth were centrifuged to collect the cell pellet and washed with phosphate buffer solution (PBS - 19 mL 0.2 M $NaH_2PO_4$ + 81 mL 0.2 M $Na_2HPO_4$, pH 7.4) and further stabilized by using 2.5% gluteraldehyde for 4 hr. Then, pellets were washed again thrice with phosphate buffer solution (PBS). Further, the dehydration of cell biomass was done by using ethanol of various concentrations ranging from 30% to 100% and dried in freeze for 48 h before observing in SEM (Wang l et al., 2016, Ammar, 2017). Then, isolated strains were further screened for PGPR and biosurfactant activity. Based on the potentiality, strain SA3 was selected for the present study among all the strains.

### 2.2. Screening of plant growth promoting (PGP) activities

For qualitative analysis of phosphate solubilizing bacteria, modified pikovskaya agar method was employed (Pikovskaya, 1948; Mehta and Nautiyal, 2001). Quantitative estimation of tricalcium phosphate solubilizing ability of the strain was estimated through the method described by King (1932). The siderophore production test was done on CAS (Chrome Azural S) agar media by following a standard protocol of Schwyn and Neilands (1987). Production of IAA was checked by following the method of Brick et al. (1991.) SA3 was screened for the production of hydrogen cyanide HCN by using the standard method of Lorck (1948). The ammonia production test was checked by Cappucino and Sherman (1992).

### 2.3. Screening for biosurfactant production

For biosurfactant production, strain SA3 was grown on the 100 ml Bushnell-Hass media with dextrose as a carbon source for 72 h in a shaker with 150 rpm. Further estimation of biosurfactant production the various methods like oil displacement test was performed by the method of Ohno and Wang (1993), drop collapse test was done through Bodour et al. (2003) and the emulsification index E24 (%) test was measured by Cooper and Goldenberg (1987). Surface tension of biosurfactant was measured by drop weight method by staglometer (Dilmohamud et al., 2005).

### 2.4. Production, extraction and partial purification of biosurfactant

Solvent extraction method was employed for extraction of biosurfactant produced by SA3. Briefly, the strain SA3 grown in Bushnell Hass media (composition g/l $MgSO_4$ 0.20; $Cacl_2$ 0.02; $KH_2PO_4$ 1.00; $K_2HPO_4$ 1.00; $NH_4NO_3$ 1.00; $FeCl_3$ 0.05; Dextrose 15) in a shaker with 150 rpm of shaking at 37° C was centrifuged at 10,000 rpm for 20 min after 72 hrs of incubation. The culture supernatant obtained were acidified with 1 M HCL to adjust the pH at 2.0 and stored at 4°C overnight for precipitation. The precipitate was extracted twice with an equal volume of diethyl ether using a separating funnel. The organic layer was separated and collected in an empty beaker and further the solvent were evaporated to get the biosurfactant (Das and Kumar, 2018; George and jayachan, 2013, Gandhimathi et al., 2009, Pansiripat et al., 2010). Further, the amount of biosurfactant produced was estimated along with bacterial biomass, emulsification index and oil displacement ability.

### 2.5. Determination of critical micelles concentration

The CMC of the biosurfactant was analysed by measuring the change in surface tension by increasing the biosurfactant concentration from 100 mg/L to 1000 mg/L ( Das and Kumar, 2018).

### 2.6. Structural characterization of the biosurfactant using liquid chromatography–mass spectrometry (LC-MS)

For LC-MS analysis, the extracted biosurfactant (10 mg) was dissolve in 5000 μL acetonitrile / $HCOONH_4$ buffer at 5 mM $AcNH_4$ buffer, at pH 6.5 and passed through C18 250 × 4.6, 5 μm columns and then filtered using a 0.20 $\mu$m syringe filter (Behrens et al., 2016). The filtered solution was further analyzed using Waters Ultra-High-Performance Liquid Chromatography Tandem Mass Spectrometry.

### 2.7. The ability of the strain to remediate petroleum contaminated soil in the presence and absence of biosurfactant

The soil that was used for the experiment was dried and sieved with 2 mm sieve and autoclaved it for 1 h at 121 °C for three successive days. For performing the remediation experiment, various treatments were developed by mixing the crude biosurfactant and *Pseudomonas sp.* SA3 in soil (1 kg) contaminated with petrol oil in various concentrations (1%, 3%, 5%). The treatments were executed in 1000 mL Erlenmeyer flask and kept at room temperature for 60 days Table 1.

### 2.8. Exploration of reuse ability of the remediated soil for plant growth

After 60 days of experiment, the treated soil was transferred into pot. The certified seeds of *Zea mays L* (maize) were surface sterilized with distilled water followed by immersing in 0.2% mercuric chloride ($HgCl_2$) solution for 3 min and further subjected to six times washings with sterile distilled water. After that seeds were placed in each pot and
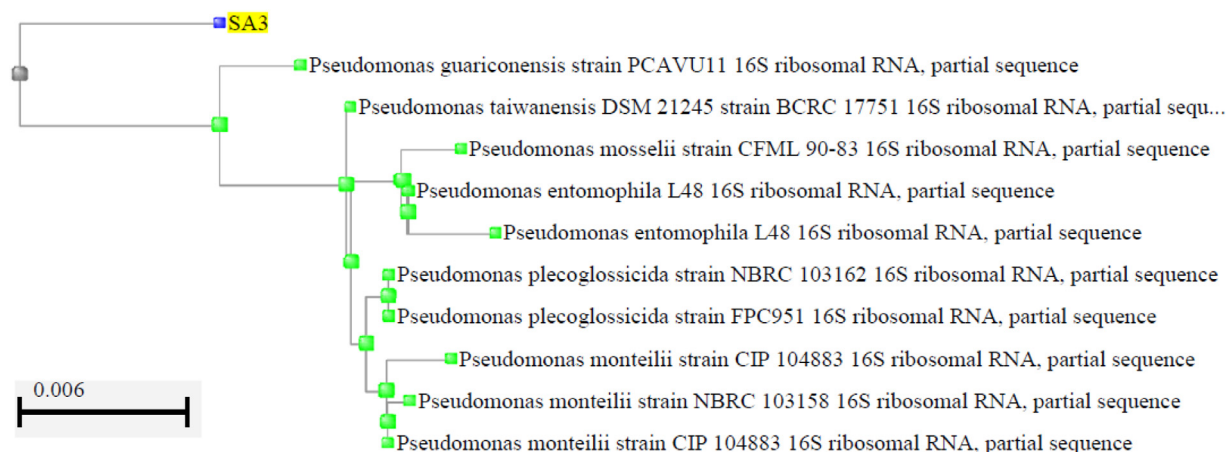
**Fig. 2.** The similarity index of the strain with its closest neighbors.

**Table 1**
Design Treatments.

| Treatments | Soil (Kg) | Water | Strain | Amount of biosurfactant used | Amount of oil used |
|---|---|---|---|---|---|
| Treatment 1 (EX1) | 1 | 1L | – | – | |
| Treatment 2 (EX2) | 1 | 1L | – | – | 1% |
| Treatment 3 (EX3) | 1 | 1L | - | – | 3% |
| Treatment 4 (EX4) | 1 | 1L | - | – | 5% |
| Treatment 5 (EX5) | 1 | 1L | *Pseudomonas sps* SA3 | – | 1% |
| Treatment 6 (EX6) | 1 | 1L | *Pseudomonas sps* SA3 | – | 3% |
| Treatment 7 (EX7) | 1 | 1L | *Pseudomonas sps* SA3 | – | 5% |
| Treatment 8 (EX8) | 1 | 1L | *Pseudomonas sps* SA3 | 300 mL | 1% |
| Treatment 9 (EX9) | 1 | 1L | *Pseudomonas sps* SA3 | 300 mL | 3% |
| Treatment 10 (EX10) | 1 | 1L | *Pseudomonas sps* SA3 | 300 mL | 5% |

kept in the greenhouse for 15 days and plant parameters were observed at interval days. Germination rate was evaluated by using the formula of Al-Ansari and Ksiksi (2016), root length (cm plant$^{-1}$) and shoot length (cm plant$^{-1}$) were measured in centimeter (cm) whereas fresh and dry weight were recorded by following the method of Huang et al. (2017). Chlorophyll and carotenoid content was estimated in leaves by following the method of Arnon (1949).

*2.9. 2.9 molecular identification of the selected isolate*

16S rRNA sequence was determined by using the forward primer 5′ – CAGGCCTAACACATGCAAGTC – 3′ and reverse complementary sequence was made by reverse primer 5′ – GGCGGATGTGTACAAGGC – 3. The PCR conditions involved an initial denaturation at 95° for 5 min followed by 35 cycles of 94 °C for 30 s, 50 °C for 30 s, 72 °C for 1:30 min and a final extension at 72°C for 7 min. The blast process was employed to investigate harmony sequences against NCBI GenBank. Phylogenetic tree builder uses sequences aligned with system software aligner. Further, a phylogenetic tree of the isolate was separately drawn to compare with the reference strain sequences deposited in NCBI GenBank (Bruno et al., 2000; Wiley et al., 1991)

*2.10. Statistical analysis*

Results were analyzed by employing single-factor ANOVA of variance and mean of three replicates with standard deviation (Means $\pm$ S.D, $n = 3$). Treatments were considered Significant at [a]p>0.01, [b]p >0.05 and [c]Not significant by comparing the treatments. Treatment (EX5 and EX8) was compared with EX2, Treatment (EX6 and EX9) was compared with EX3, Treatment (EX7 and EX10) was compared with EX4 and Treat-

ment (EX2, EX3 and EX4) was compared with EX1 and treatment EX1 was compared with (EX2, EX3 and EX4).

**3. Results and discussion**

*3.1. Characterization of the strain*

Total 8 strains were selected based on their petroleum degrading ability. Further, based on PGP and biosurfactant producing ability the strain SA3 was found to be potent and selected for the present study Table 2. The selected bacterial strain SA3 is Gram-negative, motile, and non-spore-forming and electron microscopy study reveal rod-shaped structure of the stain Fig. 1. The biochemical test indicates catalase, oxidase and citrate positive for the strain whereas negative results for indole, methyl red, voges-proskauer test, urease and b-galactosidase. Hence, the results of morphological, structural and biochemical test indicates that the strain might be *Pseudomonas sp*. Genotypic characterization demonstrates through sequencing using universal primers 27F (AGAGTTTGATCMTGGCTCAG-20) and 1492R (GGTTACCTTGTTACGACTT-20) reveal aligned sequence of 1438 bp. Further, the sequence analyzed for its closest neighbors reveal a 97.91% similarity index with *Pseudomonas taiwanensis* and 97.91% *Pseudomonas guariconensis*. The accession number allotted for the strain by NCBI was MW750216. (Fig. 2)
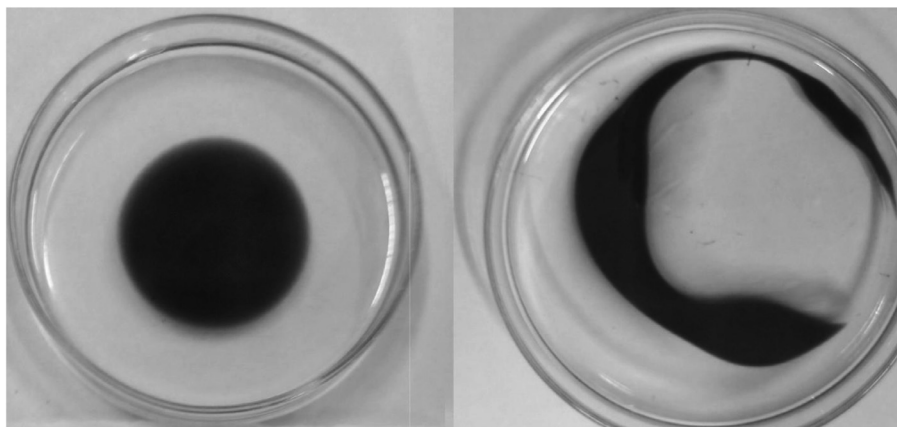
*3.2. Characterization of biosurfactant from the strain*

The strain *Pseudomonas sp.* SA3 showed the production of biosurfactant on Bushnell-Hass media with foaming property in 72 hrs. An oil displacement test of strain SA3 possessed the largest clear zone whereas

**Table 2**
Potentiality of the isolated strains based on PGP and biosurfactant producing ability.

| Strains | Gram's test | Biosurfactant producing ability | | | | | PGPR activity | | | | Oil Degrading activity |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Drop collapse | Oil displacement | Emulsification activity | Foam test | IAA | Phosphate solubilisation | Siderophore production | HCN production | Ammonia production | |
| SA1 | Gram's negative | – | + | + | + | – | + | + | – | + | + |
| SA2 | Gram's negative | + | ++ | + | + | + | – | + | + | + | + |
| SA3 | Gram's negative | + | ++ | ++ | ++ | ++ | + | + | + | ++ | + |
| SA4 | Gram's positive | + | ++ | ++ | ++ | ++ | – | – | + | + | + |
| SA5 | Gram's negative | – | + | + | + | – | – | – | – | + | + |
| SA6 | Gram's negative | + | + | + | + | + | – | + | – | ++ | + |
| SA7 | Gram's positive | + | ++ | + | + | + | + | – | – | + | + |
| SA8 | Gram's positive | + | + | + | + | + | ++ | + | + | + | + |

+ (positive), ++ (potent), -(negative).



**Fig. 3.** Oil displacement ability of the biosurfactant extracted from the strain.

**Table 3**
Characterization properties of extracted biosurfactant from the strain SA3.

| Biosurfactant activities | Qualitative results | Quantitative results |
|---|---|---|
| Emulsification test | +ve | 43% |
| Surface tension | | 34.5 ( mN/m) |
| Oil displacement test | +ve | Diameter of displaced Oil - 2.5 cm |
| Foam test | +ve | – |
| Drop collapse test | +ve | – |

collapsing of a drop in drop collapse test shows positive for surfactant production. In drop collapse test liquid drop get collapse as a result of a reduction in interfacial tension among the hydrophobic surface and the liquid (Bodour et al., 2003). Further, the biosurfactant extracted was characterized and the results were depicted within Table 1. Emulsification index of biosurfactant produced by the strain SA3 is 43.75 for engine oil, which indicates that the strain SA3 can emulsify the petroleum engine oil signifying its potential use in petroleum oil remediation and enhanced oil recovery. The extracted surfactant is found efficient in the oil displacement test Fig. 3. The surface tension of non inoculated broth (Bushnell-Hass media) was 61.9 mN/m, whereas the bacterial inoculated broth was 34.5 mN/m, this signifies that the biosurfactant extracted from strain SA3 has the ability to reduce surface tension from 61.9 mN/m to 34.5 mN/m. Table 3

*3.3. Critical micelle concentration (CMC) and structural characterization of the biosurfactant*

CMC of the extracted biosurfactant synthesized from the strain SA3 is represented within Fig. 4. LC-MS mass spectra of the extracted biosurfactant from strain SA3 indicates that the strain produces rhamnolipids biosurfactant with various rhamnose congeners Fig. 5 (a, b, c, d and e). Peak at $m/z$ 645.3 depicts RhaRhaC$_{10}$C$_8$/RhaRhaC$_8$C$_{10}$ and $m/z$ at 671.8 RhaRhaC$_{10}$C$_{10}$ congeners indicates presence of dirhamnolipids ($\alpha$ -L-rhamnopyranosyl -$\alpha$ -L-rhamnopyranosyl -$\beta$ -hydroxydecanoyl -$\beta$ -hydroxydecanoate), whereas peak at $m/z$ 555.4 depicts RhaC$_{12}$C$_{10}$/RhaC$_{10}$C$_{12}$ congeners and peak at $m/z$ 527.3 depicts RhaC$_{10}$C$_{10}$ congeners indicates the presence of olefinic rhamnolipids and monorhamnolipid (L-rhamnosyl -$\beta$ -hydroxydecanoyl- $\beta$ -

hydroxydecanoate). The above reported results were also complemented with the previous findings of Arora et al. (2016).

### 3.4. Plant growth promoting activities of the strain

The isolated strain SA3 was found positive for phosphate solubilization, siderophore production test, IAA, HCN production test and ammonia production test **Table 4**. In phosphate solubilization assay the red-dish zone around the colony in pikovskaya agar media with bromophenol dye indicates that the strain has efficiency for solubilizing phosphate (Pikovskaya, 1948; Mehta and Nautiyal, 2001). The quantification analysis demonstrated that the strain SA3 solubilizes the tri-calcium phosphate up to 93.46 µg/mL. Siderophore production on Chrome Azurol-S agar (CAS agar) plates was found to be positive as indicated by the change in color of the media around the colonies (Schwyn and Neilands, 1987). The quantitative analysis of siderophore production was
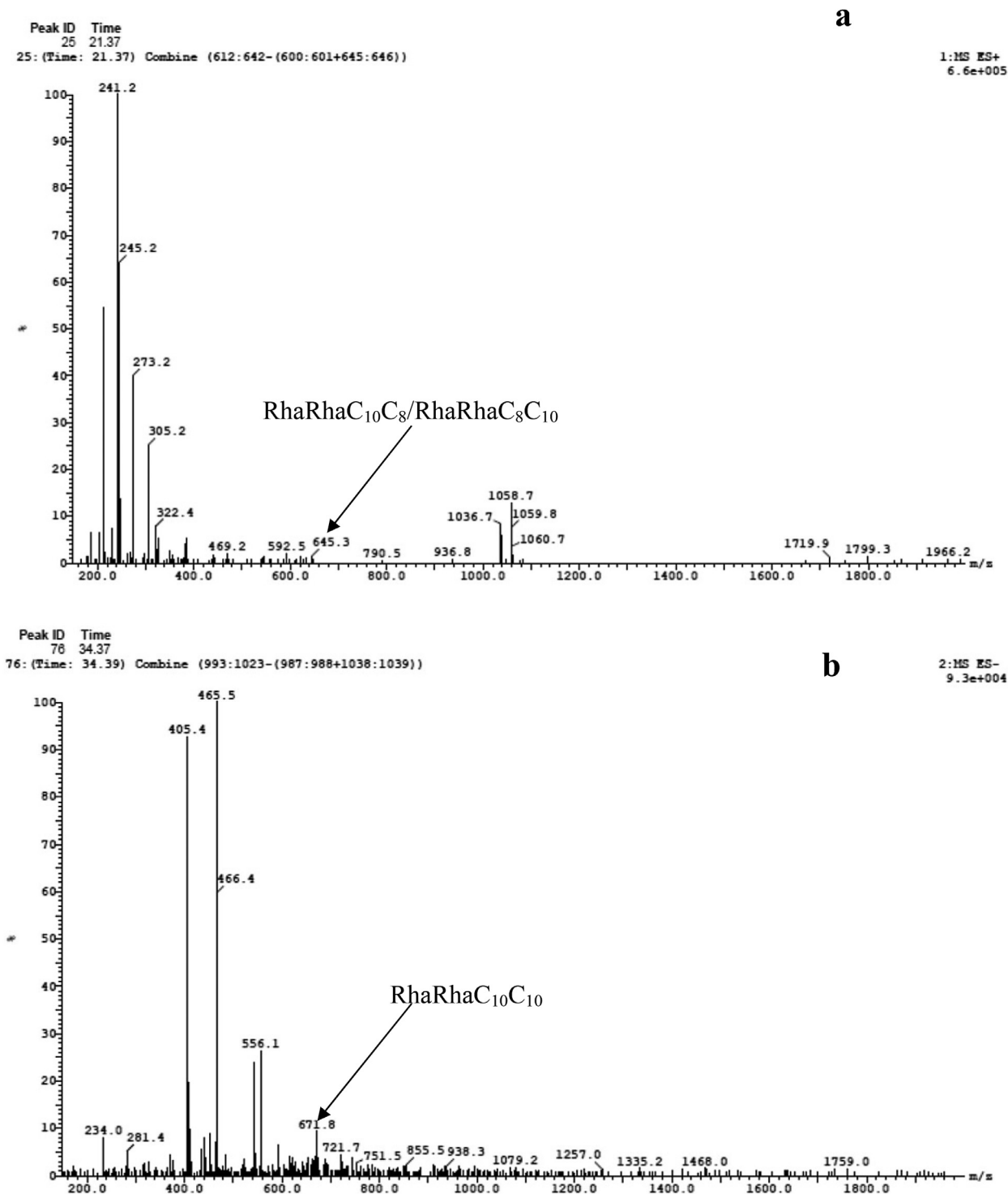
**Fig. 5.** LC-MS analysis of the biosurfactant extracted from the strain SA3 depicting various rhamnose congeners **(a)** peak at $m/z$ 645.3 depicts $RhaRhaC_{10}C_8/RhaRhaC_8C_{10}$ congeners, **(b)** peak at $m/z$ 671.8 depicts $RhaRhaC_{10}C_{10}$ congeners, **(c)** peak at $m/z$ 673.6 depicts $RhaRhaC_{10}C_{10}$ congeners, **(d)** peak at $m/z$ 555.4 depicts $RhaC_{12}C_{10}/RhaC_{10}C_{12}$ congeners and **(e)** peak at $m/z$ 527.3 depicts $RhaC_{10}C_{10}$ congeners.
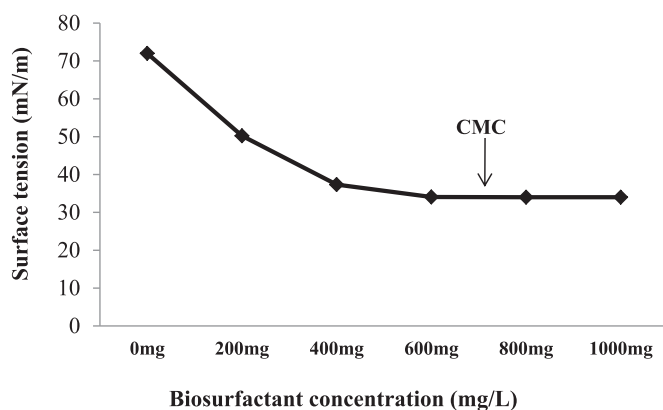
**Fig. 4.** CMC of the biosurfactant produced by *Pseudomonas sp.* SA3.

**Table 4**

Plant growth promoting traits of the test strain (SA3).

| PGP Activities | Qualitative results | Quantitative results |
| --- | --- | --- |
| Phosphate Solubilisation | +ve | 93.46 µg/ml |
| Siderophore production | +ve | 69.41% SU |
| IAA production | +ve | 81.41 µg/ml |
| Ammonia Production | +ve | – |
| HCN test | +ve | – |

done by CAS-shuttle assay. The percentage of siderophore production of SA3 has estimated as the proportion of CAS color shifted i.e. up to 69.41 siderophore unit (SU). Ammonia production test showed positive by developing brown color in 48 h grown cultural broth after adding a few drops of Nessler's reagent (Cappucino and Sherman, 1992). Positive result were observed in HCN production test, as the filter paper which was soaked in picric acid and sodium carbonate solution turns brown from yellow (Lorck, 1948). Quantitative analysis of indole acetic acid in presence of 100 µg/mL concentration of tryptophan showed 81.41 µg/mL of IAA production by the isolate SA3. All the PGP activity signifies that the strain SA3 can stimulate the growth of plant if employed in soil.

*3.5. Effect of treatment in seed germination*

Germination test indicates the toxicity level of the soil (Kumar et al., 2015; Das and Kumar, 2018; Banks and Schultz, 2005). Different plant species may function differently in petroleum-contaminated soil (Banks and Schultz, 2005; Plaza et al., 2005; Das and Kumar, 2018). In this study, cereal crop (maize) is examined to demonstrate the effects of petroleum contamination **Fig. 6**. The present study depicts that treatment EX2, EX3, and EX4 exhibits high toxic effects on germination as compared to other treatments. This reduction in germination rate might be due to petrol oil that forms an oily film layer in the vicinity interfering air water relations (Adam and Duncan, 2002; Ziółkowska and Wyszkowski, 2010; Hawrot-Paw et al., 2015). The better germination rate was observed in the treatment EX8, EX9, and EX10 indicating the effectiveness of the strain and the biosurfactant amended in the treatment. Similar results were previously reported by Das and Kumar (2018),(2016), Tang et al. (2011) and Adam and Duncan (2002). .

*3.6. Effect of treatment on plant growth parameters*

All the treatments showed an immense effect on the plant growth parameters **Fig. 7**. The plants that arose from the treatments EX5, EX6, EX7, EX8, EX9, and EX10 shows better plant parameters as compared to the treatments EX2, EX3, and EX4. But, the plants from the treatments EX8, EX9, and EX10 showed better parameters. The treatment EX2, EX3, and EX4 shows retardation of growth in the plants. The retardation in

the growth of plant might be due to the anaerobic and hydrophobic condition developed by pollutants, which interferes soil, plant and water interaction reducing the soil oxygen level and creates anaerobiosis condition that causes root stress (Smith et al., 1989; Shukry et al., 2013). Similar reports were also reported in Małachowska-Jutsz and Miksch (2004). They demonstrated that rye, white mustard and red clover grown in the engine oil contaminated soil induces toxic effects which increases with the increase in the concentration of engine oil. The highest growth observed in EX8, EX9 and EX10 treatments were due to the bacteria and biosurfactant as the biosurfactant enhanced the hydrophobicity of bacteria towards the petroleum by formation of an emulsion that increases the degradation rate. The results obtained were similar to the previous findings of Das and Kumar (2018) who demonstrated the usefulness of the treatment with biosurfactant producing bacteria and its effects on the growth parameters of wheat, maize, mustard and moong. The better results were reported in EX5, EX6, and EX7 treatments were due to bacterial inoculum in the treatment that enhanced the plant parameters as compared to the nontreated plant (EX2, EX3 and EX4) as it has the efficiency to degrade the wholesome oil as a carbon source in the treatment and reducing the petroleum phyto-toxicity but its less than EX8, EX9, and EX10 which proved more effective due to presence of both bacteria and biosurfactant.

*3.7. Effects of the soil treatments on the fresh and dry weight of plant biomass*

The treatments augmented fresh and dry biomass of plant. The plant arose from the treatments EX5, EX6, EX7, EX8, EX9, and EX10 exhibit higher plant biomass **Fig. 8**. The higher plant biomass was due to the stimulatory effects of PGP activity which might have enhanced mineral solubilization and phytohormones production (Das and Kumar, 2016), moreover, biosurfactant release by the bacterial inoculants intensify the solubilization process of pollutant increasing the degradation rate. The results obtained were found similar to Liu et al. (2014), Houab (2015), Hong et al. (2011), Das and Kumar (2016).

*3.8. Effects of the soil treatments on plant pigments*

Chlorophyll (Chl) is a significant photosynthetic pigment of the plant that demonstrates photosynthetic capacity, plant growth and productivity (Li et al., 2018; Baruah et al., 2014). The petroleum contaminated soil displayed a negative effect on chlorophyll content. The higher values of chlorophyll were recorded in the plants grown in treated soil with bacteria and biosurfactant **Fig. 9**. The higher chlorophyll content in the plants were observed in treatments amended with strain SA3 and biosurfactant whereas low chlorophyll content reported in the plants arose from the soil without strain SA3 and biosurfactant (**Fig. 9**). This reduction of photosynthetic activity is due to the toxicity of petroleum that causes osmotic stress and partial closure of stomata (Meloni et al., 2003) or due to the inhibition of enzymes required for the synthesis of chlorophyll by the crude oil (Baruah et al., 2014). The increase in chlorophyll values in the plants from the treated pots may be due to the PGP activity of the strain or might be due to the reduction in petrol oil concentration in the soil. These results were complemented by the previously published report of Das and Kumar (2016) and Baruah et al. (2014).

Carotenoid pigments are essential plant pigments that play a vital role in photosynthesis and plant protection (Young, 1991). The carotenoid content in the plants arose from the treated soil with bacteria and biosurfactant is better than the nontreated plants. The treatments EX5, EX6, EX7, EX8, EX9, and EX10 exhibits high carotenoid content in the plants **Fig. 9**. Al-Hawas et al. (2012) and Das and Kumar (2016) demonstrate that the total chlorophyll and carotenoids content decreases as the concentration of the oil increases. The results were also similar to the previous findings of (Das and Kumar, 2016) who reported the effects of bacterial treatments in enhancement of carotenoid content as compared to the non treated pots.
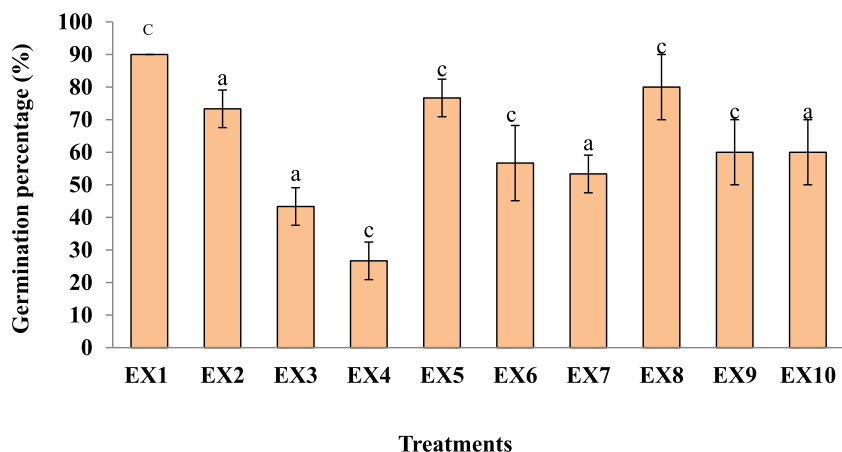
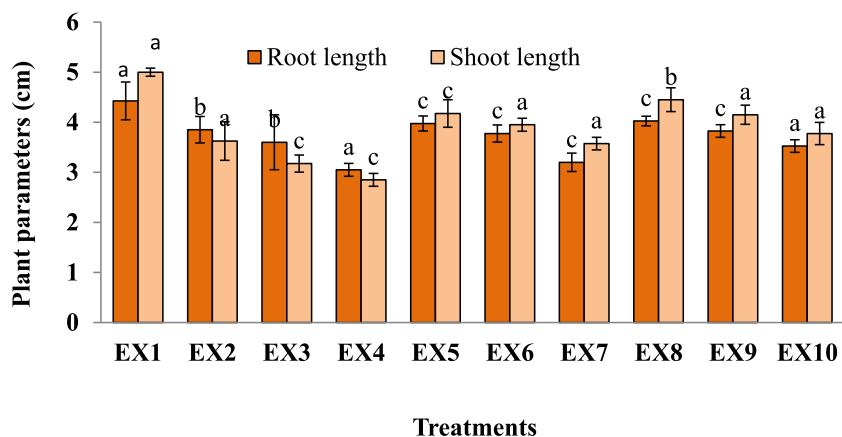**Fig. 6.** Effect of treatment in seed germination.



**Fig. 7.** Effects of the soil treatments on plant health parameters.

Petrol oil exhibits toxic effects in plants due to the presence of various hazardous hydrocarbons. The plant microbe interaction in petroleum contaminated soil is a complex phenomenon (Das and Chandran, 2011). In the present study the treatments reveal positive results in reduction of toxic effects of petroleum pollutants. The possible hypothetical explanations regarding the treatments are mentioned below:

- Petroleum oil pollution induces conditions that make basic supplements such as nitrogen, phosphorus and iron inaccessible for plant growth, adversely affecting plant growth, yield and leaf chlorophyll

content (Cooney, 1984; Atlas, 1985; Mitsch and Gosselink, 1993; Das and Chnadran, 2011; Alzahrani and Rajendran, 2019). Hence similar effects can be seen in the treatment 2, 3 and 4 which were polluted with petroleum oil (Fig. 10a).

- In the treatment 5, 6, and 7 the PGPR have been used as an inocula to promote degradation by rhizoremediation process. The plant growth promoting bacteria Pseudomonas *sp.* (SA3) degrade the petroleum pollutants by using it as a carbon source and increase the nutrient availability like phosphorus through phosphorus solubilization, iron
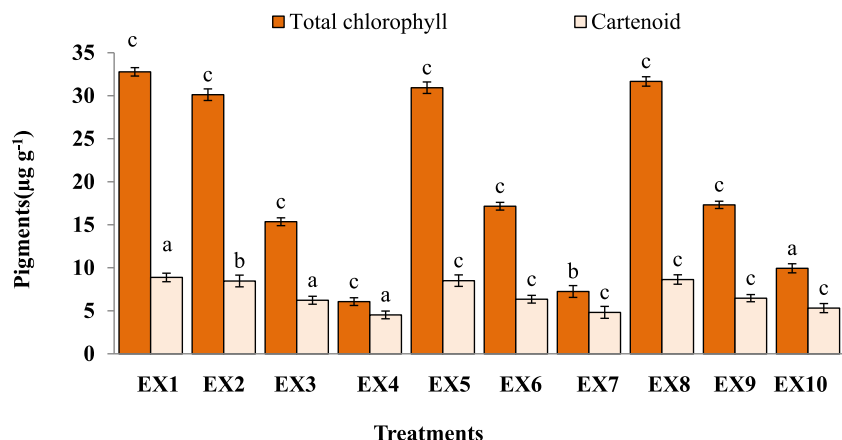


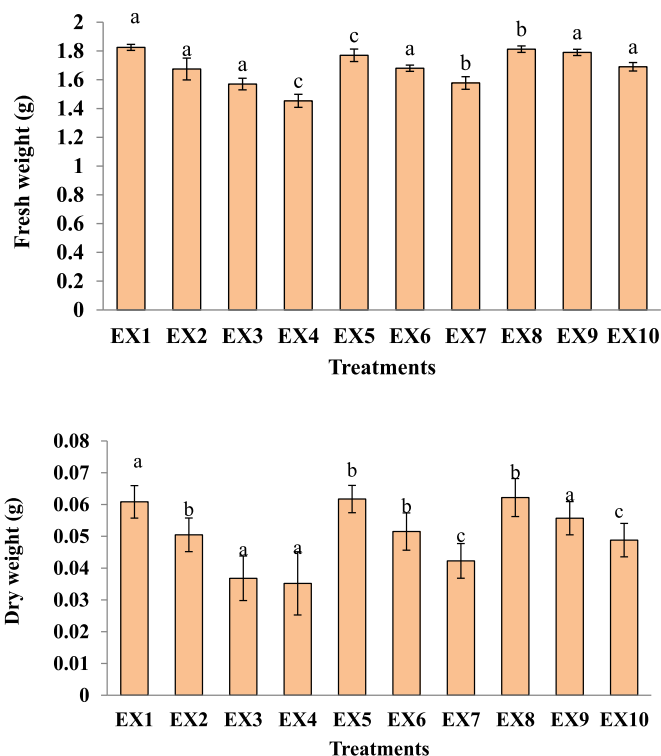**Fig. 9.** Effects of soil treatments on plant pigments.

**Fig. 8.** Effects of the soil treatments on plant weight.

properties, thus promoting their adhesion to pollutants. Biosurfactant acts as efficient emulsifiers which promote desorption and solubilization of hydrocarbons in soil and such events greatly increase the mobility and penetration of hydrocarbons (Kaczorek et al., 2018) (**Fig. 10c**).

There are a few reports that portray the success story of biosurfactant producing plant growth promoting rhizobacterial strains in remediation of petroleum contaminated soil. Many tactics were adopted for management of petroleum contaminated soil but using biosurfactant producing PGPR strain can open a new vista in future for management of contaminated wastelands . Some of the published studies that demonstrate the potentiality of various strains are depicted within **Table 5**.

## 4. Conclusion

The result of the present study signifies that developed treatments are effective in management of petroleum contaminated soil. The plants arose from the treatments consisting bacteria and biosurfactant displayed better plant parameters namely shoot and root length, plant fresh and dry weight and photosynthetic pigments. The effectiveness of the treatments is due to *Pseudomonas sp.* (SA3) which produces biosurfactant and can exhibit plant growth promoting activity. The results of present study depicts that the developed treatments can be efficaciously used for the management of petroleum contaminated soil for agronomy.

**Declaration of Competing Interest**

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availability through siderophore production and by production of plant growth hormone (IAA) (**Fig. 10b**).

- In treatment 8, treatment 9 and treatment 10, a secondary metabolite called biosurfactant has been used to increase the bioavailability of petroleum pollutants towards PGPR by modifying cell surface
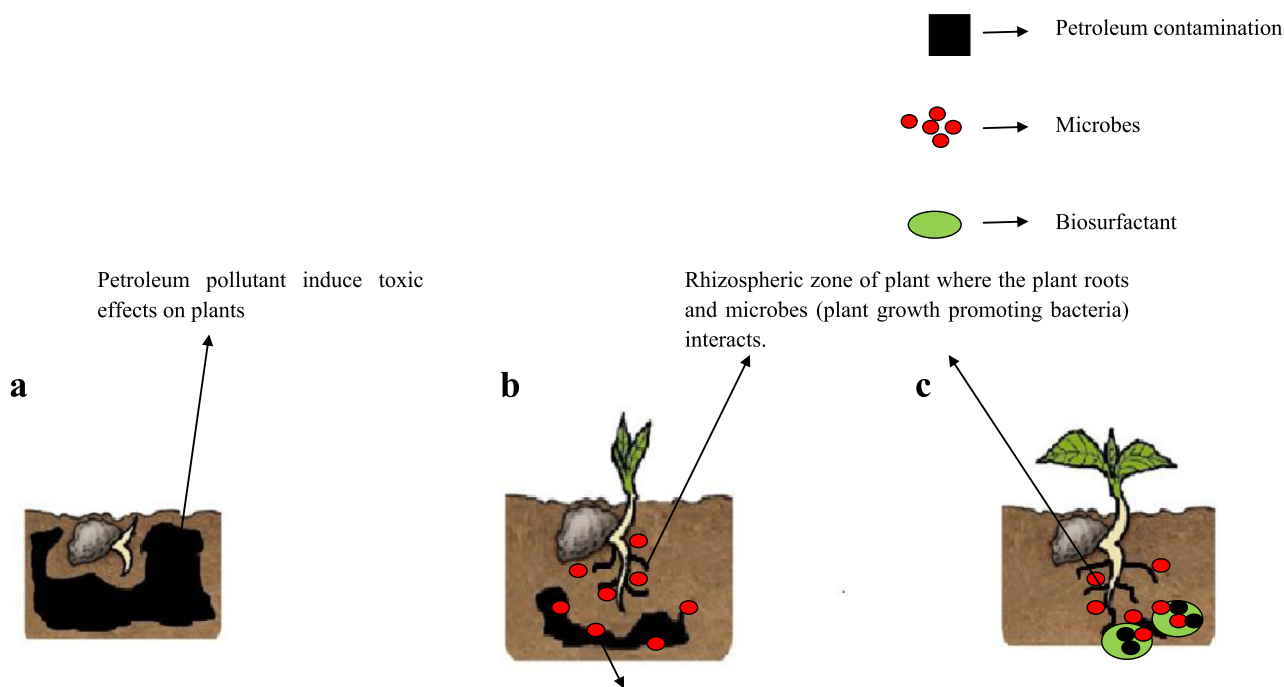


**Fig. 10.** Diagrammatic explanation of possible hypothetical mechanism regarding the treatments.

**Table 5**
Various published study depicting the comparison of various strain and their success story in ability to remediate petroleum contaminated soil.

| Strains | PGPR activity | | Biosurfactant producing ability | Ability to degrade hydrocarbons | References |
|---|---|---|---|---|---|
| *Pseudomonas sp.* AJ15 | Phosphate Solubilization | +ve | +ve | +ve | Das and Kumar 2016 |
| | Siderophore production | +ve | | | |
| | Indole acetic acid (IAA) | +ve | | | |
| *Pseudomonas sp.* (VI4T1 and VI4.1) | Siderophore production | +ve | +ve | +ve | Imperato et al., 2019 |
| | Indole acetic acid (IAA) | +ve | | | |
| *P. aeruginosa PG1* | NM | | +ve | +ve | Patowary et al., 2017 |
| P. aeruginosa L10 | Siderophore production | +ve | +ve | +ve | Wu et al., 2018 |
| | Indole acetic acid (IAA) | +ve | | | |
| | ACC deaminase activity | +ve | | | |
| P. aeruginosa strains (T4, T27, T30 &E1) | NM | | +ve | +ve | Ebadi et al., 2017 |
| *Pseudomonas sp.* | Phosphate Solubilization | | +ve | +ve | Ferjani et al., 2019 |
| | Siderophore production | +ve | | | |
| | Indole acetic acid (IAA) | +ve | | | |
| | Ammonia production | +ve | | | |
| *P. aeruginosa* (DKB1) | NM | | +ve | +ve | Deivakumari et al., 2020 |
| *Pseudomonas sp.* (SA3) | Phosphate Solubilization | +ve | +ve | +ve | Present study |
| | Siderophore production | +ve | | | |
| | Indole acetic acid (IAA) | +ve | | | |
| | Ammonia production | +ve | | | |
| | HCN production | +ve | | | |

NM=not mentioned.

## Declaration of Competing Interest

There is no conflict of interest associated with this publication.

## CRediT authorship contribution statement

**Shweta Ambust:** Conceptualization, Investigation, Writing - original draft. **Amar Jyoti Das:** Conceptualization, Writing - original draft. **Rajesh Kumar:** Conceptualization, Supervision.

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