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Full Length Research Paper

Bioremediation of crude oil contaminated tea plantation soil using two *Pseudomonas aeruginosa* strains AS 03 and NA 108

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Crude oil contamination of soil is a major concern for tea industry in Assam, India. Crude oil is a persistent organic contaminant which alters soil physical and biochemical characteristics and makes tea plants more susceptible against crude oil contamination. Therefore, two native bacterial strains designated as AS 03 and NA 108 having crude oil degradation ability was isolated from crude oil contaminated soil. Isolates were evaluated for reduction of crude oil phytotoxicity and soil bioremediation. Biochemical and 16s ribosomal ribonucleic acid (rRNA) analysis confirmed that the bacterial strains belong to *Pseudomonas aeruginosa*. Under *in vitro* evaluation, it was found that both the strain could tolerate crude oil up to 40% (v/v). However, structural changes including morphology, difference in number of colonies were found in the presence of hydrocarbon in both AS 03 and NA 108. Also, an improvement in growth of bacterized tea plants was observed compared to non-bacterized plants grown in crude oil contaminated soil. The cumulative increment in height was 5 to 42%, compared to non-bacterized plants and with significantly higher root and shoot dry biomass accumulation. Soil treatment with both AS 03 and NA 108 improved soil quality including organic carbon, conductivity, pH and degradation of total petroleum hydrocarbon (TPH) of the contaminated soil. These findings suggest that the tested bacteria can be exploited for bioremediation of crude oil contaminated soil in the geographical region of Assam.

Key words: *Pseudomonas*, tea plant, total petroleum hydrocarbon, crude oil.

INTRODUCTION

Tea and crude oil drilling industries in Assam, India started since eighteenth century and are vital for the economic prospective of the state. Occurrence of several crude oil drilling sites in and around tea plantation area is common in Assam. As a result, crude oil contamination in such tea gardens is very much prevalent due to spillage, tank failures, transport and abandonment of drilling sites etc. Crude oil contains numerous components including polar hydrocarbons, n-alkanes, aromatics, resin and asphaltene residuals (Killops and Al-Jaboori, 1990; Oudot et al., 1998). Crude oil affects the soil physical, biological properties, reduces the growth and resistance of the plants to biotic and abiotic factors thus making them more vulnerable to pathogen infestation (Udo and Fayemi, 1995; De Jong, 1980; Schutzendubel and Polle, 2002). Crude oil contamination is severely affecting the tea industry in Assam, as it reduces the production and quality of the produced tea. To restore the crude oil contaminated sites, various physical, chemical and thermal processes are the common techniques in use (Frick et al., 1999).

These processes are quite expensive and require site restoration moreover absolute remediation is also not ensured (Chaillan et al., 2006; Cunningham and Ow, 1996). Therefore, eco-friendly and cost effective means of bioremediation using native plants, bacteria, fungi and yeasts are drawing attention and have already been reported by

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several research groups (McGarth, 2001; Glick, 2010).

For bacteria, it is well known that they work synergistically to degrade the different hydrocarbon constituents (Alkhatib et al., 2011; Mukred et al., 2008). Among the different bacterial types, use of plant growth promoting rhizobacteria (PGPR) for bioremediation activity is gaining imputes due to their differential abilities to degrade and detoxify contaminants and also multiple effect on plant growth promotion (Glick et al., 2010). In light of the aforementioned conditions, this work focus to assess the two bacterial strains isolated from crude oil contaminated soil of Geleky, Assam for *in vitro* bioremediation potential, tea plant health improvement under crude oil stress along with comparison of soil quality improvement after the bioremediation.

MATERIALS AND METHODS

Soil samples and characterization

Crude oil contaminated soil was collected from Geleky, Assam, India (26.59 N latitude and 94.38 east longitude). The sample collection site has an average annual rainfall of 98.5 inches, mean temperature is 32°C and elevation is 318 feet. Soil pH was determined in soil-water suspension (1:2.5) with a pH meter (Eutech, Malaysia), while soil conductivity was determined by digital conductivity meter (IKON, India). Soil organic carbon content was estimated according to Jackson (1972). Soil dehydrogenase activity was determined according to Camina et al. (1998) and quantified using an iodonitro tetrazolium formazon (INTF) calibration curve and expressed in µg g^{-1}soil h^{-1}.

Chemicals and reagents

All the chemicals used were of analytical grade and procured from Sigma Aldrich; media used in this study were obtained from Himedia, India.

Isolation of crude oil degrading bacterial strains

Isolation of bacterial strains from crude oil contaminated soil was done by enrichment culture method using mineral media ($NaNO_3$ 4.0, Na_2HPO_4 3.61, KH_2PO_4 1.75, $MgSO_4.7H_2O$ 0.2, $FeSO_4$ 0.01, $CaCl_2$ 0.05, each in g L^{-1}, trace element solution (1 ml L^{-1}) amended with 2% (v/v) crude oil, pH was maintained at 7.0. The culture incubation temperature was 37° C for 48 h. From the enrichment culture morphologically distinct single colonies were purified by dilution technique on solid mineral media.

Hydrocarbon degradation ability of the isolated bacteria

Crude oil degradation ability of the bacterial isolates were screened in liquid mineral media containing different hydrocarbon sources for example, crude oil, asphaltene fraction, aliphatic fraction and aromatic fraction up to 40% (v/v), hexadecane, dodecane, naphthalene, fluroanthene, phenanthrene and anthracene (200 ppm each) as the sole carbon. The degradation ability was verified at 37°C after 48 h onwards by observing the emulsification, turbidity of the media and breakdown of the crude oil layer.

Identification of the bacterial isolate

Gram staining and biochemical characterization of the studied bacteria was performed according to Bergy's manual of systematic determinative bacteriology and Cappuchino and Sherman (1983). Molecular identification was done by polymerase chain reaction (PCR) amplification of 16s rRNA gene by using universal forward primer 2A (5′-AGAGTTTGATCCTGGCTCAG-3′) and reverse primer 2B respectively (5′-ACGGCTACCTTGTTACGACTT-3′). The PCR product was confirmed by deoxyribonucleic acid (DNA) sequencing and subsequently deposited in NCBI database.

Characterization of PGPR properties

Indolacetic acid (IAA) production was detected in Luria Bertini (LB) broth in the absence and presence of tryptophan according to Brick et al. (1991). Hydrogen cyanide (HCN) production and phosphorous solubilization was adapted from Lorck (1948) and Gaur (1990), respectively. *In vitro* antagonism of the two strains was tested against the fungal root pathogen *Fomes lamoensis* according to the method of Deka Boruah and Dileep Kumar (2002) on potato dextrose agar medium (PDA).

Scanning electron microscopy studies

Scanning electron microscopy (SEM) was done to observe the hydrocarbon uptake and surface modifications of AS 03 and NA 108 in the presence of hexadecane. For this, 72 h old cultures of AS 03 and NA 108 were grown at 37°C in solid mineral media with 4% hexadecane were used. A small piece of solid mineral media with active bacterial growth was taken and fixed using 3% glutaraldehyde for 4 h followed by 3 times washing with 0.1 M sodium cacodylate buffer.

Further, gradual drying of the samples was done in different acetone grades, followed by critical point drying using tetramethylsilane twice for 5 h at 4°C. The samples were then brought to room temperature, mounted on aluminum stubs and approximately 35 nm thick gold coating was done over the samples. The observation was done in different magnification range (3000 X to 20,000 X) in a SEM instrument (JEOL JSM-6360).

Bioremediation under green house condition

The experiments were laid in completely randomized block design under green house condition with normal light and temperature. The crude oil contaminated soil were homogenized, sieved and taken in earthen pots (bottom diameter × height × top diameter) (18 × 30 × 20 cm). Each tube was filled up with 8 kg of crude oil contaminated soil. The soil was then allowed to acclimatize for one week and one year old tea clones (TV1 type) were transplanted. For induction of disease resistance in tea plants, soil was contaminated with five days old *F. lamoensis* at 10% concentration (w/v). The collar region of the plants was treated with bacterial suspension (AS 03 and NA108) by spraying until the soil was wet enough. The bacterial cell suspensions were prepared from 48 h old culture broth cultivated in nutrient broth media and finally adjusted to 3.0 CFU ml^{-1}. All the treatments were repeated three times with five replications each. Effect of different treatments on tea plant growth was studied after two months by recording shoot height, root length, root diameter and biomass accumulation. Root, shoot and leaf dry biomass of tea plants was also recorded by drying at 100°C until a constant weight was obtained.

Effect on physiological behavior

Measurement of relative water content (RWC) of leaf tissue was done by Kramer (1983) using the following formulae:

RWC (%) = (Fresh weight - Dry weight) / (Turgid weight - Dry weight) × 100

Similarly, the moisture content of the leaf sample was also measured by the formulae given below.

Moisture content (%) = (Fresh weight - Dry weight)/Fresh weight × 100

Effect on biochemical activity

Total protein content of the leaf was calculated by Lowry et al. (1951). Total chlorophyll content was measured by using the dimethylsulfoxide (DMSO) method (Hiscox and Israelstam, 1979). Total carbohydrate estimation was done by the anthrone reagent method (Sadasivam and Manickam, 1992). Catalase enzyme activity was measured by the method given by Sinha (1972). The peroxidase enzyme activity was studied by the method given by Mali et al. (1989).

Effect on soil quality

To compare the bioremediation efficiency and soil fertility improvement of the two strains; TPH, pH, conductivity, organic carbon content and dehydrogenase activity was assessed. TPH was compared in different treatments by standard methods. Briefly, 5 g of soil sample was taken in an extraction thimble (Whatman, 25 × 80 mm) and heated in reflux condition in a Soxhlet's apparatus with chloroform for 8 h at 60 to 70°C, The chloroform content in the residual oil was evaporated using rotary evaporator (Buchi, Switzerland) and remaining crude oil was converted to percentage.

Statistics

All the experiments were repeated at least three times with five replications each. The data were subjected to analysis of variance and significant means further compared by tukey's test. All the data were analyzed by using SPSS software. The 16s rRNA sequence data were analyzed using BLAST, Clustal W and Mega 5 softwares.

RESULTS

Identification of the isolates

Both the bacteria were Gram negative, rod shaped (Table 1). The two isolates were designated as AS 03 and NA 108, respectively. The pigment pattern was fluorescent green for AS 03 while normal green was noticed for NA 108. Both AS 03 and NA 108 showed negative in starch hydrolysis, H_2S production, indole test, methyl red test and voges proskauer test (Table 1). However, both the strains showed positive results in gelatin hydrolysis, catalyses test, and oxidase test. AS 03 showed urea and citrate positive result while NA 108 were negative (Table 1). 16s rRNA sequencing data confirmed both AS 03 and NA108 to be *Pseudomonas aeruginosa* and NCBI accession number (JN410947) and (JN410948) was obtained for the respective strains. The relatedness of the two *P. aeruginosa* strains with NCBI reference strain is showed in Figure 1.

Hydrocarbon degradation activity of the isolates

Both the strains were able to grow in crude oil, asphaltene, aliphatic and aromatic fractions in liquid mineral media (Table 1). Both AS 03 and NA 108 were able to grow at 40% (v/v) crude oil concentration in mineral media. Both the strains also showed good growth in the standard aliphatic hydrocarbons hexadecane and dodecane. AS 03 was able to grow up to a concentration of 200 ppm/ml of mineral media with all the five different polycyclic aromatic hydrocarbon (PAH) compounds tested (Table 1). However, NA 108 did not show growth in phenanthrene, anthracene and fluroanthene at the same concentration.

PGPR properties of the bacterial isolates

Both NA 03 and NA 108 strains showed positive result in phosphorous solubilization, indole-3-acetic acid (IAA) production and HCN production with antifungal activity against the pathogenic fungus *F. lamoensis* (Table 2, Figure 2). Strain NA 108 produces higher amount of HCN, IAA and siderophores compared to AS 03 (Data not shown) but showed a significantly less (26.7%) antifungal activity.

Scanning electron microscopic studies

Scanning electron micrographs of both AS 03 and NA 108, showed structural deformation when grown in the presence of hexadecane compared to the control the bacterial cell appeared to be elongated and distorted at the ends in the presence of hexadecane. The micrographs showed a significant less bacterial population in hexadecane (Figure 3). Some web like fibrous projections from the cell, and also deposition of hydrocarbon granular materials on the surface and matrix of the bacterial cells were also observed in higher magnifications beyond 20,000 X (Data not shown).

Effect on shoot height

The different sets of treatment given to the tea plants are described in Table 3. Effect of crude oil and bacterial treatments on growth of tea plants is described in Table 4. Compared to non-contaminated soil T_1, the plants grown in crude oil contaminated soil showed a significantly lowest growth. Among the treatments, the cumulative highest shoot height was found in T_5 followed by $T_1 > T_6 > T_4 > T_{12} > T_{10} > T_9 > T_2 > T_8 > T_3 > T_7$. The overall increment of height was 46.4% (T_5) compared to plant grown in crude oil contamination and fungal pathogen together (T_7). However, lowest shoot growth was observed for the treatment T_7.

Effect on physiological behavior and biochemical activity

The comparative effect of crude oil, microbial treatment and induced disease resistance by *F. lamoensis* on the tea plants have been studied by monitoring the percentage moisture content, relative water content, plant pigment and other biochemical changes (Table 5).

Table 1. Biochemical, morphological and hydrocarbon degradation characters of the AS 03 and NA108.

Biochemical characterization	Bacterial isolate	
	AS 03	NA 108
Gram staining	-	-
Cell shape	Rod	Rod
Pigmentation	Fluorescent Green	Green
Spores	-	-
Motility	+	+
Indole test	-	-
Methly red test	-	-
Voges proskauer test	-	-
H_2S Production	-	-
Citrate utilization	+	-
Urea hydrolysis	+	-
Gelatin liquefaction	+	+
Starch hydrolysis	-	-
Catalase	+	+
Oxidase	+	+
Nitrate reductase	+	+
Different hydrocarbon degradation		
Crude oil (40% v/v)	+	+
Aliphatic fraction (40% v/v)	+	+
Aromatic fraction (40% v/v)	+	+
Asphaltene fraction (40% v/v)	+	+
Hexadecane (200 ppm/ml)	+	+
Dodecane (200 ppm/ml)	+	+
Napthalene (200 ppm/ml)	+	+
Phenanthrene (200 pm/ml)	+	-
Anthracene (200 ppm/ml)	-	-
Fluroanthene (200 pm/ml)	+	-

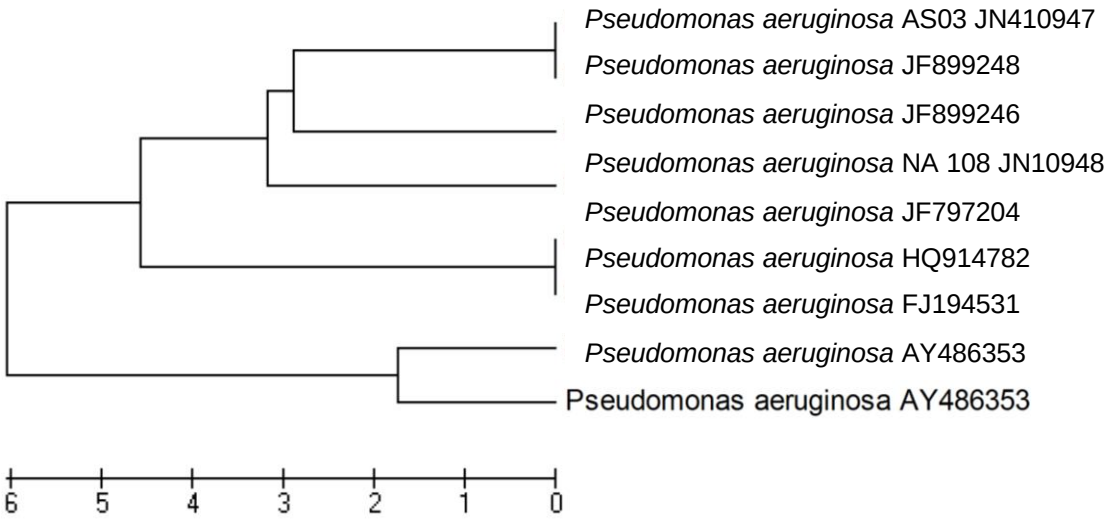

Figure 1. Phylogenetic relationship of AS 03 and NA 108 strains with reference strains.

Table 2. Some PGPR properties and hydrocarbon degradation of the test isolates.

Strain	HCN Production	IAA production	Phosphorous solubilization	Siderophore Production	Antifungal activity (mm)
AS 03	+	+	+	+	19.9 ± 0.1
NA108	+	+	+	+	15.7 ± 0.2

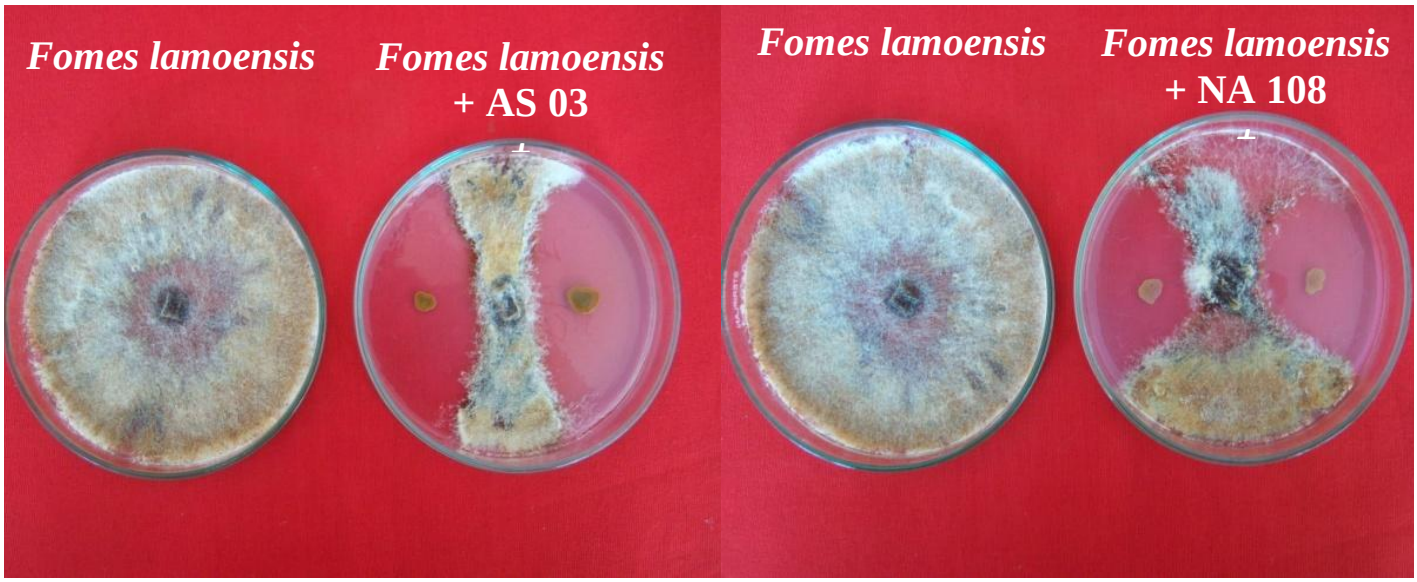

Figure 2. *In vitro* antagonism of AS 03 and NA 108 respectively against *Fomes lamoensis* in PDA.

Significantly, highest moisture content of leaf was found for the treatment T_5 and lowest was observed for T_7. Except for T_7, all the other treatments showed a significant increase in leaf moisture over T_2. The increment of moisture content compared to T_2 was between 4.8 to 42.9%. On the other hand, all the plants under crude oil stress had lower moisture content than the plants growing in normal soil. However, in the plant growing in the crude oil contaminated soil and treated with AS 03/NA 108 showed an increase in their moisture content as compared to plants grown in crude oil contamination alone.

In case of RWC of the leaf samples of plants were found highest in T_6 (99.80%) followed by T_4 (97.2%) and T_1 (96.6%) while it was lowest in T_9 (72.1%). Likewise, among all the treatments, total chlorophyll content was found highest in T_1 and T_2 (2.4 mg g^{-1}) followed by T_{12} (2.5 mg g^{-1}) and lowest in T_8 (1.8 mg g^{-1}). On the other hand, T_1 showed highest total protein content (9.3 mg g^{-1}) while lowest in treatment T_9 (2.5 mg g^{-1}). Similar to total protein, the highest carbohydrate content was also found in treatment T_1 (21.6 mg g^{-1}) and lowest in treatment T_9 (4.4 mg g^{-1}). In case of oxidative enzyme responses of plants grown in crude oil contaminated soil, no significant variation was observed in catalase and peroxidase enzyme activities.

Effect on overall growth

Comparison of effect of crude oil, microbial treatment and induced disease resistance by *F. lamoensis* on root length, root diameter and dry biomass of tea plant is described in Tables 4 and 6. Significant lower root length, root diameter and dry biomass accumulation were found for the tea plants grown in crude oil contaminated soil along with the *F. lamoensis* (T_7) and crude oil contamination alone (T_2) compared to other treatments. Highest root length was observed in T_4 (18.3 cm) followed by T_5 (16.2 cm), lowest root length was observed in case of T_2 (8.5 cm) and T_7 (12.0 cm), respectively. Similarly, root diameter was also found highest in T_5 (0.53 mm) followed by T_4 (0.48 mm) and lowest was observed in case of T_7 (0.09 mm) and T_2 (0.12 mm), respectively. The dried biomass leaf (5.2 g), shoot (3.9 g) and root biomass (2.8 g) was found highest in T_1 and lowest in the treatments T_7 (0.4 g), (0.8 g) and (0.3 g), respectively. The uprooted roots from different treatments T_2, T_7, T_8, T_9, T_{10} and T_{11} showed crude oil adherence to root tips and root hairs with overall reduced root growth and root branching observed for the treatment T_2 and T_7 (Figure 4).

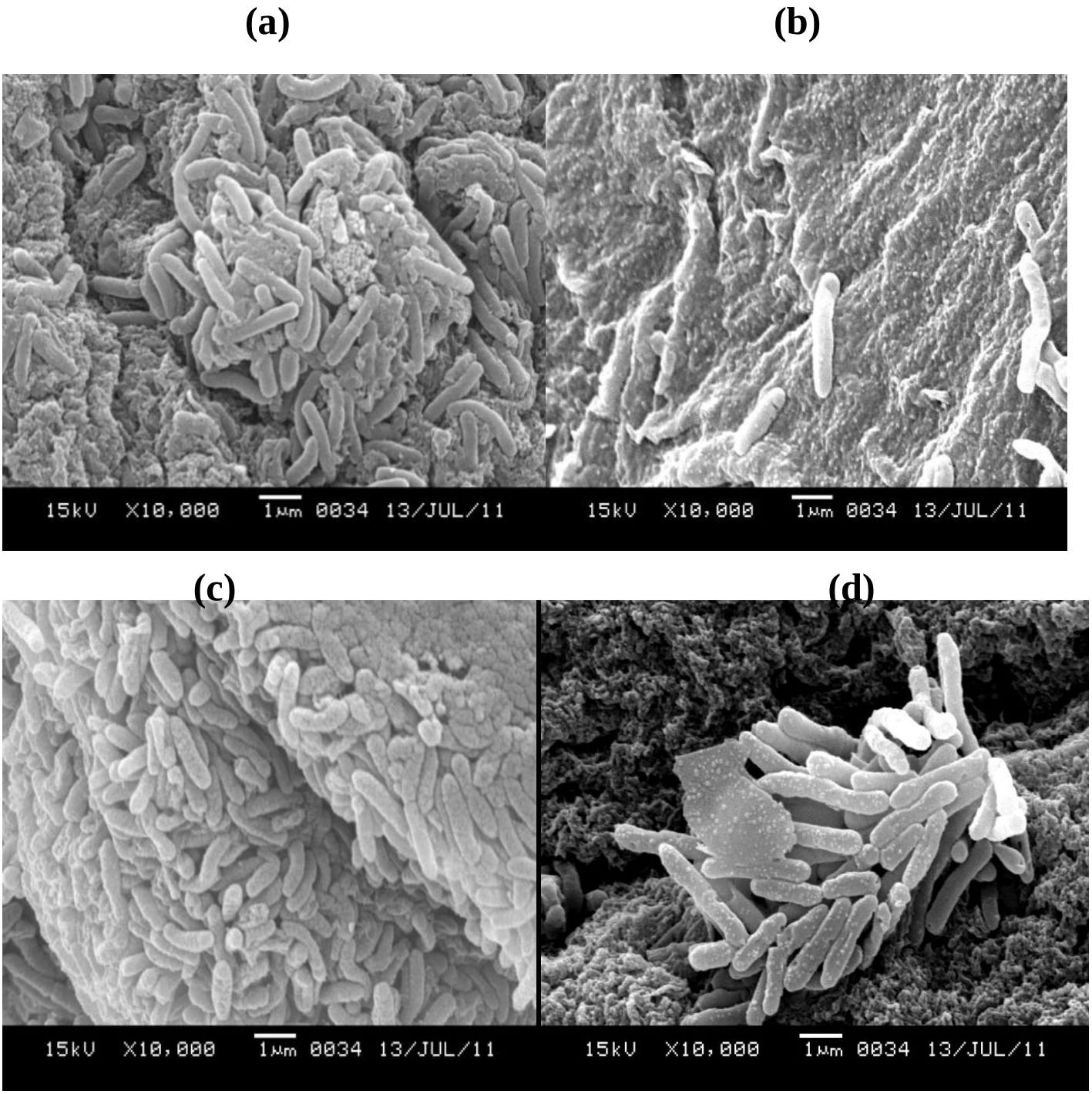

Figure 3. Scanning electron micrographs of the two isolates AS 03 and NA 108 in normal condition and in presence of hexadecane (a) AS 03 (Control on Nutrient Agar), (b) AS 03 (4% v/v, hexadecane), (c) NA 108 (Control on Nutrient agar) (d) NA108 (4% v/v, hexadecane).

Comparison of soil quality improvement

The comparison of soil pH and soil conductivity after the bacterial treatment is described in Figure 5. It was observed after completion of experiment that pH of the crude oil contaminated soil came down nearly 4.0 to 5.5. However, in treatment T_7 and T_{11} it was near neutral after the experiment was over. On the other hand, the soil conductivity of the crude oil contaminated soil was improved for all the treatment. It was found highest for treatment T_4, T_5 and lowest for the crude oil contaminated soil. There were also enhancement of soil organic carbon and reduction of dehydrogenase activities except T_9, and T_{12} for organic C,

Table 4. Effect of different treatments on growth parameters of tea plants grown in crude oil contaminated soil 60 days after treatment.

Treatment	Shoot height (cm)	Root length (cm)	Root diameter (mm)	Observation of the root after uprooting
T_1	29.0 ± 3.5^{a}	15.0 ± 1.5^{a}	0.4 ± 0.1^{a}	Normal root growth was observed.
T_2	18.7 ± 0.4^{c}	8.5 ± 2.2^{d}	0.12 ± 0. 6^{b}	Reduced root growth, crude oil adhered in roor tips, with reduced root branching.
T_4	23.6 ± 0.4^{b}	18.3 ±1.3^{c}	0.48 ± 0.1^{a}	Improved root growth, profuse branching.
T_5	29.5 ± 3.6^{a}	16.2 ± 0.3^{a}	0.53 ± 0.3^{a}	Good root growth
T_6	24.3 ± 3.2^{b}	16.0 ± 1.7^{a}	0.23 ± 0.3^{c}	Normal Growth
T_7	15.8 ± 3.1^{d}	12.0 ± 1.1^{b}	0.09 ± 0.1^{d}	Reduced root growth, crude oil adhered in roor tips, with reduced root branching.
T_8	17.6 ± 2.4^{c}	13.3 ± 1.7^{b}	0.24 ± 0.2^{d}	Reduced root growth, crude oil adhered in roor tips, with reduced root branching, root growth was however better then T_2 and T_7
T_9	20.3 ± 2.4^{e}	13.0 ± 0.5^{b}	0.25 ± 0.14^{c}	Reduced root growth, crude oil adhered in roor tips, with reduced root branching, root growth was however better then T_2 and T_7
T_{10}	22.0 ± 3.5^{eb}	14.2 ± 3.7^{ab}	0.32 ± 0.1^{c}	Reduced root growth, crude oil adhered in roor tips, with reduced root branching.
T_{11}	16.8 ± 3.9^{c}	13.6 ±1.3^{b}	0.1 2 ± 0.1^{b}	Reduced root growth, crude oil adhered in roor tips, with reduced root branching.
T_{12}	23.2 ± 2.1^{b}	15.6 ± 2.7^{a}	0.26 ± 0.2^{c}	Normal growth, but less profuse branching compared to T_1, T_4 and T_5

Data are mean of three observations with three replications each; Standard error bars followed by similar letter are not significantly from each other at according to Tukey's test at $p < 0.01$.

Table 5. Biochemical parameters in leaf two months after treatment.

Treatment	Effect on physiological change		Effect on biochemical change				
	Moisture content (%)	Relative water content (%)	Total chlorophyll (mg g^{-1})	Total protein (mg g^{-1})	Total carbohydrate (mg g^{-1})	Catalse (µg g^{-1} h^{-1})	Peroxidase (µg g^{-1} h^{-1})
T_1	77.8 ± 1.3b^{c}	96.6 ± 1.0^{b}	2.5 ± 0.1^{a}	9.3 ± 0.1^{a}	21.6 ± 2.8^{a}	0.6 ± 0.006^{a}	0.1 ± 0.004^{a}
T_2	58.7 ± 0.3^{g}	89.1 ± 1.2^{c}	1.8 ± 0.1^{a}	3.2 ± 0.1^{c}	5.1 ± 0.1d^{e}	0.6 ± 0.008^{a}	0.2 ± 0.009^{a}
T_3	63.4 ± 1.4^{f}	82.4 ± 1.4^{e}	2.0 ± 0.1^{c}	4.4 ± 0.1^{bc}	11.8 ± 0.2^{c}	0.4 ± 0.007^{a}	0.1 ± 0.006^{a}
T_4	81.1 ± 1.6^{a}	97.2 ± 1.2^{c}	1.9 ± 0.1^{c}	4.3 ± 0.1^{bc}	5.1 ± 0.2^{de}	0.4 ± 0.004^{a}	0.1 ± 0.004^{a}
T_5	82.9 ± 0.9^{a}	89.7 ± 0.7^{c}	2.3 ± 0.1^{a}	3.1 ± 0.1^{c}	6.6 ± 0.1^{d}	0.58 ± 0.004^{a}	0.1 ± 0.003^{a}
T_6	79.7 ± 0.9^{ab}	99.8 ± 3.3^{a}	2.3 ± 0.1^{a}	4.3 ± 0.1^{bc}	5.7 ± 0.3^{de}	0.5 ± 0.006^{a}	0.1 ± 0.002^{a}
T_7	58.0 ± 1.7^{g}	82.8 ± 1.8^{cd}	1.6 ± 0.1^{d}	3.7 ± 0.1^{c}	6.1 ± 0.5^{d}	0.5 ± 0.003^{a}	0.2 ± 0.010^{a}
T_8	63.7 ± 0.9^{f}	83.1 ± 2.3^{d}	1.8 ± 0.1^{d}	3.3 ± 0.1^{c}	5.5 ± 0.1^{de}	0.5 ± 0.002^{a}	0.2 ± 0.007^{a}
T_9	69.3 ± 1.2^{d}	72.5 ± 1.01^{f}	2.3 ± 0.1^{a}	2.5 ± 0.1^{c}	4.9 ± 0.1^{ef}	0.5 ± 0.003^{a}	0.2 ± 0.001^{a}
T_{10}	67.0 ± 1.7^{e}	94.8 ± 3.7^{eb}	2.2 ± 0.1^{b}	5.3 ± 0.1^{b}	4.4 ± 0.1^{ef}	0.5 ± 0.003^{a}	0.1 ± 0.005^{a}
T_{11}	76.7 ± 0.7^{c}	94.5 ± 1.4^{c}	2.2 ± 0.1^{b}	3.7 ± 0.6^{c}	6.5 ± 0.6^{d}	0.5 ± 0.003^{a}	0.1 ± 0.004^{a}
T_{12}	79.3 ± 0.7^{ab}	85.2 ± 2.4^{e}	2.5 ± 0.1^{e}	5.6 ± 0.1^{a}	13.3 ± 1.3^{b}	0.5 ± 0.013^{a}	0.2 ± 0.004^{a}

Data are mean of three observations with three replications each. Standard error bars followed by similar letter are not significant different from each other according to Tukey's test at $p < 0.0$.

Table 6. Biomass of the different treatments after 60 days.

Treatment	Leaf		Shoot		Root	
	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)
T_1	13.2 ± 0.1^{a}	5.2 ± 0.1^{a}	10.3 ± 0.1^{a}	3.9 ± 0.1^{a}	7.7 ± 0.1^{a}	2.8 ± 0.1^{a}
T_2	2.1 ± 0.1^{b}	0.9 ± 0.1^{b}	3.8 ± 0.1^{b}	1.1 ± 0.1^{b}	1.2 ± 0.1^{f}	0.7 ± 0.1^{b}
T_3	2.3 ± 0.3^{c}	1.7 ± 0.1^{b}	3.9 ± 0.1^{b}	1.7 ± 0.1^{b}	1.5 ± 0.1^{b}	0.5 ± 0.1^{b}
T_4	13.1 ± 0.1^{a}	3.9 ± 0.1^{c}	8.7 ± 0.1^{d}	2.1 ± 0.7^{c}	3.1 ± 0.1^{c}	1.2 ± 0.1^{c}
T_5	14.8 ± 0.1^{a}	4.8 ± 0.2^{a}	9.9 ± 0.1^{a}	3.4 ± 0.2^{a}	3.8 ± 0.1^{c}	1.7 ± 0.1^{c}
T_6	9.3 ± 0.1^{d}	2.7 ± 0.1^{d}	6.2 ± 0.1^{c}	3.2 ± 0.4^{a}	2.4 ± 0.1^{d}	1.2 ± 0.1^{c}
T_7	1.2 ± 0.1^{e}	0.4 ± 0.1^{e}	3.3 ± 0.1^{b}	0.8 ± 0.3^{c}	0.6 ± 0.1^{b}	0.3 ± 0.6^{d}
T_8	6.6 ± 0.1^{f}	1.6 ± 0.1^{b}	6.7 ± 0.1^{c}	1.6 ± 0.2^{b}	3.1 ± 0.1^{c}	0.8 ± 0.1^{d}
T_9	3.7 ± 0.1^{b}	1.3 ± 0.1^{b}	5.9 ± 0.1^{c}	2.3 ± 0.1^{c}	1.8 ± 0.1^{b}	1.0 ± 0.1^{c}
T_{10}	2.5 ± 0.1^{c}	1.1 ± 0.1^{b}	3.8 ± 0.1^{b}	1.8 ± 0.1^{b}	1.4 ± 0.1^{b}	0.8 ± 0.1^{d}
T_{11}	4.6 ± 0.1^{b}	6.7 ± 0.1^{f}	6.7 ± 0.1^{c}	1.9 ± 0.1^{b}	4.8 ± 0.1^{e}	1.0 ± 0.1^{c}
T_{12}	2.5 ± 0.1^{c}	1.8 ± 0.1^{a}	5.8 ± 0.1^{c}	2.7 ± 0.1^{c}	2.3 ± 0.1^{d}	0.9 ± 0.1^{d}

Data are mean of three observations with three replications each; Standard error bars followed by similar letter are not significantly different from each other according to Tukey's test at $p < 0.01$.

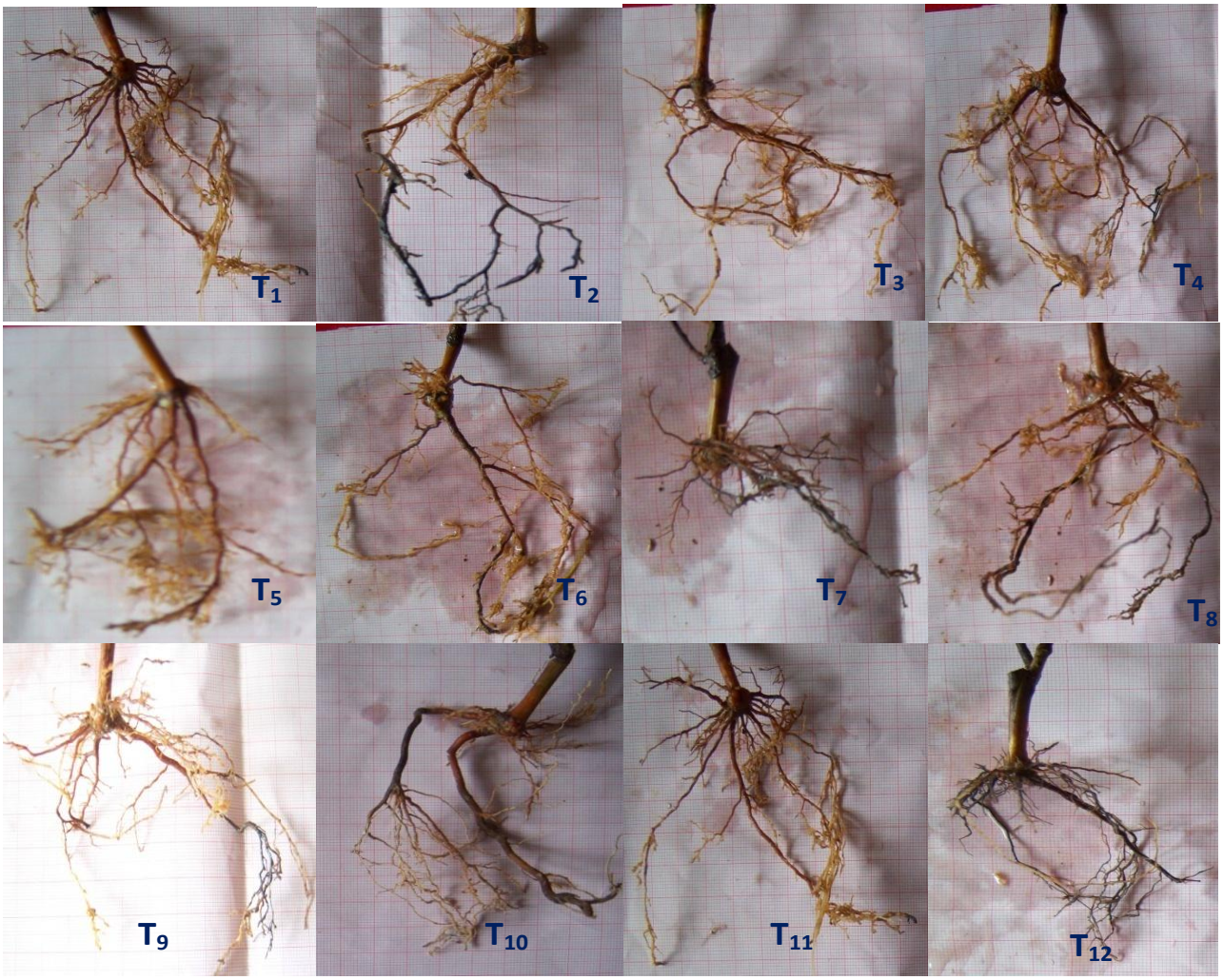

Figure 4. Morphological changes in roots of tea plants grown for 60 days with various treatments T_1 to T_{12} (as mentioned in Table 3).

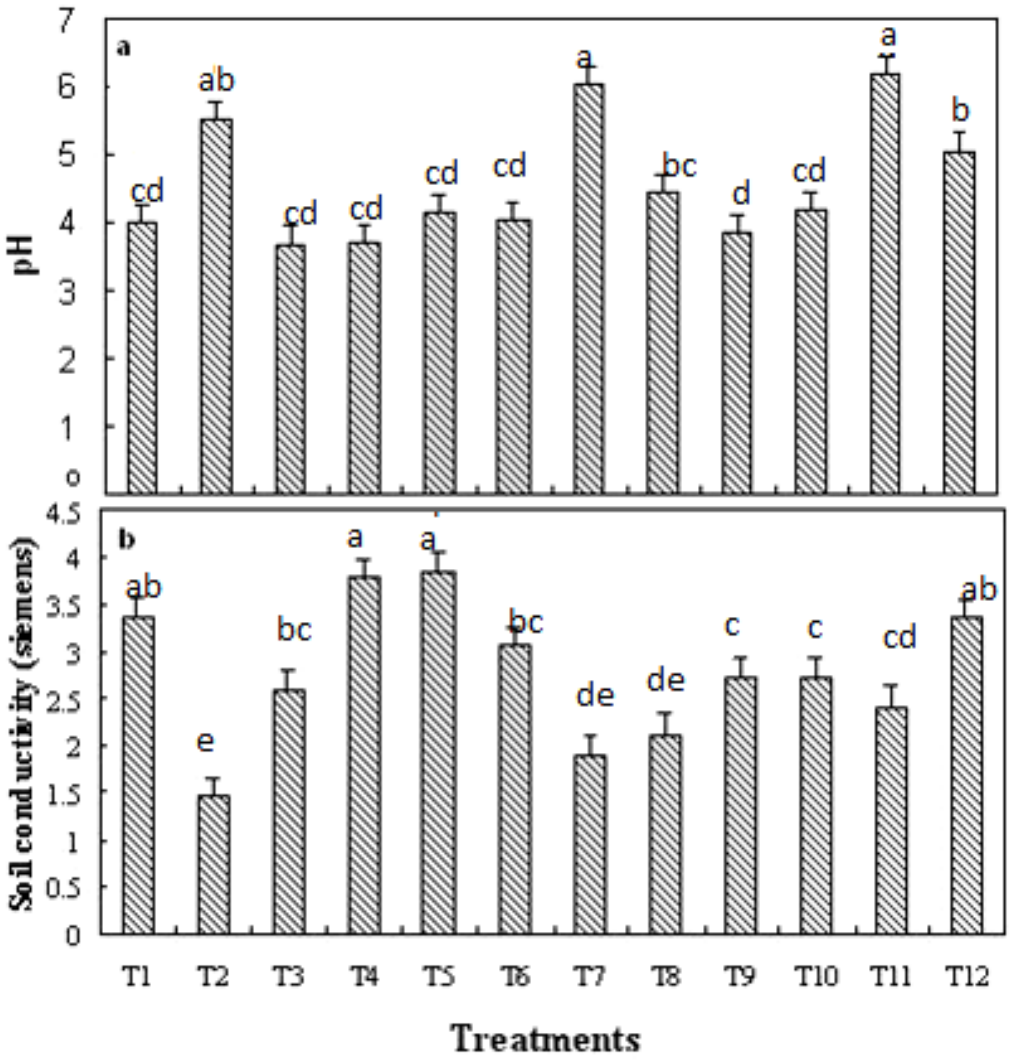

Figure 5. Comparison of effect of different treatment on improvement of soil pH and conductivity. Data are mean of three observations with three replications each; Standard error bars followed by similar letter are not significantly different from each other according to Tukey's test at $p < 0.01$.

and T_9 for the dehydrogenase activity (Figure 6). The enhancement of organic C were more than double for the treatment T_5 and reduced to half in case of the dehydrogenase activity for the treatment T_9. TPH reduction was observed in both AS 03 and NA 108 treatment as compared to the control (Figure 7).

DISCUSSION

Crude oil contamination is a serious emerging problem in tea plantation sites of Assam, which makes soil unsuitable for plant growth. It reduces the availability of plant nutrients and increases the toxic level of elements like zinc and iron (Udo and Fayemi, 1995). Further use of plant growth promting regulator (PGPR) in bioremediation of crude oil and PAH contaminated sites have been reported by several research groups (Zhuang et al., 2007; Gerhardt et al., 2009). For this study, native crude oil degrading bacterial strains were isolated from crude oil contaminated soil. Isolates were able to tolerate aliphatic, aromatic, asphaltene fractions and polycyclic aromatic hydrocarbons. This signifies the potential of these bacteria designated as AS 03 and NA 108 for crude oil degradation and also for bioremediation. Morphological, biochemical and molecular characterization confirmed that both the isolates were *P. aeruginosa* (Table 1). Isolation, identification and characterization of crude oil degrading bacteria and their remediation efficacy from different ecological niches were earlier reported by several other researchers (Gunderson et al., 2008; Li et al., 1997; Glick, 2003).

Several researchers also stressed that beside crude oil degradation ability, assessment of their PGPR properties

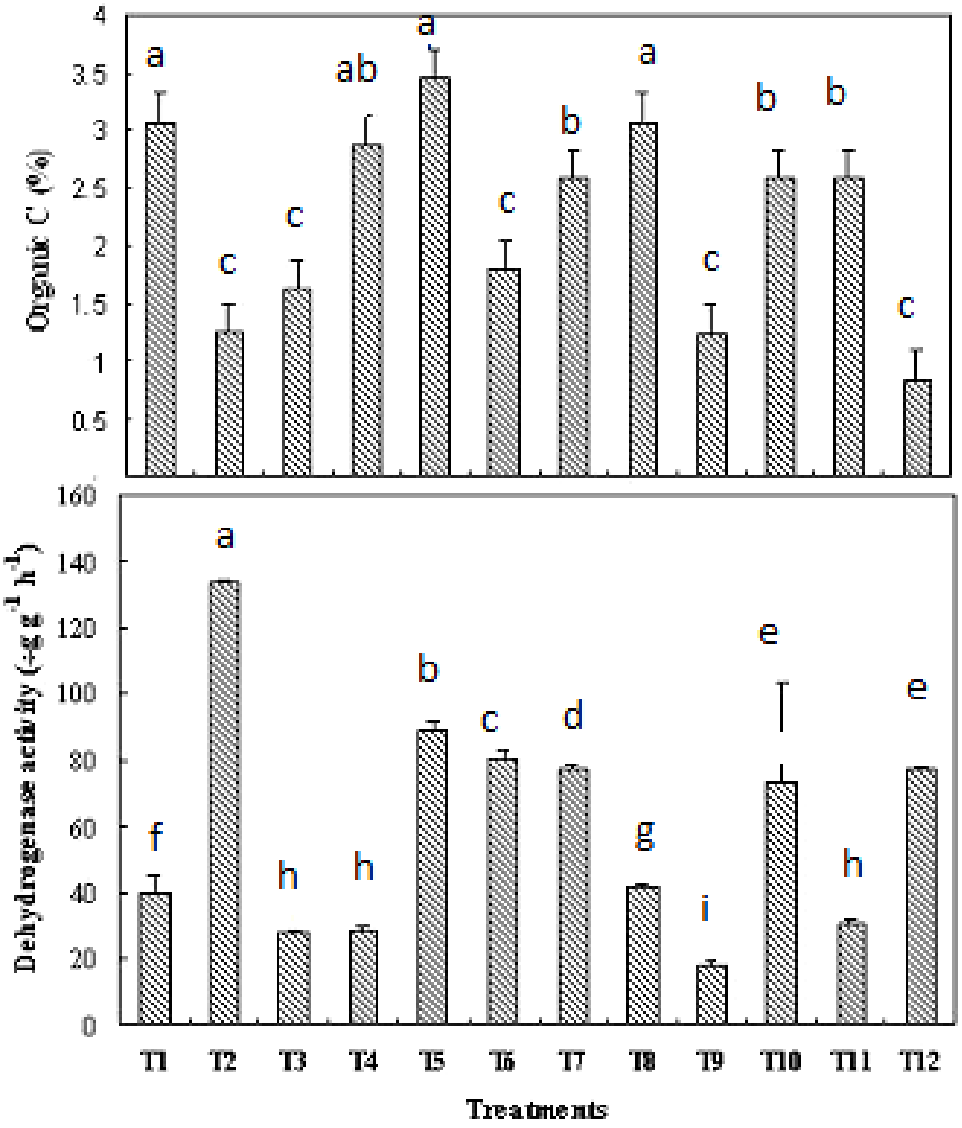

Figure 6. Comparison of effect of different treatment on improvement of soil organic carbon and dehydrogenase activity. Data are mean of three observations with three replications each; Standard error bars followed by similar letter are not significantlydifferent from each other according to Tukey's test at $p < 0.01$.

is vital before selection of a bacterium for bioremediation (Glick and Pasternak, 2003; Zhuang et al., 2007). In the present investigation, both the bacterial strains were found to produce IAA, siderophore production, phosphorrus solubilization, HCN production, phosphorus solubilization and *in vitro* anti-fungal activity which confirms their PGPR properties. Studies also reported that crude oil contamination reduces plant growth to various extents and also affects their biochemical and physiological parameters (Ogbo et al., 2009; Omosun et al., 2008). Similar observations were also made in tea plants, however, tea plants bacterized with AS 03 and NA 108 promote overall higher plant growth and alleviate the effect of crude oil to a considerable extent. This may be due to multifold effects of AS 03 and NA 108 on degradation of crude oil and also the PGPR activities. Soil quality improvement was also observed in this study by improvement in soil pH, organic carbon, conductivity. Soil-water relation for assessing the end point of biore-mediation is an important factor and was earlier reported by Li et al. (1997), which well supports the present findings.

Conclusion

From this investigation, it can be concluded that the two PGPR bacterial isolates used in the study can be used for bioremediation of crude oil contaminated soil. They are particularly helpful in plant health improvement grown in crude oil contaminated site.

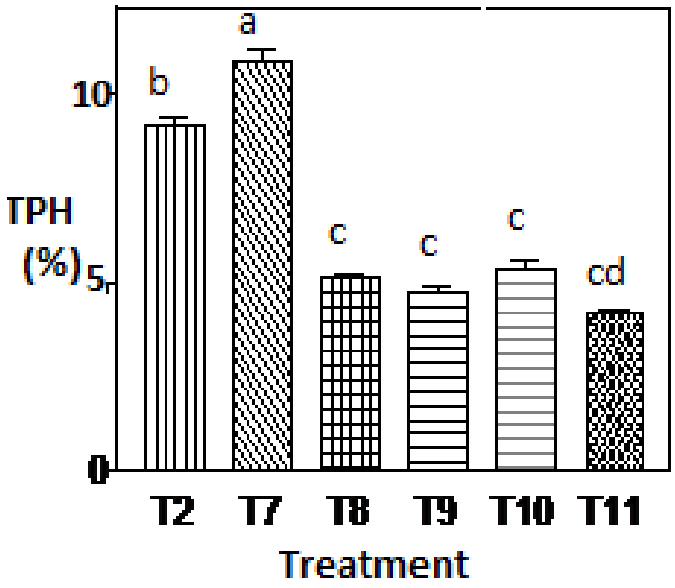

Figure 7. Comparison of effect of different treatments on soil TPH. Data are mean of three observations with three replications each; Standard error bars followed by similar letter are not significantly different from each other according to Tukey's test at $p < 0.01$.

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