CONCLUSION
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In the present study, the crude oil contaminated soils collected from Amguri, Borhulla, Gelaky, Lakowa and Rudrasagar drill sites of upper Assam were found alkaline in nature with high concentration of TPH(15.0-32.8%). Moisture content in the contaminated soil was found less ranging from 7.3-10.6% compared to uncontaminated soil. Conductivity (1.0-4.6 mMhos) of the crude oil contaminated soil was also not as much of uncontaminated soil. Different elements essential for plant growth are detected in low concentration in the contaminated soil. While, concentration of heavy metal was found to be considerably higher in the contaminated soil before remediation. Apart from chemical characteristics, low microbial population and soil enzymatic activities are the biological characteristic of the contaminated soil. Such degraded lands of upper Assam are not suitable for plant growth and survivability. The chief motive of the present study is characterization, screening and evaluation of hydrocarbon degrading bacteria which can provide a passive bioremediation potential of the bacterial strains isolated from the crude oil contaminated soil. Total 50 nos. of bacterial strains were isolated from the crude oil contaminated soil of various drill sites of Assam using skinner’s medium, thiosulphate medium, nitrogen free medium and soil extract medium. Liquid mineral media (LMM) supplemented with different concentration of crude oil has been used for screening of hydrocarbon degrading bacteria. The degree of diversity of bacteria in crude oil contaminated soil was estimated by means of morphological and biochemical characterization of the isolates. The microscopic observation of the isolates showed that 28 numbers of isolates were gram positive while the other 22 numbers are found gram negative.

Out of 50 nos., 5 nos. of isolates designated as 3SG, 4SG, 18ML, 22ML and 26ML showed growth in LMM with 5% (v/v) crude oil in 48-72h. The morphological, biochemical and molecular characterization of amplified 16S rDNA confirmed that the strains 18ML, 22ML
and 26ML belong to the genus *Microbacterium* (GeneBank accession no. KF732707), *Brevundimonas* (GeneBank accession no. KF732706) and *Cellulosimicrobium* (GeneBank accession no. KF732709) respectively. While both the strain 3SG and 4SG were identified as *Brevibacillus laterosporus* (GeneBank accession no. KF732710 and KF732708 respectively). Hydrocarbon degradation potential of the 5 isolates was studied in liquid media with crude oil concentration 10-30%. Among the 5 isolates, two isolates *Cellulosimicrobium* sp. strain 26ML and *B. laterosporus* strain 3SG isolated from Lakowa and Gelaky drill site respectively showed significant growth in LMM with 30% crude oil. Apart from the two strains, growth of another two previously isolated hydrocarbon degrading bacterial strains *Pseudomonas aeruginosa* N3 and N4 (Saikia et al. 2007) were studied in LMM with crude oil concentration 10-30%. Growth study of the respective strains was evaluated in liquid media containing model hydrocarbon compounds (200ppm, v/v) and in soil condition. As *Cellulosimicrobium* sp. strain 26ML and *B. laterosporus* strain 3SG showed best growth which was indicated by highest log_{10}CFU/ml after 360h, hence these two strains were selected for the present study. The growth activities of *Cellulosimicrobium* sp. strain 26ML and *B. laterosporus* strain 3SG are analyzed in aliphatic hydrocarbon viz., n-heptane, n-dodecane, n-hexadecane and polycyclic aromatic hydrocarbons (PAHs) like naphthalene, anthracene and fluoranthene. The degradation kinetics of the n-heptanes, n-dodecane, n-hexadecane, naphthalene and anthracene was studied using gas chromatography (G/C) analysis of biodegradation products of the respective aliphatic and polycyclic aromatic hydrocarbons. The result confirmed that between the two types of hydrocarbons, both the strains showed best growth in aliphatic compounds than the polycyclic aromatic hydrocarbon compounds. In the present study degradation potential of *Cellulosimicrobium* sp. strain 26ML and *B. laterosporus* strain 3SG was evaluated in soil condition with respect to time. Concentration of crude oil in soil was estimated below 14% after 3 months of bacterial treatment. After 1 year of treatment with *Cellulosimicrobium* sp.
strain 26ML concentration of crude oil was estimated 4.1% in Rudrasagar soil followed by Amguri (4.0%), Borhulla (8.0%), Lakowa (9.0%) and Gelaky (9.3%). Concentration of crude oil was determined 4.3% in Rudrasagar soil which is considered as the highest gain compared to the contaminated soil of the remaining drill sites treated with *B. laterosporus* strain 3SG.

Efficacy of *Cellulosimicrobium* sp. 26ML and *B. laterosporus* 3SG in germination of seeds in crude oil contaminated soil was evaluated on four plant species viz., *Vigna unguiculata* (Long Bean), *Zea mays* (Maize), *Vigna catjung* (French bean) and *Cicer arietinum* (Gram). Result of seed germination experiment showed that germination of seeds of the particular plant species did not respond to crude oil level 15.0-32.8%. However, germination of seeds of the plant species occurred which were introduced in soil after 3 months of application of bacterial inocula and the crude oil concentration was <10.08-13.8%. Root and shoot development of the respective plant species were impressive in the crude oil contaminated soil treated with *Cellulosimicrobium* sp. strain 26ML and *B. laterosporus* strain 3SG separately. Application of bacterial inocula has also increased the quantity of plant dry biomass.

Similarly, effect of bacterial treatment on physiological and biochemical parameters of *Thevetia peruviana* was studied. *T. peruviana* planted in contaminated soil under control condition showed that *T. peruviana* could not survive in soil contaminated with 15.0-32.8% crude oil. However, *T. peruviana* showed a slight growth and survivalability in contaminated soil of Amguri (15.1% oil) and Rudrasagar drill site (15.0%) up to 3 months of plantation. Though after 3 months, no plants survived. In contrast, contaminated soil treated with *Cellulosimicrobium* sp. strain 26ML and *B. laterosporus* strain 3SG showed encouraging survival rate of *Thevetia peruviana*. Data recorded on height of *T. peruviana* grown in treated soil at 1 month interval indicated that application of both the bacterial treatments led to the higher increase in the height compared to the plants of untreated soil. As compared with untreated soil, *Cellulosimicrobium* sp. strain 26ML and *B. laterosporus* strain 3SG treated soil
showed higher plant height with maximum number of leaves. Rate of transpiration and photosynthesis of *T. peruviana* was determined to be increased with decrease in concentration of crude oil in case of both the treatments. Biochemical changes of plants due to contaminated environment were assessed in two parameters viz. concentration of total chlorophyll content carbohydrate. Application of *Cellulosimicrobium* sp. strain 26ML and *B. laterosporus* strain 3SG showed significantly higher amount of chlorophyll and carbohydrate in leaves of *T. peruviana* grown in Rudrasagar soil followed by Amguri, Lakowa, Borhulla and Gelaky drill site after 1 year of plantation of *T. peruviana*.

In 1 year old *T. peruviana* plants the metal accumulation pattern was estimated in different parts (leaf, root and shoot) of *T. peruviana*. Both treatments i.e., *Cellulosimicrobium* sp. strain 26ML and *B. laterosporus* strain 3SG showed higher amount of heavy metal in roots compared to shoot and leaves of *T. peruviana*. Among the different drill sites, highest amount of heavy metal was detected in roots of *T. peruviana* grown in contaminated soil of Gelaky drill site treated with *Cellulosimicrobium* sp. strain 26ML. On the other hand, treatment of *B. laterosporus* strain 3SG showed highest metal Pb that was found in Gelaky (300µgKg$^{-1}$) soil. Finally, physical, chemical and biological changes of the treated soil were estimated after 1 year of bioremediation of crude oil contaminated soil. The result revealed that application of the two individual bacterial inocula *Cellulosimicrobium* sp. strain 26ML and *B. laterosporus* strain 3SG in crude oil contaminated soil led to the decrease of soil pH, concentration of TPH and heavy metal and as well as improvement of soil biological characteristics and available nutrient. Bioassay performed with the remediated soil improvement in soil physical, chemical and biological characteristics which supports Both the *Cellulosimicrobium* sp. strain 26ML and *B. laterosporus* strain 3SG as a proficient agent in bioremediation of hydrocarbon contaminated soil. Finally, the present study concludes the importance of screening,
characterization and evaluation of potential hydrocarbon degraders for decontamination of crude oil contaminated soil in environmental restoration.