Remediation of hydrocarbons contaminated soil using Plant Growth Promotory Rhizobacteria and *Ricinus communis* (L.)

SUMMARY OF

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Submitted By

Shweta Ambust

(Enrolment No. 024/13)

Under the Supervision of

Prof. Rajesh Kumar

DEPARTMENT OF ENVIRONMENTAL MICROBIOLOGY

SCHOOL FOR ENVIRONMENTAL SCIENCES

BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY

(A CENTRAL UNIVERSITY)

VIDYA VIHAR, RAEBARELI ROAD, LUCKNOW-226025

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Summary

Energy is primarily obtained from petroleum-based products in industry and daily life. Petroleum currently poses a hazard to the environment in today's contemporary, mechanized civilization because of its large scale extraction and waste dumping, oil spillages during transportation. Petroleum products have contaminated soil globally as a result of their widespread use. Soil biocenosis is negatively affected by petroleum pollution, causing serious chemical changes in soil structure, properties, and reducing soil fertility. Furthermore, they decrease agricultural productivity and pollute groundwater, which has a detrimental effect on health and the economy. Oxygen, water, and nutrients (phosphorus and nitrogen) in soil can be adversely affected by this type of contamination. A further consequence of these contaminants is a reduction in soil's hydrophobicity, an increase in trace element content, and a reduction in the oxygen level, thereby reducing the soil's ability to hold water. As a result, this pollution needs focus on a large scale for remediation of soil contaminated with petroleum hydrocarbons. There are two major processes for bioremediation that are: a) biostimulation and b) bioaugmentation. Bioaugmentation and Biodegradation are the subject of this study, occurs primarily through the soil microbiome obtained from nature (rhizosphere of *Ricinus communis* L.). Compared to physical and chemical remediation methods, these methods are highly effective, low cost, and produce harmless products (mainly CO_2 and water). Plant Growth Promotory Rhizobacteria (PGPR) residing in the rhizospheric zone interact closely with the roots of the plant and synthesize metabolites like siderophore, indole acetic acid, phosphatases, ACC deaminase activity, hydrogen cyanide production etc. Besides this, they also produce biosurfactants that help in mitigating abiotic and biotic stress. Different classes of biosurfactants have been reported in literature that play a role and

in the present study also they have been found to play a major role in bioremediation of petroleum hydrocarbons in the contaminated soil. This study was therefore focused on bioremediation potential of PGPR bacterial strains isolated from rhizosphere of *Ricinus communis* L. which was the plant of choice as it has been reported to be a good remediator but scanty literature is available on this plant for bioremediation, so this plant was selected for the study. These biosurfactants producing bacterial strains having plant growth promoting traits are an added advantage in bioremediation and is a popular and eco friendly method gaining attention worldwide. The key findings of the study are summarized below:

- Present study has been based on four bacterial strains that were isolated from petroleum-contaminated sites at Lucknow (26°55' N latitude and 80° 59' E longitude), Uttar Pradesh, India. Studies involving morphological, biochemical, and 16S rRNA parameters revealed that the isolated bacterial strains were similar to *Pseudomonas taiwanensis* (SA3) with accession number MW750216, *Pseudomonas aeruginosa* (SA9) with accession number MW730535, *Pseudomonas plecoglossicida* (A1) with accession number OP143795 and *Bacillus subtilis* (T1) with accession number MW730518. All the four bacterial strains were screened for Plant growth-promoting characteristics. The four selected strains produced a clear zone on pikovaskaya agar plates around the bacterial colony, indicating a positive result. Quantitative tests for tricalcium phosphate solubilisation by the test strain in liquid revealed that strain was able to solubilize tricalcium phosphate. On Chrome Azurol-S media, the qualitative and quantitative siderophore tests showed positive results for strains SA3, SA9, A1 and T1 as evidenced by the colour change of the media around the colonies. IAA

was measured qualitatively, quantitatively in the presence of 100 g/ml tryptophan concentration and found that all strains (SA3, SA9, A1 and T1) were able to generate good amounts of IAA. Abiotic stress conditions are well tolerated by all 4 strains, which is demonstrated by their strong production of HCN and activity of ACC deaminase. These PGPR strains also produce biosurfactant on Bushnell Hass media. All these four strains produce foam with the biosurfactant production and were positive for drop collapse test. Various tests are carried out on biosurfactants isolated from the selected bacteria, including testing for oil displacement, emulsification index, surface tension reduction and cell surface hydrophobicity. If the biosurfactant is present in the supernatant, the oil is displaced and a clear zone is formed. The quantitative estimation is measured by the diameter of this clear zone on the oil surface correlates to surfactant activity, also called oil displacement activity. Diameter of displaced oil by SA3 biosurfactant is 2.5±0.1, Diameter of displaced oil by SA9 biosurfactant was 7.9±0.1, Diameter of displaced oil by A1 biosurfactant is 6.3±0.2 and Diameter of displaced oil by T1 biosurfactant was 5.6±0.1. Emulsification index of biosurfactant produced by the strain SA3 was 43±2.6%, SA9 was 92±2%, A1 was 84±2% and T1 54±1%, respectively. The surface tension of non inoculated broth was 65.8 mN/m. After 72 h, the surface tension of the broth was 34.5±0.4 mN/m for SA3, 28.79±0.3 mN/m for SA9, 33.44±0.4 mN/m for A1 and 31.6±0.5 mN/m for T1. Based on these results, it appears that the strains produce biosurfactants that reduce surface tension effectively. An analysis of the extracted biosurfactants by FTIR and Liquid Chromatography-Mass Spectrometry (LC-MS) reveals that SA3

biosurfactant is a mono-rhamnolipid, while SA9 biosurfactant is both a mono-rhamnolipid and a di-rhamnolipid, whereas A1's biosurfactant is a mono-rhamnolipid, and T1's is a surfactin type of biosurfactant. Root exudates, extracted from the healthy plant of *Ricinus communis* L. and characterized by LC-MS analysis, revealed presence of sugar compounds such as glucose, sucrose and fructose. The effects of sugars found in root exudates of *Ricinus communis* (L.) on PGP potential (IAA, siderophore production and phosphate solubilization) of selected isolates illustrated that PGP activity gets enhanced after adding sugars (glucose, sucrose, and fructose) found in root exudates as compared to the normal media. However, the PGP activities are less with maltose, a sugar that was not found in root exudates and used for comparison. Thus, this shows the interaction study between selected bacteria (SA3, SA9, A1 and T1) and plants. As part of the consortium development process, compatibility tests were conducted on all four strains and it was observed that SA3, SA9 and A1 were compatible with one another, while T1 was not. Thus, strains SA3, SA9, and A1 were chosen for the pot study and degradation test. Using a pot experiment, studied the effects of PGP and biosurfactant metabolites produced by bacteria and plant (*Ricinus communis*) growing on contaminated soil for the detoxification of petroleum hydrocarbons contamination (bioremediation). First, garden soil was autoclaved and then used in the remediation experiment. After that, crude biosurfactant and bacterial culture were added to soil contaminated with petrol engine oil (7.5 kg) at concentrations of 1% to 3%, respectively, in each pot over a period of 10 days, and the mixture stabilized and evaporated in accordance with the experimental design.

- **Treatment 1**

EX C (Positive control) = 7.5 kg soil + castor plant

EX C (Negative control) = 7.5 kg soil + 1% spent engine oil + castor plant

EX 1(A) = 7.5 kg soil + 1% spent engine oil + 1% *Pseudomonas taiwanensis* (SA3) + castor plant

EX 1(B)= 7.5 kg soil + 1% spent engine oil +1% *Pseudomonas aeruginosa* (SA9) + castor plant

EX 1(C) = 7.5 kg soil + 1% spent engine oil + 1% *Pseudomonas plecoglosscida* (A1) + castor plant

EX 1(D) = 7.5 kg soil + 1% spent engine oil + 1% consortia + castor plant

- **Treatment 2**

EX C (Positive control) = 7.5 kg soil + castor plant

EX C (Negative control) = 7.5 kg soil + 3% spent engine oil + castor plant

EX 2(A) = 7.5 kg soil + 3% spent engine oil + 1% *Pseudomonas taiwanensis* (SA3) + castor plant

EX 2(B)= 7.5 kg soil + 3% spent engine oil +1% *Pseudomonas aeruginosa* (SA9) + castor plant

EX 2(C) = 7.5 kg soil + 3% spent engine oil + 1% *Pseudomonas plecoglosscida* (A1) + castor plant

EX 2(D) = 7.5 kg soil + 3% spent engine oil + castor plant + 1% consortia

- **Treatment 3**

EX C (Positive control) = 7.5 kg soil + castor plant

EX C (Negative control) = 7.5 kg soil + 1% spent engine oil + castor plant

EX3A= 7.5 kg soil + 1% spent engine oil + 1% *Pseudomonas taiwanensis* SA3 + 2% crude biosurfactant + castor plant

EX3B= 7.5 kg soil + 1% spent engine oil +1% *Pseudomonas aeruginosa* SA9+ 2% crude biosurfactant + castor plant

EX3C= 7.5 kg soil + 1% spent engine oil + 1% *Pseudomonas plecoglosscida* A1 + 2% crude biosurfactant + castor plant

EX3D= 7.5 kg soil + 1% spent engine oil + 1% consortia + 2% crude biosurfactant + castor plant

- **Treatment 4**

EX C (Positive control) = 7.5 kg soil + castor plant

EX C (Negative control) = 7.5 kg soil + 3% spent engine oil + castor plant

EX4A= 7.5 kg soil + 3% spent engine oil + 1% *Pseudomonas taiwanensis* SA3 + 2% crude biosurfactant + castor plant

EX4B= 7.5 kg soil + 3% spent engine oil +1% *Pseudomonas aeruginosa* SA9+ 2% crude biosurfactant + castor plant

EX4C= 7.5 kg soil + 3% spent engine oil + 1% *Pseudomonas plecoglosscida* A1 + 2% crude biosurfactant + castor plant

EX4D= 7.5 kg soil + 3% spent engine oil + 1% consortia + 2% crude biosurfactant + castor plant

In comparison with the treatment EX C - negative control (1% and 3%) i.e. untreated pots, plants treated with bacteria strains and bacteria strains with biosurfactant in pots reported high values for all parameters studied, i.e. germination, shoot length, root length, fresh and dry weight.. In addition, phytochemicals and antioxidant activity indicate a variation in the stress effect of petroleum contaminated soil under bacterial and nonbacterial treatment. Gas chromatography-mass spectrometry analysis was performed on soil that was contaminated with spent engine oil as well as soil that had been treated with gas chromatography-mass spectrometry (GCMS), a method that

reveals the presence of hydrocarbons. The detected hydrocarbon chromatogram is identified using (The National Institute of Standards and Technology) mass spectral library. Thereafter, the contaminated soil was subjected to bioremediation treatment within lab scale using the selected bacterial strains. Based on the results of the GC-MS study, bacterial strains are capable of remediating petroleum-contaminated soil, and they are also capable of degrading some compounds in 90 days. Hence, Microbial remediation of oil-contaminated soils can be improved by using bacterial consortium to effectively degrade petroleum hydrocarbon compounds and utilize them as carbon and energy sources as compared to single strain.

Based on the above findings, it can be concluded that *Ricinus communis* L. in combination with bacterial consortium developed in the present study can be effectively used for bioremediation of petroleum hydrocarbons contaminated soil and there is a positive interaction (quorum sensing) between castor plant root exudates and microbial for remediation.