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# Bioremediation Potential of Plant Growth Promoting Rhizobacteria (PGPR) to Promote *Zeamays* L. growth under Petrol Stress

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## ABSTRACT

**Introduction:** Bacteria produce various extracellular substances with variable properties and applications. Among these microbial products, bacteria possessing plant growth promoting traits together with biosurfactants may improve plant growth in petroleum contaminated areas.

**Aims:** The aim of the current study is the potential use of biosurfactant producing plant growth promoting rhizobacteria (PGPR) to enhance the physio-chemical traits of maize plants grown under petrol stressed soil.

**Methodology:** For the current study, a strategy has been shaped for plant growth improvement using biosurfactant producing PGPR. Initially ten bacteria were taken which were screened for biosurfactant production vis-à-vis plant growth promoting attributes. After selecting the most efficient bacteria on the basis of screening results, *in vitro* plant assay was conducted to observe the ameliorating response of maize plant growth, grown under 1 and 2% of petrol stress (v/w) in terms of physio-chemical traits of plants.

**Results:** Three bacteria were selected as most potent surfactant producing PGPR i.e., *Enterobactercloacae* (A9G), *Exiguobacterium* sp. (A13G) and *Bacillus pumilus* (ALa). These bacterial strains revealed their amphiphilic nature by showing high efficiency for flattening and collapsing property and degrading ability for hydrocarbons (HC) and showed emulsification capability more than 50%. In alliance with this, these strains exhibited auxin and ammonia production potential and showed zinc solubilizing ability. The dual nature of these bacteria made the work worthy for assessing their potential to enhance the plant growth under petrol stress. The results of plant assay imparted a coherent picture of increment in growth and biochemical attributes of treated plants while among selected strains, *Bacillus pumilus* (ALa) exhibited tremendous increase in all studied parameters.

**Conclusion:** Current study envisages an eco-friendly biological method to improve the plant growth under petrol contaminated conditions by showing biosurfactant production, hydrocarbon degradation and plant growth improving attributes.

**Key Words:** Bioremediation, Biosurfactants, Emulsification, Hydrocarbon, PGPR, *Zea mays* L

## INTRODUCTION

Global industrialization and rapid increase in population caused increase in global demands of energy which is being fulfilled by exploiting various natural resources specifically hydrocarbons.[1] Inadvertent release of petroleum products are of specific concern in the environment. For deterioration of such compounds, chemical surfactants are being in use but causing severe threat to the environment.[2] The paradigm shift from synthetic approaches to biological means give an insight to develop some innovative and eco-friendly technique emphasizing on indigenous soil microbiota.[3] Usage of biosurfactants is an effective way to meet the challenges regarding hydrocarbons contamination in ecosystem rather than using chemical surfactants.

It has been reported that bacteria may produce biosurfactants and bioemulsifiers and act as natural tool to remediate hydrocarbon or petrol pollution.[4,5] Biosurfactants are microbial metabolites having amphiphilic nature showing both hydrophilic and hydrophobic moieties and tend to accumulate at interface of hydrocarbons and water or any other surface.[6]Bacterial surfactants possess lipids,polysaccharides, fatty acid complexes, lipopeptides,

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proteins and other biological compounds containing carboxyl, phosphate or amino groups.[7] On the other hand, bacteria residing in rhizosphere may have potential to secrete some plant growth promoting substances that may involve in rapid stimulation of plant growth, such kind of bacteria are known as plant growth promoting rhizobacteria (PGPR). These bacteria stimulate plant growth by regulating the synthesis of phytohormones, ammonia, enzymes, siderophores, by fixing nitrogen or by solubilizing minerals, thus may be used as bioinoculants, biofertilizers and biocontrol agents.[8] These traits when combined with bacterial capability to produce extracellular biosurfactants may facilitate the productive growth of plants in hydrocarbons, oil, petrol or diesel contaminated soil environments.

*Zea mays* L. is an important economical staple food and carries a major part of global food production, thus, it is essential to get high yield of maize plants. Instead of wasting the petroleum contaminated areas, this land can be utilized for agricultural productivity. For that purpose, the present research has been designed which aimed at the biological green way to make possible the growth of maize plants in those areas which are being contaminated with petroleum hydrocarbons making the land infertile for plants.

## MATERIALS AND METHODS

### Bacterial growth conditions

The current study comprised of bacterial assessment for biosurfactant production potential together with plant growth stimulating capability. In order to do so, ten bacteria were used isolated from the rhizosphere of different plants [i.e., AAL1, AL2, AB8 (*Solanum nigrum*), A3E, A9G, A12G, (*Malvestrum tricuspidatum*), P4 (*Populus* sp.), A7B (*Sonchus oleraceae*) and A13G (*Cassia occidentalis*)] which were already characterized and identified.[9]Also, rhizospheric bacterial isolate (ALa) was used for the current research study, which was also previously identified.[10]Isolates were routinely grown on LB-agar medium following incubation at 37°C for 24 hours.

### Screening profile for bacterial biosurfactants

Biosurfactants production potential of bacterial strains was detected using two different sources of hydrocarbons (HC) i.e., *n*-hexane and toluene as hydrophobic substrates. Bacteria were primarily observed for their ability to produce surfactants. For which, strains were observed for hemolytic activity using 5% fresh sheep blood and after incubation for 48 hours, α, β and γ-type of hemolysis was noted. Similarly, drop collapsing ability of bacterial strains was detected following Mahalingam and Sampath[11] while oil spreading assay was carried out following Thavasi et al.[12] Also, HC degrading potential was observed following 2,6-dichlorophenol indophenol (DCPIP) method[13] using 1 and 2% of HC as sole carbon source. For secondary screening, emulsification capacity assay and bacterial adhesion to hydrocarbon (BATH) assaywas performed following Shoeb et al.[14] and Thavasi et al.[12] respectively.

### Screening profile for PGPR attributes

For plant growth promoting attributes, bacterial IAA (indole-3-acetic acid) was determined following Habib et al.[15] Similarly, bacterial ammonification assay and zinc solubilization assay was carried out following Goswami et al.[16] and Khanghahi et al.[17] respectively.

### *In vitro* plant assay

On the basis of screening profile of bacteria, most efficient bacterial strains were further used for plant growth evaluation under petrol stress using randomized complete block design. *Zea mays* L. was used as test plant. Certified seeds (var. DK-6714) were surface sterilized and inoculated with bacterial cultures after adjusting optical densities at 600nm to $10^6$ to $10^7$ CFU/ml for one hour. Experiment was accomplished with four replicates while seven seeds were sown per pot containing 165g of sterilized sieved soil. Two different stress conditions were used for petrol i.e., 1% and 2% on the basis of soil dry weight. Petrol treatment was given to soil at seedling stage of maize plants, after three days of germination. Treatments used for the experiment were; control (without petrol stress and bacterial treatments), negative control (with 1% and 2% petrol stress without bacterial treatments), bacterial treatments (without petrol stress) and bacterial treatments with 1% and 2% petrol stress respectively. After 24 days of growth, seedlings were removed from their respective pots and analyzed for growth attributes i.e., plant height, fresh weight, root length and number of leaves while for biochemical analysis, plants were observed for chlorophyll content, total soluble sugars (TSS), free aminoacid and proline content following Lichtenthaler and Wellburn,[18] Tiwari et al.,[19] Khanna et al.[8] and Karthik et al.[20] respectively.

### Statistical analysis

All data attained were statistically analyzed by Duncan's multiple range (DMR) test using SPSS v.16 software with 95% confidence level (P = 0.05).

## RESULTS

### Screening profile for bacterial biosurfactants

Taking screening profile of bacterial strains into critical consideration, three most efficient bacteria were selected for the current research study i.e., *Enterobactercloacae* (A9G) [Accession no. HQ202888], *Exiguobacterium* sp. (A13G) [Accession no. HQ202890] and *Bacillus pumilus*(ALa) [Accession

no. MG988291] out of ten isolates. Among these, *Enterobactercloacae* (A9G) showed β type of hemolysis while other exhibited α-type of hemolysis, primarily confirming the presence of surfactants. Also, all these strains displayed flattening property of cell-free broth and showed moderate oil displacement zones while other strains were negative for drop collapse and oil spreading methods. These two assays are sensitive and convenient methods corroborating with each other. Further, selected strains exhibited complete decolorization in the presence of 1% HC when DCPIP was used as redox indicator while in the presence of 2% HC, incomplete decolorization was recorded showing bacterial potential for HC degradation (Fig. 2B). Likewise, emulsification capacity assay indicated 58.4, 53.9 and 50.9% emulsification exhibited by *Bacillus pumilus* (ALa), *Exiguobacterium* sp. (A13G) and *Enterobactercloacae* (A9G) using hexane as hydrophobic substrate while with toluene, emulsification percentagewas 62.5, 61.3 and 60.3% for *Enterobactercloacae* (A9G), *Bacillus pumilus* (ALa) and *Exiguobacterium* sp. (A13G) respectively (Fig.1A and 2A). Other strains have shown minor emulsification activity lesser than 20%. Also, bacterial adhesion to hydrocarbons (BATH) assay revealed that when hexane was used as hydrophobic substrate, percentage of hydrophobicity of bacterial cells was 56.3, 54.3 and 53.1% exhibited by *Enterobactercloacae* (A9G), *Exiguobacterium* sp. (A13G) and *Bacillus pumilus* (ALa) respectively, while 53.2, 51.4 and 47.7% hydrophobicity of cells with toluene was shown by *Exiguobacterium* sp. (A13G), *Bacillus pumilus* (ALa) and *Enterobactercloacae* (A9G) respectively (Fig. 1B). On the contrary, other isolates demonstrated less than 15% adherence to HC.

## Screening profile for PGPR attributes

Bacterial strains *Bacillus pumilus* (ALa), *Enterobactercloacae* (A9G) and *Exiguobacterium* sp. (A13G) synthesized maximum IAA i.e., 195.7, 159 and 155µg/ml respectively, while isolates AAL1, A12G and A7B were moderately IAA producing. Similarly, *Bacillus pumilus* (ALa), *Enterobactercloacae* (A9G) and *Exiguobacterium* sp. (A13G) produced 12, 11.1 and 9.4 millimoles of ammonia respectively, while lower amount of ammonia was produced by other isolates. Further, analyzing the results of zinc solubilizing assay, it was revealed that *Bacillus pumilus* (ALa) and *Exiguobacterium* sp. (A13G) showed slight increment in zinc solubilization after seven days of incubation till fourteen days while *Enterobactercloacae* (A9G) exhibited prominent rise in solubilization when incubated for fourteen days. This time-dependent increase suggested variation in bacterial solubilizing efficiency at different intervals of time. It was found that *Enterobactercloacae* (A9G), *Bacillus pumilus* (ALa) and *Exiguobacterium* sp. (A13G) showed 286.7, 226.7 and 146.7% zinc solubilizing efficiency (ZSE) when bacteria were incubated for seven days, however ZSE was increased upto 446.7, 240 and 226.7% upon incubation of fourteen days respectively (Fig. 3).

## *In vitro* plant assay

### Plant growth attributes

Based on the outcomes of screening profile of bacterial biosurfactants and PGPR attributes, three best bacterial strains were selected and utilized for *in vitro* plant assay. Analysis of growth attributes of treated plants showed that *Bacillus pumilus* (ALa), *Enterobactercloacae* (A9G) and *Exiguobacterium* sp. (A13G) showed 54.8, 33.1 and 25.6% increase in plant height respectively, over control when plants were grown without petrol stress. Under 1% petrol stress, 136.7, 99 and 34.5% increment was recorded in plants with treatments of *Bacillus pumilus* (ALa), *Exiguobacterium* sp. (A13G) and *Enterobactercloacae* (A9G) respectively, while *Bacillus pumilus* (ALa), *Exiguobacterium* sp. (A13G) and *Enterobactercloacae* (A9G) exhibited esteemed proliferation in plant height upto 57.4, 37.9 and 31.4% under 2% of petrol stress respectively, in contrast with plant height of respective control. Likewise, when plants were grown without petrol stress, root lengths were increased upto 21.3, 18.1 and 6.9% upon treatments with *Bacillus pumilus* (ALa), *Enterobactercloacae* (A9G) and *Exiguobacterium* sp. (A13G) respectively, over non-inoculated control. On contrary, plants inoculated with *Exiguobacterium* sp. (A13G), *Bacillus pumilus* (ALa) and *Enterobactercloacae* (A9G) caused significant increment in root length i.e., 66.3, 63.7 and 49.9% in the presence of 1% petrol, whereas increase in root length was 80.1, 70.8 and 40.9% in plants treated with *Bacillus pumilus* (ALa), *Exiguobacterium* sp. (A13G) and *Enterobactercloacae* (A9G) under 2% petrol stress respectively, over their respective control.

In addition, 9.09% increase in leaf number was observed for all bacterial treatments without petrol stress, over control. However, with 1% petrol stress, *Enterobactercloacae* (A9G), *Bacillus pumilus* (ALa) and *Exiguobacterium* sp. (A13G) triggered 10.5, 8.7 and 1.7% increase in leaf number while with 2% petrol stress, the number of leaves were increased upto 44.7, 39.4 and 31.5% when treated with *Enterobactercloacae* (A9G), *Bacillus pumilus* (ALa) and *Exiguobacterium* sp. (A13G) respectively, oer their respective control plants. Further, fresh weight of treated plants was also significantly increased upto 96.3, 88 and 87.2% in plants inoculated with *Bacillus pumilus* (ALa), *Enterobactercloacae* (A9G) and *Exiguobacterium* sp. (A13G) respectively, over non-inoculated plants. Significant improvement in fresh weight was also recorded in plants with treatment of *Bacillus pumilus* (ALa), *Exiguobacterium* sp. (A13G) and *Enterobactercloacae* (A9G) under 1% petrol stress, which was 145.1, 60.4 and 48.5% whereas, 75.5, 38.5 and 18.7% enhancement was caused by *Bacillus pumilus* (ALa), *Enterobactercloacae* (A9G) and *Exiguobacterium* sp. (A13G) when plants were grown under 2% petrol stress respectively, compared with respective control treatments (Fig. 4).

### Plant biochemical attributes

Plants were analyzed biochemically showing increase in biochemical features with respect to control treatments. Bacterial application with *Enterobactercloacae* (A9G), *Bacillus pumilus* (ALa) and *Exiguobacterium* sp. (A13G) exhibited 200, 198.9 and 44.1% elevation in chlorophyll 'a' content of treated plants while 91.1, 87.2 and 67.7% increase in chlorophyll 'b' content was seen in treated plants upon *Exiguobacterium* sp. (A13G), *Bacillus pumilus* (ALa) and *Enterobactercloacae* (A9G) treatments respectively, comparing with control. Similarly, carotenoid content was significantly increased upto 86.3%, when plants were treated with *Bacillus pumilus* (ALa), while *Exiguobacterium* sp. (A13G) and *Enterobactercloacae* (A9G) failed to increase carotenoid content over respective control plants. Under 1% petrol stress, increase in chlorophyll 'a' content was 209.4, 87.6 and 10.7% in *Enterobactercloacae* (A9G), *Bacillus pumilus* (ALa) and *Exiguobacterium* sp. (A13G) treated plants, whereas 88.1, 76.9 and 9.94% improvement in chlorophyll 'b' content was recorded upon inoculations with *Bacillus pumilus* (ALa), *Enterobactercloacae* (A9G) and *Exiguobacterium* sp. (A13G) respectively in treated plants, over control. Prominent enhancements in carotenoid content was shown by *Bacillus pumilus* (ALa), *Enterobactercloacae* (A9G) and *Exiguobacterium* sp. (A13G) inoculated plants i.e., 140.5, 11.6% (significant increase) and 1.2% (insignificant increase) respectively, compared to control. When plants were grown under 2% petrol stress, *Enterobactercloacae* (A9G), *Exiguobacterium* sp. (A13G) and *Bacillus pumilus* (ALa) treatments showed 207.7, 106.4% (significant) and 16.5% (insignificant) enhancements in chlorophyll 'a' content, treatments with *Bacillus pumilus* (ALa), *Enterobactercloacae* (A9G) and *Exiguobacterium* sp. (A13G) exhibited 117.1, 103.8 and 39.1% improvement in chlorophyll 'b' content while *Bacillus pumilus* (ALa) and *Enterobactercloacae* (A9G) inoculations caused significant enhancements in carotenoid content upto 113 and 36.5%, respectively whereas *Exiguobacterium* sp. (A13G) failed to increase carotenoid content, when compared with their respective control treatments (Fig. 5). Similarly, bacterial treatments with *Bacillus pumilus* (ALa), *Exiguobacterium* sp. (A13G) and *Enterobactercloacae* (A9G) tended to enhance total soluble sugars (TSS) of treated plants upto 81.2, 62.6 and 58% when seedlings were grown without petrol stress. In the presence of 1% petrol stress, 143.9, 84.2 and 73.7% increment in TSS was observed with *Bacillus pumilus* (ALa), *Enterobactercloacae* (A9G) and *Exiguobacterium* sp. (A13G) supplementation in treated plants while *Bacillus pumilus* (ALa), *Enterobactercloacae* (A9G) and *Exiguobacterium* sp. (A13G) inoculations tended to increase soluble sugars upto 211.1, 120.4 and 93.5% respectively in treated plants, over control plants, in the presence of 2% petrol stress (Fig. 6). Moreover, prominent increase in free amino acid content was recorded with treatments of *Bacillus pumilus* (ALa), *Exiguobacterium* sp. (A13G) and *Enterobactercloacae* (A9G) exhibiting 89.6, 51.2 and 20.9% increase respectively in treated plants, over non-inoculated control. Likewise, 81.3, 66.2% (significant) and 0.6% (insignificant) increase in free amino acid content was observed upon supplementation of *Bacillus pumilus* (ALa), *Exiguobacterium* sp. (A13G) and *Enterobactercloacae* (A9G) to the treated plants under 1% petrol stress while 88.5% (significant) 40.1 and 35.5% (insignificant) enhancements were shown by *Bacillus pumilus* (ALa), *Enterobactercloacae* (A9G) and *Exiguobacterium* sp. (A13G) treated plants when grown under 2% petrol stress conditions respectively, in comparison with their respective control treatments (Fig. 5). Further, there was 56, 56 and 39.3% increment in proline concentration of treated plants when supplemented with *Enterobactercloacae* (A9G), *Exiguobacterium* sp. (A13G) and *Bacillus pumilus* (ALa) respectively, over non-treated plants. On contrary, proline content was significantly increased upto 93.9, 62.2 and 20.3% in treated plants triggered by *Bacillus pumilus* (ALa), *Enterobactercloacae* (A9G) and *Exiguobacterium* sp. (A13G) treatments while 72.1, 56.9 and 30.5% increment was recorded by *Enterobactercloacae* (A9G), *Bacillus pumilus* (ALa) and *Exiguobacterium* sp. (A13G) inoculations under 1 and 2% petrol stress respectively, compared with respective control plants (Fig. 6).

## DISCUSSION

The present study was designed to meet the challenge of petroleum hydrocarbons pollution in a cost-effective and environmental friendly manner by using surfactants-producing plant growth promoting rhizobacteria (PGPR). Three bacteria [*Enterobactercloacae* (A9G), *Exiguobacterium* sp. (A13G) and *Bacillus pumilus* (ALa)] were used for the current study which were firstly screened for biosurfactants production. Previous studies revealed the potential of bacteria to release extracellular components that may have some lipid or fatty acid components having low surface tension, thus can degrade hydrocarbons owing to their amphiphilic nature.[21,22] The toxicity of petroleum hydrocarbons for ground water and soil microbiota have been studied widely, but still very little is known about plants cultivation in petrol polluted lands.

Bacteria with dual properties for biosurfactants and PGPR traits were used in the current study as bioinoculants in order to observe the growth pattern of *Zea mays* plants grown under 1 and 2% of petrol stress. Significant increase in plant height, root length, leaves number and fresh weight was recorded when bacteria were inoculated in the presence of petrol stress in comparison with non-inoculated treatments (Fig. 7). A study was presented in which growth improvement in *Withania somnifera* grown in petrol engine oil polluted soil-was observed, when inoculated with biosurfactants producing PGPR showing potential for IAA synthesis, siderophore production and phosphate solubilization.[23,24] Similarly, significant

increase in growth and biochemical traits of treated plants after bacterial application might be attributed to the surfactants production potential of bacteria which might accumulate at the soil-petrol interface due to their amphiphilic nature. Once the bacteria were able to break this barrier, they might be capable to colonize plant roots and owing to their IAA synthesizing ability and synthesis of ammonia and mineral solubilizing efficiency, they might have increased the growth of plants without experiencing any damage due to the petrol. The data showed decrease in growth and biochemical traits of plants, when plants were grown in presence of petrol stress without bacterial inoculations indicating that petrol toxicity damaged the roots making the nutrients and minerals unavailable for plant uptake, thus spoiled all plants' machinery. Petrol when present in soil, wrap around soil particles which can only be removed through microbial activity or by using chemicals. The late method being toxic to plant growth directs to the use of microbes for remediation purpose.[25]

## CONCLUSIONS

Bacterial degradation of petrol is one of the major natural mechanisms to remediate petrol pollution. Two-folded type of bacteria having potential for biosurfactants production and carrying PGPR traits were effectively utilized to enhance the plant growth in *Zea mays* L., grown under petrol stressed conditions. Metabolic capabilities and amphiphilic nature of these bacteria [*Enterobactercloacae* (A9G), *Exiguobacterium* sp. (A13G) and *Bacillus pumilus* (ALa)] gave encouraging results in terms of significant improvement in growth and biochemical features of plants when compared with respective control treatments while *Bacillus pumilus* (ALa) strain was good for all studied features. The current strategy for plant growth improvement in petrol contaminated sites is economically feasible showing environmental compatibility and thus will contribute in safeguarding the environment from petrol pollution to make plants' cultivation possible in such areas in better way. This aspect was not studied in detail in previous researches, thus present study gives an initiative to go for analyzing the petroleum biodegradation mechanisms using microbes for future studies.

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### Conflict of interest

Authors declare no conflict of interests.

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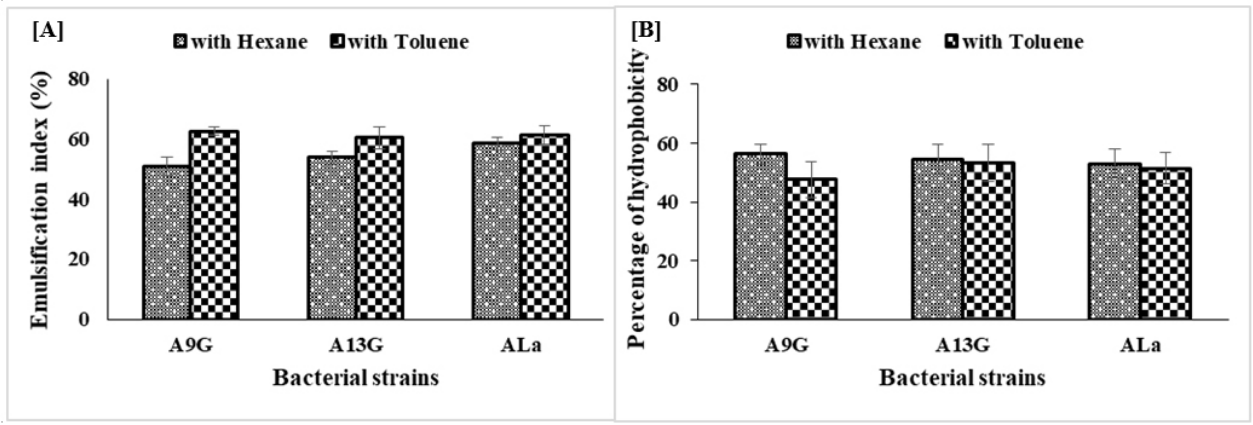


**Figure 1:** Emulsification index ($E_{24}$) of bacterial strains showing percentage of emulsification. Error bars are indicating error in reported measurements. [A]; Bacterial adhesion to hydrocarbons (BATH) showing percentage of hydrophobicity of bacterial strains when hexane and toluene were used as hydrophobic substrates [B] [A9G = *Enterobactercloacae*, A13G = *Exiguobacterium* sp. and ALa = *Bacillus pumilus*]

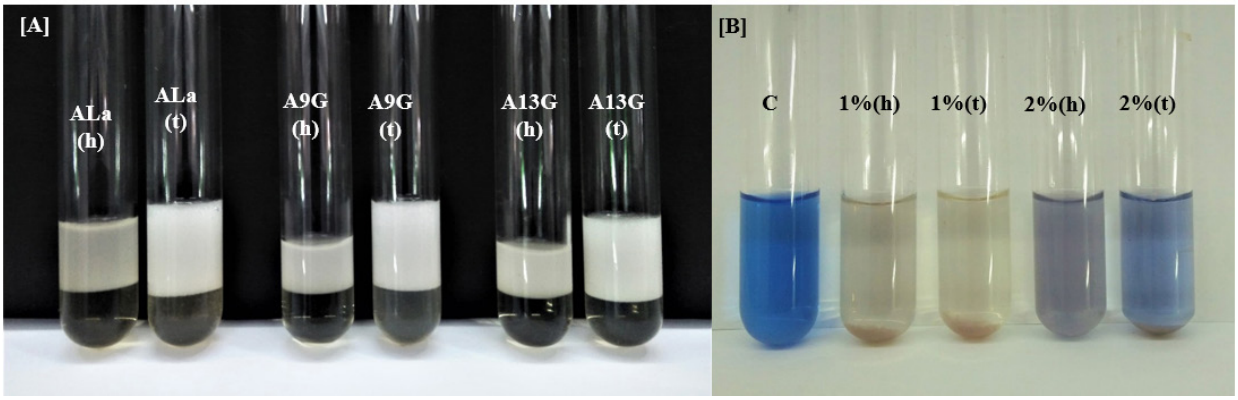


**Figure 2:** Emulsion layer of bacterial strains with hexane (h) and toluene (t) [A]; Biodegradation of *Exiguobacterium* sp. (A13G) at 1 and 2% of hexane (h) and toluene (t) [B] [C = Control, ALa = *Bacillus pumilus*, A9G = *Enterobactercloacae* and A13G = *Exiguobacterium* sp.]

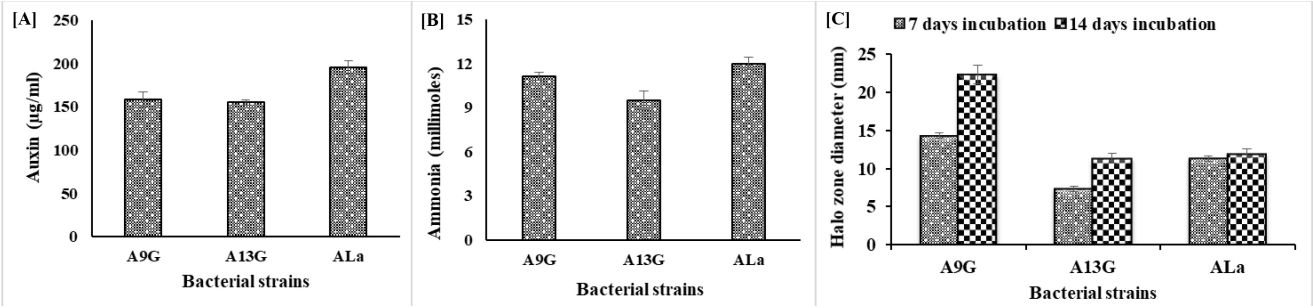


**Figure 3:** Plant growth promoting attributes of bacterial strains. Error bars are indicating error in reported measurements. Auxin production [A]; ammonia production [B] and Zinc solubilizing diameter at seven and fourteen days on incubation [C] [A9G = *Enterobactercloacae*, A13G = *Exiguobacterium* sp. and ALa = *Bacillus pumilus*].

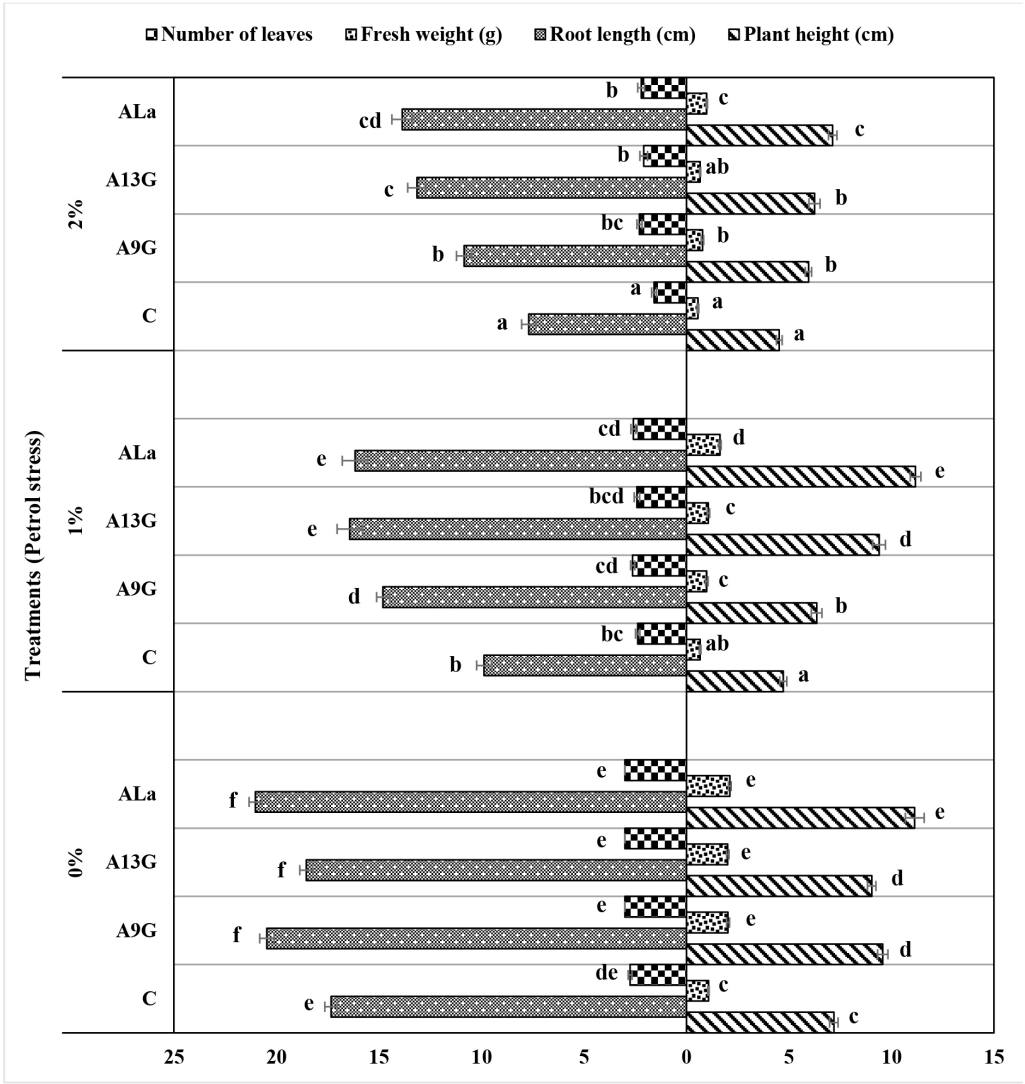


**Figure 4:** Effect of bacterial treatments with and without petrol stress (0, 1 and 2%) on plant height, root length, leaves number and fresh weight of *Zea mays*. Data represent mean of twenty four replicates. Different letters indicate significant differences between treatments using Duncan's multiple range test (P = 0.05) [Control – C, Bacterial strains: *Enterobacter cloacae* (A9G), *Exiguobacterium* sp. (A13G), *Bacillus pumilus* (ALa)].

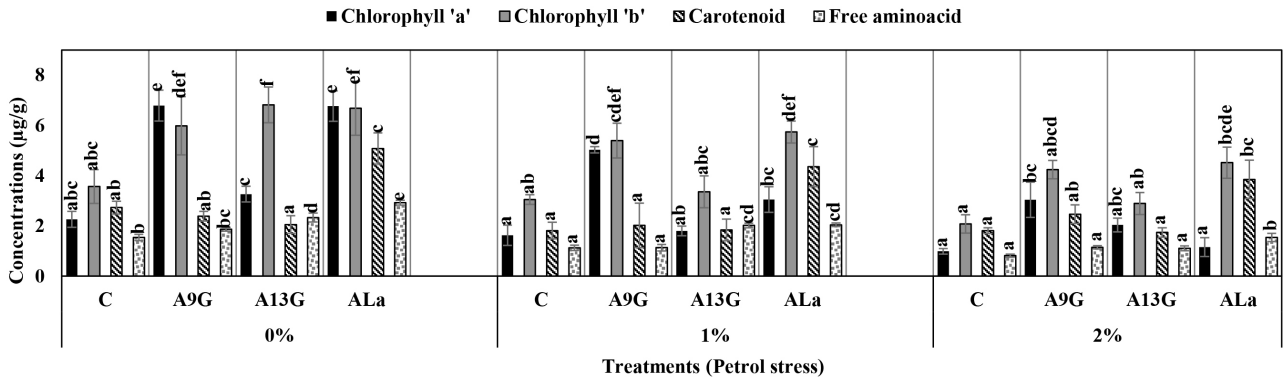


**Figure 5:** Effect of bacterial treatments with and without petrol stress (0, 1 and 2%) on chlorophyll 'a', chlorophyll 'b', carotenoid and free amino acid contents of *Zea mays*. Data represent mean of twenty four replicates. Different letters indicate significant difference between treatments using Duncan's multiple range test (P = 0.05) [Control – C, Bacterial strains: *Enterobacter cloacae* (A9G), *Exiguobacterium* sp. (A13G), *Bacillus pumilus* (ALa)].

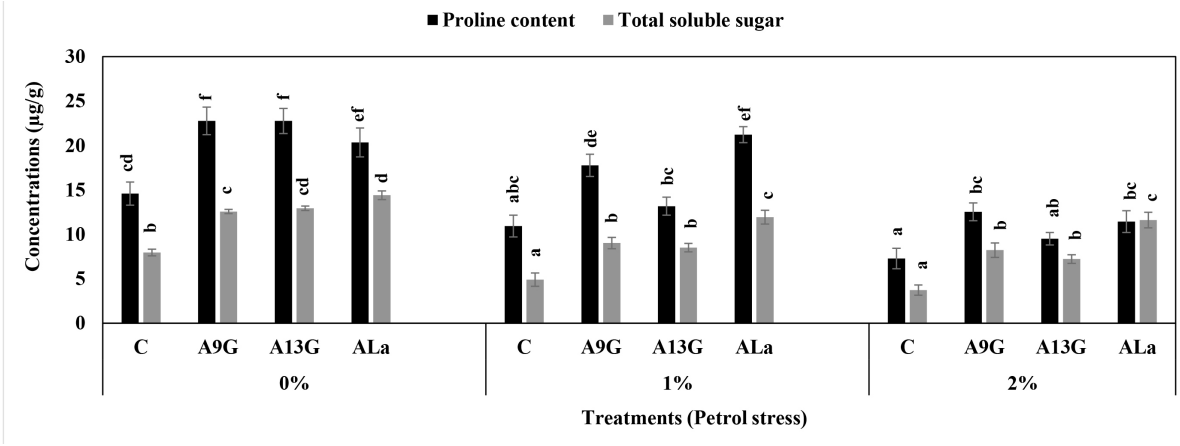


**Figure 6:** Effect of bacterial treatments with and without petrol stress (0, 1 and 2%) on proline and total soluble sugar content of *Zea mays*. Data represent mean of twenty four replicates. Different letters indicate significant difference between treatments using Duncan's multiple range test (P = 0.05) [Control – C, Bacterial strains: *Enterobacter cloacae* (A9G), *Exiguobacterium* sp. (A13G), *Bacillus pumilus* (ALa)].

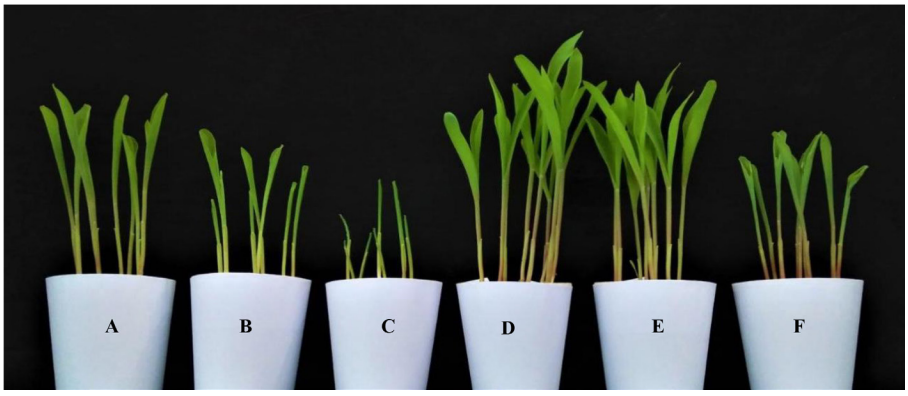


**Figure 7:** Effect of *Enterobacter cloacae* (A9G) inoculation on the growth of *Zea mays* plantswith and without petrol stress (0, 1 and 2%) [A =non-inoculated control without bacterial treatment, B = non-inoculated control with 1% petrol stress, C = non-inoculated control with 2% petrol stress, D = *Enterobacter cloacae* (A9G), E =*Enterobacter cloacae* (A9G) + 1% petrol stress and F = *Enterobacter cloacae* (A9G) + 2% petrol stress].