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Biofertilizer Assay of Phosphate Solubilizing Bacteria Isolated from Western Ghats

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Abstract: Soil samples from wild variety of crop plants in Western Ghats were subjected to serial dilution (up to $10^{-3}$and 10^{-4}) and plated on Luria bertani agar and later streaked on Pikovskaya's medium and further screened for isolation of potential biofertilizer agent. The isolates were subjected to morphological and biochemical tests and was identified to be Phosphate Solubilizing Bacteria. The biofertilizer property of the isolate was studied on a leguminous plant in a flowering pot and the assay was based on percentage of seed germination, primary root length, number of secondary roots, shoot length, number of leaves and average leaf length, plant water content, protein and sugar content estimation and chlorophyll content respectively. The study was done in triplicates and un-inoculated control were used, the results showed that the isolate had higher influence in plant growth and further mass cultivation and field study was required.

INTRODUCTION

Phosphorus is a macroelement essential for plant growth. The visual symptoms of deficiency of this element in plants include young plants become stunted, leaves turn dark blue-green; stems slendor; often anthocyanins in veins and may become necrotic; fruits ripen slowly and plants often dwarfed at maturity [7]. Phosphate is an inorganic form of phosphorus utilized by plants for growth. Phosphorus is an important constituent of ATP which plays a vital role in energy metabolism of the cell. It is also a constituent of sugar phosphates, nucleic acids, nucleotides, coenzymes, phospholipids, phytic acid [7].

Biofertilizers are capable of mobilizing nutritive elements from non-usable form to usable form through biological processes. Biofertilizer production also helps in reducing the input of chemical fertilizer to an extent of 25% for obtaining the same yield. They are less expensive and eco-friendly. Rapid urbanization has lead to environmental pollution and further usage of chemical fertilizers may add up to the present problems of environmental degradation [9, 10]

Phosphate solubilization is very important plant growth promoting activity though soluble forms of phosphate is added to soil as a fertilizer still the greater proportion of those inorganic phosphates are unavailable to the autotrophs[1,5]. Phosphate solubilizers basically employ their fermentative end products for solubiilizing phosphates mostly organic acids like butryric, lactic, propionic, acetic and formic acids, sometimes succinic, fumaric or glycolic acids. Most soil bacteria including genea *Bacillus* and *Psuedomonas* have the propensity to convert insoluble form of phosphate to soluble forms[8]. They

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are quite adaptive to different crops and different soil atmospheres and climatic conditions [3].

METHODS

Phosphate Solubilization:

Potential isolate grown on Luria Bertanimedium was later streaked onto Pikovskaya's medium and incubated at30°C for 1 to 2 weeks. The plates were observed for ClearP-zone formation around the colonies[2]. Quantification of available phosphorus solubilized by the bacterial isolate was quantifiedby the molybdate blue color method [3]. Fresh bacterial culture was grown in Pikovskaya brothon a rotary shaker for 2 weeks at 30°C. The suspension wascentrifuged and the supernatant wasdecanted and filtered and absorbance determined at 660nm.

Phosphorus estimation:

The plant material is dried using a carbonate-nitrate mixture and the ash extracted with tri-chloro acetic acid. An aliquot of the extract is treated with an acid moybdate solution and 1,2, 4- amino-naphthol sulphonic acid reagent. Blue coloured product could be seen with the intensity of which is proportional to the phosphorous content is produced. The intensity of the colour is read photometrically at 660nm [3, 7].

Biofertilizer assay:

Garden soil and sand were mixed in the ratio 3:1 and sterilized and dispensed into small pots.The mehti plant seeds were treated with different combination of biofertilizeralong with urea and were sown. The seeds untreated with Biofertilizers were used as control.These were grown for a period of about 45 days.Following growth, various morphological and biochemical parameters were carried out with the control and treated plants and the results were tabulated.

Total Protein assay:

The plant extract was taken onto centrifuge tube and spun for 10 min at 5000 rpm. One gram of culture sample was taken & homogenized with 10ml of distilled water. This homogenate is used as a source of protein. Estimation of the protein was performed by Lowry method whereby a stock solution of 100µg/ml of tyrosine was prepared by dissolving 10mg of tyrosine in 100ml of distilled water in a standard volumetric flask. Using this stock solution, dilution ranging from 10-100µg/ml was prepared. To 1ml of each of the dilution, 5ml of the alkaline reagent was added & the test tube was incubated at 40°C for 15 min. To the incubated tubes, 0.5ml of Folin's reagent was added & tubes were further incubated at 40°C for 15 min for the reaction to be completed. The OD was then taken at 640nm. Using a blank solution, the colorimeter was adjusted. The OD of the protein sample was plotted on the standard graph & extrapolated to get the concentration of protein.

Total sugar estimation:

The plant extract was collected for sugar estimation.Each of the broth was diluted to obtain three concentration of 1/10, 1/25 and 1/50 dilution respectively.To 1ml of starch solution, 0.5ml of the above dilutions were added and incubated at room temperature for 5 min.The reaction was stopped using 2ml of 1% DNS reagent.The tubes were boiled in the boiling water bath for 5 min, cooled immediately and 5ml of water was added to it.They were then mixed thoroughly and absorbance was read of 540nm.A stock

solution of 1mg/ml was prepared by dissolving 100mg of glucose in 100ml of water using a volumetric flask. The stock was further diluted with water to obtain a final sugar concentration of 100µg/ml to 1000 µg/ml. the above mentioned method was followed and the concentration of glucose was plotted o the X axis against the absorbance (OD) on Y axis and a straight line was derived from the point of origin using standard values.

Chlorophyll content:

The plant photosynthesis is essentially dependent on chlorophylls and their concentration decides the plants efficiency in growth and development. This parameter helps directly to evaluate the plant health and robustness and their shortfall could lead to poorly developed plants and indicates plant diseases. Chlorophyll content can be easily analyzed by simple extraction protocol wherein they are found to be loosely bound and solvents like ether and/or acetone can help in extraction. Though the chlorophyll type varies among prokaryotic and eukaryotic autotrophs the extraction mechanism is almost similar wherein they are characterized by porphyrin nucleus with a chelated magnesium atom. One gram of plant material would be fine for analysis, it has cut finely and macerated with organic solvent, 80% of acetone was used, 20 ml of solvent was used to grind 1g of plant material, then centrifuged followed by collection of supernatant and absorbance was taken at 645, 663 and 652nm against the solvent as blank [7].

Results and Discussion

The potential isolate was tested for biofertilizer properties by conducting a laboratory trail in comparison with standard Phosphate solubilizing culture and an un-inoculated control. Based on the morphological parameters tested for the test plant the performance of the potential isolate was far better than the control phosphate solubilizing bacteria. The efficacy of the biofertilzer was clearly evident from the results obtained for the un-inoculated control (**Fig 1**).

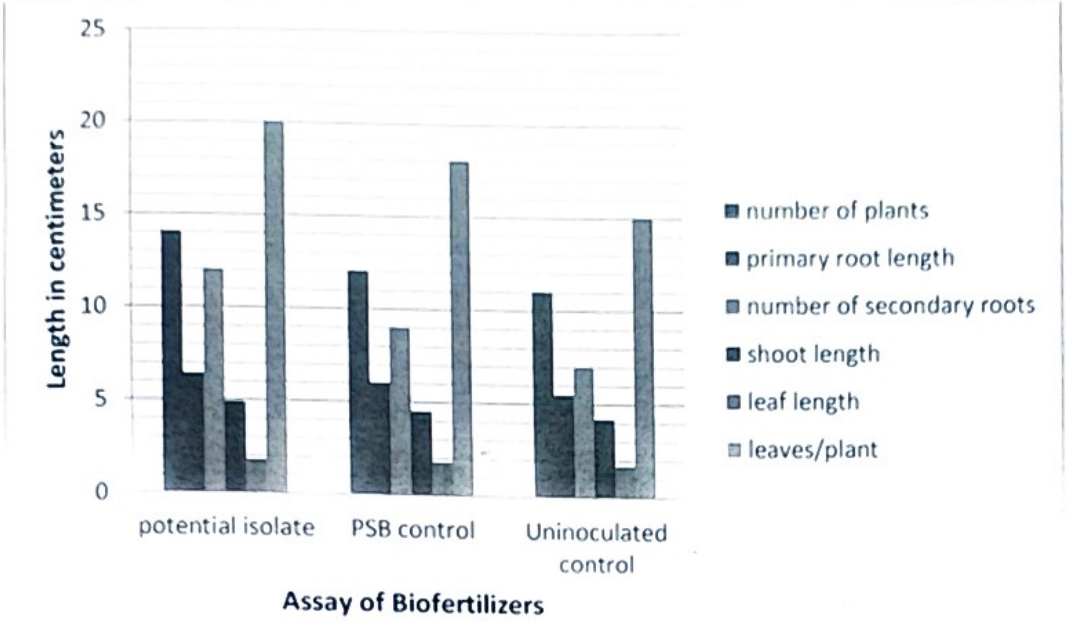

Figure 1: Morphological parameters

Better root development was noted in PSB treated pots than the uninoculated control indicating the positive influence of phosphate solubilizing bacteria. The isolate inoculated pots showed maximum seed germinations, further the number of secondary roots development was far higher than control though the primary root length seemed to be same for all three categories. The number of leaves was also slightly higher in average number of plants considered. The height of the plant was almost same for all the three varieties of plants. The measurements were taken in centimeters and apart from the morphological traits of the mehti plants the biochemical aspects were also estimated. Among the biochemical aspects few parameters were analyzed which are essential for evaluating the performance of the biofertilzers.

The methti plants were evaluted for total chlorophyll content, total protein content, sugar content and water content. Biofertilizers such as *Azospirillum* promote plant growth, productivity and increase the nutrient status of the host plant have internationally been accepted as an alternative source of chemical fertilizers [8].The robust growth of the plant depends on the chlorophyll content and the concentration of the total chlorophyll was found to be more in PSB isolate treated pots and was least in un-inoculated pots. The standard PSB control performed better than the un-inoculated control (**Table 1**)

Table 1: Chlorophyll content of the various biofertilizers agents

Sl. No.	**Biofertilizers**	**Chlorophyll content mg/100mg**
1	Potential isolate (PSB)	23.4
2	Standard PSB control	22.8
3	Un-inoculated control	22.1

The nutrients play an important role in the crop productionbut under intensive cultivation use of chemical fertilizerscould result in deterioration of soilfertility and quality of produce and hence the use of organic manurein combination with biofertilizershelps in balancing soil

fertility[6]. The protein content was comparatively higher in isolate and un-inoculated control plants yielded lesser concentration of protein and reducing sugar content. The combination of isolate and standard biofertilizers always exhibited a positive influence on plant growth and yield (**Table 2**).

Table 2: Chlorophyll content of the various biofertilizers agents

Sl. No.	Biofertilizers	Protein content mg/g^{-1}	Reducing sugar content mg/g^{-1}
1	Potential isolate (PSB)	5.9	19.1
2	Standard PSB control	5.6	19.0
3	Un-inoculated control	5.5	19.0

Atpresent, nutrition for methi is being provided by manuresand chemical fertilizers, which are in short and costly and therefore, it has become imperative to conduct forcomplementary resources which can minimize the use ofchemical fertilizers. In this regard the potential isolate based biofertilizers may pave way for further enhancing the use and yield of methi plants.

CONCLUSION

Rapid depletion of soil quality can be attributed to indiscriminate use of chemical fertilizers and the possible shift in beneficial microbial load could have a grave consequences in near future. A balanced approach is preferable that could mean to controlled use of chemical fertilizers along with organic manure opening up a scope for role of biofertilizers that could bring back the balance required for soil rejuvenation. In the present study the role of PSB isolate can help crop plant to reduce usage of chemical fertilizer.

ABBREVIATIONS

PSB – Phosphate solubilizing bacteria, OD – optical density, DNS – Dinitro salicylic acid.

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