Enrichment, Isolation & Screening of *Pseudomonas spp.* & it's Encapsulation Techniques to Study Effect on Plant Growth

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Abstract: Plant growth-promoting rhizobacteria (PGPR) showcase a diverse subgroup of rhizosphere-colonizing bacteria. PGPR were first described for root crops, when the use of antibiotic resistance made possible the monitoring of introduced bacteria of interest in soil. Reported mechanisms of action for PGPR have focussed on the indirect mechanisms of Phosphate-solubilisation, Protease & Cellulase activity. One such example of PGPR is *Pseudomonas fluroscens* and it is the bacteria of interest for this particular project. After Enrichment & Isolation, desirable characters were obtained from as many as 7 isolates. These isolates were found to be *Pseudomonas spp*. Out of 7 isolates, 4 isolated showed promising activities. The isolates found to be effective were BGS 10^{-3}, NPS 10^{-2}, KJS 10^{-3}, CS 10^{-3} and 3 controls were used as well. These isolates were encapsulated into beads with the help of 4% Na-Alginate & 8% $CaCl_2$, these isolates were chosen to perform the pot assay and the pot assay was done in triplicates to ensure safety of isolates. Out of these 15 pots, the pots of BGS 10^{-3}, NPS 10^{-2}, KJS 10^{-3} showed overwhelming growth while CS 10^{-3} showed less growth and Controls without Encapsulated beads showed no growth at all.

Keywords: Bacteria, Biological-control, Growth-promotion, PGPR.

I.INTRODUCTION

Soil is a sole substrate for plants to grow in. Soil facilitates support, nutrients, and a network of water and air to the plant's roots. But, the use of chemical fertilisers hampers the plant growth because of poor soil quality. Poor soil quality disturbs seed germination, reduces nodule formation, retards plant development and reduces crop yield. Due to overuse of undesired chemicals, the microbial flora which helps plants in using necessary growth factors like siderophores, proteases, Indole Acetic Acid etc. also gets killed. All the harmful changes in soil quality hinders the root and shoot development and also the overall plant growth [10].

Plant growth promoting rhizobacteria (PGPR) are a class of bacteria that can enhances plant growth. One such example of PGPR is *Pseudomonas fluorescens*. In fact, PGPR are biologically quite unstable and the bacterial-activity degrades over time due to environmental factors. The application of delivery system based on encapsulation technology shows a promising and sustainable technique to store and deliver PGPR [9].

Application of microbial inoculants into the soil can enhance or improves soil properties and plant nutrient acquisition/uptake and increase the efficiency of mineral fertilizers and manures. The encapsulated cells are spread or distributed around the rhizosphere of plants and allowed to work with an association with the plants symbiotically you can say. This PGPR-Plant association helps the plant to utilise all the essential growth factors and thus it helps in root & shoot development and of course overall plant growth as well [2].

II.MATERIALS & METHODS

1. Enrichment: Five different samples of soil will be collected & 1 composite soil sample. Samples are labelled as BGS, DPS, NPS, KJS, KNS, CS. Sterile King's B Agar Media will be prepared for enrichment of collected rhizosphere soil sample. 1g of each collected soil sample will be inoculated in Sterile King's B Agar Media. Post inoculation, the broths will

be incubated at RT under shaker conditions for 3 days. [11]

2. Isolation: Isolation will be done on Sterile King's B Agar Media. For spreading, the inoculants are diluted up to 10^{-1} to 10^{-3} marks to get well isolated colonies. The plates will be kept for incubation at RT for 2-3 days. [11]

3. Morphological and Biochemical characterisation of the isolates: Colonies of each isolate will be observed, it's size, shape, colour, elevation etc. will be noted down. Biochemical tests of isolates will be done. [9]

4. Screening of isolates for production of different metabolites:
Phosphate Solubilization: Isolates are streaked on Pikovaskaya's agar and plates will be incubated at 28°C for 3 days. The colonies showing the clear halo zone around them will indicate P-solubilization [12]
Protease Activity: Gelatin Agar is used. Plates will be inoculated and incubated at 28C and checked daily for transparent halo zone formation.[13] Cellulase Activity: Isolates were spot inoculated on CMC agar. Plates will be incubated at 28°C for 2-3 days and clear halo zone will indicate the positive result for cellulase activity. [13]
Techniques for encapsulation of *Pseudomonas spp.* Cells into beads: Prepare 500 ml each of 4% Na-Alginate and 6% CaCl2 and autoclave them. Grow *Pseudomonas spp.* on King's B agar at 28°C for 48 hours. Cool the sodium alginate and add culture to it in proportion of 1 ml of suspension in 100ml of sodium alginate. The beads formed are of calcium alginate with entrapped *Pseudomonas* cells.[10]
Assay of plant growth promoting factors in *Pseudomonas Spp.*: The wheat seeds will be sowed in the soil. Encapsulated cells will be also sowed. At an interval of 7 days, shoot, root and total plant height will be measured for 21 days.[12]

III.RESULTS & DISCUSSIONS

Enrichment & Isolation of *Pseudomonas spp.* From different soil samples
Five soil samples were collected-Karanjade (KJS), Karnala Sports (KNS), New Panvel (NPS), Botanical Garden (BGS), Dapoli (DPS). The samples were serially diluted and plated onto Sterile Kings B medium.

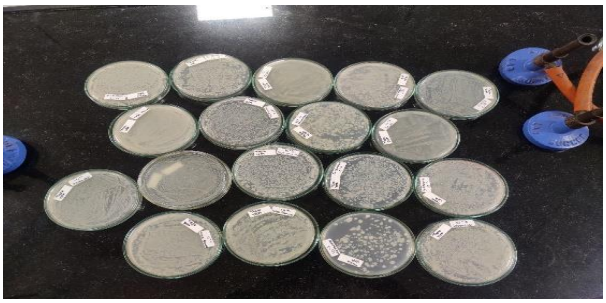


Fig 1.1: Isolation of bacterial colonies from soil samples

Determining colony characteristics of the isolates
Most of the isolates developed small to medium, smooth, glistening colonies, convex elevation and these isolates were Gram negative, rods when observed under microscope.

Screening of isolates for production of different metabolites
The isolates were screened for various growth factors like Phosphate solubilisation, Protease activity and Cellulase activity. Phosphate Solubilisation activity was performed on Pikovaskaya's Medium. On incubation for 2-3 days at room temperature, the isolates showed good activity were BGS 10^{-2}, CS PDA, CS 10^{-3}, KJS 10^{-3} AND NPS 10^{-2}.

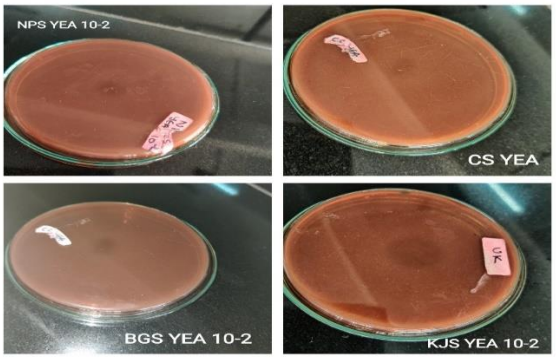
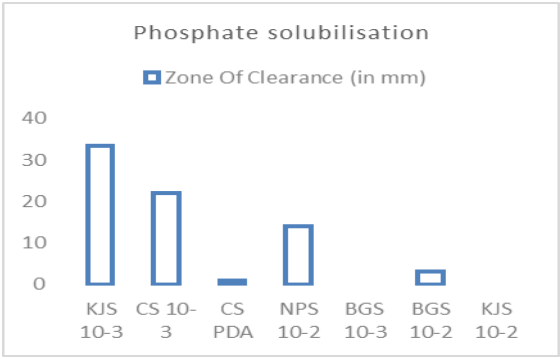


Fig 1.2: P-Solubilisation activity of isolates



Graph 1.1: P-Solubilisation activity of isolates

Protease activity was performed on Gelatin agar Medium. On incubation for 2 days at RT, the isolates showed good activity were BGS 10^{-2}, CS 10^{-3}, KJS 10^{-3}, BGS 10^{-3}, NPS 10^{-2}.

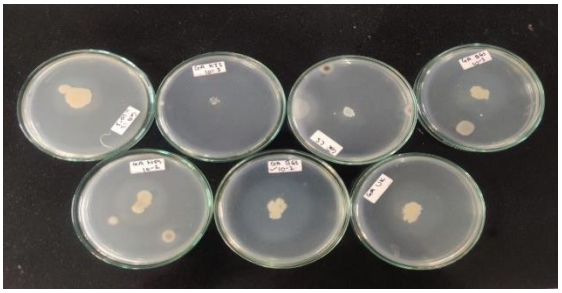
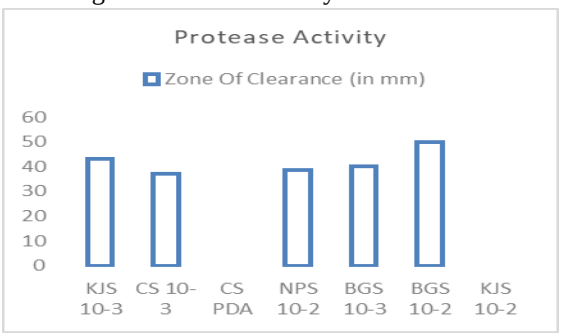


Fig 1.3: Protease activity of the isolates



Graph 1.2: Protease activity of the isolates

The Cellulase activity was performed on Carboxy methyl cellulose agar medium. On incubation for 2 days at RT, the isolates showed good activity were BGS 10^{-2}, CS 10^{-3}, KJS 10^{-3}, BGS 10^{-3}, NPS 10^{-2}, CS PDA.

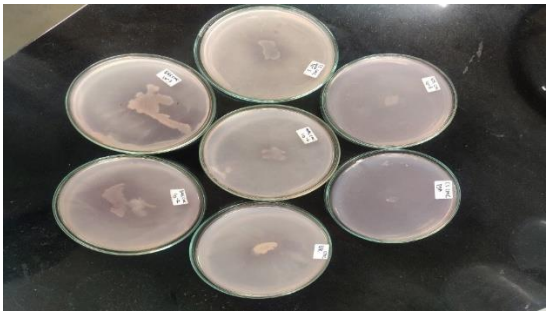
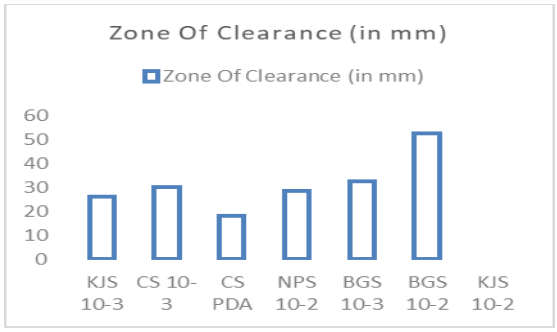


Fig 1.4: Cellulase activity of the isolates



Graph 1.3: Cellulase activity of the isolates

Determining biochemical characteristics of the efficient isolates

The efficient isolates underwent biochemical tests like Citrate utilisation test, Cetrimide test, Nitrate reduction, Gelatin liquification test, Catalase test and Oxidase test. All these isolates gave positive results for performed test. Hence, we can conclude that isolates BGS 10^{-2}, KJS 10^{-3}, CS 10^{-3} and NPS 10^{-2} were the most efficient isolates.

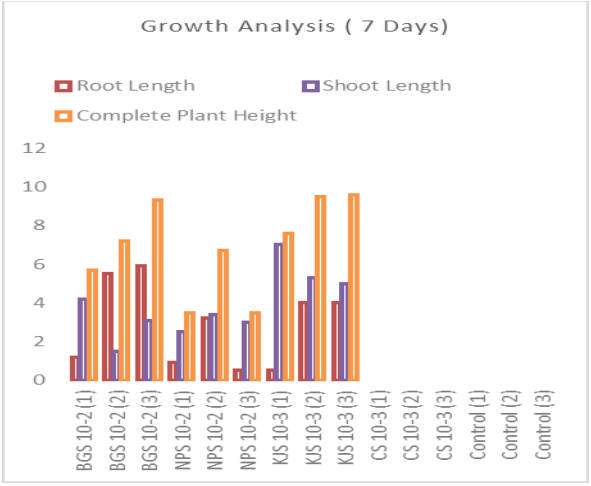
Techniques of encapsulation of *Pseudomonas* cells into beads

Four efficient isolates were selected for encapsulation. Encapsulation was done using Sodium alginate & Calcium Chloride. Culture was mixed with Sodium alginate & further beads formed were of Calcium alginate. Prepared beads were kept in activation medium.

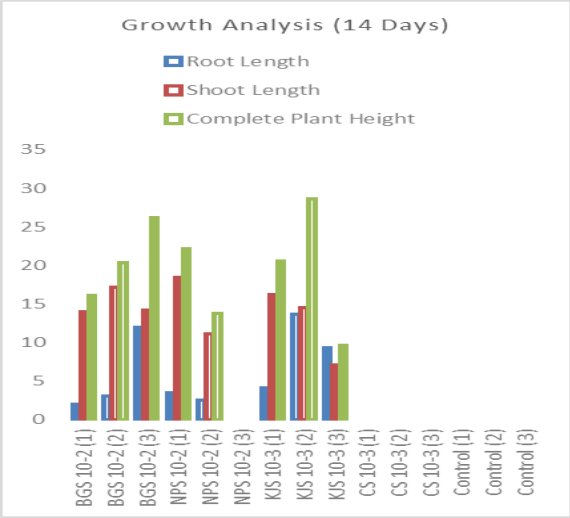
Fig 1.5: Encapsulation of *Pseudomonas* cells into Calcium Alginate beads

Assay of plant growth promoting factors in *Pseudomonas spp.:*

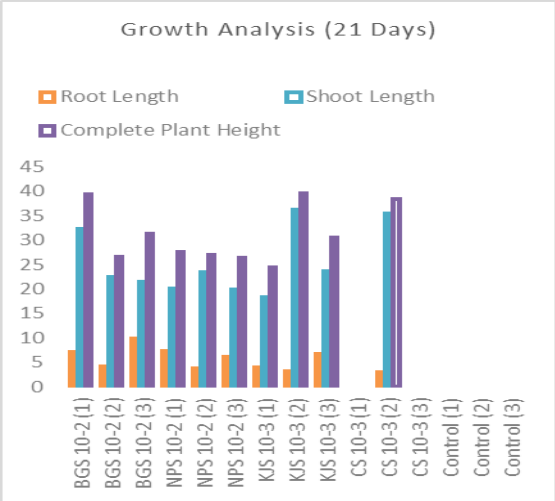
Pot assay was performed in triplicates along with controls. Sterile soil was used for the assay. Growth measurements like root length, shoot length and total plant height was measured at intervals of 7 days.



Graph 1.4: Growth Analysis of wheat plant after 7 days



Graph 1.5: Growth Analysis of wheat plant after 14 days



Graph 1.6: Growth Analysis of wheat plant after 21 days

IV.CONCLUSION

PGPRs are interesting in sustainable agriculture; it has been proven to be an environmentally sound way of increasing crop yields through either direct or indirect mechanism including regulating hormonal and nutritional balance. The exploitation of PGPRs in sustainable agriculture as biofertilizers, which enhance agriculture productivity, enrich the soil fertility, and enhance the agriculture yield without harming the ecosystem, is the key challenge for the agronomist. PGPRs have good impact in crop productivity in terms of biofertilizers, biocontrol, bioremediation, and ecosystem functioning. In this research project, the bacteria of interest were isolated from different soil samples. The bacteria of interest is *Pseudomonas spp*. *Pseudomonas spp.* Acts as a PGPR. Hence, the bacteria were screened for various Growth Promotion Activities. Biochemical tests were performed to determine the efficient isolates for further studies. Once screened for various activities as earlier mentioned, it was found that all four isolates were efficient in producing large amount of our desired result. These four isolates were then encapsulated into the beads for the further application part. These encapsulated beads of our bacteria of interest were sown along with the wheat seeds and were observed for the growth promotion. At the interval of 7 days, root length, shoot length and total plant height was measured. The pots with encapsulated seed showed encouraging growth while the Control pots showed no growth at all. Hence, here we can conclude that *Pseudomonas spp*. Do act as a plant growth promoting rhizobacteria and is efficient enough to practise in field purposes for better and sustainable agriculture.

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