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# Isolation and Screening of Rhizosphere Bacteria from Sugarcane Roots for Plant Growth Promoting Traits: IAA Production and Phosphate Solubilization

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**Abstract: This study aimed to isolate, characterize, and identify indole-3-acetic acid (IAA)-producing bacteria from the sugarcane (*Saccharum officinarum*) rhizosphere, as bacterial IAA is a key trait that facilitates plant growth promotion. The level of auxin production was characterized by calorimetric estimation of IAA and the ability to solubilized calcium phosphate was evaluated by a qualitative analysis of phosphate solubilization in NBRIP Agar assay. Bacterial strains designated A, B, C, D, E, H, J, L, M, N, O, R, and S were isolated from the rhizosphere soil of sugarcane and were pure cultured. These strains produced IAA at detectable levels and out of these strain "C" produced the maximum amount of IAA per gram of the bacterial cell pellet, i.e. 2.72 mg of IAA per gram of dry cell pellet. Bacterial strains A, D, M, E, J, R, O and N had the capacity of solubilize the insoluble calcium phosphate in NBRIP agar. 3 strains - M, N, and R were capable producing both plant growth promoting traits. This study concludes that bacterial strains capable of IAA production, phosphate solubilization, or—most promisingly—both functions, show significant potential as effective bio-inoculants and optimal components for plant growth-promoting biofertilizer consortia."**

***Keywords:*** *Rhizosphere, Phosphate Solubilization, IAA, Sugarcane, PGPR.*

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## I. INTRODUCTION

For decades, the application of chemical fertilizers has played a crucial role in enhancing agricultural productivity and supporting the agronomic development of nations worldwide.The unchecked use of these has led to widespread soil degradation. Furthermore, the increasing global population pressure has driven an excessive increase in the use of chemical fertilizers and pesticides to boost yields. While these agrochemicals deliver immediate, satisfactory results in crop production, their accumulating disadvantages now pose a significant threat to agricultural sustainability. These detrimental effects include eutrophication, the eradication of beneficial soil microorganisms, increased soil acidity, and a consequent decline in overall soil fertility. To maximize fertilizer efficiency and plant growth, Plant Growth-Promoting Rhizobacteria (PGPR) inoculation should be integrated with optimized fertilization. This strategy is vital for sustainable agriculture, as it reduces reliance on chemical fertilizers, mitigating their environmental impact [1]. Various bacterial genera, including *Pseudomonas, Azospirillum, Azotobacter, Bacillus, Klebsiella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia* and *Serratia*, are widely reported as Plant Growth-Promoting Rhizobacteria (PGPR). These species enhance plant growth through direct mechanisms such as nitrogen fixation, phosphate solubilization, and production of phytohormone IAA [2].

PGPR are a type of bacteria that live in plants' rhizospheres and help them grow both directly and indirectly [3 - 9]. Plant growth regulators (PGR) such as auxins (Indole-3-Acetic Acid ), cytokinins, and gibberellins promote root hair formation, resulting in increased branching, length, and surface area of roots and root hairs. This boosts the plant's ability to absorb water and nutrients, increasing its resilience, productivity, and ability to withstand adverse weather conditions [4]. PGPR also helps solubilize phosphorus, the second-most important nutrient for crop growth after nitrogen, become more mobile in the soil [5]. These soil microbes also help to manage plant diseases naturally by increasing the plant's defenses. Guided by the aforementioned rationale, this research was carried out to achieve the following aims: (i) the isolation and cultural characterization of bacteria from the sugarcane rhizosphere, (ii) the qualitative and quantitative

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assessment of the isolated strains for indole-3-acetic acid production, and (iii) the evaluation of their capacity for tricalcium phosphate solubilization.

*Saccharum officinarum* L. (sugarcane) is a tall, perennial grass of the Poaceae family, cultivated globally as a primary source of sucrose. Indigenous to the tropical and warm temperate regions of South Asia, it is a major cash crop whose yield is highly sensitive to climatic conditions, soil type, drainage, fertilizer inputs, and biotic stresses such as pests and diseases [10]. As mentioned earlier, sugarcane is South-Asian in origin, different species of *Saccharum* are found to be having different origin. For example, *Saccharum barberi* was found to have originated in India and *S.officinarum* from New Guinea [11]. In India, after Maharashtra and Uttar Pradesh; Tamil Nadu has emerged as one of the largest other states producing sugar in India in recent times, other states include Andhra Pradesh, Karnataka, and Punjab etc. In Karnataka, the Mandya Belt and adjoining parts are the major sugarcane production areas.

Plant-microbe interactions within the rhizosphere are fundamental determinants of plant health, productivity, and soil fertility. PGPR enhance plant growth and confer resistance against diseases and abiotic stresses through diverse metabolic mechanisms. Notably, endophytic PGPR, which colonize internal plant tissues, often demonstrate exceptional efficacy in promoting plant growth [12]. PGPR employ both direct and indirect mechanisms to promote plant growth: direct methods involve the secretion of plant growth regulators, whereas indirect methods occur through the production of antimicrobial compounds that mitigate the deleterious effects of phytopathogens [13].

Agricultural practices may lead to noticeable alterations in soil parameters that may affect the rhizospheric environments. Physical changes in soil structure after tillage [14], crop rotation [15], and wastewater irrigation were shown to impact upon soil and root-associated bacteria. Crop rotation increased bacterial diversity in the rhizosphere [14, 15]. Nevertheless, continuous grassland sustained more diverse soil bacteria than rotation of cereals and potatoes [16].

Plant Growth Promotion (PGP) traits of PGPR include the production of growth regulators, fixing N2, activity of ACC deaminase, phosphate solubilisation etc. PGPR inoculants can help to increase agronomic efficiency, reduce production costs and environmental pollution. If the PGPR inoculants applied to the soil are efficient, then they can serve as better substitutes for chemical fertilizers. The exploitation of chemical fertilizers in agriculture necessitates sustainable alternatives like microbial inoculants. Research evaluating a reduced fertilizer regimen (75% of recommended dose) amended with a select inoculant on tomato—used as a model system—revealed that this combination achieved statistically equivalent results in growth, yield, and nutrient (N, P) uptake compared to the full fertilizer dose, highlighting the potential of inoculants to reduce chemical inputs without compromising productivity. [1]. Plant age, species, genotype, and root exudates directly shape microbial community structure. Furthermore, sensitivity to changes in exudate composition, for example during plant development, varies significantly among different root-associated populations. [18]. The efficiency of PGPR largely depends on the plant roots health i.e. stress can influence the roots to secrete components that may interfere with the rhizosphere microbe interactions with the plant roots [19].

- *PGPR Produces Auxins*

Bacterial species associated with plants are also known for their active production of indolic auxins like indole-3-acetic acid which present great physiological significances like pathogenesis, phytostimulation, and many more. It is reported that 80% of rhizosphere-resident microbes of various crops possess the ability to synthesize and release auxins as secondary metabolites [20]. Generally, IAA functions to aid plant cell division, elongation, and differentiation. IAA functions as a germination stimulant, increases the rate of xylem and root development; regulates vegetative growth; initiates lateral and adventitious roots [25]; Auxin (IAA) mediates plant responses to light, gravity, and fluorescence, and plays crucial roles in photosynthesis, pigment formation, metabolite biosynthesis, and resistance to stress conditions. IAA produced by rhizobacteria can interfere with these physiological processes by altering the plant's endogenous auxin pool. In addition, rhizobacterial IAA promotes root elongation and increases root surface area, thereby enhancing the plant's ability to access soil nutrients. It also loosens plant cell walls, which leads to greater root exudation, providing additional nutrients that support the proliferation of rhizosphere bacteria [13]. Studies reveal that rhizosphere bacteria and endophytic bacteria (Enterobacteriaceae family) produce more indolic compounds (IC) than local soil bacterial genera. L-tryptophan, a root exudate, has been identified as the main precursor for the route of IC biosynthesis in bacteria and the different pathways of IAA synthesis in bacteria show a high degree of similarity with the IAA biosynthesis pathways in plants. [21]. The amino acid tryptophan, identified as the main precursor for IAA and plays a role in modulating the level of IAA biosynthesis [22, 23]. Tryptophan stimulates IAA production, whereas its precursor anthranilate reduces it. This fine-tuning occurs because tryptophan negatively regulates anthranilate synthase, thereby indirectly promoting IAA biosynthesis. Beneficiary PGPR use the indole-3-pyruvic acid pathway, an alternative pathway dependent on L-tryptophan [29].

- *PGPR Can Solubilize Phosphates*

After nitrogen and potassium, phosphorus is very essential for plant growth, being a structural component of nucleic acids, phospholipids and nucleoside triphosphates, important particularly for biological nitrogen fixation and photosynthesis. Plants absorb P as the monobasic ($H_2PO_4^-$) and the dibasic ($HPO_4^{2-}$) forms. Phosphate-solubilizing bacteria solubilize inorganic soil phosphates, such as $Ca_3(PO_4)_2$, $FePO_4$, and $AlPO_4$, through the production of organic acids, siderophores, and OH- ions [27].

Microorganisms can solubilize fixed phosphorus by the release of organic acids, chelating, competing phosphorous for adsorption sites, and forming soluble complexes with metal ions. At low pH (soil is made acidic by the release of

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organic acids), $H_2PO_4^-$ (monovalent anion) is the most soluble form of phosphate. As pH rises, it converts to divalent and trivalent ions, which are highly insoluble. Research indicates that phosphate-solubilizing bacteria produce gluconic, oxalic, tartaric, and lactic acids that lower soil pH, facilitating the availability of $H_2PO_4^-$ ions [28]. Othman and Panhwar (2014) [29] reported that phosphate-solubilizing bacteria produce enzymes like phosphatase, phytase, and C–P lyase, which convert organic phosphorus into plant-available forms.

Phosphate solubilization is also influenced by soil factors like organic content, physicochemical properties, vegetation type, environmental conditions, agronomic practices, and interactions between phosphate-solubilizing microbes and other microbial communities in the soil [30].

Microbes can also release enzymes such as phytase to solubilize phosphate. This enzyme releases phytate, a type of phosphorus linked to organic compounds found in soil. The mechanism degrades phytate and releases phosphorus in a form that plants can utilize. Plants cannot absorb phosphorus directly from phytate, but phosphate-solubilizing microorganisms breakdown it and make it available for uptake [31]. Understanding enzymes, soil microbial communities, plant P uptake, root exudation, and other rhizospheric activities can aid in investigating soil phosphorous transformations [32].

Bacterial genera like *Enterobacter, Pantoea* and *Klebsiella* solubilize $Ca_3(PO_4)_2$ to a greater extent than $FePO_4$ and $AlPO_4$ [33]. *Burkholderia, Cedecea, Cronobacter, Enterobacter, Pantoe*a, and *Pseudomonas* associated with rice roots were able to solubilize $Ca_3(PO_4)_2$.

➢ *Factors that Affect the Efficiency of PGPR Inoculants*

Secretion of plant origin components via roots is an environmental response of a plant. These compounds may or may not be of benefit to the soil colonizing bacteria. These secretions can be ionic species, oxygen, moisture, carbon based primary and secondary metabolites. Studies show that flavonoids exudation is different at different plant life stages. These compounds behave as a gene inducer in Rhizobium species. The nodD gene transcription influences thee root nodule formation by rhizobia in legumes. Also, L-malic acid attract Bacillus subtilis FB17 to *Arabidopsis thaliana* infected by $H_2PO_4^-$ [34]. Apart from benefiting soil microbes, these metabolites can also behave as antimicrobials and antimetabolites for PGPR. Canavanine secretions from leguminous seeds affect the colonization of beneficial rhizobia. The success of PGPR inoculants depends on their root colonization efficiency. In response to plant exudates, associative and endophytic PGPR regulate the expression of genes linked to exopolysaccharide (EPS) synthesis and biofilm development [34]. Comprehensive studies and reviews highlight that quorum sensing (via AHLs or AI-2) regulates critical PGPR functions, including biofilm formation, motility, exopolysaccharide production, siderophore and volatile synthesis, and IAA production—traits that enhance root colonization, stimulate plant growth, and suppress pathogens. Moreover, mechanisms such as chemotaxis, motility, pili formation, surface components, and particularly quorum sensing are widely regarded as central molecular factors governing effective root colonization. [51]

## II. MATERIALS AND METHODS

➢ *Collection and Isolation of PGPR from Sugarcane Rhizosphere.*

A sugarcane growing patch in Malavalli-Mysore road, (12°20'53.4"N 76°54'09.9"E) was chosen. The field was divided into 4 unequal quadrants. One sugarcane sapling (in the tillering stage) was chosen at random in each of the 4 quadrants. These saplings were uprooted and shaken to remove the soil debris that remained on the root systems. The closely adhering soil (rhizosphere soil) was carefully placed in clean sample vials and were transported to the lab.

In the lab, 1 gram of each of the 4 rhizosphere soil was weighed and was taken in autoclaved test tubes. To each of the above, 9 ml of sterile water was added and the test tubes were shaken vigorously so as to suspend the soil particles and the adherent bacterial cells. The contents of each of the test tubes were transferred into alcohol-washed Tarson's tubes and were subjected to centrifugation at 5000 rpm for 10 minutes. The supernatant containing the bacterial cells were used to culture. A loopful of the supernatant was streaked onto Soil Extract Agar (SEA) plates and the plates were incubated at 37ºC for 2 days until colonies have grown.

➢ *Preparation of samples and strain isolation.*

Rhizosphere soil samples were taken aseptically from the fields and placed in sterile polypropylene bags. They were then brought to the lab for microbiological isolation in less than five hours. To create a rhizospheric soil suspension, the roots were rotary agitated for 20 minutes in 90 mL of sterile phosphate buffer, pH 7, after being cleaned in sterile distilled water to remove loosely adherent soil.

For the preparation of soil extraction agar (SEA), 400g of bulk soil from the collection site t was autoclaved at 121o C, 15lbs pressure for 15 minutes. The sterilized soil was mixed well with 1000mL distilled water. This soil-water mixture was allowed to undergo sedimentation for 4-5 hours and the supernatant was carefully filtered into a clean conical flask. To this, 0.5g of disodium phosphate, 1g of glucose, and 15g of bacteriological-grade agar was added. The mixture was heated to ensure the dissolution of agar, and was autoclaved. The finished medium was pour into 6 sterile glass petri plates (10-15ml per plate), cooled to allow the medium to set and the soil suspensions were steaked on them. Once the colonies were established, they were characterized based on the types and sizes of the colonies. Around 5-6 colonies were found growing on the SEA plates. A control plate for each of the 6 plates were maintained.

The bacterial colonies were pure cultured on nutrient agar (HiMediaTM) plates. The resultant mixture was heated to dissolve the agar particles. This solution was autoclaved. Meanwhile, 3 sterile, dry petri plates were divided into 4 quadrants by drawing horizontal and vertical lines on the

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under-side of the plate base. The agar medium was allowed to cool and set, and poured into the divided petri plates.

Into each of the 4 quadrants, a new colony of bacteria from the mother culture was streaked with the help of a heat sterilized inoculation loop. The plates were sealed air tight with a cling film and were incubated at 37oC for 2 days. Each of these pure cultures were inoculated into 400 □L of Yeast Extract Mannitol Broth which was taken in 1.5 mL Eppendorf tubes. To these 50% glycerol (2.5mL pure glycerol in 5mL distilled water) was added. The resultant glycerol stocks were vortexed and were stored at -20oC. The shape and the cell wall status of the bacteria in pure cultures were determined by this differential staining technique for characterizing them into Gram + and Gram - bacteria.

- *Estimation of Indole-3-Acetic Acid Using Colorimetry*

This protocol was elucidated by Gordon and Weber in 1951 [35]. 1g of yeast extract, 10g of mannitol, 0.5g of disodium phosphate, 0.2g of magnesium heptahydrate and 0.1g of sodium chloride were weighed and were added into a clean and dry conical flask. Dissolve these contents in a small volume of distilled water and was made up to 1000 mL using distilled water. The pH was set to 7.0 ± 0.2. A volume of 100 mL of YEM was prepared and amended with 0.1% L-tryptophan (0.1 g or 100 mg of L-tryptophan in 100 mL of YEM). A volume of 5 mL of YEM was taken into clean, sterile test tubes, and a loopful of the pure cultured bacteria was aseptically added. The test tubes were plugged with cotton and incubated in an orbital shaker incubator at 32°C at 100-110 rpm for 5 days. In a fume hood, 2 mL 0.5M $FeCl_3$ was added to 40 mL of 70% perchloric acid and 49 mL of distilled water to make the Salkowski Reagent which will be used to estimate IAA. 10mg of pure IAA was weighed carefully and was transferred into a glass stoppered bottle. Under the fume hood, 10mL of acetone was measured using a glass measuring cylinder. The acetone was emptied into the stoppered bottle containing the weighed IAA, and complete dissolution of IAA was ensured. This is a 1mg/mL stock solution.

From the IAA stock solution, 0.2 – 1.0 mL was transferred into 5 different clean, dry test tubes using a clean pipette. The volume in each of the test tubes was made up to 2 mL with the YEM that had been amended with 0.1% L-Tryptophan. To each of the test tubes, 0.4 mL of Salkowski reagent was added. The contents were mixed to uniformity and incubated at room temperature for 25 minutes until a bright pink color developed. A blank was maintained with all the required reagents except for the stock solution, which was substituted with the amended YEM. Post incubation, the contents of the blank tube were transferred into a clean cuvette. The cuvette was placed into the cavity of a warmed-up colorimeter. The wavelength knob was set to 540 nm, and 'zero' was set to read the blank. The blank was removed, the cuvette was cleaned, and 0.2 mg/mL of the tube contents was transferred into it to read the absorbance. The absorbance of the other tubes was read in the same manner.

The unknown samples consisted of bacterial cultures that were grown for five days in 0.1% L-Tryptophan YEM medium. A volume of 2 mL of the liquid culture was taken in Eppendorf tubes and centrifuged at 5000 rpm for 5-7 minutes. The clear supernatant containing the synthesized IAA was transferred into a test tube. To this, 0.4 mL of Salkowski reagent was added, and it was incubated at room temperature for 25 minutes until the pink color had developed. This was transferred into a clean cuvette, and the absorbance was read against the blank at 540 nm. The above steps were repeated for all the liquid cultures. Before centrifugation of the Eppendorf tubes, they were weighed and the weight was recorded. Once the contents (2mL) were emptied, the Eppendorf tubes with the cell pellets were allowed to dry in the hot air oven for 3 days. The Eppendorf tubes were weighed with the dried cell pellets. The weights of the bacterial cell pellets were obtained by calculating the differences in weight, this data will be used to assess the amount of IAA produced per unit weight of the cell pellets.

The above information was fed into MS Excel 2013. Using the scatter plot and add trendline options, a standard curve was generated.

The isolates that produced color on incubation were only considered for calculating the amount of IAA produced. The other isolates did show some value for the absorbance (between 0.2-0.56 OD) but failed to produce the characteristic pink color (an indication that IAA in the medium reacted with FeCl3 in the presence of perchlorate ions to yield a soluble pink complex).

- *Qualitative Analysis of Phosphate Solubilization in NBRIP Agar (Nautiyal 1999) [42]*

Preparation of National Botanical Research Institute's Phosphate Growth Medium Devoid of Yeast Extract (NBRIP): 10g of glucose, 5g of Ca3(PO4)2[insoluble calcium source], 5g of MgCl.7H2O, 0.25g of MgSO4.7H2O, 0.2g of KCl, 0.1g of (NH4)2SO4 were weighed and added to a clean, dry conical flask. The contents were dissolved in a minimal volume of distilled water. To this 15g of agar was added. The contents were mixed and the total volume was made up to 1000mL with distilled water. The resultant mixture can be heated to ensure the complete dissolution of agar particles. The flask was cotton plugged and autoclaved. This recipe was downsized to suit our needs to prepare a 300mL medium. Post autoclaving, the medium was allowed to cool and was poured into sterilized petri plates that were divided into 4 quadrants (10-15mL medium per plates). The medium was allowed to set and in each of the 4 quadrants, 4 different pure cultured bacteria were streaked. The plates were sealed with a cling film and were incubated at 37oC for 3 days. The isolates that showed a zone of clearance were capable of solubilizing the insoluble Ca3(PO4)2. This is a very important trait for a PGPR. The zone of clearance around each positive colony was recorded and tabulated in millimeters

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## III. RESULTS

In total 26 isolates, named A to Z were successfully isolated from SEA and were maintained on NA. From the gram staining results, 17 isolates were negative of which, 8 were cocci, 5 rods and 4 irregular. 9 positive isolates were identified out of which, 5 cocci, 3 rods and 1 irregular.

A protocol table (Table 1) was generated based on the requirements and the order of the estimation of the secreted IAA from the YEM and 0.1% L-Tryptophan, depicting the standard concentrations of IAA stock solution, their volumes used for the estimation, volumes of the Salkowski reagent, incubation procedure and the respective absorbances at 540 nm.

Using the data from Table 1, a standard calibration curve was generated on MS Excel 2013. From the graph, the amount of IAA produced per unit weight of the dry cell pellet of each of the 11 isolates were determined based on their respective absorbances at 540nm. The supernatant of the YEM with the isolates were used as unknowns to estimate the amount of IAA. This information is enumerated in the Table 2 and is also represented graphically in Graph 1

From the Graph 1 it is evident that strain “C" produced the maximum amount of IAA per gram of the bacterial cell pellet, i.e. 2.72mg of IAA per gram of dry cell pellet, following which is strain “M” producing 2.61mg per gram of dry cell pellet.

The strains of PGPR that solubilized insoluble phosphates in NBRIP agar were tabulated in Table 3. Of the 26 bacterial species that were isolated from the rhizosphere, IAA was produced in detectable levels by only 11 of them, and only 8 out of the 26 isolates were found to be capable of solubilizing the insoluble calcium source, Ca3(PO4)2, in NBRIP agar. These results are graphically represented in Graph 2. 3 out of the 26 bacterial isolates were able to produce IAA and also solubilize insoluble phosphate, namely R, M and N.

Based on the results of IAA production and the respective colony characteristics and gram staining, the isolates were identified to be Acinetobacter spp, Pseudomonas spp, Azotobacter spp. and Enterobacter spp. Bacillus spp, Azotobacter spp, Rhizobium spp, Enterobacter spp and Pseudomonas spp. were identified to solubilize the phosphates. However, nuclei acid-based strain assessment methods such as PCR amplification of 16S rRNA genes is required to warrant the taxonomy of these plausible isolates [36 and 37].

Table 1 Protocol Table for Colorimetric Estimation of IAA

| **Serial no:** | **1** | **2** | **3** | **4** | **5** | **6 (Blank)** | **7 (Unknown)** |
|---|---|---|---|---|---|---|---|
| **Concentration of stock soln (mg/mL)** | 0.2 | 0.4 | 0.6 | 0.8 | 1 | 0 | - |
| **Volume of stock taken (mL)** | 0.2 | 0.4 | 0.6 | 0.8 | 1 | 0 | 0 |
| **Volume of Amended YEM added (mL)** | 1.8 | 1.6 | 1.4 | 1.2 | 1 | 2 | 2 |
| **Total volume (mL)** | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| **Volume of Salkowski reagent added (mL)** | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 |
| **Incubation at Room Temp** | 25min | | | | | | |
| **Absorbance [O.D] at 540nm** | 0.52 | 0.65 | 0.74 | 0.83 | 0.91 | 0.00 (as set) | ? |

Table 2 Amount of IAA Produced Per Dry Weight of the Cell

| **Sl No:** | **Isolate** | **Absorbance at 540nm** | **Determined concentration of IAA from the standard curve** | **Weight of dry cell pellet (g)** | **Amount of IAA produced per gram of the cell pellet (mg/g)** |
|---|---|---|---|---|---|
| **1** | N | 0.49 | 0.1 | 0.32 | 0.31 |
| **2** | H | 0.54 | 0.21 | 0.29 | 0.72 |
| **3** | B | 0.51 | 0.15 | 0.28 | 0.53 |
| **4** | S | 0.5 | 0.13 | 0.36 | 0.46 |
| **5** | M | 0.83 | 0.81 | 0.31 | 2.61 |
| **6** | L | 0.51 | 0.15 | 0.24 | 0.62 |
| **7** | R | 0.53 | 0.19 | 0.32 | 0.59 |
| **8** | C | 0.8 | 0.74 | 0.29 | 2.72 |
| **9** | X | 0.55 | 0.23 | 0.3 | 0.76 |
| **10** | V | 0.6 | 0.33 | 0.29 | 1.13 |
| **11** | U | 0.73 | 0.6 | 0.34 | 1.76 |
| **Plausible bacterium based on the colony morphology and Gram staining** | | | *Acinetobacter spp* *Pseudomonas spp* *Azotobacter spp* *Enterobacter spp* | | |

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Table 3 Isolates that Solubilized Phosphates.

| Sl No. | Designated Isolated that Solubilized Ca3(PO4)2 in NBRIP Agar | Plausible Bacterium Based on the Bacterial Characterization |
|---|---|---|
| **1** | A | *Bacillus spp Azotobacter spp Rhizobium spp* |
| **2** | D | |
| **3** | O | |
| **4** | E | |
| **5** | J | |
| **6** | R | *Enterobacter spp Pseudomonas spp* |
| **7** | N | |
| **8** | M | |

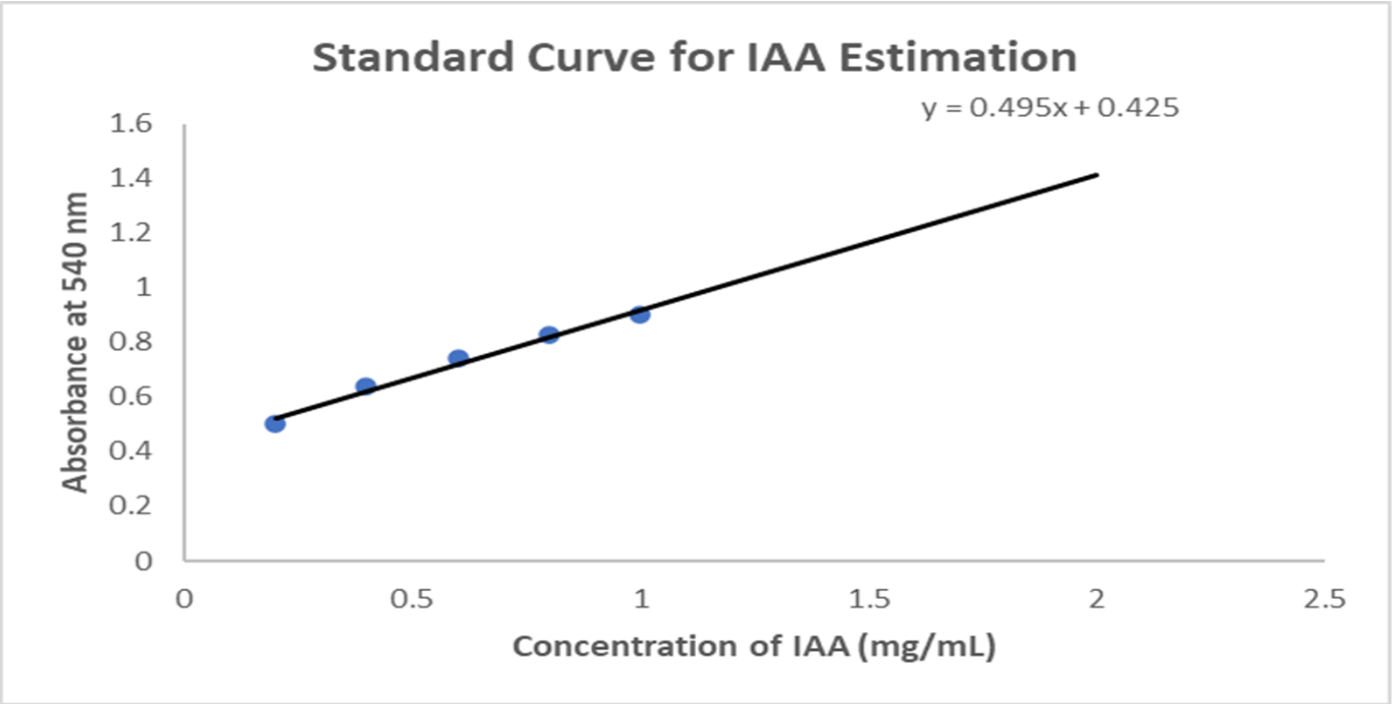


Fig 1 Comprehensive Representation of IAA Produced Per Gram of the Cell Pellet

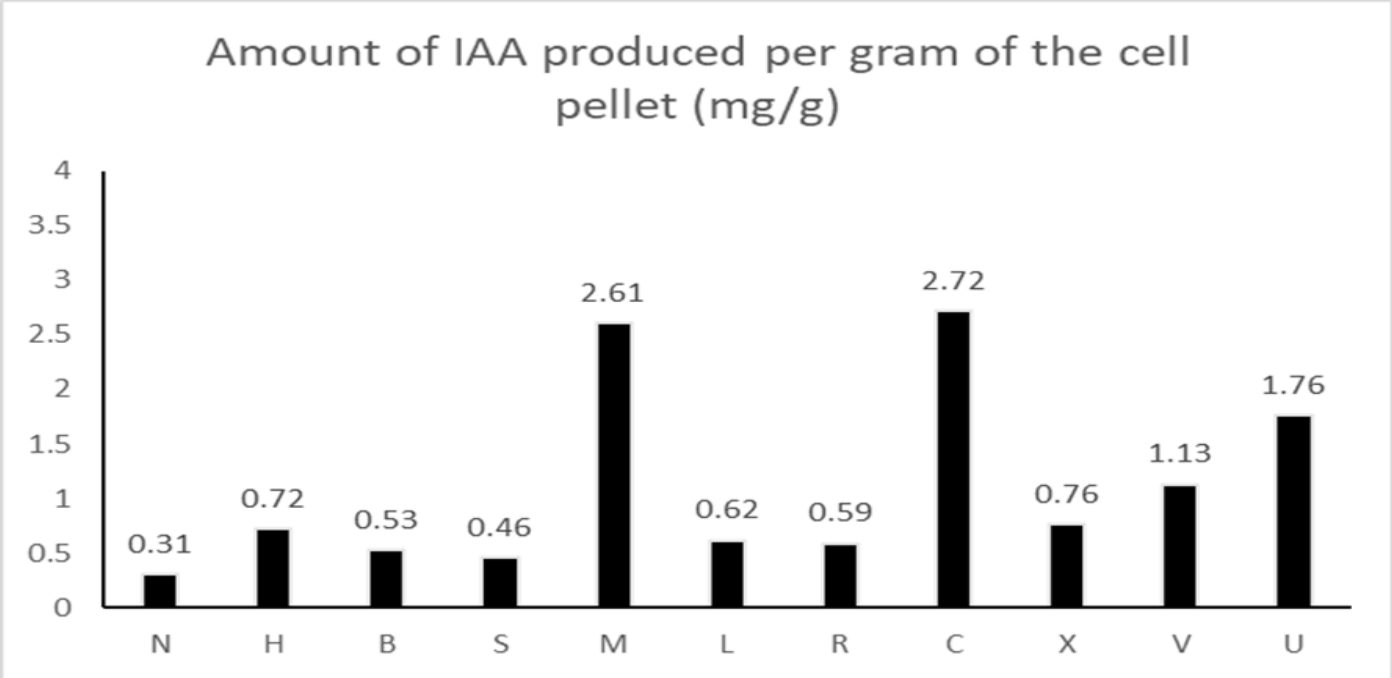


Fig 2 Comprehensive Representation of IAA Produced Per Gram of the Cell Pellet

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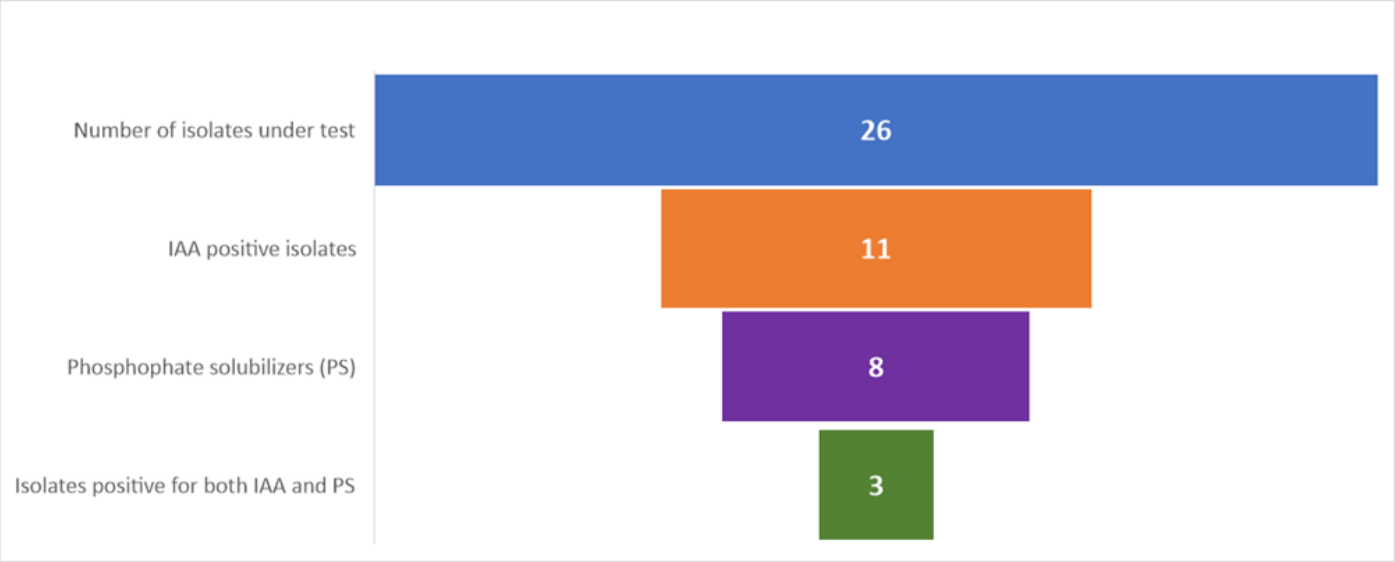


Fig 3 Status of the Isolates Procured in This Study

## IV. CONCLUDING REMARKS

Conclusions from the results that we drew was that strains M and C producing the highest amount of IAA can best serve to be an efficient soil inoculum as far as the other strains that were tested are concerned. Also the 8 strains of isolates, A, D, O, E, J, R, M and N can support plant growth by making the insoluble soil phosphates to a soluble form. A more potential option for soil inoculum would be to use either individually or as a consortium the strains M, N and R, due to the dual ability to provide IAA and soluble phosphates.

From the above facts we could conclude that applying PGPR inoculants to soils would possibly be a novel approach to reduce the use of chemical fertilizers, which have deleterious impacts on soil health and natural diaspora of beneficial soil microbes. Apart from plant growth promotion, these PGPR inoculates tend to defend the plant from pathogens, which also is a growth promoting strategy. This venture also opens doors for the use of technologies that improve the efficiency of the inoculants. Thus, the introduction of beneficial bacteria in the soil tends to be less aggressive and cause less impact to the environment than chemical fertilization, which makes it a sustainable agronomic practice and a way of reducing the production costs.

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