# ISOLATION AND APPLICATION OF PLANT GROWTH PROMOTING RHIZOBACTERIA FROM MANGROVES OF GOA

A Dissertation

Course code and course title: MMD 412 Dissertation

Credits: 08

Submitted in partial fulfilment of Master's Degree

M.Sc. in Marine Microbiology

**BY**

**AFSHA A. MEMON**

21P039013

Under the Supervision of

**DR. CHANDA BERDE**

School of Earth, Ocean and Atmospheric Sciences

Marine Microbiology


GOA UNIVERSITY

DATE: APRIL 2023

Examined by: Seal of the school

## DECLARATION

I hereby declare that the work incorporated in this dissertation, titled as "Isolation and application of plant growth promoting rhizobacteria from mangroves of Goa" is based on the

results of investigations carried out by me in the Marine Microbiology at the School of Earth, ocean and Atmospheric Sciences, Goa University under the Supervision of Dr. Chanda Berde and the same has not been submitted elsewhere for the award of degree or diploma by me. I understand that Goa University or its authorities will be not be responsible for the correctness of observations or other findings given the dissertation.

I hereby authorize the University authorities to upload this dissertation on the dissertation repository or anywhere else as the UGC regulations demand and make it available to any one as needed.

Afsha A. Memon

21P039013

M.Sc. Marine Microbiology

School of Earth, Ocean and Atmospheric Sciences

Date

Place: Goa University

## COMPLETION CERTIFICATE

This is to certify that the dissertation entitled as "Isolation and application of plant growth promoting rhizobacteria from mangroves of Goa" is a bonafide and an authentic record of the research carried out by Ms. Afsha A. Memon, student of M.Sc. Marine Microbiology under the supervision and guidance of Dr. Chanda Berde, Assistant Professor, at the School of Earth Ocean and Atmospheric Sciences, Goa University, Goa, in partial fulfilment of the requirement of M.Sc. Marine Microbiology Degree of the University and that no part has been submitted before for any other degree or diploma in any other university.

Chanda

Dr. Chanda Berde

Marine Microbiology

Date: 02/05/2023

Prof. Chandrashekher U. Rivonker

Dean

School of Earth, Ocean and Atmospheric Sciences

Date:

Dr. C. U. Rivonker
Senior Professor and Dean,
School of Earth, Ocean
& Atmospheric Sciences,
Goa University,
Goa - 403 206.

SCHOOL OF EARTH, OCEAN & ATMOSPHERIC SCIENCES
GOA UNIVERSITY

III

**COMPLETION CERTIFICATE**

This is to certify that the dissertation entitled as “Isolation and application of plant growth promoting rhizobacteria from mangroves of Goa” is a bonafide and an authentic record of the research carried out by Ms. Afsha A. Memon, student of M.Sc. Marine Microbiology under the supervision and guidance of Dr. Chanda Berde, Assistant Professor, at the School of Earth Ocean and Atmospheric Sciences, Goa University, Goa, in partial fulfilment of the requirement of M.Sc. Marine Microbiology Degree of the University and that no part has been submitted before for any other degree or diploma in any other university.

Dr. Chanda Berde

Marine Microbiology

Date:

Prof. Chandrashekher U. Rivonker

Dean

School of Earth, Ocean and Atmospheric Sciences

Date:

**ACKNOWLEDGEMENT**

I am overjoyed as I express my gratitude towards all the people who contributed towards the completion of my project. I express my deep sense of gratitude to our Dean Dr. Chandrashekhar U. Rivonker. I would like to express my gratitude and appreciation to our Programme Director Dr. Priya M. D'costa for her support. Special thanks to my guide Dr. Chanda Berde for her encouragement, guidance and for having the time and patience to solve my queries throughout the duration of the project.

I would also like to thank all the staff (teaching and non-teaching) of School of Earth Ocean and Atmospheric Sciences, Goa University for helping me with everything that was required whenever approached. I extend my science thanks to our Lab Assistant- Ms Vaishali and Ms Sitam for constantly helping me throughout my dissertation.

Afsha Memon

**TABLE OF CONTENTS**

## ABBRIVATIONS

| | |
|---|---|
| **g** | **Grams** |
| **◦ C** | **Degree Celsius** |
| **mm** | **Milli meter** |
| **RT** | **Room temperature** |
| **cm** | **Centimeter** |
| **EPS** | **Exopolysaccharide** |
| **$GA_3$** | **Gibberellin** |

| | |
|---|---|
| **IAA** | **Indole acetic acid** |
| **ml** | **Milli litre** |
| **µg** | **Microgram** |
| **YMD** | **Yeast Malt Dextrose** |
| **OD** | **Optical Density** |

## LIST OF TABLES



## LIST OF FIGURES

| | |
|---|---|
| **14** | **40** |

# APPENDIX

**Appendix 1: Media composition of Ashby's Mannitol Agar for Nitrogen Fixation**

| | |
|---|---|
| Mannitol | 20g/l |
| Dipotassium hydrogen phosphate | 0.200g/l |
| Magnesium sulphate | 0.200g/l |
| Sodium chloride | 0.200g/l |
| Potassium sulphate | 0.100g/l |
| Calcium carbonate | 5.000g/l |
| Agar | 15.000g/l |
| pH | 7.4 |

**Appendix 2: Media composition of Nutrient broth for Gibberellin production, Exopolysaccharide production (Nutrient broth + glucose) and IAA production**

| | |
|---|---|
| Beef extract | 5g |
| Peptic digest of animal tissue | 5g |
| Sodium chloride | 1.5g |
| Yeast extract | 1.5g |
| Distilled water | 1000ml |
| Glucose | 1% |

**Appendix 3: Composition of Salkowski reagent for IAA production**

| | |
|---|---|
| $Fecl_3$ | 0.5 M |
| Perchloric acid | 35% |

**Appendix 4: Media composition of Pikovskaya's Agar for Phosphate solubilization**

| | |
|---|---|
| Yeast extract | 0.500g/l |
| Dextrose | 10.000g/l |
| Ammonium sulphate | 0.500g/l |
| Potassium chloride | 0.200g/l |
| Calcium phosphate | 5.000g/l |
| Magnesium sulphate | 0.100g/l |
| Manganese sulphate | 0.0001g/l |
| Ferrous sulphate | 0.0001g/l |
| Agar | 15.00g/l |
| pH | 7.4 |

**Appendix 5: Selected cultures for Gibberellin production**

| Culture no | Absorbance at 254nm |
|---|---|
| 12 | 0.774 |
| 28 | 0.953 |
| 32 | 0.761 |
| 7s | 0.824 |
| 8s | 0.903 |

**Appendix 6: Selected cultures for EPS production**

| Culture no. | EPS Production |
|---|---|

| | |
|---|---|
| **38** | **0.04** |
| **32** | **0.04** |
| **13s** | **0.04** |
| **9s** | **0.04** |
| **14s** | **0.04** |
| 6s | 0.03 |
| 7s | 0.03 |

**Appendix 7: Growth parameters for seed treatment**

| Culture pots | 32 | 36 | 25 | Mixture | Control |
|---|---|---|---|---|---|
| Germination days | **2** | **2** | 3 | 3 | 2 |
| Germination no. | 15 | 19 | **20** | 10 | 9 |
| Root length | 2 | 6 | 5.5 | **4.5** | 3.8 |
| Leaf length | 2 | 2 | 2.3 | 2.5 | 2.9 |
| Leaf number | 2 | 2 | 2 | 2 | 2 |
| Shoot length | 22 | 18.5 | **21.4** | 18.5 | 15 |
| Shoot number | 1 | 1 | 1 | 1 | 1 |

**Appendix 8: Growth parameters for soil treatment**

| Culture pots | 32 | 36 | 25 | Mixture | Control |
|---|---|---|---|---|---|
| Germination days | 3 | 3 | 4 | 4 | 2 |
| Germination number | 14 | 18 | 20 | 10 | 9 |
| Root length | 2.5 | 6.5 | 5.5 | 2 | 3.8 |
| Leaf length | 2 | 1.8 | 2.2 | 1 | 2.9 |
| Leaf number | 2 | 2 | 2 | 2 | 2 |
| Shoot length | 15.5 | 18 | 16.9 | 7 | 15 |

| | | | | | |
|---|---|---|---|---|---|
| Shoot number | 1 | 1 | 1 | 1 | 1 |

**Appendix 9: Germination days for seed treated cultures used in pot assay**

| Cultures used in pot assay | 32 | 36 | 25 | Consortium | Control |
|---|---|---|---|---|---|
| Germination days | 2 | 2 | 3 | 3 | 2 |

**Appendix 10: Count of each parameter for seed treated cultures used in pot assay**

| Cultures used for pot assay | 32 | 36 | 25 | Consortium | Control |
|---|---|---|---|---|---|
| Germination number | 15 | 19 | 20 | 10 | 9 |
| Leaf number | 2 | 2 | 2 | 2 | 2 |
| Shoot number | 1 | 1 | 1 | 1 | 1 |

**Appendix 11: Length of each parameter for seed treated cultures used in pot assay**

| Cultures used for pot assay | 32 | 36 | 25 | Consortium | Control |
|---|---|---|---|---|---|
| Root length | 2 | 6 | 5.5 | 4.5 | 3.8 |
| Leaf length | 2 | 2 | 2.3 | 2.5 | 2.9 |
| Shoot length | 22 | 18.5 | 21.4 | 18.5 | 15 |

**Appendix 12: Germination days for soil treated cultures used in pot assay**

| Cultures used in pot assay | 32 | 36 | 25 | Consortium | Control |
|---|---|---|---|---|---|
| Germination days | 3 | 3 | 4 | 4 | 2 |

**Appendix 10: Count of each parameter for seed treated cultures used in pot assay**

| Cultures used for pot assay | 32 | 36 | 25 | Consortium | Control |
|---|---|---|---|---|---|
| Germination number | 14 | 18 | 20 | 10 | 9 |
| Leaf number | 2 | 2 | 2 | 2 | 2 |
| Shoot number | 1 | 1 | 1 | 1 | 1 |

**Appendix 11: Length of each parameter for seed treated cultures used in pot assay**

| Cultures used for pot assay | 32 | 36 | 25 | Consortium | Control |
|---|---|---|---|---|---|
| Root length | 2.5 | 6.5 | 5.5 | 2 | 3.8 |
| Leaf length | 2 | 1.8 | 2.2 | 1 | 2.9 |
| Shoot length | 15.5 | 18 | 16.9 | 7 | 15 |

## INTRODUCTION

The twenty-first century has been experiencing rapid rise in human population along with critical issues in global agro ecosystems, leading to decreased productivity and decline in sustainable agro ecosystem. The population exhibits pressure on arable land to increase crop yield, leading to indiscriminate use of chemical fertilisers, pesticides and insecticides by the farmers. Agrochemical runoffs from such land adversely affect life on earth through bioaccumulation and biomagnifications through the food chain. Also, the pesticides used to fight against plant illness affect the beneficial natural insect, soil fertility and soil microbiota and additionally have an impact on humans. Alongside, the soil acidity due to these strong chemicals is also altered. Anthropogenic activities are responsible to cause ecological damage and discourage soil health which ultimately leads to deplete the non-renewable assets. It is therefore essential to adopt various environment-friendly ways.

In the present circumstance, sustainable agriculture is essential as it offers the capacity to meet not only our present needs but also ensure a healthy future, something that can't be realised based on the harmful cultivation practices. Crop yields have been affected by a wide range of

ecological disruption emerging from complex issues. For example, salinity as a significant environmental abiotic stress is a serious global agricultural concern, turning the cultivated areas into uncultivated regions. Salinity brings about extreme change in the development and metabolism in plants (e.g., physiology, morphology, and biochemistry). Drought is another abiotic stress in most arable lands, particularly in the arid and semiarid regions affecting crop productivity. Drought affects the crop adversely by influencing water association, photosynthetic assimilation, and supplement take-up. In present times, heavy metal contamination may pose a threat to ecology. Since metals are hard to remove, environmentalists are worried about their harm to the ecology and biological systems. Some of the metals are needed at very low quantities for various plant metabolic processes, but at very high levels these metals affect the phytological and microbial network adversely. Heavy metals add to the inadequacy and irregularity of basic plant supplements. Likewise, flooding is another stress factor that affects plant productivity. All these non-biological stresses are increasing day by day due to global climatic change. Therefore, it is important to reduce environmental stress by employing eco-friendly strategies, including eco-accommodating and sustainable utilisation of beneficial microbes. The biotic stress in plants is brought by the living forms, particularly bacteria, fungi, viruses, nematodes and insects. The biotic stress directly interferes with host nutrients leading to death of the plants. The pre- and the postharvest loss occur due to biotic stress. Although few microbes participate in the biological control of pathogens, yet PGPR is known to create protection from many diseases following various mechanism including antibiosis bacteriocin, Volatile Organic Compound (VOC) production and lysis through extracellular enzyme. The microbial stimulants are found to be effective in the suppression of a variety of plant-pathogen which ultimately leads to development in the harvest. Antibiosis produced by PGPR is more efficient than others due to their antimicrobial, insecticidal, antiviral, phytotoxic, cytotoxic properties. The bacteriocin (bacterial toxins against bacteria) is very specific in their action and eliminates competitor bacterial species. The bacteriocins are produced by Gram-negative (e.g., colicin) and Grampositive (e.g., nisin) bacteria. In VOC

Production, PGPR secretes numerous VOC, which are biocontrol specialists for certain nematodes and microbes. Hydrogen cyanide (HCN) is one of the VOC which is delivered by rhizospheric microbes having the capacity in the biocontrol of some phytopathogens. The extracellular enzyme of rhizobacteria (chitinase and B-1, 3- glucanase) is associated with lysis of the cell wall. These two can degrade the fungal cell wall made of chitin and B-1, 4-N-acetyl-glucosomine and act as strong antifungals (Mohanty et al 2021).

Mangrove ecosystem a natural resource has importance to mankind by virtue of its utility and aesthetic value. This ecosystem is one of the most productive ecosystem of tropical and sub-tropical regions of the world and serves as nursery, feeding and spawning grounds for commercial living organisms. Mangroves have attained great importance both in terms of economic and ecologic aspects. In India, mangroves are found in West Bengal, Orissa, Andhra Pradesh, Tamil Nadu, Kerala, Karnataka, Goa, Maharashtra, Gujarat and Andaman and Nicobar Islands (Kannahi & Dhivya 2015). New and novel solutions for plant growth enhancements are required to ease the burden imposed on our environment and other resources. Here we can look at potential solutions to these issues by examining some of the biological applications of free-living plant growth promoting rhizobacteria (PGPR) (Morshed 2018).

**PLANT GROWTH PROMOTING RHIZOBACTERIA**

Plant growth promoting rhizobacteria (PGPR) are the group of bacteria that enhances growth of plant. The rhizosphere refers to the volume of soil surrounding roots where plant root is influenced chemically, physically and biologically (Morshed 2018)**.** The term rhizosphere was introduced for the first time by Hiltner. The rhizosphere microorganisms have become an important tool to guard the health of plants in an eco-friendly manner. These microorganisms can have effect on plant growth often referred to as a plant growth promotory rhizobacteria. They are involved in various biotic activities of the soil ecosystem to make it dynamic for nutrient turnover and sustainable for crop production (Gupta et al 2015). Microorganisms

proliferate in suitable habitats and help to improve soil health and soil fertility. Root exudates serve as the main source of nutrients which is composed of amino acids, monosaccharides and organic acids. In rhizosphere, this root exudate addresses the dynamic growth of plant and function of microorganisms. These root-colonizing microorganisms could be free-living, parasitic or saprophytic and their diversity becomes different with a continuous changing in community structure, and species abundance. Joseph Kloepper and Milton Schroth (Kloepper & Schroth, 1978) first reported these microbial communities and coined the name plant growth-promoting rhizobacteria (PGPR). They enhance crop growth and can help in the sustainability of a safe environment and crop productivity. Examples of PGPR are given in Table 1:

**Table 1: PGPR and their effect on growth parameters/ yields of crops/ fruit plants (Morshed 2018)**

| PGPR | Crop parameters |
|---|---|
| *Rhizobium leguminosarum* | Direct growth promotion of canola and lettuce |
| *Pseudomonas pudita* | Early development of canola seedlings, growth stimulation of tomato plant |
| *Azospirillum brasilense and A. irakense* | Growth of wheat and maize plants |
| *P. flurescens* | Growth of pearl millet, increase in growth, leaf nutrient contents and yield of banana |
| *Azotobacter and Azospirillum* spp. | Growth and productivity of canola |
| *Pseudmonas, Azotobacter and Azospirillum* spp. | Stimulates growth and yield of chick pea |
| *R. leguminosarum and Pseudomonas* spp. | Improves the yield and P uptake in wheat |
| *P.putida, P. flurescens, A.brasilense* and *A. lipoferum* | Improves seed germination, seedling growth and yield of maize |
| *P. alcaligens, Bacillus polymyxa,* and *Mycobacterium phlei* | Enhances uptake of N,P and K by maize crop |

**Plant Growth Promoting Rhizobacterial Forms:**

Plant growth promoting rhizobacteria can be classified into

- extracellular plant growth promoting rhizobacteria (ePGPR)
- intracellular plant growth promoting rhizobacteria (iPGPR).

The ePGPRs may exist in the rhizosphere, on the rhizoplane or in the spaces between the cells of the root cortex while iPGPRs locates generally inside the specialized nodular structures of root cells. (Gupta, G et al., 2015).

**Plant Growth Promotion Mechanism:**

There are a number of mechanisms used by PGPR for enhancing plant growth and development in diverse environmental conditions. (Gupta et al 2015).

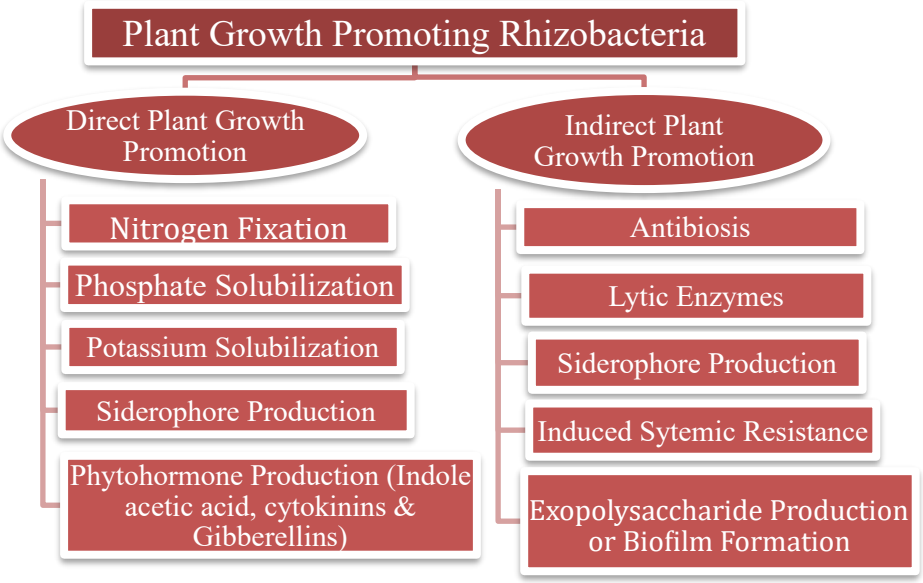



**Fig 1: Schematic diagram of plant growth promoting bacteria directly and indirectly**

**a. Direct mechanisms**

Plant growth promoting rhizobacteria have direct mechanisms that facilitate nutrient uptake or increase nutrient availability by nitrogen fixation, mineral nutrients solubilisation, mineralize organic compounds and production of phytohormones (Gupta et al 2015).

## 1. Nitrogen fixation

Nitrogen is an essential for all forms of life and it is the most vital nutrient for plant growth and productivity. Although the nitrogen presents 78 % of the atmosphere, it remains unavailable to the plants. Regrettably no plant species is capable for fixing atmospheric dinitrogen into ammonia and expend it directly for its growth. Thus the atmospheric nitrogen is converted into plant utilisable forms by biological nitrogen fixation (BNF) which changes nitrogen to ammonia by nitrogen fixing microorganisms using a complex enzyme system known as nitrogenase. Plant growth promoting rhizobacteria have the ability to fix atmospheric nitrogen and provide it to plants by two mechanisms: symbiotic and nonsymbiotic.

Symbiotic nitrogen fixation has a mutual relationship between a microbe and the plant. The microbe first enters the root and later forms nodules in which the fixation of nitrogen occurs. Non-symbiotic nitrogen fixation is carried out by free living diazotrophs and this can stimulate non-legume plants growth such as radish and rice. Inoculation by biological nitrogen fixing plant growth promoting rhizobacteria on crop provide an integrated approach for disease management, growth promotion activity, maintain the nitrogen level in agricultural soil (Gupta et al 2015).

**Table 2: Nitrogen fixing bacteria and relationship with host plants (Morshed 2018)**

| PGPR | Relationship | Host Plant |
|---|---|---|
| *Azospirillum* sp. | Non-symbiotic | Rice, wheat, maize, sugarcane |
| *Azotobacter* sp. | Non-symbiotic (aerobic) | Paspalumnotatum grass, maize, wheat |
| *Azoarcus* sp. | Non-symbiotic (aerobic) | Kallar grass, sorghum |
| *Acetobacter* | Non-symbiotic (obligatory aerobic) | Sugarcane |
| *Rhizobium leguminosarum* | Symbiotic (endo-symbiotic) | Wheat, maize, barely |
| *Bradyrhizobium betae* | Symbiotic | Sugar beets |
| *Bradyrhizobium japonicum* | Symbiotic | Cowpeas, mungbeans, soybeans |

| | | |
|---|---|---|
| *Burkholderia* sp. | Symbiotic (endo) | Rice |

**2. Phosphate solubilization**

Phosphorus is the most important key element in the nutrition of plants, next to nitrogen (N). It plays an important role in metabolic processes in plant including photosynthesis, energy transfer, signal transduction, macromolecular biosynthesis and respiration. The main phosphate solubilization mechanisms employed by plant growth promoting rhizobacteria include:

- release of complexing or mineral dissolving compounds e.g. organic acid anions, protons, hydroxyl ions, $CO_2$,
- liberation of extracellular enzymes (biochemical phosphate mineralization)
- the release of phosphate during substrate degradation (biological phosphate mineralization). (Gupta et al 2015).

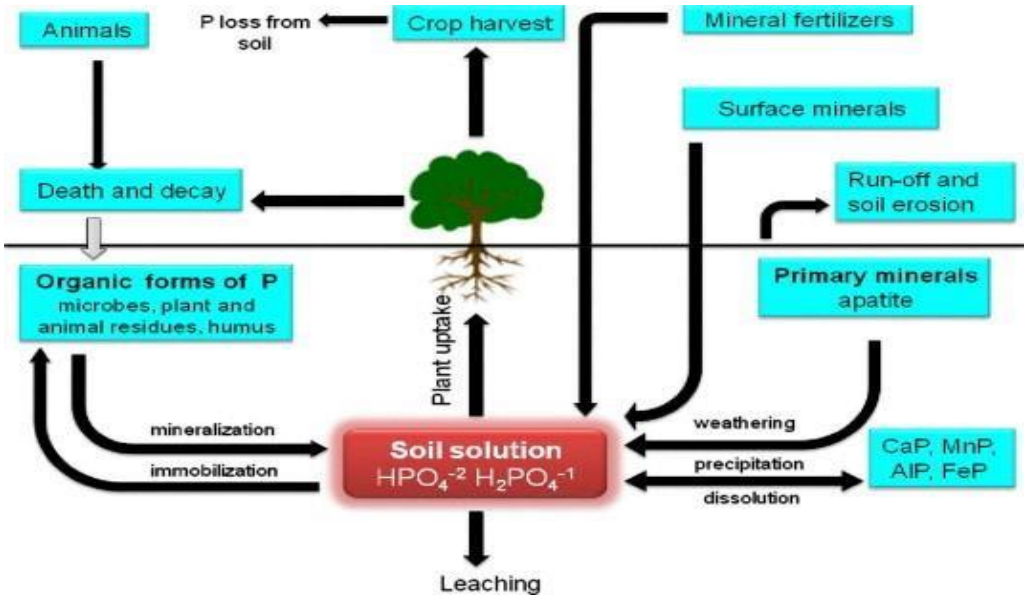



**Fig 2: Movement of phosphorus in soil (Morshed 2018)**

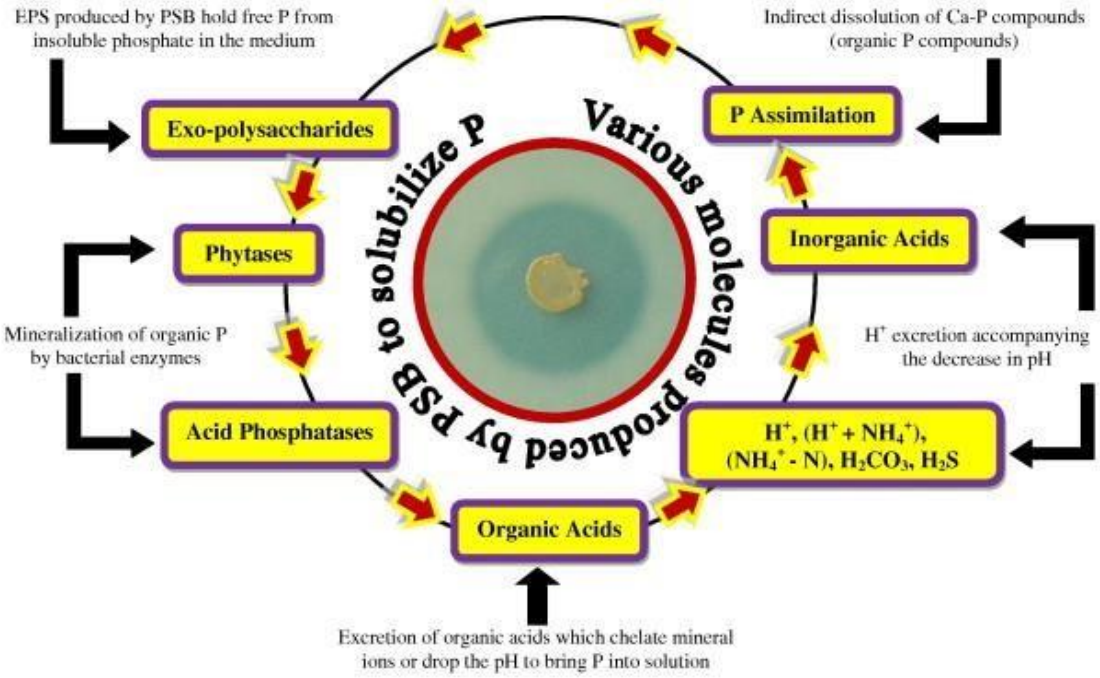



**Fig 3: Organic/inorganic substances produced by Phosphate Solubilizing Bacteria (PSB) responsible for P solubilization in soil (Morshed 2018)**

### 3. Phytohormone production

A wide range of microorganisms found in the rhizosphere are plant growth regulators Plant growth promoting rhizobacteria produce phytohormones such as indole-3-acidic, cytokinins, gibberellins and ethylene can affect cell proliferation in the root by overproduction of lateral roots and root hairs with a subsequent increase in nutrient and water uptake (Morshed 2018), (Gupta et al 2015).

**Table 3: Examples of different Phytohormone-producing PGPR (Morshed 2018)**

| PGPR | Phytohormones |
|---|---|
| *Acetobacter diazotrophicus* and *Herbaspirillum seropedicae* | Indole-3-acetic acid |
| *Azospirillum* sp. | Zeatin and ethylene |
| *Azospirillum lipoferum* | Gibberellic acid |
| *Azospirillum brasilense* | Abscisic acid |

### 3.1 Indole Acetic Acid (IAA)

Indole acetic acid (IAA) is the natural auxin found in plants having positive effect on root growth. Tryptophan is an amino acid commonly found in root exudates, has been identified as

main precursor molecule for biosynthesis of IAA in bacteria. The biosynthesis of indole acetic acid by plant growth promoting rhizobacteria involves formation via indole-3-pyruvic acid and indole-3-acetic aldehyde. (Gupta et al 2015).

### 3.2 Cytokinins and gibberellins

Several plant growth promoting rhizobacteria can produce cytokinins or gibberellins or both can produce either cytokinins or gibberellins. Some strains of phytopathogens can also synthesise cytokinins. It appears that plant growth promoting rhizobacteria produce lower cytokinin levels compared to phytopathogens so that the effect of the plant growth promoting rhizobacteria on plant growth is stimulatory while the effect of the cytokinins from pathogens are inhibitory. Ethylene is a key phytohormone has a wide range of biological activities can affect plant growth and development in a large number. The enzyme 1-aminocyclopropane-1 carboxylic acid (ACC) is a pre-requisite for ethylene production, catalysed by ACC oxidase. (Gupta et al., 2015).

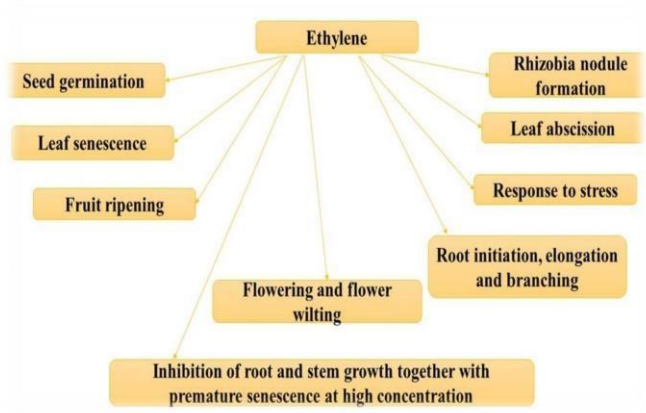



**Fig 4: The phytohormones ethylene affects a large no of different processes in the growth and development in the plant (Morshed 2018).**

**b. In-direct mechanisms**

Phytopathogenic microorganisms are a major and chronic threat to sustainable agriculture and ecosystem stability worldwide destabilises the soil ecology, degrade soil fertility, disrupt the environment and consequently show harmful effects on human health, along with contaminating ground water. PGPR is a promising sustainable and environmentally friendly

approach to obtain sustainable fertility of the soil and plant growth indirectly. (Gupta, G et al., 2015).

**1. Exopolysaccharides production or biofilm formation**

Production of exopolysaccharides is generally important in biofilm formation; root colonisation can affect the interaction of microbes with roots appendages. Effective colonisation of plant roots by EPS-producing microbes helps to hold the free phosphorus from the insoluble one in soils and circulating essential nutrient to the plant for proper growth and development and also protecting it from the attack of foreign pathogens. Plant growth promoting rhizobacterial producing exopolysaccharides are highly important in promoting plant growth due to work as an active signal molecule during beneficial interactions, and provide defence response during infection process (Gupta et al 2015).

To assess the diversity of genes involved in nitrogen fixation, fragments (360 bp) of the *nifH* gene, which encodes the nitrogenase reductase enzyme, were amplified by PCR using genomic DNA of each bacterial strain using the primers PoIR. Out of the 57 selected isolates, a 360 bp *nifH* PCR product was observed in 34 isolates showing 60% of nitrogen fixation (Do Carmo et al 2011). Five species of *Azospirillum* isolated from mangrove ecosystem were subjected for their efficiency to out the growth parameters potential for the successful establishment of mangrove seedlings. In order to determine the rate of nitrogen fixation,
medium from each saline level (0, 10, 15, 20, 25, 30 and 35 g $l^{-1}$ Nacl) were transferred into serum bottles and autoclaved. Liquid cell suspension of each *Azospirillum* sp was inoculated into medium. After sealing bottles 10% of gaseous phase was removed and same volume of acetone was injected. After 12 hours of incubation the amount of ethylene was analysed in the gas chromatograph. Nitogenase activity was expressed in Nano moles of ethylene, $10^8$ $cells^{-1}$ $ml^{-1}$ $hr^{-1}$ (Ravikumar et al 2012).

In the (Sharma et al 2018) investigation thirty isolates of Fluorescent *Pseudomonas* sp. were isolated from apple and pear rhizosphere. They were investigated for Gibberellins production. The Gibberellins were extracted and separated from supernatant with diethyl ether and detected for homogeneity by Thin Layer Chromatography (TLC). The range of production of Gibberellins was found 116.1-485.8µg/ml. *Burkolderia gladiola* was isolated from the roots of *Zea mays*, and the plant growth promoting properties were evaluated. The Gibberellins production by *Burkholderia gladioli* was 5.57±0.03 µg/ml evident in Luria Bertani (LB) broth. Quantitative estimation was done in LB broth. The samples were incubated for 72 hours and centrifuged. The concentrations were calculated by extrapolating from standard graph prepared using pure Gibberellins $GA_3$ as a standard range from 10-100µg/ml. this was the first study on production of Gibberellins by *Burkholderia gladioli* (Gunjal & Kapadnis 2013).

*Burkolderia gladiola* was isolated from the roots of *Zea mays*, and the plant growth promoting properties were evaluated. The study aimed for estimation of exopolysaccharide (EPS) production, where the cultures grown in yeast extract broth at 28◦C for 72 hours. The culture broth was centrifuged for 20 min and the supernatant was mixed with two volumes of chilled acetone. The crude polysaccharide developed was collected and centrifugation for 30 min. The EPS was washed with distilled water and acetone alternately, filtered and weighed after overnight drying in oven. The isolate produced about 0.98 g of exopolysaccharide

(Gunjal & Kapadnis 2013). Total Heterotrophic Bacteria (THB) was screened from mangrove sediment. Eight isolates were selected based on colony morphology, gram and biochemical character. All the isolates were screened for producing Exopolysaccharide (EPS). The standard seed and production media were contained with its composition. To access the effect of cultural conditions on bacterial exopolysaccharide production the standard medium were supplemented with glucose and sodium acetate. All the media used were autoclaved. The maintenance culture was transferred to sterile medium. The seed culture was incubated at 35◦C for 24 hours. Vegetative seed culture was used to inoculate same medium at different shaking speeds (25 and 450 $min^{-1}$) and temperatures (23-42◦C) for 48 to 120 hours. Off all EPS producing isolates,

isolate1 showed the maximum with 6.8mg/ml, followed by isolate 6 with 5.3mg/ml and isolate 7 showed the minimum i.e. 3.0 mg/ml level of EPS production (Lakshmipriya & Sivakumar 2012).

Plant growth promoting rhizobacteria have been identified in influencing the growth and yield of many plants. In search of efficient PGPR strains with multiple activities, microbial isolates belonging to *Bacillus* spp*, Pseudomonas* spp, *Azotobacter* spp, *Azospirillium* spp, *Phosphobactera* spp, *Glucanacetobacter* spp, *Aspergillus niger* and *Penicillium* spp were isolated from rice field, mangrove and effluent soil. These test isolates were screened for plant growth promoting traits like Indole acetic acid (IAA). The isolates were grown for 72 hours except *Bacillus* spp and *Psedomonas* which were grown for 48 hours. Fully grown cultures were centrifuged for 30 min. The supernatant was mixed with two drops of Salkowski reagent. Development of pink colour indicated IAA production. All the isolates were able to produce 100% IAA (Samuel & Muthukkaruppan 2011). IAA production is a major property of rhizosphere bacteria that stimulate and facilitate plant growth. The work dealed with isolation, characterization and identification of IAA producing bacteria from rhizospheric soil. Out of ten isolates, five were selected as efficient producers. Isolates were grown in yeast malt dextrose broth (YMD) and incubated at 28◦C for 4 days. The broth was centrifuged after incubation. Supernatant was mixed with Salkowski reagent (2% 0.5 $FeCl_3$ in 35% $HCLO_4$ solution) and kept in dark. The optical density (OD) was recorded at 530nm after 30 and 120 min. IAA production was compared in YMD and LB media. YMD medium was compared with and without tryptophan. Extraction and purification of IAA was done by centrifuging the isolates, collecting the supernatant was collected and mixed with ethyl acetate (1:2). After vigorous shaking it was allowed to stand for 10 min. IAA was extracted in solvent layer. L- Tryptophan is used as a precursor for IAA biosynthesis by various bacteria. Five isolates (br1, br2, br3, mr2, mwr2) grew well in medium containing tryptophan. Maximum IAA production was found in medium containing 0.1% for br1, br2 and br3, 1.5% for mr2 and 0.05% for wr2. IAA was not produced or the amount was negligible in medium without tryptophan (Mohite 2013).

The (Do Carmo et al 2011) study aimed to compare the bacterial structure, isolate and evaluate bacteria able to degrade oil and stimulate plant growth, from the rhizospheres of the three mangrove plant species. Fifty-seven isolates from the mangrove rhizospheres were inoculated and supplemented with oil as a sole carbon source and tested for plant growth promotion. The phosphate solubilisation test was used to determine the ability of the isolates to solubilise phosphate (calcium phytate). Strains were inoculated as spot on the Pikovskaya medium and incubated for 5 days at 32◦C. clear zones around the bacterial colonies were taken as evidence for phosphate solubilisation. The ability to solubilize phosphate was detected in 29 isolates with 51%. Four hundred and forty eight actinomycetes were isolated from water and sediment samples of mangrove areas at Andaman Coastal Research Station. Assessment of plant growth promoting activities was done having 448 isolates, of which 174 isolates showed 38.8% of phosphate solubilisation forming a clear zone around the colony on

Pikovskaya's medium. (Suksaard 2017).

(Soldan et al 2019) hypothesis was that mangroves, being able to grow into seawater, should harbour bacteria able to interact with the host and to exert positive effects under salt stress, which could be exploited to improve crop production. Bacterial endophytes were isolated from mangrove propagules with the aim to test whether these bacteria have potential on their natural host and on different crops such as barely and rice, cultivated under salt stress. The strains were tested on mangrove proagule germination and on rice growth. The most effective strain was *G. terrae* which enhanced the root length of mangrove seedlings and the biomass of salt-stressed rice under axenic conditions upto 65% and 62%, respectively. The study demonstrated that propagules, the reproductive units of mangroves, host beneficial bacteria that enhance the potential of mangrove seedlings establishment and confer salt tolerance to cereal crops.

Unsterile soil was used pot experiment. A single bacterial colony of bacterial isolate was inoculated in the nutrient broth and incubated in a rotary shaker at 120 rpm for 2-5 days. Jaggery slurry was used as an adhesive agent and mixed to the bacterial culture for seedling pelleting. Seeds mixed with broth without inoculum but jiggery slurry served as control. Seeds were dried

on a clean and sterilized blotting paper. The experiment for plant growth was planned in a randomized block design with six treatments with and without tri-calcium phosphate (TCP) which were as follows: i) Soil, uninoculated control (UC), ii) UC+TCP, iii) UC+ SSP (single super phosphate), iv) UC+ Biogold (a commercial fertilizer), v) UC+ *O. intermedium,* vi) UC + *O. intermedium* +TCP. The pot experiment was performed in pots containing 5kg unsterile and sieved clay soil. Eight mung bean seeds were sown in each pot. The plants were sprinkled with water daily as per the requirements. The plants were plucked carefully 15 days after sowing for measuring growth parameters such as shoot and root lengths and fresh and dry mass, root nodules, and chlorophyll contents, whereas at harvest time and yield parameters such as number and weight of pods and seeds were studied. The samples were dried in hot air oven at 60◦C for 48 hours for calculation dry mass. O. intermedium CP-2 acted as beneficial bioinoculant and showed increase in growth and yield of mung bean crop. Addition of TCP to the experimental soils resulted better performance for most of the growth parameters. The number of nodules formed within plant roots increased treatments as compared to the UC control. The maximum increase was observed when CP-2 was inoculated in presence of TCP. The highest significant values of shoot length was 37.6 cm and root length was 8.7 cm observed in the soil + CP-2+ TCP treatment, and the same was true for fresh and weights of roots having 0.07 and 0.06g and shoots 0.81 and 0.70g. soil treated with Biogold showed significant increase as compared to the control but lesser then *O. intermedium* CP-2 treated plants. The bacterial inoculated plants showed significantly enhanced number of pods i.e. 6, weight of pods were 2.43g, and number of seeds was 47.3 per plant in absence of TCP, followed by treatment in the presence of TCP. Unlike growth parameters, the yield was non-significant when Biogold or SSP was added as compared to other uninoculated treatments. As regarding the leaf chlorophyll contents, the highest chlorophyll-a was 1.24mg $g^{-1}$ fresh weight and total chlorophyll was 2.68 mg $g^{-1}$ fresh weight and the levels were achieved by the inoculation of bacterial strain in the presence of TCP (Saini et al 2017).

## AIMS AND OBJECTIVES

**AIM:** To enhance plant growth by mangrove organisms in a wide variety of mechanisms and to increase the availability of nutrient concentration in the rhizosphere.

**OBJECTIVES:**

→ To isolate plant growth promoting bacteria from mangrove ecosystem.

→ Morphological and Gram characteristics of bacterial isolates.

→ Screening of PGPR for various attributes: nitrogen fixation, phosphate solubilisation, production of IAA, gibberellin, and exopolysaccharide (EPS).

→ Characterisation of IAA and EPS.

→ To promote growth of mung bean plants using selected strains of PGPR.

## MATERIALS AND METHODS

### Sampling

The rhizosphere soil sample and water sample was collected across four corners of the mangrove opposite to the Mantry Villa (15◦29'59.5"N and 73◦52'55.3"E) Old Goa. The soil samples were collected from 4 - 5 cm depth under the root surface of the mangrove plant and transferred aseptically into laboratory using polythene bags, also the collected water samples were filled in plastic bottles.

### Viable count and Isolation of cultures

A ten-fold serial dilution from $10^0$ to $10^{-5}$ was done to reduce bacterial concentrations to a required concentration for the test method. 1ml of aliquot of the stock solution (soil sample) was added to the labelled tube $10^0$ which contained 9ml of 0.85% of normal saline . 1ml of aliquot from $10^0$ was transferred to $10^{-1}$ containing 9 ml of saline. 1ml from $10^{-1}$ was transferred to $10^{-2}$ and the steps were repeated till it reached up to $10^{-5}$. The last 3 dilution factor tubes i.e. $10^{-5}$, $10^{-4}$ and $10^{-3}$ were taken into consideration as the least concentrates, which were spread with 0.1 ml of each dilution onto a Nutrient agar medium. The plate was incubated at room temperature for 24 hours.

0.1 ml of water sample collected was directly spread plated on the nutrient agar plate with incubation at room temperature for 24 hours. The colonies were counted to determine the viable count, morphological character and Gram character of each isolates. Total 51 rhizobacterial isolates from the mangrove soil and water sample were isolated on the Nutrient agar slants.

**Screening of Nitrogen fixing microorganisms**

A total of 51 rhizobacteria were screened to show the fixation of nitrogen using Ashby's Mannitol agar (Appendix 1). The culture was streaked onto the plates and growth was observed for 1 week.

**Screening of Gibberellin producing microorganisms**

All the isolates obtained were screened for the production of gibberellins. The culture was inoculated in the tubes containing nutrient broth. The tubes were incubated at room temperature for 48 hours. The culture was then centrifuged at 8000 rpm for 10 min. The supernatant was taken and pH was adjusted to 2.5 using 0.1N HCl. The culture supernatants were extracted using ethyl acetate. Amount of gibberellic acid in ethyl acetate was measured by UV spectrophotometer at 254nm against the control blank.

**Screening of Exopolysaccharide producing microorganisms**

The bacterial cultures were inoculated in nutrient broth with 1% of glucose. The culture tubes were incubated at room temperature for 48 hours. The tubes grown were centrifuged at 8000 rpm 10 min. The supernatant was treated with thrice the volume of chilled ethanol (i.e. 4ml of culture and 12ml of ethanol). Precipitation observed indicated exopolysaccharide production.

**Screening of Indole acetic acid (IAA) producing microorganisms**

Isolates were grown in nutrient broth with 0.1% tryptophan and incubated at room temperature for 48 hours. The isolates were centrifuged at 8000 rpm for 10 min and to determine amounts of IAA production by each isolate, the supernatant was mixed with 2ml of Salkowski reagent (Appendix 3). On developing pink colour, optical density (OD) was recorded at 530 nm. The procedure was repeated in absence of tryptophan. The supernatant was mixed with ethyl acetate vigorously and allowed it to stand for 10 minutes. IAA was extracted within solvent layer. The procedure was repeated 2 times.

In order to confirm Thin Layer Chromatography slide was prepared with silica gel. Chloroform:Methanol (5:1) was used as a solvent system. The extracted samples were spotted on TLC plate. Chromatogram was developed with the Salkowski's reagent.

**Screening of Phosphate solubilizing microorganisms**

All the bacterial cultures obtained were screened for phosphate solubilization. The cultures were spot inoculated on the Pikovskaya agar (Appendix 4). The plates were incubated for 4872 hours and clear zones around the bacterial colonies were taken as evidence for phosphate solubilisation.

**Pot Assay**

Unsterile soil was used pot experiment. A single bacterial colony of bacterial isolate from mangrove was inoculated in the nutrient broth and incubated in a rotary shaker at 120 rpm for 2-4 days. After growth the broth was centrifuged at 8000rpm for 10 min. Suspension was made for each of the culture pellets and used for pot experiment. For seed treatment of experimental cultures, 40 mung seeds were soaked overnight in each suspension; also a mixture of all suspension was made in which seeds were soaked overnight. Control was maintained with same number of seeds soaked in water. Approximate 1kg of soil was weighed for experimental and control to which the treated seeds and the seeds just soaked in water was introduced into each pot with appropriate labels. The plants were sprinkled with water daily as per the requirements. For soil treatment same number of seeds were taken and soaked in water overnight. The weighed soil was mixed with the culture suspension thoroughly and the mung seeds were introduced to each pot. Plant Growth promotion of mangrove isolates was observed for 15 days considering parameters such as germination time and number, root length, leaf length leaf number, shoot length and shoot number of each pot.

# RESULTS

## Isolation of cultures

Fifty one morphologically distinct colonies were isolated on sterile nutrient agar. These isolates were purified in 3 cycles and were given culture codes. The isolates were characterized based on their morphology and Gram characteristics.

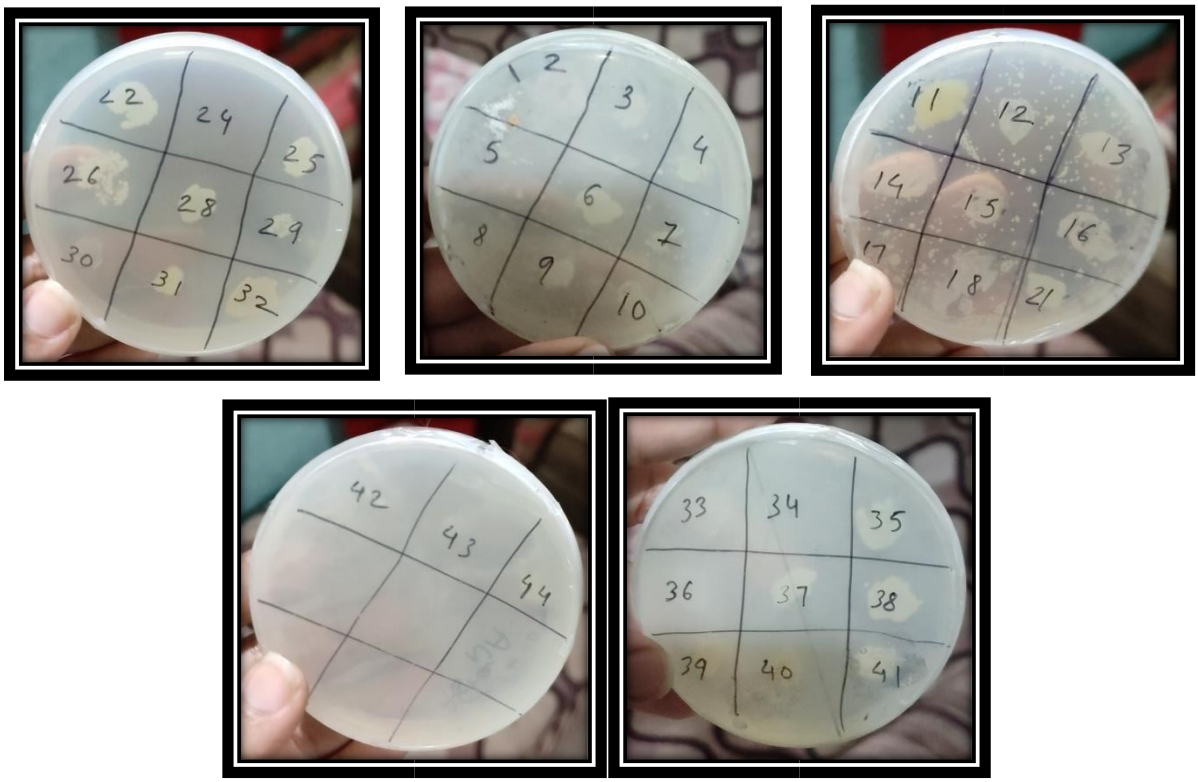



**Fig 5: Bacterial isolates on Nutrient agar plates**

**Colony characteristics of the selected cultures.**

**Table 4: Colony characteristics of Nitrogen fixing cultures**

| **Culture** | **12** | **16** | **18** | **25** | **36** |
|---|---|---|---|---|---|
| **Size** | 2mm | 2mm | 1mm | 1mm | 3mm |
| **Shape** | Circular | Oval | Circular | Circular | Circular |
| **Time** | 24hr | 24hr | 24hr | 24hr | 24hr |
| **Temperature** | RT | RT | RT | RT | RT |
| **Colour** | White | White | White | White | White |
| **Elevation** | Flat | Raised | Raised | Raised | Raised |
| **Margin** | Entire | Entire | Entire | Entire | Entire |
| **Opacity** | Transparent | Opaque | Opaque | Opaque | Opaque |
| **Consistency** | Sticky | sticky | Sticky | Non sticky | Sticky |

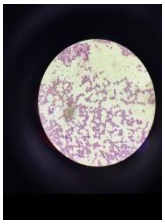
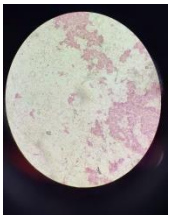
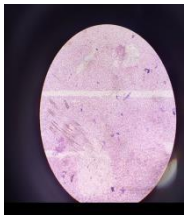
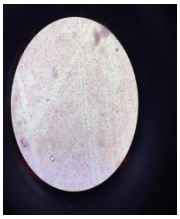
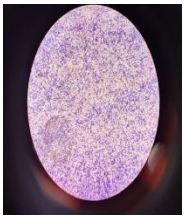
| **Gram character** | Gram positive cocci | Gram negative rods | Gram positive cocci in clusters | Gram positive rods in clusters | Gram positive rods in clusters |
|---|---|---|---|---|---|
| **Image** |  |  |  |  |  |

**Table 5: Colony characteristics of $GA_3$ producing cultures**

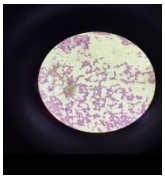
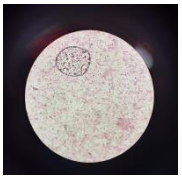
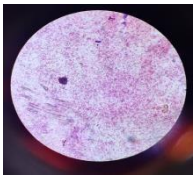
| **Culture** | **12** | **28** | **32** |
|---|---|---|---|
| **Size** | 2mm | 1mm | 1mm |
| **Shape** | circular | Circular | Circular |
| **Colour** | white | white | white |
| **Time** | 24hours | 24hours | 24hours |
| **Temperature** | RT | RT | RT |
| **Elevation** | Flat | Raised | Raised |
| **Margin** | Entire | Entire | Entire |
| **Opacity** | Transparent | Opaque | Transparent |
| **Consistency** | Sticky | Non sticky | Non sticky |
| **Gram character** | Gram positive cocci | Gram negative rods in clusters | Gram positive cocci in clusters |
| **Image** |  |  |  |

**Table 6: Colony characteristics of EPS producing cultures**

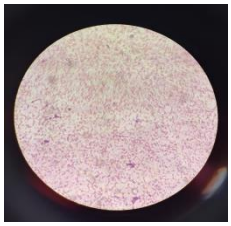
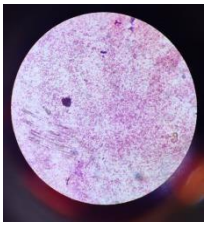
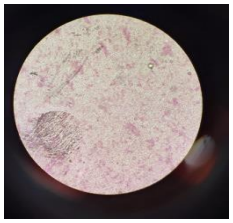
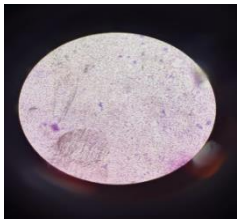
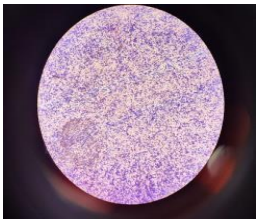
| **Culture number** | **38** | **32** |
|---|---|---|
| **Size** | 1mm | 1mm |
| **Shape** | Circular | Circular |
| **Colour** | white | White |
| **Time** | 24hr | 24hr |
| **Temperature** | RT | RT |
| **Elevation** | raised | Raised |
| **Margin** | Entire | Entire |
| **Opacity** | Opaque | Transparent |
| **Consistency** | Sticky | Non sticky |
| **Gram character** | Gram negative rods in clusters | Gram positive cocci in clusters |
| **Image** |  |  |

**Table 7: Colony characteristics of IAA producing cultures**

| **Culture** | **34** | **35** | **36** |
|---|---|---|---|
| **Size** | 2mm | 2mm | 3mm |
| **Shape** | Circular | Oval | Oval |
| **Colour** | white | white | White |
| **Time** | 24hr | 24hr | 24hr |
| **Temperature** | RT | RT | RT |

| | | | |
|---|---|---|---|
| **Elevation** | Raised | Raised | Raised |
| **Margin** | Entire | Entire | Entire |
| **Opacity** | Transparent | Transparent | Opaque |
| **Consistency** | Non sticky | Sticky | Sticky |
| **Gram character** | Gram negative rods in clusters | Gram positive cocci in clusters | Gram positive rods in clusters |
| **Image** |  |  |  |

| **Culture** | **37** | **38** | **40** |
|---|---|---|---|
| **Size** | Pinpoint | 1mm | 1mm |
| **Shape** | Circular | Circular | Circular |
| **Colour** | white | white | White |
| **Time** | 24hr | 24hr | 24hr |
| **Temperature** | RT | RT | RT |
| **Elevation** | Raised | Raised | Raised |
| **Margin** | Entire | Entire | Entire |
| **Opacity** | Opaque | Opaque | Opaque |
| **Consistency** | Sticky | Sticky | Sticky |
| **Gram character** | Gram negative rods | Gram negative rods in clusters | Gram negative rods in clusters |

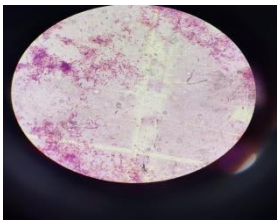
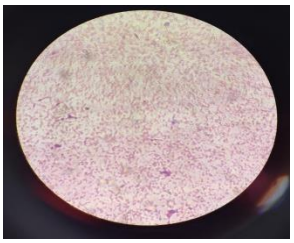
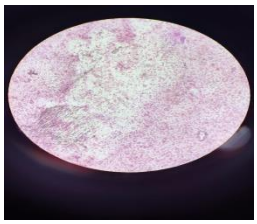
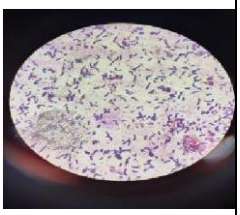
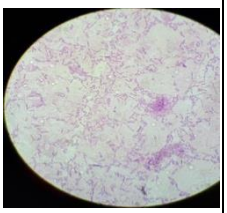
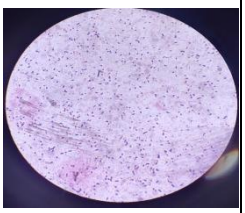
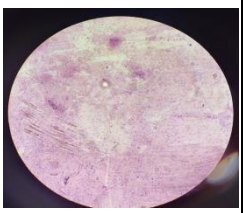
| | | | |
|---|---|---|---|
| **Image** |  |  |  |

**Table 8: Colony characteristics of Phosphate solubilizing cultures**

| **Culture** | **4** | **10** | **11** | **25** |
|---|---|---|---|---|
| **Size** | 1mm | 4mm | 4mm | 1mm |
| **Shape** | Circular | Circular | Circular | Circular |
| **Colour** | Yellow | White | White | White |
| **Time** | 24hr | 24hr | 24hr | 24hr |
| **Temperature** | RT | RT | RT | RT |
| **Elevation** | Flat | Raised | Flat | Raised |
| **Margin** | Entire | Entire | Entire | Entire |
| **Opacity** | Opaque | Opaque | Opaque | Opaque |
| **Consistency** | Sticky | Non sticky | sticky | Non sticky |
| **Gram character** | Gram positive rods | Gram positive rods in chains | Gram positive rods | Gram negative rods |
| **Image** |  |  |  |  |

**Screening of Nitrogen fixing microorganism**

The bacterial isolates showed growth on Ashby's Mannitol agar media, out of 51 isolates, 5 bacterial isolates grew well on the media as shown in Table 9 therefore this five isolates were nitrogen fixing microorganisms.

**Table 9: Bacterial isolates showing nitrogen fixing organisms**

| Culture no. | Nitrogen fixation | Culture no. | Nitrogen fixation | Culture no. | Nitrogen fixation |
|---|---|---|---|---|---|
| 2 | + | 21 | + | 41 | ++ |
| 3 | _ | 22 | + | 1s | - |
| 4 | _ | **25** | +++ | 2s | - |
| 5 | _ | 26 | + | 3s | + |
| 6 | + | 28 | + | 4s | - |
| 7 | + | 29 | + | **5s** | **-** |
| 8 | _ | 30 | _ | 6s | + |
| 9 | _ | 31 | + | 7s | - |
| 10 | + | 32 | + | 8s | + |
| 11 | ++ | 33 | + | 9s | - |
| **12** | +++ | 34 | + | 10s | - |
| 13 | ++ | 35 | + | 11s | + |
| 14 | ++ | **36** | +++ | 12s | + |
| 15 | ++ | 37 | _ | 13s | + |
| **16** | +++ | 38 | + | 14s | + |
| 17 | ++ | 39 | + | 15s | + |
| **18** | +++ | 40 | _ | 16s | - |

Key: **+, growth; ++, good growth; +++, very good growth; -, No growth**

**Screening of $GA_3$ producing microorganisms**

Out of 51 bacterial cultures, 5 were selected that produced higher Gibberellic acid on extraction shown in Fig 7. Hence the activity was found more in culture 28 and lowest in culture 12 as shown in table 10. Graph of absorbance was plotted for 5 selected cultures as shown in Fig 6. More the absorbance more the amount of $GA_3$ is produced.

**Table 10: Bacterial isolates showing absorbance at 254nm for $GA_3$ production**

| Culture no. | Absorbance at 254nm | Culture no. | Absorbance at 254nm | Culture no. | Absorbance at 254nm |
|---|---|---|---|---|---|
| 2 | 0.351 | 21 | 0.744 | 41 | 0.517 |
| 3 | 0.642 | 22 | 0.476 | 1s | 0.316 |
| 4 | 0.299 | 25 | 0.323 | 2s | 0.732 |
| 5 | 0.657 | 26 | 0.539 | 3s | 0.647 |
| 6 | 0.678 | **28** | **0.953** | 4s | 0.572 |
| 7 | 0.355 | 29 | 0.451 | 5s | 0.532 |
| 8 | 0.667 | 30 | 0.492 | 6s | 0.549 |
| 9 | 0.421 | 31 | 0.52 | 7s | 0.824 |
| 10 | 0.401 | **32** | **0.761** | 8s | 0.903 |
| 11 | 0.548 | 33 | 0.667 | 9s | 0.481 |
| **12** | **0.774** | 34 | 0.534 | 10s | 0.345 |
| 13 | 0.372 | 35 | 0.466 | 11s | 0.64 |
| 14 | 0.643 | 36 | 0.352 | 12s | 0.435 |
| 15 | 0.475 | 37 | 0.468 | 13s | 0.498 |
| 16 | 0.447 | 38 | 0.407 | 14s | 0.524 |
| 17 | 0.37 | 39 | 0.499 | 15s | 0.316 |
| 18 | 0.478 | 40 | 0.542 | 16s | 0.721 |

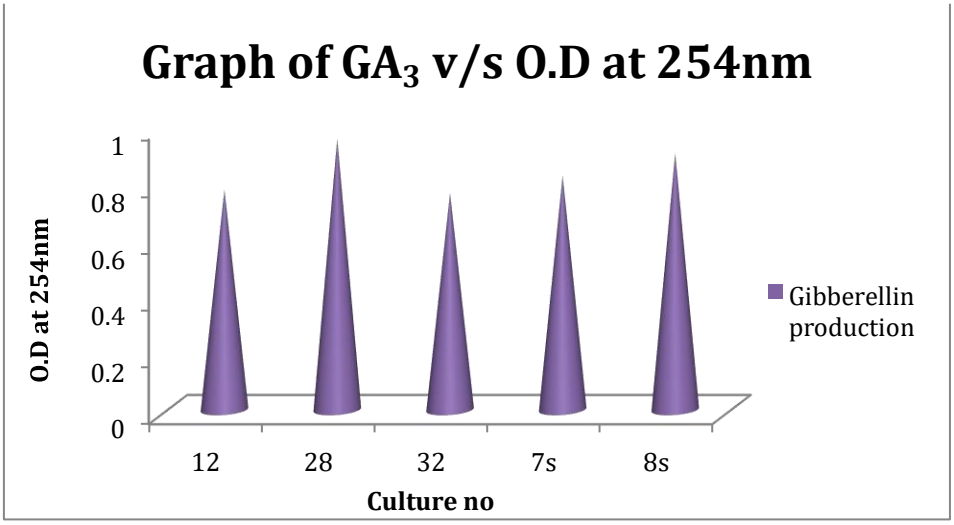



**Fig 6: Graphical representation of bacterial isolate showing highest absorbance value at 0.953nm in culture 28 for $GA_3$ production.**

**Fig 7: Extraction using ethyl acetate**

**Screening for Exopolysaccharide producing microorganisms**

Among 51 bacterial isolates 7 isolates were selected as best EPS producers as shown in Table 11. Culture 38, 32, 13s, 9s and 14s produced high amount of precipitate (0.04g) as result more EPS was produced than culture 6s and 7s (0.03g) respectively as shown in the graph (Fig 8).

**Table 11: Bacterial isolates showing presence and absence of precipitation**

| Culture no. | Precipitation | Culture no. | Precipitation | Culture no. | Precipitation |
|---|---|---|---|---|---|

| | | | | | |
|---|---|---|---|---|---|
| 2 | | 21 | | 41 | × |
| 3 | × | 22 | | 1s | × |
| 4 | × | 25 | × | 2s | × |
| 5 | × | 26 | | 3s | × |
| 6 | | 28 | × | 4s | |
| 7 | | 29 | | 5s | × |
| 8 | × | 30 | × | **6s** | |
| 9 | × | 31 | × | **7s** | |
| 10 | | **32** | | 8s | |
| 11 | | 33 | | 9s | |
| 12 | × | 34 | × | 10s | × |
| 13 | | 35 | × | 11s | × |
| 14 | × | 36 | × | 12s | |
| 15 | × | 37 | × | **13s** | |
| 16 | × | **38** | | **14s** | |
| 17 | | 39 | | 15s | × |
| 18 | × | 40 | × | 16s | |

Key: precipitate; × No precipitate

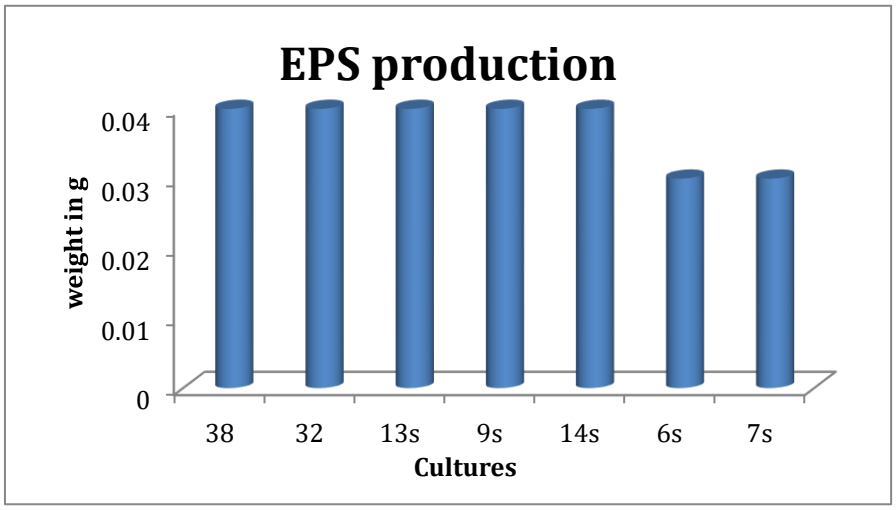



**Fig 8: Graph of culture 38, 32, 13s, 9s, showing maximum precipitation as compared to culture 6s and 7s.**

**Screening of IAA producing microorganisms**

Out of 51 isolates obtained, 6 cultures showed positive results with tryptophan for the production of IAA whereas without tryptophan the six cultures did not produce IAA as shown in Table 12. After adding Salkowski's reagent to the supernatant of the IAA producing culture it turned pink as shown in Fig 8, absorbance was measured at 530nm. The IAA after extraction was dried to powder form to measure the amount of extracted product which showed the maximum IAA production of 0.02g for culture number 34, 38 and 40 respectively whereas the lowest amount of IAA was produced 0.01g for culture number 35, 36 and 37 respectively shown in graph (Fig 10). TLC was performed to confirm the presence of IAA and Rf was calculated using the formula i.e. Distance travelled by solute /distance travelled by solvent wherein 0.7 Rf value was maximum of extracted IAA culture number 36. Also there was no colouration seen on TLC plate for extracted IAA culture number 32 and extracted IAA culture number 35, 38 and 40 showed an orange colour due to the

concentration of the product as shown in Fig 11.

**Table 12: Bacterial isolates showing presence and absence of IAA**

| Culture no. | IAA production | Culture no. | IAA production | Culture no. | IAA production |
|---|---|---|---|---|---|
| 2 | × | 21 | | 41 | × |
| 3 | × | 22 | | 1s | × |
| 4 | × | 25 | | 2s | × |
| 5 | × | 26 | | 3s | × |
| 6 | × | 28 | | 4s | × |
| 7 | × | 29 | × | 5s | × |
| 8 | × | 30 | × | 6s | × |
| 9 | × | 31 | × | 7s | × |
| 10 | × | 32 | × | 8s | × |
| 11 | × | 33 | × | 9s | × |
| 12 | × | **34** | | 10s | × |
| 13 | × | **35** | | 11s | × |
| 14 | × | **36** | | 12s | × |
| 15 | × | **37** | | 13s | × |
| 16 | × | **38** | | 14s | × |
| 17 | × | 39 | × | 15s | × |
| 18 | × | **40** | | 16s | × |

Key: Presence of IAA; × Absence of IAA

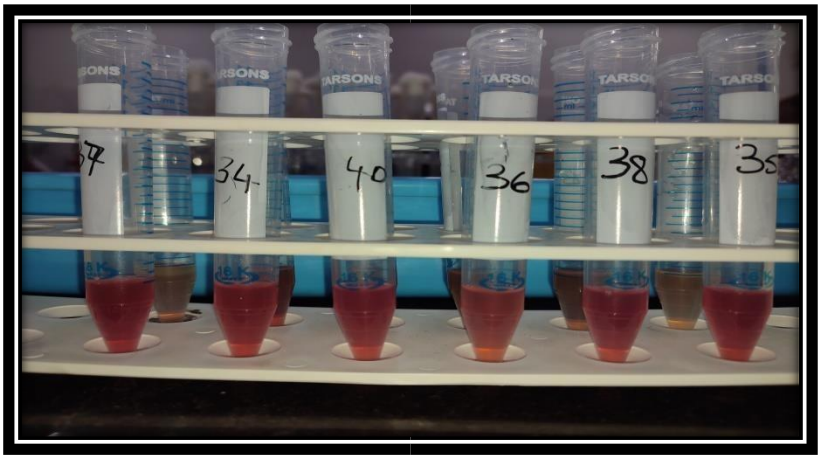



**Fig 9: Isolates showing pink colouration after adding Salkowski reagent for IAA**

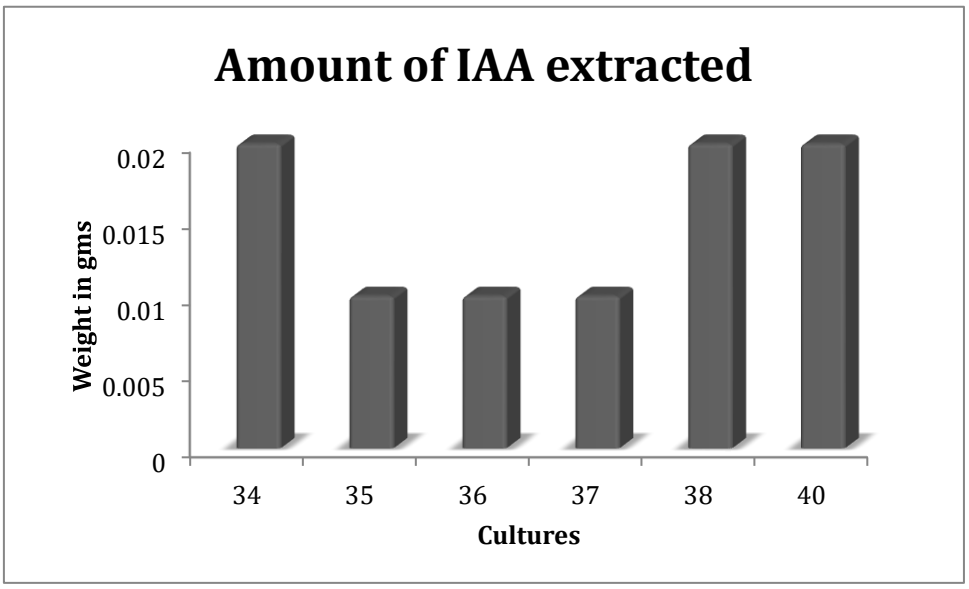
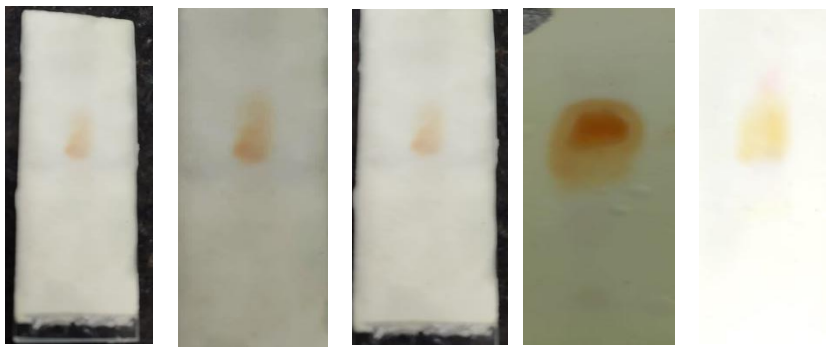



**Fig 10: Graphical representation of IAA production of bacterial isolates**



Culture 35 Culture 36 Culture 37 Culture 38 Culture 40

**Fig 11: TLC of IAA isolated cultures**

**Calculations for Retention factor (Rf)**

Rf value of extracted IAA culture number 34

Distance travelled by solute /distance travelled by solvent

**No colouration on the TLC**

Rf value of extracted IAA culture number 35

Distance travelled by solute /distance travelled by solvent
= 1.8/5.0

= 0.36

Rf value of extracted IAA culture number 36

Distance travelled by solute /distance travelled by solvent

= 3.5/ 5.0

= 0.7

Rf value of extracted IAA culture number 37

Distance travelled by solute /distance travelled by solvent

= 2.0/5.0

= 0.4

Rf value of extracted IAA culture number 38

Distance travelled by solute /distance travelled by solvent

= 2.5/5.0

= 0.5

Rf value of extracted IAA culture number 40

Distance travelled by solute /distance travelled by solvent

= 2.0/5.0

= 0.4

**Screening of Phosphate solubilization**

Out of 51 bacterial isolates, 6 isolates showed zone of clearance, and phosphate solubilisation efficiency (Fig 12). The highest P-solubilization activity was found in culture 25 having 1.35mm clearance zone and 245% of phosphorous solubilisation efficiency as compared to others (table 14).

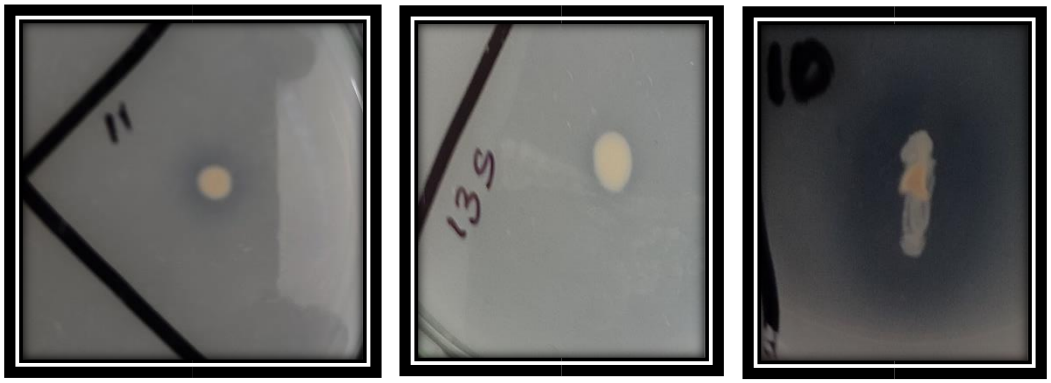



**Fig 12: Bacterial isolates showing phosphate solubilisation**

**Table 13: Bacterial isolates showing zone of clearance.**

| Culture no. | Zone of clearance | Culture no. | Zone of clearance | Culture no. | Zone of clearance |
|---|---|---|---|---|---|
| 2 | No clearance | 21 | No clearance | 41 | No clearance |
| 3 | No clearance | 22 | No clearance | 1s | No clearance |
| **4** | **Clearance** | **25** | **Clearance** | 2s | No clearance |
| 5 | No clearance | 26 | No clearance | 3s | No clearance |
| 6 | No clearance | 28 | No clearance | 4s | No clearance |
| 7 | No clearance | **29** | **Clearance** | 5s | No clearance |
| 8 | No clearance | 30 | No clearance | 6s | No clearance |
| 9 | No clearance | 31 | No clearance | 7s | No clearance |
| **10** | **Clearance** | 32 | No clearance | 8s | No clearance |

| | | | | | |
|---|---|---|---|---|---|
| **11** | **Clearance** | 33 | No clearance | 9s | No clearance |
| 12 | No clearance | 34 | No clearance | 10s | No clearance |
| 13 | No clearance | 35 | No clearance | 11s | No clearance |
| 14 | No clearance | 36 | No clearance | 12s | No clearance |
| 15 | No clearance | 37 | No clearance | **13s** | **Clearance** |
| 16 | No clearance | 38 | No clearance | **14s** | **Clearance** |
| 17 | No clearance | 39 | No clearance | 15s | No clearance |
| 18 | No clearance | 40 | No clearance | 16s | No clearance |

**Table 14: Bacterial isolates showing Phosphate solubilization efficiency**

| Culture no. | Solubilization Diameter | Growth diameter | Phosphate solubilization % |
|---|---|---|---|
| 4 | 0.9 | 0.55 | 163% |
| **10** | **1.35** | **0.55** | **245%** |
| 11 | 0.8 | 0.4 | 200% |
| 25 | 1.1 | 0.4 | 275% |
| 13s | 0.8 | 0.6 | 133% |
| 14s | 1.5 | 0.8 | 187% |

**Pot assay**

From table 21, it is concluded that culture 36, 32 and 25 were giving (N2 + IAA, GA3 + EPS, N2 + Phosphate solubilization) respectively and were considered for pot assay.

The time taken for each plant to germinate was maximum 2-3days of which pot with culture 32 and 36 germinated in faster (2 days) as compared the to the pots containing culture 25 and mixture. The growth parameters of each experimental pot were compared with control and was seen that each plant grew well in comparison with control. Pot 32 and 36 germinated faster as that of control. Likewise pot 25 and pot consortium grew a little slower than the other pots. 20

seeds were germinated maximum in pot 25 and the rest pots gave better results than the control. The root length with 5.5 cm showed best in pot 25 as compared to others and pot 32 showed short root length as compared to control. The experimental pots showed poor leaf lengths as compared to control. The leaf number was same for all in comparison with control. The shoot length was seen maximum in pot 32 with 22 cm in height as compared to other experimental pots and control. The shoot number was seen same in all the experimental and control pot. For soil treatment The time taken for each plant to germinate was maximum 3-4 days of which pot with culture 32 and 36 germinated in faster (3 days) as compared the to the pots containing culture 25 and mixture. The growth parameters of each experimental pot were compared with control and was seen that each plant grew well with some parameters in comparison with control. Pot 32 and 36 germinated faster but slower than control. Likewise pot 25 and pot consortium grew much slower than the other pots. 20 seeds were germinated maximum in pot 25 and the rest pots gave better results than the control. The root length with 6.5 cm showed best in pot 36 as compared to others and pot consortium showed short root length as compared to control. The experimental pots showed poor leaf lengths as compared to control. The leaf number was same for all in comparison with control. The shoot length was seen maximum in pot 36 with 18 cm in height as compared to other experimental pots and control. The shoot number was seen same in all the experimental and control pot.

**Table 15: Culture selection for pot assay**

| Nitrogen fixation | Giberrellin production | Exopolysaccharide production | Indole acetic acid production | Phosphate solubilization |
|---|---|---|---|---|
| 12 | 12 | 38 | 35 | 10 |
| 16 | 28 | **32** | 40 | 29 |
| 18 | **32** | 13s | **36** | 13 |
| **25** | 7s | 9s | 34 | 4 |

| | | | | |
|---|---|---|---|---|
| **36** | 8s | 14s | 38 | 11 |
| | | 7s | 37 | **25** |


**Fig 13: Growth of mung plants with seed treatment**

**Fig 13.1: Graphical representation of cultures used for pot assay v/s number of days**

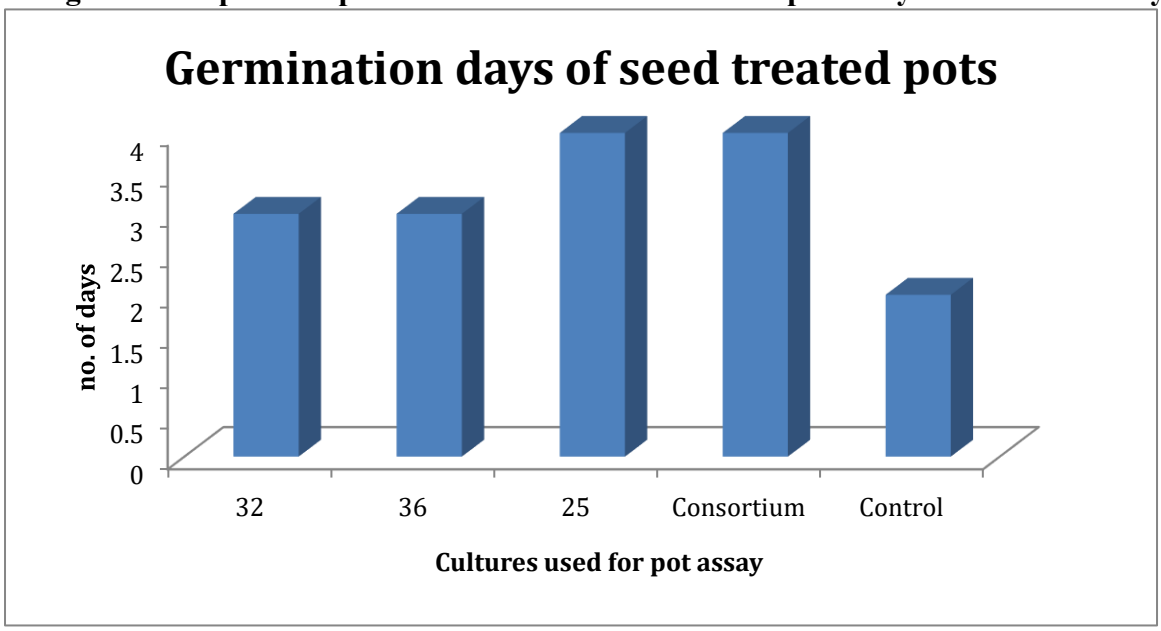


**Fig 13.2: Graphical representation of cultures used for pot assay v/s count in numbers**

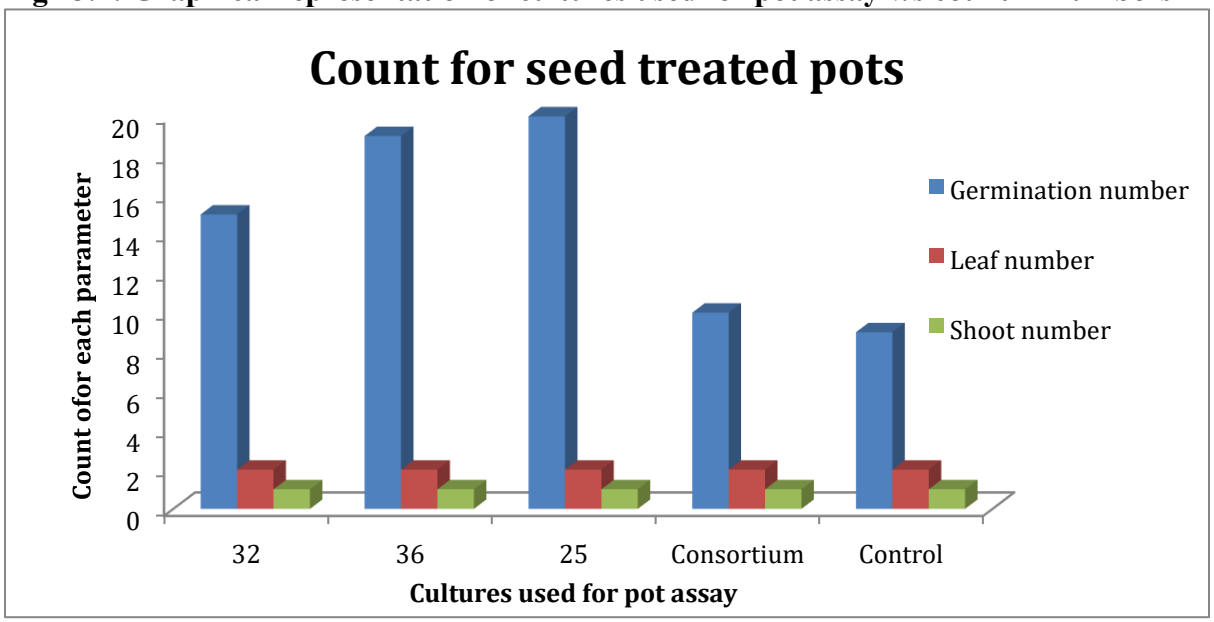



**Fig 13.3: Graphical representation of cultures used for pot assay v/s length in cm**

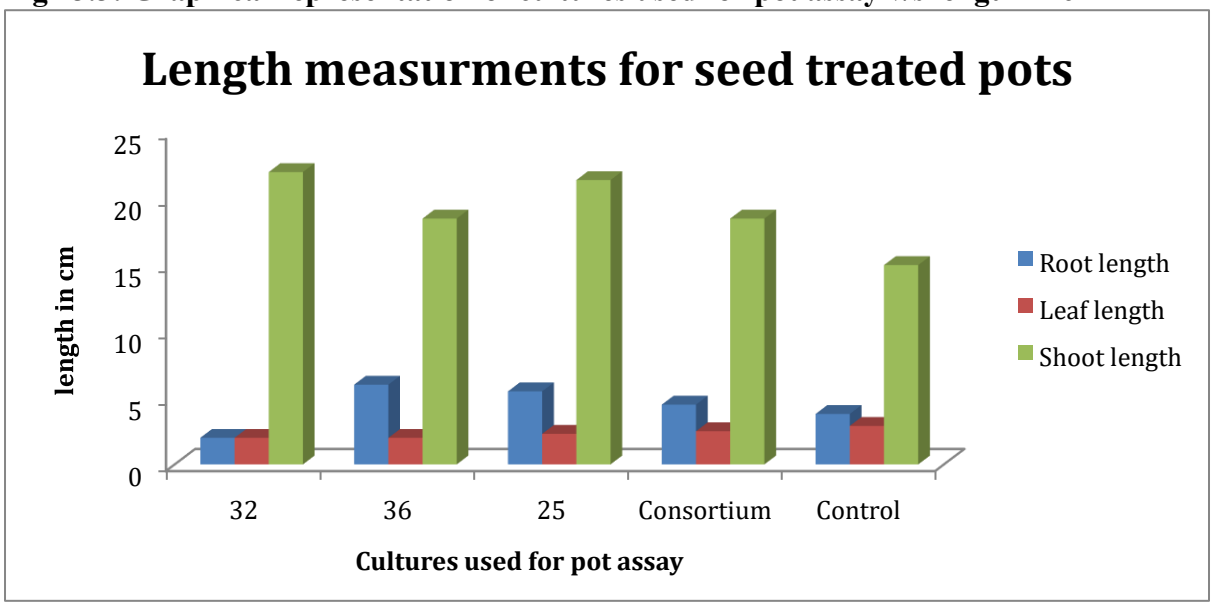



**Fig 14: Growth of mung plant with soil treatment**

**Fig 14.1: Graphical representation of cultures used for pot assay v/s number of days**

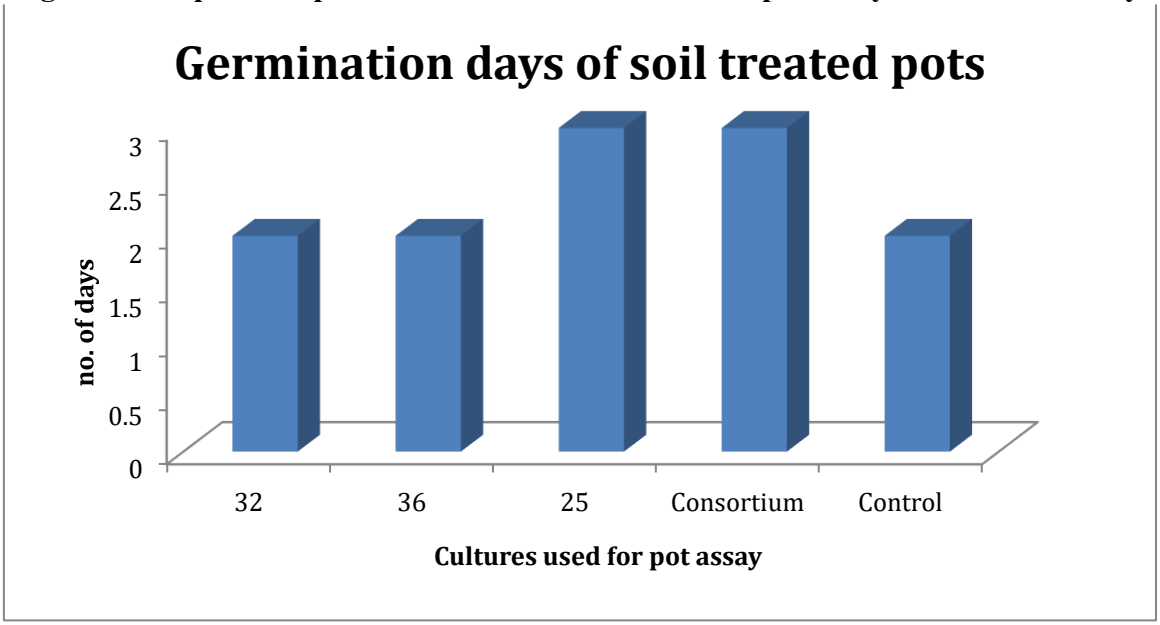


**Fig 14.2: Graphical representation of cultures used for pot assay v/s count in numbers**

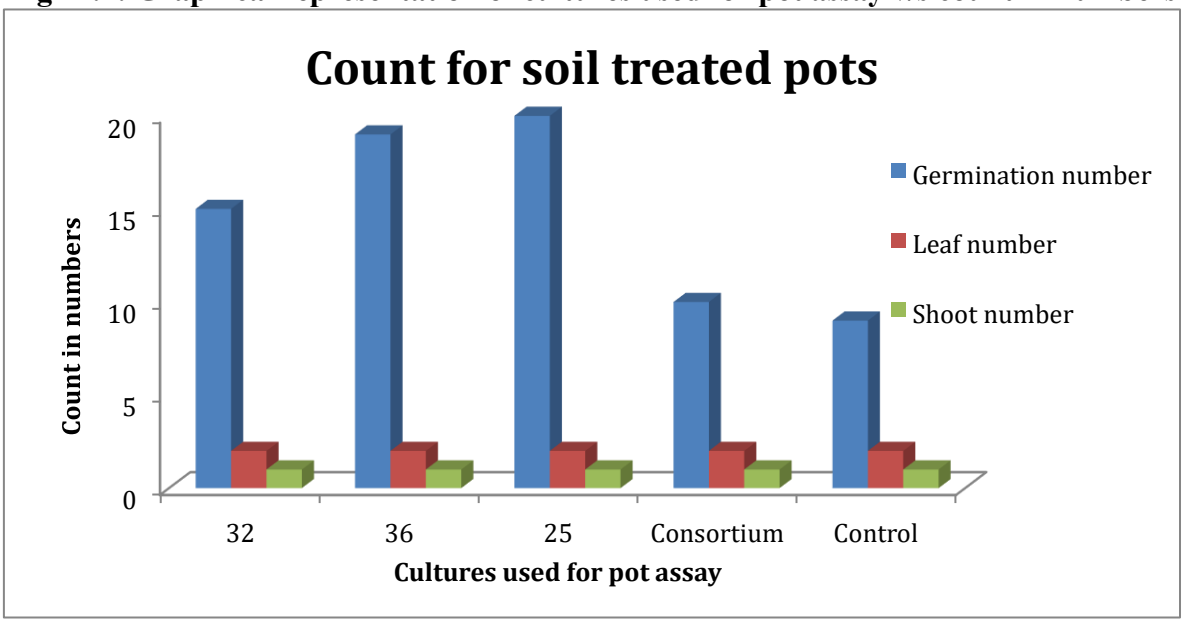
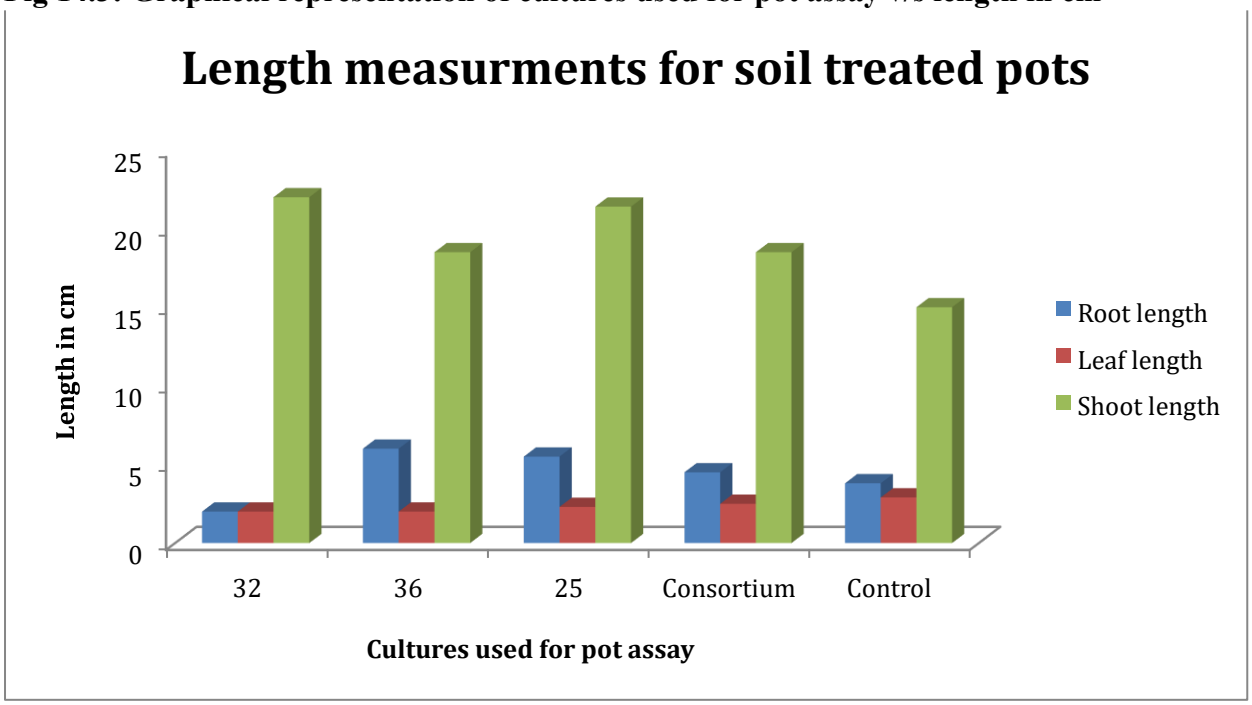



**Fig 14.3: Graphical representation of cultures used for pot assay v/s length in cm**




## DISCUSSION

Five bacterial isolates were examined as best nitrogen fixers as compared to 51 isolates. In (Do Carmo et al 2011) study showed the nitogenase reductase enzyme were amplified by

PCR using genomic DNA of each bacterial strain. Out of 57 selected isolates a 360bp *nifH* PCR product was observed in 34 isolates showing 60% of nitrogen fixation. In (Ravikumar et al 2012) the nitrogen fixation of mangrove seedling was analysed in the gas chromatograph. The nitrogenase activity was expressed in Nano moles of ethylene $10^8$ cells$^{-1}$ ml$^{-1}$ hr$^{-1}$. In comparison of all both these studies showed good results as compared to my data.

Gibberellin production was highest in culture 28 with 0.953nm. In (Sharma et al 2018) investigation the $GA_3$ was extracted and separated from supernatant with diethyl ether and was detected by TLC and the product formed was 116.1-485.8µg/ml. (Gunjal and Kapadnis 2013) study showed that *Burkolderia gladiola* concentration were calculated by standard graph prepared using Gibberellins as a standard range from 10-100µg/ ml. In comparison of both these studies neither TLC nor the standard graph was calculated in my data. To assume these two studies are considered to be better than my study. Maximum precipitate of EPS was 0.04g/ml in culture 38, 32, 13s, 9s and 14s. (Lakshmipriya and Sivakumar 2012) determined that off all the EPS producing isolates, isolate 1 showed the maximum with 6.8mg/ml i.e. 0.0068 g/ml levels of EPS production. (Gunjal and Kapadnis 2013) determined *Burkolderia gladiola* isolate which produced about 0.98g of exopolysaccharide. More exopolysaccharide production was seen in the two research studies as compared to my data achieved. In IAA production 3 cultures were able to produce the maximum amount of IAA i.e. 0.02% In

(Samuel and Muthukkaruppan 2011) study the isolates all the 8 isolates were able to produce 100% of IAA. The (Mohite 2013) study screened IAA with and without tryptophan in the medium which indicated positive results with 0.1% for br1, br2 and br3, for mr2 and 0.05% for wr2. IAA was not produced without tryptophan. Comparing the above two research papers with my data of 0.02g they have achieved more amount of IAA product. The highest Phosphate solubilization activity was found in culture 25 having 1.35mm clearance zone and 245% of phosphorous solubilisation efficiency. In the (do Carmo 2011) study the ability to solubilize phosphate was detected in 29 isolates with 51% phosphate solubilization efficiency. The (Suksaard 2017) investigated 448 isolates of which 174 isolates showed 38.8% of phosphate

solubilization efficiency. In order to compare the results of the two research studies with my data, the phosphate solubilization is much better in my data with 245% than the other two. For pot assay, culture 25 was showing maximum growth with treatment of seeds and soil as compared to others. (Soldan et al) hypothesis, the strains were tested on mangrove propagule, germination and rice growth. The most effective strain was *G. terrae* which enhanced the root length of mangrove seedlings and biomass of salt-stressed rice under axenic up to 65% and 62% respectively. (Saini, A. et al., 2017) determined the number of nodules formed within plant roots increased treatments as compared to the UC control. The maximum increase was observed when CP-2 was inoculated in presence of TCP. The highest significant values of shoot length was 37.6 cm and root length was 8.7 cm observed in the soil + CP-2+ TCP treatment, and the same was true for fresh and weights of roots having 0.07 and 0.06g and shoots 0.81 and 0.70g. Soil treated with Biogold showed significant increase as compared to the control but lesser then *O. intermedium* CP-2 treated plants. The bacterial inoculated plants showed significantly enhanced number of pods i.e. 6, weight of pods were 2.43g, and number of seeds was 47.3 per plant in absence of TCP, followed by treatment in the presence of TCP. Unlike growth parameters, the yield was nonsignificant when Biogold or SSP was added as compared to other uninoculated treatments. As regarding the leaf chlorophyll contents, the highest chlorophyll-a was 1.24mg $g^{-1}$ fresh weight

and total chlorophyll was 2.68 mg $g^{-1}$ fresh weight and the levels were achieved by the inoculation of bacterial strain in the presence of TCP. In comparison of all, the two research studies and my data gave good results with respect to parameters aimed.

## CONCLUSION

- The bacterial isolates of mangroves showed beneficial effects of PGPR. PGPR improves the soil fertility through increase plant nutrients such as nitrogen, gibberellin, exopolysaccharide, indole production and phosphate solubilization

  available in soil.

- Total 51 morphologically distinct colonies were isolated on sterile nutrient agar and the isolates were characterized based on their morphology and Gram character.
- Culture 12, 16, 18, 25 and 36 were the best nitrogen fixing organisms.
- The production of Gibberellin was high for culture number 28 i.e. 0.953 at absorbance 254nm among all the isolates.
- The amount of EPS produced was more for isolate number 38, 32, 13s, 9s and 14s.
- Screening of IAA was determined maximum in culture 38, 34 and 40. TLC was performed to confirm the presence of IAA and Rf value was calculated. The highest phosphate solubilization was found in culture number 25 having 1.35mm clear zone and 245% of phosphate solubilization efficiency as compared to others.
- For pot assay, culture 32 was showing maximum growth with treatment of seeds and pot 36 for soil treatment.
- These rhizobacteria are the best alternatives to use instead of chemical fertilizers and pesticides that generate many problems such as groundwater and crop products contamination by heavy metals. Thus, this technology based on the PGPR use, should be integrated into agricultural production strategies of all countries to a healthy and sustainable agriculture.

## BIBLIOGRAPHY

Mohanty, P., Singh, P. K., Chakraborty, D., Mishra, S., & Pattnaik, R. (2021). Insight into the role of PGPR in sustainable agriculture and environment. *Frontiers in Sustainable Food Systems*, *5*, 667150.

Kannahi, M., & Dhivya, S. (2015). Plant Growth Promoting Potentiality Of Mangrove Rhizospheric Organisms. *World Journal of Pharmacy and Pharmaceutical Sciences*

*(WJPPS)*, *4*(9), 1470-1476.

Singh, J. S. (2013). Plant growth promoting rhizobacteria: potential microbes for sustainable agriculture. *Resonance*, *18*(3), 275-281.

Morshed, M. (1706). *Plant Growth Promoting Rhizobacteria (PGPR): Current & Future Prospective for Sustainable Agriculture*. Doctoral thesis. Bangabandhu Sheikh Mujibur Rahman Agricultural University, Dhaka, Bangladesh.

Gupta, G., Parihar, S. S., Ahirwar, N. K., Snehi, S. K., & Singh, V. (2015). Plant growth promoting rhizobacteria (PGPR): current and future prospects for development of sustainable agriculture. *J Microb Biochem Technol*, *7*(2), 096-102.

Lanteigne C, Gadkar VJ, Wallon T, Novinscak A, Filion M (2012) Production of DAPG and HCN by Pseudomonas sp. LBUM300 contributes to the biological control of bacterial canker of tomato. *Phytopathology* 102: 967-973.

Singh, J. S. (2013). Plant growth promoting rhizobacteria: potential microbes for sustainable agriculture. *Resonance*, *18*(3), 275-281.

Samuel, S., & Muthukkaruppan, S. M. (2011). Characterization of plant growth promoting rhizobacteria and fungi associated with rice, mangrove and effluent contaminated soil. *Current Botany*, *2*(3).

Mohite, B. (2013). Isolation and characterization of indole acetic acid (IAA) producing bacteria from rhizospheric soil and its effect on plant growth. *Journal Of Soil Science And Plant Nutrition*, *13*(3), 638-649.

Do Carmo, F. L., Dos Santos, H. F., Martins, E. F., van Elsas, J. D., Rosado, A. S., & Peixoto, R. S. (2011). Bacterial structure and characterization of plant growth promoting and oil degrading bacteria from the rhizospheres of mangrove plants. *The Journal of Microbiology*, *49*, 535-543.

Suksaard, P., Pathom-aree, W., & Duangmal, K. (2017). Diversity and plant growth promoting activities of actinomycetes from mangroves. *Chiang Mai J Sci*, *44*(4), 1210-1223.

Ravikumar, S., Ignatiammal, S. T. M., Gnanadesigan, M., & Kalaiarasi, A. (2012). Effects of saline tolerant *Azospirillum* species on the growth parameters of mangrove seedlings. *Journal of Environmental Biology*, *33*(5), 933.

Gunjal, A. B., & Kapadnis, B. P. (2013). *Burkholderia gladioli*, an endophyte with plant growth promoting potential. *Journal of Chemo and Biosphere*, 43-53.

Sharma, S., Sharma, A., & Kaur, M. (2018). Extraction and evaluation of gibberellic acid from *Pseudomonas* sp.: Plant growth promoting rhizobacteria. *Journal of Pharmacognosy and Phytochemistry,* 7(1), 2790-2795.

Lakshmipriya, V. P., & Sivakumar, P. K. (2012). Isolation and characterization of total heterotrophic bacteria and exopolysaccharide produced from mangrove ecosystem. *International Journal of Pharmaceutical & Biological Archives*, *3*(3), 679-684.

Soldan, R., Mapelli, F., Crotti, E., Schnell, S., Daffonchio, D., Marasco, R., & Cardinale, M. (2019). Bacterial endophytes of mangrove propagules elicit early establishment of the natural host and promote growth of cereal crops under salt stress. *Microbiological research*, *223*, 33-

43.

Saini, A., Nain, L., Garg, V., & Saxena, J. (2017). Improvement of growth, yield, and pigmentation of mung bean plants using *Ochrobactrum intermedium* CP-2 as bioinoculant. CLEAN–Soil, Air, Water, 45(6), 1500670.