ISOLATION AND CHARACTERIZATION OF BENEFICIAL RHIZOSPHERE MICROORGANISMS FROM RAGI GROWN IN ATTAPPADY HILL TRACT OF KERALA

by

GAYATHRI M

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THESIS

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requirements for the degree of

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VELLAYANI, THIRUVANANTHAPURAM-695 522

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DECLARATION

I, hereby declare that this thesis entitled "**ISOLATION AND CHARACTERIZATION OF BENEFICIAL RHIZOSPHERE MICROORGANISMS FROM RAGI GROWN IN ATTAPPADY HILL TRACT OF KERALA**" is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Vellayani

Date: 10-12-2021

GAYATHRI M

(2019-11-266)

CERTIFICATE

Certified that this thesis entitled **"ISOLATION AND CHARACTERIZATION OF BENEFICIAL RHIZOSPHERE MICROORGANISMS FROM RAGI GROWN IN ATTAPPADY HILL TRACT OF KERALA"** is a record of research work done independently by Ms. GAYATHRI M under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Vellayani,
Date: 10-12-2021

Dr. Chitra N.
(Major advisor)
Assistant Professor
Department of Agricultural Microbiology
RARS(SZ), College of Agriculture
Vellayani, Thiruvananthapuram – 695 522

CERTIFICATE

We, the undersigned members of the advisory committee of Ms. GAYATHRI M a candidate for the degree of Master of Science in Agriculture with major in Agricultural Microbiology, agree that the thesis entitled "**ISOLATION AND CHARACTERIZATION OF BENEFICIAL RHIZOSPHERE MICROORGANISMS FROM RAGI GROWN IN ATTAPPADY HILL TRACT OF KERALA**" may be submitted by Ms. GAYATHRI M in partial fulfilment of the requirement for the degree.

Dr. Chitra N.
Assistant Professor
Department of Agricultural Microbiology
RARS (SZ)College of Agriculture,
Vellayani,
Thiruvananthapuram-695 522

Dr. Anith K. N.
Professor and Head
Department of Agricultural Microbiology
College of Agriculture,
Vellayani,
Thiruvananthapuram-695 522

Dr. Anu Rajan S
Assistant Professor
Department of Agricultural Microbiology
College of Agriculture, Vellayani
Thiruvananthapuram-695 522

Dr. Nishan M. A.
Assistant Professor
Department of Agronomy
College of Agriculture, Vellayani
Thiruvananthapuram-695 522

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et al.	And other co-worker
cm	Centimeter
cfu	Colony forming unit
CRD	Completely randomized design
°C	Degree celsius
Fig.	Figure
g	Gram
ha	hectare
h	hours
kg	Kilogram
L	Liter
µg	Microgram
µL	Microliter
µm	Micrometer
mg	Milligram
mL	Milliliter
mm	Millimeter
Min	Minutes
M	Molar
viz.,	Namely
nm	Nanometer
pH	Negative logarithm of hydrogen ions

No.	Number
OD	Optical density
ppm	Parts per million
%	Percentage
rpm	Rotations per minute
Sl.	Serial
sp. or spp.	Species (Singular and plural)
i.e.	That is
t	Tonnes
NFB	Nitrogen fixing bacteria
PSB	Phosphate solubilizing bacteria
IAA	Indole acetic acid
GA	Gibberellic acid
dia	Diameter

INTRODUCTION

1. INTRODUCTION

Finger millet or Ragi (*Eleusine coracana* (L.) Gaertn) is a self-pollinated tetraploid crop (2n = 36). It is also known as African millet. It is one of the most important small millet grown in many parts of Africa and Asia. It can perform well under adverse soil and climatic condition in comparison with other crops. India is one among the major producers of Ragi in world. In India, Ragi is grown in an area of 2 million hectares with an average yield of 1483 kg ha^{-1} and production of about 1.85 million tonnes (Annual report 2016-2017) which contributes nearly 45 percent of the world's cultivated area and 55 percent of the World's production (Mahanthesha *et al.*, 2017).

The striking feature of ragi is its drought tolerance, disease resistance, weed growth suppression, and ability to grow in low fertile marginal lands (Thilakarathna and Raizada, 2015).

Ragi is an important staple food in many parts of Africa and Asia. It is processed by milling, germinating or sprouting, malting, fermentation and cooking, which makes the final product more attractive and modifies its nutritive quality (Singh and Raghuvanshi, 2012). Ragi grains are highly nutritious containing high quality protein of about 7-10 percent, also the richest source of calcium (344 mg per 100g), iron (3.9 mg per 100g) and other minerals (Mahanthesha *et al.*, 2017). Ragi gains are high in cysteine, methionine, tryptophan, and total aromatic amino acids content compared to the other cereals and millets, and thus it plays a major role in the diet of malnutritional population in developing and underdeveloped nations.

Millets are known to be the ancient grains to be domesticated even before rice and wheat. Rice and wheat gained its importance by green revolution and thus leads to the loss of millet cultivated areas to rice and wheat cultivation. In India and all over the world, there is increasing incidence of malnutrition which can be mitigated by including millets in regular diet (Weber and Fuller, 2008). Thus, millets gaining its popularity due to its rich micronutrient and superior nutritional quality over other staple cereal crops (Das *et al.*, 2019).

The alarming increase in the human population is a great threat to food security due to the limited availability of land for agricultural purpose. Therefore, it is necessary to meet the ever-increasing demand of food by enhanced agricultural productivity. Increased reliance on chemical fertilizers to boost crop output has serious consequences for both the environment and human health. Thus, use of microorganisms, as biofertilizers in agriculture sector can promote crop production and food safety to some extent. Microorganisms including bacteria, fungi, cyanobacteria, etc., have shown biofertilizer-like and plant growth promotion activities. Biofertilizers are known to exert a variety of mechanisms in order to enhance plant development as well as give protection against various plant diseases, resulting in increased crop output (Mahant *et al.*, 2016).

A biofertilizer is a biologically active substance containing living micro-organisms. When applied to the seeds, plants or soil it promotes plant growth by increasing nutrients supply to plant. The plant-microbe interaction occurs by means of colonizing the rhizosphere or the interior of the plant parts like root (Malusa and Vassilev, 2014).

Biofertilizers improve soil fertility by fixing atmospheric nitrogen (N) in both symbiotic and non-symbiotic relationships with plant roots, solubilizing insoluble soil phosphates and minor nutrients, and producing plant growth promoting substances in the soil. N fixers, P and K solubilizers, S oxidizers, and organic matter decomposers are the important groups of microorganisms used in biofertilizer formulations. As a result, they are known as bio inoculants, which boost plant growth and yield when given to them. The N fixing biofertilizers (NFB) includes family Rhizobiaceae, which are mainly symbiotic with legumes and fix N 50-100 kg ha^{-1}. Family Azotobacteraceae, includes *Azotobacter* spp. that colonize the root and the root tissues in harmony with plants. They are non-symbiotic free-living aerobic bacteria can fix N upto 25 kg ha^{-1} under optimum condition and promotes yield upto 50 percent. Family Spirillaceae includes *Azospirillum* spp., beneficial for non-leguminous plant, heterotopic and associative in nature. *Azospirillum* spp. can fix N of about 20-40 kg ha^{-1} and produces growth regulating substances. Associative symbiont, *Herbspirillum* spp. is responsible for atmospheric nitrogen fixing on sugarcane roots. *Acetobacter* spp. is best used as an

endophyte in the sugarcane ecosystem because it can tolerate high sucrose concentrations and fix up to 15 kg of nitrogen per hectare per year (Mazid and Khan, 2014).

Uptake of phosphorus (P) by plants stimulates root development, enhance flower formation, improve the quality and quantity of fruits, and seed setting. Majority of soil types around the world is deficient in P, and only 1.5 percent of total soil P is available to plants. In order to eliminate deleterious environmental impacts of phosphatic fertilizers, Phosphate solubilizing micro-organisms (PSM), a microbial community which can transform insoluble P into soluble and plant available form of P is used. Many of PSM belongs largely to the genera *Pseudomonas*, *Bacilli*, *Rhizobium* and *Azotobacter* (Khan *et al.*, 2013).

In 2015-2016, India reports a total biofertilizer production of about 88,029 MT carries base and 6240 kL liquid base. Out of which, the biofertilizer production contributed by Kerala alone is reported to be 4926 MT carries base and 57 kL liquid base (Yadav and Pandey, 2020). The beat approach to meet the goal of sustainable agriculture is organic farming, which can be done by exploitation, development and application of biofertilizers. The increase in the area of organic farming over the past two decades accelerated the production of biofertilizer.

Attappady, one of the prominent forest regions of Kerala is situated in the north eastern part of Palakkad. Attappady block is one of the 43 tribal development blocks in India having predominant tribal population. Traditionally tribal communities in Attappady depended on agriculture based on indigenous knowledge gained through oral tradition and practice over generations for their livelihood. Major traditional crops cultivated included millets, pulses and oilseeds such as makkacholam or maize, ragi or finger millet, chama or little millet, thuvara or red gram, groundnut and castor. Here ragi is extensively grown in an area of about 215 ha during the month of May to August and September to December every year.

Coastal sandy soils, soils of southern Thiruvananthapuram, cultivated soils of Attappady and the soils of eastern Palakkad have extensive deficiency of N to the tune of more than 40 percent (Kerala State Planning Board, 2014). Majority of tribal farms

still depend on traditional indigenous practices such as the use of traditional seeds, hand weeding, inter-cultural operations using traditional tools. Thus, Attapady hill tract farming soils will have a lesser exposure towards chemical fertilizers, which makes the soil rich in microbial diversity and with increased load of microbial communities which help in maintaining the soil fertility (Sachana and Bonny, 2020). The chance of getting efficient strains of biofertilizers is very high is such soils.

Realising the need for developing newer and better stains of biofertilizer microorganisms which are capable of fixing atmospheric nitrogen, solubilizing phosphorous and having plant growth promotion activity for enhancing sustainable agriculture practice, the present study was taken up with the following objectives.

1. Isolate and characterize beneficial rhizosphere microorganisms *viz.* nitrogen fixers and phosphate solubilizers from ragi grown in Attappady hill tract of Kerala.
2. Estimate the plant growth promotion activities of the isolates.
3. *In vitro* assessment of nitrogen fixing and phosphate solubilizing capacity of the bacterial isolates.
4. Study the potential of selected bacterial isolates for plant growth promotion in *in vitro*.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The ecological balance of nature is highly influenced by the soil microorganisms as they play an important role in Carbon (C), Nitrogen (N), Sulphur (S) and Phosphorus (P) cycles. The fast-growing world population will reach a peak value of 9.8 billion by the year 2050 according to the United Nation Food and Agriculture Organization (FAO) prediction (Reetz, 2016). Food crisis and malnutrition in many parts of the world resulting from the exponential growth of human population can be sorted out by increasing the overall food production. The soil microbiome which includes plant growth promoting rhizobacteria can promote overall food production by making nutrients available for plant uptake.

Progressive decline of earth's natural resources and excess use of chemical fertilizer remain significant challenges for the future of agriculture. The surplus application of chemical fertilizers caused reduction of soil organic matter and also drastically reduced the microbial activity in soil. Biofertilizers are used as a supplement to chemical fertilizer (Yadav and Sarkar, 2019). Biofertilizers act as key components in maintaining soil fertility, in turn increasing crop yield and sustainability of agriculture for a longer period. The essential nutrients such as Nitrogen, Phosphorus, Potassium, Zinc and Silica are naturally available in insoluble or complex form. Certain microorganisms convert this insoluble form of nutrients into soluble form and makes them available for plant growth. Biofertilizers are cost effective, environmentally safe, non-toxic and are easy to apply. As a result, they are a good alternative to hazardous chemical fertilizers (Nosheen *et al.*, 2021).

Attappady is a tribal village area where subsistent farming is being practiced. Cereals and millets are the major crops grown in this area. Finger millaet or ragi is one among the major crop grown in hill tract of Attappady. Farming practice followed by tribal farmers does not encourage the use of chemical fertilizer and hence the fertility of soil is mainly based on the available farm inputs and microbial population present in these soils. A greater number of microorganisms are present in the plant rhizosphere region, which are known to fix N and solubilize P and make them available to plants (Karpagam and Nagalakshmi, 2014). These microorganisms when applied to seed, plant surface or soil improved the plant growth and yield by 10-40 percent. Apart from improving the crop

yield and soil fertility, they also provided protection to the plants against pest and diseases. They have been reported to enhance the root growth, extend its life, degrade the toxic materials, increase seedling survival, and shorten the time taken to flower (Prasad *et al.*, 2015). Additional beneficial aspect of biofertilizer is that after prolonged use of biofertilizer for about 3-4 years, there is no need for further application, as mother culture is sufficient for its growth and multiplication (Bumandalai and Tserennadmid, 2019).

2.1. NITROGEN

Nitrogen is the most abundant gas in atmosphere comprising 78 percent among total atmospheric gas, but it is unreactive in nature. The formation, growth and functioning of all plant tissues depends on the availability of nitrogen (Sprent, 1987). Nitrogen is the primary element and often limits the growth and development of plants. Nitrogen is the basic and essential element found in amino acids, proteins and many other organic compounds (Egamberdieva and Kucharova, 2008). Nitrogen is also essential for ATP, NADH, NADPH, membrane lipid, co-enzymes, photosynthetic pigments and secondary metabolites (Ohyama, 2010). Thus, the requirement of nitrogen is comparatively higher than all other essential elements (Khalofah *et al.*, 2021). The biological process that converts the nitrogen gas in atmosphere into ammoniacal form is known as nitrogen-fixation, which is mainly done by means of soil microbial activity. The most important part of soil microbial process is Biological Nitrogen Fixation, which is carried out by prokaryotes, either symbiotic or free living in nature (Sridhar, 2012).

Nitrogen deficit stunts the development of all plant organs, roots, stems, leaves, flowers, and fruits (including seeds). The restricted growth of the vegetative organs in a nitrogen-deficient plant makes it appear stunted. Nitrogen-deficient foliage is light green or yellow coloured. The loss of green colour is consistent throughout the leaf blade. The entire plant will become pale, stunted, or spindly if nitrogen deprivation occurs throughout its life cycle. When nitrogen insufficiency develops throughout a plant's growth cycle, it is mobilised from lower leaves and translocated to young leaves, causing the lower leaves to become pale in colour and, in the case of severe deficiency,

brown (firing) and abscise (Barker and Bryson, 2016). Proper maintenance of nitrogen supply to the plant is to be ensured to obtain high yield.

Production and procurement of nitrogen fertilizer is highly costly and can have detrimental environmental impacts. Nitrous oxide and ammonia emissions from too much nitrogen fertiliser can damage air quality, and extremely nitrogenous run-off water from arable land can cause ecological problems such as eutrophication of inland lakes, rivers, and coastal waters. Nitrous oxide emission occurs as a result of overfertilization. It is an extremely harmful greenhouse gas produced as a result of production and use of nitrogen fertilisers. (Hawkesford and Howarth, 2018). Nitrogen fixing biofertilizers are a better alternative to these chemical fertilizers and thus the demand for nitrogen fixing biofertilizers is continuously increasing.

2.2. PHOSPHORUS

Phosphorus is the second most important macronutrient next to nitrogen for the plant growth and development. It is absorbed by plants only in soluble forms of phosphate ion i.e., HPO_4^{2-} or $H_2PO_4^-$. Phosphorus plays a major role in improving crop quality, resistance towards plant disease and N-fixation in legumes which ultimately result in high crop yield. Also, phosphorus is a key nutrient in the functioning of many enzymes that catalyze the metabolic pathways (Padmavathi Tallapragada, 2010). Phosphorus availability in soil is very low because of its fixation and precipitation as insoluble phosphates of iron, aluminium and calcium.

When phosphorus is deficient the leaves turn dark green in colour and the plant appears stunted. Older leaves show symptoms earlier and may develop a purple discoloration because of accumulation of sugars in P deficient plants which aids anthocyanin synthesis. Also, P deficiency delays maturity. Leaf expansion is inhibited making the leaves small and curled (McCauley *et al.*, 2009).

Approximately 95-99 percent of phosphorus in soil is present in insoluble form and hence cannot be utilized by plants. The use of chemical phosphatic fertilizer is practiced in order to achieve optimum yield. Rock phosphate is the main phosphate source used in the manufacturing of most commercial phosphate fertilizers available in market. The application of phosphorus fertilizers obtained from phosphate rock is

critical to agricultural production. The soluble form of P fertilizers is rapidly precipitated into the insoluble form and made unavailable to plants, so it is necessary to provide P fertilizer to crop plants on a regular basis (Kannapiran and Ramkumar, 2011).

Due to persistent demand and limited inventories, current global phosphate rock sources are expected to be depleted within a few hundred years (Cordell *et al.*, 2009). With better knowledge on plant-soil-microbial P cycle the dependence on phosphate fertilizer can be reduced with the use of P solubilizing biofertilizers. This increased the interest in harnessing of microorganisms to support P cycling in agroecosystems. Soil microorganisms reported to have greater potential to improve the rate of organic or inorganic P cycling i.e., by solubilizing insoluble organic- and mineral-bound P (Jones and Oburger, 2011).

2.3. PLANT GROWTH PROMOTING BACTERIA

Many microorganisms that are found around plant rhizosphere are known to facilitate plant growth promotion and are therefore known as Plant Growth Promoting Bacteria (PGPB). PGPB promotes plant growth directly by the production of auxin, ACC deaminase, cytokinin, gibberellin, nitrogen fixation, phosphorus solubilization and sequestration of iron by siderophore production. They are also involved in inhibiting the growth and activity of one or more pathogenic fungi or bacteria indirectly by the production of antibiotics, cell wall degrading enzymes, ACC deaminase, hydrogen cyanide, competition and induced systemic resistance (Olanrewaju *et al.*, 2017).

Rajput *et al.* (2013) identified that *Planococcus rifietoensis* SAL-15 strain is capable of protecting wheat against salt stress with enhanced plant growth promotion. The strain proved to be a potent bacterial inoculum for yield improvement of wheat under salinity stress with ability of salt tolerance, IAA production and P solubilization capacity.

Majeed *et al.* (2015) observed a significant increase in shoot and root length, shoot and root dry weight, and N content in wheat seedlings by PGPR isolates. Plant growth hormone synthesis, nitrogen fixation, and P solubilization are all aided by the

application of beneficial PGPR isolates derived from the wheat rhizosphere. The synthesis of auxin by the applied isolates is a primary technique of promoting plant growth. Suleman *et al.* (2018) screened two bacterial isolates with plant growth promoting activities for IAA production by quantifying with HPLC through ethyl acetate extraction method in tryptophan supplemented LB broth medium. The isolated *Enterobacter* sp. MS32 exhibited higher IAA production of 28.1 μg mL^{-1} followed by *Pseudomonas* sp. MS16 (25:6 μg mL^{-1}). Gibberellic acid production in both of the tested strains were reported to be 2.5±0.70 (*Pseudomonas* sp. MS16) and 11.8±3.11 (*Enterobacter* sp. MS32). Further, GC assay for nitrogen-fixing activity in these isolates confirmed that higher nitrogenase activity is seen in *Enterobacter* sp. MS32 (19 nmol ethylene h^{-1} mg $protein^{-1}$) followed by *Pseudomonas* sp. MS16 (8 nmol ethylene h^{-1} mg $protein^{-1}$).

Sekar *et al.* (2018) reported the potential of indigenous rhizobacterium *Pseudomonas* spp. MSSRFD41 in blast disease management and plant growth promotion. Strain MSSRFD41 produced IAA, siderophore, hydrolytic enzymes and solubilized phosphate. In addition, the strain showed a 22.35 mm zone of inhibition against *Pyricularia grisea* causing blast disease with the production of antifungal metabolites. The strain MSSRFD41 was compatible with different group of rhizobacteria which as evidenced by the cross streaking and RAPD analysis. The field application and the bio-primed seeds of finger millet with MSSRFD41 showed notable reduction in blast disease and improved plant growth compared to the control and other treatment.

Shankar (2009) reported symbiotic bacterium *Bradyrhizobium* have potential ability to promote plant growth in non-legume crops like tomato and ragi. Four root nodulating bacteria were isolated from cowpea, pigeon pea, chick pea and soybean out of which *Bradyrhizobium* spp. reported to produce maximum amount of IAA, gibberellic acid and was also involved in P solubilization. Ragi and tomato plants showed increased shoot weight, root weight and biomass production in field experiment with inoculation of *Bradyrhizobium* spp.

Perrig *et al.* (2007) investigated the phytohormone and polyamine biosynthesis, as well as siderophore production and phosphate solubilization in two strains Cd and

Az39 of *Azospirillum brasilense.* Both the strains Cd and Az39 showed greater phytohormone activity with higher potential for directly promoting plant growth and as a result involved in increased agronomical yield of the plant.

The bacterial strains *Azotobacter chroococcum* (AU-1), *Bacillus subtilis* (AU-2), *Pseudomonas aeruginosa* (AU-3) and *Bacillus pumilis* (AU-4) were isolated from the rhizosphere of *Cicer arietinum* (chickpea). The IAA production of all the four strains ranged between 20 and 35.34 $\mu g\ mL^{-1}$ and also the ACC degrading potential of strains was estimated in the range of 600–1700 nmol α-ketobutyrate per mg of cellular protein per hour. The strains *P. aeruginosa* and *B. pumilis* shows maximum phosphate solubilizing activity of 78–87.64 mg soluble P/L (Pandey *et al.*, 2019).

2.4. NITROGEN FIXING BACTERIA

A wide range of bacteria are known to interact with plants by colonizing rhizosphere region and thereby promoting the growth of crop plants. Legumes and actinorhizal plants fix nitrogen in association with *Rhizobium* and *Frankia* on their respective host plants through specialized structures called root nodules (Bhat *et al.*, 2015).

Endophytic diazotrophic bacteria use biological nitrogen fixation to supply nitrogen to plants, which is an important source of nitrogen in agriculture and a promising alternative to chemical fertilizers. Endophytic bacteria can synthesize plant growth hormones like auxin and gibberellin, help in nutrient uptake, and boost the plant's resistance to biotic and abiotic challenges in addition to fixing nitrogen. (Puri *et al.*, 2018).

Romero-Perdomo *et al.* (2017) reported two *Azotobacter chroococcum* strains AC1 and AC10 able to fix nitrogen with acetylene reduction (AR) ranging between 1033 and 1408 nmol ethylene mL^{-1}. Also, the strains were able to solubilize P from tricalcium phosphate. The strain AC1 was noticeable in promoting the growth of shoots than on roots, while AC10 promoted root growth of cotton plants. A positive correlation between root length and N content was observed by the presence of AC10 alone.

Ahmadi-Rad *et al.* (2016) observed that the strain *Azotobacter chroococcum* (Strain 5) and *Azospirillum lipoferum* (Strain 21) showed noticeable improvement in plant growth promotion upon its foliar application to canola. The collective application of *Azotobacter* spp. and *Azospirillum* spp. along with nitrogen fertilizer enhanced canola productivity related to their individual application. The outcomes showed that the efficacy of nitrogen fertilizer increased in the presence of rhizobacteria.

Rafi and Charyulu (2016) found that the *Azospirillum* isolates A1 (45 C2) and A2 (45 L1) and one isolate of PSB (45 LR3) were significant in increasing plant height, dry weight of shoot and root over control plants. The enhanced nitrogen fixation by *Azospirillum*, together with the availability of phosphorus solubilized by phosphate-solubilizing bacteria, resulted in a higher yield in foxtail millet.

Bambharolia *et al.* (2020) isolated 30 endophytic bacteria out of which two most potent isolates were identified as *Bacillus subtilis* (EP 6) and *Achromobacter xylosoxidans* (EP 17) by assessing plant growth promoting activities *viz.* phosphate solubilization, nitrogen fixation, IAA production and 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity. EP 6 shows highest phosphate solubilization zone ratio, IAA production and positive growth on 1-aminocyclopropane-1-carboxylate medium through ACC deaminase activity. EP17 from finger millet exhibited nitrogen fixing activity as presence of growth on Norris glucose nitrogen free medium plates.

Ramakrishnan and Bhuvaneswari (2014) showed that combined inoculation of AM Fungi with bacterial isolates of *Azospirillium* spp. and *Bacillus polymyxa* pointedly increased all the growth parameters and yield in ragi. *Azospirillum*, an associative symbiotic nitrogen fixing bacteria have a tendency to increase the uptake of P and N content in shoots, per cent root colonization and spore count of AM fungi over the control ones. The application of dual inoculum of AM fungi and *Azospirillium* to ragi exhibited higher P uptake, plant height, root and shoot dry weight, per cent root colonization, spore count when related to control plants. Tripartite inoculation of mycorrhizal fungi, *Azospirillium* and phosphate solubilizing bacteria stimulated upsurge in plant growth parameters.

Kumar *et al.* (2018) isolated psychrotolerant and psychrophilic N fixing bacteria using N deficient Burk's medium from the soil of temperate region of Western Indian Himalayas. Bacterial strain isolated was identified as *Dyadobacter* spp. through 16S rDNA sequencing. The inoculum of *Dyadobacter* spp. was applied to chickpea, black gram, green gram, pigeon pea, and finger millet. After 30 days of the treatment, the plants indicated increase in agronomical parameters, which was highest for pigeon pea followed by green gram, finger millet, black gram, and chickpea. When plants treated with *Dyadobacter* spp. were compared to their respective controls, both leaf nitrate reductase and chlorophyll content increased. The activity of plant nitrate reductase was found to be positively correlated with soil nitrate concentrations.

2.5. PHOSPAHATE SOLUBILIZING BACTERIA

A highly preferred method for preliminary screening and isolation of potential phosphate solubilizing microorganism (PSM) was first given by Pikovskaya (1948). Isolation of potential P solubilizing organisms can be done by plating 0.1 mL or 1 mL of serially diluted rhizosphere soil suspension on sterilized Pikovskaya's agar medium added with insoluble tricalcium phosphate (TCP). On incubating these plates at appropriate temperature, colonies with clear zone around are observed, which are selected as PSM. Pure cultures of such colonies will be obtained and subjected to further identification through biochemical and molecular characterization (Kalayu, 2019).

FZB 24 *Bacillus subtilis* was reported to be an efficient biofertilizer in increasing the yield and growth of cotton in comparison to the use of commercial fertilizers containing N, P and K on its application. Biofertilizer FZB 24 *B. subtilis* accelerated the growth and physiological activities of plants, resulting in proper utilization of important nutrients by making them available to plants. FZB 24 *B. subtilis* produced phytase integrating phosphorus from organic bound phytate (Yao *et al.*, 2006).

Karpagam and Nagalakshmi (2014) isolated 37 bacteria on Pikovskaya's agar medium, amended with insoluble Tri- Calcium Phosphate (TCP) from tomato rhizosphere soil. Among 37 isolates 3 strains showed maximum Phosphate Solubilization Index (PSI) of psm1(2.23), psm2 (2,15) and psm6 (2.11) in agar plates

along with high soluble phosphate production of 0.37 mgL^{-1}, 0.30 mgL^{-1} and 0.28 mgL^{-1} in broth culture. Also, they identified that the highest PSI producing isolates psm1 (*Pseudomonas*), psm2 (*Bacillus*) and psm6 (*Rhizobium*) respectively.

The effect of *Pseudomonas* strain 54RB was studied by Afzal *et al.* (2010) in soybean. The *Pseudomonas* strain 54RB exhibited a PSI of 4.1 and also produced 8.034 $\mu g\ mL^{-1}$ IAA and 1766 $\mu g\ mL^{-1}$ gibberellic acid. It was observed that this strain improved growth and yield of soybean.

SAFA-2, phosphorus solubilizing bacterium (PSB) isolated from rhizosphere of vegetable roots had a phosphate solubilizing potential of 151 $\mu g\ mL^{-1}$ and a solubilization index of 2.31 with the drop in pH from 7.02-3.55(Alia *et* al., 2013).

Yi *et al.* (2008) studied the effect of three phosphate solubilizing bacteria *Enterobacter* sp. EnHy-401, *Arthrobacter* sp. ArHy-505, *Azotobacter* sp. AzHy-510 producing exopolysaccharide (EPS) have a stronger ability for P-solubilization. P-solubilization was the highest in EnHy401, followed by ArHy-505 and AzHy-510 with 632.6 mg l^{-1}, 428.90 mg l^{-1} and 229.03 mg l^{-1} Soluble-P respectively.

Emami *et al.* (2020) studied the role of the endophytic and rhizospheric bacterial inoculum for increasing the phosphorus acquiring efficiency (PAE) and the efficiency of utilization of phosphorus (PUE) through the solubilization process of phosphate as well as the expansion of root systems. The highest inorganic-PSI was detected by rhizospheric bacterium R185 (4.0 + 0.1) and then by R193 (3.9 + 0.1). Additionally, the isolate R185 had the highest organic-PSI (3.8 + 0.1). The rhizospheric strain, *Pseudomonas* sp. R185 was able to dissolve around 299.0 mg mL^{-1} and also produced 19.2 mg per mL^{-1} IAA. In contrast, endophytic bacterium E240 was able to dissolve P on Sperber's agar plates (5 mm) and produce IAA (22 mg mL^{-1}) in the nutrient broth medium, which was supplemented with L-tryptophan. The effects of rhizospheric strain increased with the addition of endophytic bacterial strains: In one instance up to 67.3 percent growth in the surface of the root was observed during co-inoculation treatments.

Panhwar *et al.* (2014) obtained three potential isolates with greater P solubilizing characters. The isolate PSB17 (70.23 percent), followed by PSB7 (57.5

percent) reported highest P solubilizing activity in National Botanical Research Institute's Phosphate growth medium (NBRIP). On the other hand, on Pikovskaya media plates, PSB17 (76.03 percent) reported the highest P solubilizing activity. Similarly, in NBRIP broth the highest P solubilization activity was recorded by PSB7 (43.65 percent). The three potential isolates were identified as PSB7 (*Burkholderia thailandensis*), and PSB17 (*Sphingomonas pituitosa*) by Molecular analysis with the 16S rDNA.

Suleman *et al.* (2018) isolated 15 bacterial isolates from wheat rhizospheric soils of Peshawar and southern Punjab region, Pakistan among which two bacterial strains i.e., *Pseudomonas* sp. MS16 and *Enterobacter* sp. MS32 showed maximum phosphate solubilization activity. *Enterobacter* sp. MS32 reported higher zone of solubilization in both media (Pikovskaya and NBRIP). In quantitative assay, maximum P solubilization activity was observed in *Pseudomonas* sp. MS16 showed (280 $\mu g\ mL^{-1}$) followed by *Enterobacter* sp. MS32 (136 $\mu g\ mL^{-1}$). A sharp drop in pH from 7 to 3.53 was noticed in culture filtrate inoculated with *Pseudomonas* sp. MS16 which is due to the organic acid production by the bacterium.

Harinathan *et al.* (2016) found that the strains of *Bacillus* sp. (C2) and *Pseudomonas* sp. (C7) were capable of solubilizing phosphate. The effect of these strains was studied on the growth of pearl millet and finger millet after 30 and 45 days of inoculation. The assessment of fresh weight, dry weight, starch, chlorophyll and IAA were done in the PSB inoculated and control plantlets. As the treated finger millet and pearl millet plants showed greater improvement in plant height, root length and shoot length. The isolate *Bacillus* sp. (C2) treated plants recorded maximum IAA, starch, fresh and dry weight in both ragi and pearl millet plants compared to *Pseudomonas* sp. (C7). Finger millet plant recorded IAA, starch, fresh and dry weight than pearl millet.

Ibanez *et al.* (2021) screened 9 phosphate solubilizing isolates out of which some isolates including *Advenella mimigardefordensis, Bacillus cereus, Bacillus megaterium* and *Burkholderia fungorum* were observed to be significant in improving levels of assimilated phosphate. *B. megaterium* PSB1 exhibited higher levels of both acidic (80.83 $mU\ \mu g^{-1}$ protein) and alkaline phosphatase (94.10 $mU\ \mu g^{-1}$ protein). Also, this isolate reported the maximum IAA production of 41.62 $\mu g\ mL^{-1}$ among others.

Organic acids like formic acid, citric acid and tartaric acid were produced by the isolates. The PSB inoculated barley plants showed improved growth and yield in comparison with uninoculated plants which is obtained by the presence of plant growth promoting and phosphate solubilising traits in it.

2.6. MECHANISM AND ASSESSMENT

2.6.1. Nitrogen Fixation

Biological nitrogen fixation (BNF) is the biological process in which microorganisms in plants reduce molecular nitrogen to ammonia. It is an important mechanism for sustainable agricultural production and healthy ecosystem functioning. The use of synthetic nitrogen fertilizer in agriculture is reduced by symbiotic nitrogen fixation in legumes and associative, endosymbiotic, and endophytic nitrogen fixation in non-legumes (Mahmud *et al.*, 2020).

Nitrogenase, an oxygen labile enzyme complex found in both free-living and symbiotic diazotrophs, catalysis the conversion of molecular nitrogen to ammonia. The most common type of nitrogenase, Mo-nitrogenase or conventional nitrogenase, has a molybdenum-based prosthetic group, FeMoCo. (Iron Molybdenum-Cobalt group). Photosynthetic nitrogen fixers including some cyanobacteria and bacteria such as *Azotobacter*, carry extra forms of nitrogenase whose cofactor contains vanadium (V-nitrogenase) or iron (Fe-nitrogenase) (Bhat *et al.*, 2015).

The genetics of nitrogen fixation was elucidated at first in *Klebsiella pneumoniae* where the *nif* genes are essential for the synthesis of nitrogenase in chromosome, which are clustered in a 24 kb region of the gene. This whole region was sequenced first by Arnold *et al.*, (1988). The three structural genes encoding Mo-nitrogenase proteins are *nifD, nifH* and *nifK*. The *nifD* and *nifK* for the Mo protein subunits and *nifH* for the Fe protein. The whole assembly of nitrogenase necessitates other *nif* genes such as *nifB, nifQ, nifE, nifN, nifX, nifU, nifS, nifV, nifY and nifH* which involve in the synthesis of FeMoCo. In addition, the genes involved in the assembly of iron-sulfur clusters are *nifS* and *nifU* and the genes for the maturation of the nitrogenase components are *nifW* and *nifZ*. In addition, *nifF* and *nifJ* genes are for electron transport to nitrogenase besides the regulatory *nifLA* genes are involved in the regulation of *nif*

gene expression in response to the oxygen and nitrogen status of the cell (Franche *et al.*, 2009).

2.6.2. Phosphate Solubilization

Phosphorus solubilizing microorganisms inhabiting different soil ecosystems help in converting the insoluble forms of P such as tricalcium phosphate ($Ca_3PO_4)_2$, aluminium phosphate (Al_3PO_4), iron phosphate (Fe_3PO_4), etc. to soluble P (Sharma *et al.*, 2013). The plants uptake phosphorus in the form of orthophosphate ion. The presence of iron and aluminium in acid soils and calcium in case of neutral and alkaline soil prevents the solubility of phosphate. This results in the process of fixation of phosphorus and makes it unavailable for plant uptake. The phosphorus solubilizing bacteria secrete certain organic acids such as formic acid, gluconic acid, acetic acid, malic acid, etc., which act on the insoluble phosphate compound and convert it into soluble phosphate ion form and making it available to plants (Ponmurugan and Gopi, 2006). Organic P is mineralized by the release of phosphatases and phytases by phosphate solubilizing bacteria (Kumar, 2016).

2.6.2.1. Organic Acid Production by P-Solubilizing Microorganisms

Organic acids with lower pH, chelate the cations bound to phosphate through their hydroxyl and carboxyl groups or compete with phosphate for adsorption sites, resulting in increased mineral phosphate solubility and availability (Khan *et al.*, 2007; Mander *et al.*, 2012). Various phosphate-solubilizing bacteria, on the other hand, produced a wide range of organic acids.

Suleman *et al.* (2018) reported that organic acids such as gluconic acid (102 μg mL^{-1}), acetic acid (54 μg mL^{-1}) and oxalic acid (48 μg mL^{-1}) were produced by *Pseudomonas* sp. MS16. Remarkable organic acids produced by *Enterobacter* sp. MS32 were acetic acid (34.8 μg mL^{-1}) and malic acid (31.8 μg mL^{-1}). *Pseudomonas* sp. MS16 was identified as a potential microbe for the production of phosphorus solubilizing biofertilizer for wheat under P deficient soils.

2.6.2.2. Enzyme Activity of Phosphate Solubilizing Bacteria

Hanif *et al.* (2015) isolated a phosphobacterium *Bacillus subtilis* strain KPS-11 capable of utilizing both Ca-phosphate and Na-phytate *in vitro* from potato (*Solanum tuberosum* L.) rhizosphere. The strain produced 6.48 μg mL^{-1} indole-3-acetic acid in tryptophan supplemented medium. The P-solubilization by the strain was reported as 66.4 μg mL^{-1} in 10 days inoculated broth along with the production of gluconic acid (19.3 μg mL^{-1}) and malic acid (5.3 μg mL^{-1}). It was observed that the cell-associated phytase activity (1.6×10−10 kat mg^{-1} protein) was comparatively lower than that of the extracellular phytase activity (4.3 × 10−10 kat mg^{-1} protein).

2.6.2.3. Synergistic Interaction Between Arbuscular Mycorrhizal Fungi and Phosphate Solubilizing Bacteria

Sharma *et al.* (2020) studied the physical interaction of phosphate solubilizing bacteria and mycorrhizal fungi and its synergistic effect on the uptake of plant nutrients. It was seen that phosphate solubilizing bacterium *Pseudomonas* strongly attached to the hyphae of the fungi *Rhizoglomus irregulare.* Sharma *et al.* (2020) observed that inoculation of the host plants with a combination of the mycorrhizal fungus *Rhizoglomus irregulare* and phosphate solubilizing bacterium *Pseudomonas* resulted in higher plant biomass in comparison with the single inoculation of either fungus or bacteria indicating the synergistic activities of the fungal-bacterial consortium. It was seen that phosphate solubilizing bacterium *Pseudomonas* strongly attached to the hyphae of the fungi *Rhizoglomus irregulare._Pseudomonas* showed the potential for inorganic and organic phosphate mobilization.

2.7. EFFECT OF PGPB ON BIOTIC AND ABIOTIC STRESS CONDITIONS

2.7.1. Effect on Drought

Niu *et* al. (2018) isolated drought-tolerant bacteria *Pseudomonas fluorescens, Enterobacter hormaechei,* and *Pseudomonas migulae* producing EPS (exopolysaccharide). Seeds treated with these inoculums, encouraged seed germination and seedling growth under drought stress. The highest level of ACC deaminase and EPS-producing activity was shown by *Pseudomonas fluorescens* DR7. Strain DR7, drought-tolerant PGPR from foxtail millet could improve plant development under

drought stress and serve as effective bioinoculants to support agricultural production in arid environments, according to the findings.

2.7.2. Effect on Stress

Rizvi and Khan (2018) reported that plant growth promoting nitrogen fixing rhizobacterial strain *Azotobacter chroococcum* CAZ3 increased the yield of maize grown in soil polluted by heavy metals like Copper (Cu) and Lead (Pd). The dry weight of inoculated plant root grown in presence of 2007 mg Cu kg^{-1} and 585 mg Pb kg^{-1} was improved by 28 percent and 20 percent, respectively. The strain CAZ3 improved the kernel number, kernel yield and kernel protein by 10 percent, 45 percent and 6 percent, respectively. Reduction in the levels of proline, malondialdehyde and antioxidant enzymes in foliage of maize was also observed.

Kour *et al.* (2020) studied the effect of stress - adaptive phosphorus - solubilizing microbes in rhizospheric soil of cereals, pseudo cereals as well as their roles in reducing drought stress in great millet. The isolates *Streptomyces laurentii* EU - LWT 3 - 69 and *Penicillium* sp. strain EU - DSF – 10 were effective in increasing the amount of osmolytes, including proline, glycine, sugars, a higher chlorophyll content and reducing the rate of lipid peroxidation. Plants were subjected to drought stress through the imposition of various water stresses, such as 50 percent, 75 percent and 100 percent. All growth parameters such as length of shoots/roots as well as fresh/dry biomass was observed to increase in inoculated plants in all three water regimes, compared to the plants that were not inoculated.

2.7.3. Effect on Salinity

Kadmiri *et al.* (2018) showed that two phosphate-solubilizing and auxin-producing rhizobacterial isolate *Pseudomonas fluorescens* Ms-01 and *Azosprillum brasilense* DSM1690 isolated under hypersaline conditions had a good ability to grow in high NaCl concentrations of about 600 mM. Also, the auxin production and phosphate solubilizing activities of the isolates were maintained under hypersaline conditions. A significant increase in phosphate solubilizing activity was reported by *P. fluorescens* Ms-01 with 22.6 ± 1.7 µg mL^{-1} P_2O_5 in 600 mM NaCl. Also, *A. brasilense* DSM 1690 was found to have auxin production of 32 µg mL^{-1} under saline condition.

On treating wheat plants with these isolates, the plants were shown to have increased plant height and weight under normal and saline conditions with improved defence pathway of wheat plants against salt stress resulting from the accumulation of proline and antioxidant enzymes such as peroxidase and ascorbate peroxidase.

Omer *et al.* (2016) isolated *Azotobacter salinestris* from the regions of salt affected soil in Egypt under 5 percent NaCl concentration. The strain was reported to have a maximum nitrogenase activity of 631.530 nmol C_2H_4 h^{-1} mL^{-1} with higher IAA production of 92.21 μg mL^{-1} and gibberellin production of to 86.16 μg mL^{-1} under saline condition. The presence of osmo-protectant substances such as ACC deaminase enzyme (60.4 n mole α-ketobutaric mg^{-1} $hour^{-1}$), salicylic acid (2.4 mg mL^{-1}), proline (2.75 μg mL^{-1}) and EPS (14.5 μg L^{-1}) described the tolerance of *A. salinestris* to abiotic stress conditions.

2.7.4. Effect on Disease Control

Rasul *et al.* (2019) reported that *Pseudomonas* spp. MR11, MR34 and *Bacillus* sp. MR42 isolated from rice rhizosphere were involved in the inhibition of bacterial leaf blight pathogen (18-25 mm) and also involved in solubilization of phosphorus (up to197 μg mL^{-1}) and IAA production (1.7–14 μg mL^{-1}). The presence of gluconic acid generated by phosphate solubilizing antagonistic bacteria was thought to play a dual role in P solubilization and BLB suppression. BLB disease suppression (up to 56 percent) was seen in plants treated with these three strains, as well as an increase in plant P content of 0.47 percent in straw and 1.26 percent in seed. Also increase in the activity of defence enzymes such as phenylalanine ammonia, β, 1–3 glucanase, catalase, peroxidase and polyphenol oxidase were detected in P-solubilizing antagonistic bacteria inoculated plants.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

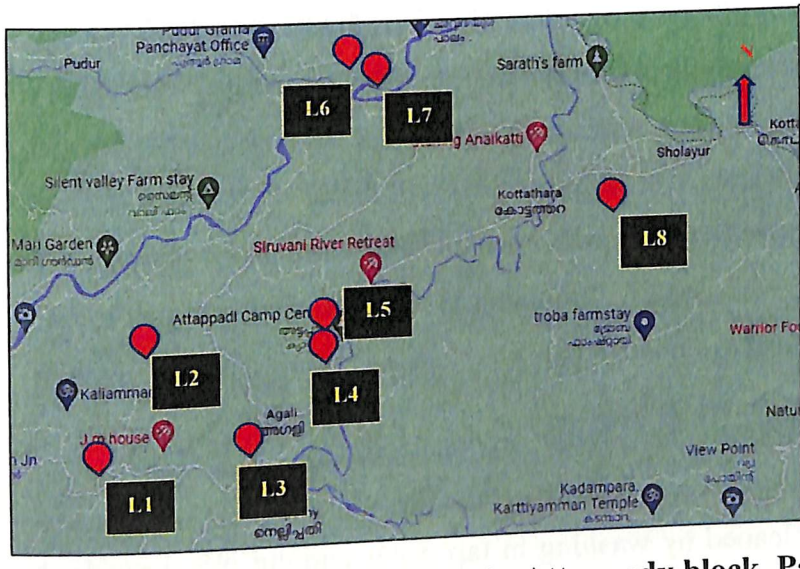
The research conducted on "Isolation and characterization of beneficial rhizosphere microorganisms from ragi grown in Attappady hill tract of Kerala" was accomplished at the Department of Agricultural Microbiology, College of Agriculture, Vellayani during the period 2019-2021.

The particulars of the materials used and methods followed throughout the course of study are mentioned below.

3.1. ISOLATION OF NITROGEN FIXING AND PHOSPHATE SOLUBILIZING BACTERIA

3.1.1. Sample Collection

Soil samples were collected from ragi fields located at different regions of Attappady hill tract, Palakkad district, Kerala. Eight ragi fields were selected in Agali, Pudur and Sholayur panchayats of Attappady block and rhizosphere soil samples were collected from 5 different locations in each of these fields.



L1(Kavundikkal)
11°05'38.7"N 76°36'56.1"E
L2(Agali)
11°06'29.4"N 76°37'12.1"E
L3(Goolikadavu)-
11°05'33.4"N 76°38'14.3"E
L4(Narasimukku1)
11°06'40.0"N 76°39'09.9"E
L5(Narasimukku2)
11°06'40.5"N 76°39'09.9"E
L6(Pudur)
11°09'20.9"N 76°39'50.4"E
L7(Goolikadavu)
11°09'20.9"N 76°39'50.4"E
L8(Sholayur)
11°07'50.7"N 76°42'09.9"E

Location map of sampling sites in Attappady block, Palakkad district, Kerala.

Soil samples of 50g each were collected from the rhizosphere region of healthy ragi plants. The plants were gently uprooted and tapped for collecting the rhizosphere soil. Samples of ragi roots were also collected separately in sterile polythene bags. Soil

samples from each field were pooled to get a representative sample, which was packed in sterile polythene bags and transported to the laboratory for further studies.

3.1.2. Isolation of Nitrogen Fixing Bacteria (NFB)

Nitrogen fixing bacteria were isolated from the rhizosphere soil samples using different N-free agar media such as Waksman Base No.77, N-free malate bromothymol blue, Jensen's and Burk's media. The different media were prepared based on their composition and sterilized by autoclaving at 121°C at 15 psi pressure for 15 minutes. The isolation was carried out by following serial dilution and plating technique (Reynolds, 2005) of rhizosphere soil and root sample.

The collected soil samples were air dried and sieved through 0.5 mm sieve to get fine soil. Ten grams of soil sample was added to 90 mL sterile water blank and mixed thoroughly by vortexing to get 10^{-1} dilution. Further dilution was made upto 10^{-6} by transferring 1 mL aliquot from each dilution to a 9 mL sterile water blank. Pour plate technique (Reynolds, 2005) was adopted for plating the aliquots in different agar-based N free media. One mL each from 10^{-4}, 10^{-5} and 10^{-6} dilutions were poured to respectively labelled sterile petri plates using 1000 µL micropipette. Sterile Waksman Base No. 77, N-free malate bromothymol blue, Jensen's and Burk's media were melted, cooled down to 45°C and poured into separate petri plates containing diluted soil samples. After pouring the plates were gently swirled thrice in clockwise and anticlockwise directions, in order to disperse the sample dilution uniformly and were allowed to solidify. Plating was done in triplicate for each dilution and media combination. These plates were incubated for 48-72 h at room temperature and observed for nitrogen fixing bacterial colonies.

Isolation of nitrogen fixing bacteria from collected root bits were done. The collected root samples were cleaned by washing in tap water and cut into 1 cm long pieces. These root bits were then surface sterilized with 1 per cent Sodium hypochlorite solution for 2-3 minutes and washed with sterile water for five times. With the help of sterilized forceps, the surface sterilized root bits were dipped into sterilized semi solid N-Free malate bromothymol blue medium contained in small vials and kept for incubation at 28±2°C for 48 h. The inoculated vials were observed for pellicle formation

just below the upper layer. The obtained mucoidal pellicles were then streaked on to solid sterile N-free malate bromothymol blue medium to obtain pure cultures.

3.1.3. Isolation of Phosphate Solubilizing Bacteria

The phosphate solubilizing bacteria were isolated from the finely processed rhizosphere soil collected from different ragi fields of Attappady region. The isolates were selectively picked after growing in Pikovskaya's medium containing tricalcium phosphate as phosphorus (P) source.

The isolation of phosphate solubilising bacteria was done by following serial dilution and plating technique. Soil samples were diluted up to 10^{-6} dilution, further one mL of each 10^{-4},10^{-5} and 10^{-6} dilutions were transferred to sterile petri dishes, to which sterile Pikovskaya's medium (45°C) was poured. The contents were mixed well and the plates were incubated for 48-72 h. The colonies with clear zone of solubilization were selected for further screening.

3.1.3. Purification of Isolates

Both Nitrogen Fixing Bacteria (NFB) and Phosphate Solubilizing Bacteria (PSB) obtained through serial dilution and plating technique were purified by picking out single colonies formed on specific media such as Waksman Base No. 77, N-free malate bromothymol blue, Jensen's, Burk's and Pikovskaya's agar and streaked on to the corresponding agar plates. The purified colonies were preserved on corresponding agar slants at 4°C in a refrigerator for further use.

3.1.4. Preliminary Screening of Isolates

The bacterial isolates were initially screened based on the colony morphology. Morphologically similar isolates from same soil sample were eliminated in order to avoid repetition.

3.2. CHARACTERIZATION OF NFB AND PSB ISOLATES

After preliminary screening, the selected NFB and PSB isolates were characterized based on morphological and biochemical characters. For this, the pure

culture of each bacterium was grown for 24 h at 28±2°C in the respective broths from which they were isolated.

3.2.1. Morphological Characterization of NFB and PSB Isolates

A loopful of pure culture of each bacterial isolate was streaked on specific agar medium using quadrant streak method and the colony pattern on thin agar plates of Waksman Base No. 77, N-free malate bromothymol blue, Jensen's, Burk's, Pikovskaya's media and were examined after incubation for 24-36 h at 28±2°C. The colony morphology including colony colour, shape, texture, margin, elevation and transparency on agar plates were observed and noted.

The other morphological characters of bacterial isolates including cell shape, motility, Gram staining and capsule staining were also studied.

3.2.1.1. Cell Shape

The log phase cultures of NFB and PSB were observed microscopically for recording the morphological character of the cells.

3.2.1.2. Gram Reaction

Gram staining (Cappuccino and Sherman, 1999) was performed and cells were observed under a microscope.

3.2.1.3. Capsule Staining

Capsule staining was done according to Anthony's staining method (Cappuccino and Sherman, 1999) using 20 per cent copper sulphate and the presence or absence of capsule was noted.

3.2.1.4. Cell Size

Size of the bacterial cells were measured using micrometry and expressed in µm.

3.2.1.5. Cell Motility

Cell motility of each isolate was checked by following hanging drop method.

3.2.2. Biochemical Characterization of NFB and PSB Isolates

Biochemical characterization of the selected isolates was done by carrying out various biochemical tests as follows

3.2.2.1. Oxidase Test

A loopful of 24 h old culture was spread on to the oxidase disc (HIMEDIA® DD018) and the reaction was observed within 5-10 seconds, the colour change after 10 seconds or no change was taken as negative reaction.

3.2.2.2. Nitrate Reductase Test

The isolates were inoculated to 10 mL of peptone broth supplemented with sterile nitrate reductase discs (HIMEDIA® DD041) as substrate and incubated for 48 h at 28±2°C. After incubation the culture was tested by adding drops of sulfanilic acid and α-naphthylamine solution. The development of red colour was taken as positive reaction.

3.2.2.3. Hydrogen Sulphide Production

The isolates were stab inoculated onto the tubes containing Sulfide, Indole, Motility (SIM) agar and are incubated at 28±2°C for 48 h. H_2S production was indicated by the presence of black coloration along the line of stab inoculation.

3.2.2.4. Catalase Activity

A loopful of 24 h old culture of the isolates maintained on specific agar plates was transferred to a glass test tube containing 0.5 ml of distilled water and thoroughly mixed with 0.5 ml of 3 per cent hydrogen peroxide solution, and effervescence was observed.

3.2.2.5. Indole Production

The isolates were stab inoculated to sterilised SIM agar test tubes and incubated at 28±2°C. After 48 h of incubation, 0.3 ml of Kovacs reagent was added and thoroughly mixed. Positive reaction was indicated by, the reddening of the alcohol layer within a few minutes.

3.2.2.6. Methyl Red and Voges Proskauer Test

The isolates were inoculated into two sets of Methyl red and Voges-Proskauer (MR-VP) broth and incubated at 28±2°C for 48 h. Few drops of an alcoholic methyl red solution were added to the first set of tubes. The appearance of a distinct red colour was indicated as positive reaction to MR test.

The second set of tubes was filled with alpha-naphthol solution (5 per cent solution in 70 per cent ethyl alcohol) and gently shaken for 15 minutes. The development of red colour indicated the positive reaction of acetyl methyl carbinol production. This indicated as positive result for VP test.

3.2.2.7. Citrate Utilization Test

The isolates were placed in test tubes containing Simmons citrate agar medium with citrate as the sole carbon source and incubated at 28±2°C for 48 h. After incubation, the colour change from green to blue due to pH change was taken as positive.

3.2.2.8. Gelatin Hydrolysis

Gelatin hydrolysis was used to test the activity of the enzyme gelatinase. The test cultures were stab inoculated into nutrient gelatin deep tubes, refrigerated for 48 h, and gelatin liquefication was observed.

3.2.2.9. Starch Hydrolysis

To investigate amylase activity, a starch hydrolysis test was performed. The isolates were streaked on nutrient agar plates containing 2 per cent insoluble starch and incubated at room temperature for 24 h. The hydrolysis of starch was tested by flooding the plates with iodine solution, and the presence of clear zone surrounding the colonies was considered as positive reaction.

3.2.2.10. Cellulose Degradation

The activity of the enzyme cellulase for cellulose hydrolysis in bacterial isolates was investigated. The isolates were streaked on CMC (Carboxymethylcellulose) agar medium containing cellulose and incubated for 72 h at 28±2°C. After incubation, plates

were flooded with 0.2 per cent aqueous Congo red solution and destained by treating with 1M NaCl for 15 minutes. After 30 minutes, the plates were examined for the presence of clear zone surrounding the colonies. Cellulase activity was indicated by the presence of clear zone around the colony.

3.2.2.11. Polysaccharide Production

In a plate assay, the isolates were tested for their ability to produce polysaccharides. A modified nutrient medium with high sucrose content (5 per cent) was used. The isolates were streaked on solidified agar plates and incubated at 28±2°C for 4 to 7 days. The thick viscous mass formed over the streak was visually scored, and isolates exhibiting viscous mass formation were considered positive for polysaccharide production.

3.3. ESTIMATION OF PGP ACTIVITY OF THE ISOLATES.

The selected isolates were grown in respective culture broths till the number of cells reached 10^8 CFU mL^{-1}. During the log phase of the bacterial growth, the culture was subjected to centrifugation. The cells were pelleted out and the supernatant was used for PGP activity estimation.

3.3.1. Quantitative Estimation of Indole Acetic Acid (IAA) by Spectrophotometry

The quantification of IAA was done according to the standard procedure described by Gordon and Weber (1951).

Twenty millilitres of sterile Waksman Base No. 77, N-free malate bromothymol blue, Jensen's and Pikovskaya's broths were prepared and supplemented with 0.1 per cent L-tryptophan (heat labile). The 24 h old bacterial isolates were inoculated to the broth and kept for incubation at 28±2°C in a shaker at 100 rpm, in dark for 48-72 h. Two replications were maintained for each isolate. The inoculated vials were wrapped with aluminium foil in order to prevent photoinactivation of the biologically active compounds. After incubation the samples were collected in centrifuge tubes and centrifuged at 4500 rpm for 20 minutes at 4°C. Two mL of the supernatant was transferred immediately to a sterile test tube and two drops of orthophosphoric acid were added and mixed well. After one minute, 4 mL of Salkowski reagent was added,

mixed well using vortex mixer and kept under dark conditions for about 25 minutes. The optical density (OD) was measured using UV-VIS spectrophotometer at 530 nm. The amount of IAA in the sample was determined by comparing to a standard curve prepared with chemical grade indole-3-acetic acid.

3.3.2. Quantitative Estimation of Gibberellic Acid (GA) by Spectrophotometry

Quantitative analysis of gibberellic acid was carried out as per the standard procedure described by Holbrook *et al.* (1961).

The 24 h old bacterial cultures were inoculated to specific broth and incubated for 48-72 h at 28±2°C. Two replications were maintained for each isolate. After incubation, 20 mL of sample was collected and centrifuged at 4500 rpm for 20 minutes at 4°C. Fifteen mL of the supernatant obtained was transferred to another centrifuge tube, to which 2 mL of zinc acetate solution was added. After two mins interval, 2 mL of potassium ferrocyanide solution was added. This mixture was then centrifuged at 2000 rpm for 15 minutes at 4°C. After centrifugation, 5 mL of the supernatant was collected in a 15 mL glass vial to which 5 mL of 30 per cent HCl was added. This solution was then kept in water bath at 20°±1°C for 75 minutes and the absorbance value was measured at 254 nm after standing time. The quantity of gibberellic acid in the sample was estimated by referring to the standard curve prepared with chemical grade gibberellic acid.

3.3.3. Quantitative Estimation of Extracellular Ammonia Production

Extracellular ammonia excretion was determined spectrophotometrically by the method described by Cappuccino and Sherman (1999).

24 h old bacterial isolate was inoculated in ten mL of peptone broth and incubated at 28±2°C for 72 h. Two replications were maintained. After incubation, the cultures were subjected to centrifugation for 15 minutes at 4500 rpm. The presence of ammonia in supernatant was detected by the development of brown colour on addition of Nessler's reagent. This was taken as positive reaction for ammonia production as described by (Cappuccino and Sherman, 1999). The absorbance of the colour developed

was measured at 450 nm and the quantity of ammonia produced was estimated by comparing to the standard curve of ammonium sulphate.

3.4. QUANTITATIVE ESTIMATION OF NITROGEN BY MICRO-KJELDAHL METHOD

3.4.1. Single Acid Digestion

Twenty-four hour old culture was inoculated to 100 mL specific broth and incubated at 28±2°C for 7 days. Two replications were maintained. After incubation, the samples were kept over a hot plate at 110°C for 5 days, until it evaporated down to a quantity of 5 mL. This 5 mL was collected and fed into tubes of digestion unit. A pinch of digestion mixture (100:10:1 ratio of potassium sulphate, copper sulphate, and selenium powder) was added to the tubes and 10 mL concentrated sulphuric acid was added. Clear solution was obtained after the completion of digestion process. Following the completion of digestion, the contents were transferred to a 100 mL volumetric flask and made up to 100 mL with distilled water.

In Kjeldahl digestion unit, 10 mL of digested sample and 10 mL of NaOH were added, and 10 mL of boric acid with a few drops of mixed indicator was taken in a conical flask. Titration of ammonia collected in a conical flask against sulphuric acid.

$$\text{Per cent of total N in the sample} = \frac{TV \times N \times 0.014 \times 100 \times 100}{V \times 10}$$

N- Normality of acid (0.02N)

V-Volume of condensed liquid culture of bacteria.

3.5. ASSESSING THE PHOSPHATE SOLUBILIZATION POTENTIAL OF BACTERIAL ISOLATES UNDER *IN VITRO* CONDITIONS

3.5.1. Plate Assay for Phosphate Solubilization by PSB Isolates

The isolates were evaluated for their ability to dissolve insoluble tricalcium phosphate present in the Pikovskaya's agar medium (Pikovskaya, 1948; Gupta *et al.*, 1994). A loop of culture grown in Pikovskaya's broth was spot inoculated at each quadrant of the Pikovskaya's agar plates (3 mm thickness). Thus, four PSB bacteria were compared and observed for P solubilization in one plate with three replications

and incubated at 28±2°C for 5 days. Phosphorous solubilization was observed as clear zone around the bacterial colonies and the diameter of the zone was measured in millimetres.

The Phosphorus Solubilizing Index (PSI) of the PSB was calculated using the formula,

$$\text{Phosphorus Solubilizing Index} = \frac{\text{Colony diameter} + \text{Halo zone diameter}}{\text{Colony diameter}}$$

3.5.2. Broth Assay for Phosphate Solubilization by PSB Isolates

The Phosphorus solubilizing potential by PSB isolates were assessed quantitatively by the method described by Clescerie *et al.* (1998).

The 24 h old bacterial cultures were inoculated to 50 mL sterile Pikovskaya's broth and incubated for 10 days at 28±2°C in shaker at 110 rpm. After centrifugation, 5 mL of supernatant was collected in a screw-capped vial. The supernatant was then treated with 5 mL of Vanadomolybdate solution. The volume was made up to 25 mL and incubated overnight for yellow colour development which was measured spectrophotometrically at 430 nm. Uninoculated broth was maintained as control. For each treatment, two replications were maintained. The phosphorus solubilization per 5 g of tricalcium phosphate amended in one litre of broth was calculated using the standard phosphorus curve and expressed in mg L^{-1}.

3.6. WEIGHTED AVERAGE RANKING

Weighted average ranking was done for identifying the best NFB and PSB isolates. For the ranking, IAA, GA and nitrogen content for NFB isolates were considered. The phosphorus solubilization potential in plate and broth assay were considered for selecting PSB isolates. The treatment of NFB and PSB showing highest value in each parameter were given 1st rank and the next lower was given 2nd rank and so on. The ranks obtained for different parameters were added to obtain weighted average rank. The isolate having the lowest value in weighted rank was assigned 1st rank, the second lowest was given 2nd and so on.

3.7. MOLECULAR CHARACTERIZATION OF THE BEST BACTERIAL ISOLATES

Molecular characterization of the best isolates selected by qualitative and quantitative screening was done.

3.7.1. Isolation of Bacterial Genomic DNA

The isolation of bacterial genomic DNA was done using NucleoSpin® Tissue Kit (Macherey-Nagel).

A loopful of bacterial culture was taken in a microcentrifuge tube to which, 180 µL of T1 buffer and 25 µL of proteinase K was added and incubated at 56°C. The incubation was carried out in a water bath until the cells were completely lysed. On completion of lysis, five µL of RNase A (100 mg mL^{-1}) was added and incubated at room temperature for five minutes. Following this, 200 µL of B3 buffer was added and incubated at 70°C for ten minutes. Further, 210 µL of 100 per cent ethanol was added and mixed thoroughly using vortex mixer. The obtained mixture was then pipetted into NucleoSpin® Tissue column placed in a two ml collection tube and centrifuged at 11000 rpm for one minute. The NucleoSpin® Tissue column was washed with 500 of BW buffer by transferring it to a new two ml tube. This was again washed with 600 µL of B5 buffer. After completion of washing the NucleoSpin® Tissue column was kept in a clean 1.5 ml tube. The elution of DNA was done using 50 µL of BE buffer.

3.7.2. DNA Quality and Quantity Check Using Agarose Gel Electrophoresis (AGE)

The quality of the isolated DNA was checked using agarose gel electrophoresis. To five µL of DNA one µL of 6X gel-loading buffer (0.25 per cent bromophenol blue, 30 per cent sucrose in TE buffer pH-8.0) was added. The samples were loaded onto a 0.8 per cent agarose gel prepared in a 0.5X TBE (Tris-Borate-EDTA) buffer with 0.5 g mL^{-1} ethidium bromide and electrophoresis was carried out with 0.5X TBE buffer at 75 V for 30 min. The gel was visualised and DNA was quantified using Gel documentation system (Bio-Rad).

3.7.3. PCR Analysis

PCR analysis was done with 2x Phire Master Mix (5 µL), 4 µL of distilled water, 0.25 µL of forward primer, 0.25 µL of reverse primer and one µL of obtained DNA.

Target	Primer name	Direction	Sequence (5'→ 3')
16S rRNA	16S-RS-F	Forward	CAGGCCTAACACATGCAAGTC
	16S-RS-R	Reverse	GGGCGGWGTGTACAAGGC

3.7.4. PCR Amplification

The PCR amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems). The PCR amplification profile as follows,

PCR Amplification profile of 16S rRNA:

95 °C	-	5 minutes	
95 °C	-	30 seconds	35 cycles
60 °C	-	40 seconds	
72 °C	-	60 seconds	
72 °C	-	7 minutes	
4 °C	-	∞	

3.7.5. Agarose Gel Electrophoresis of PCR Products

The PCR products were examined using 1.2 per cent agarose gels prepared in 0.5X TBE buffer containing 0.5 µg mL^{-1} ethidium bromide. Electrophoresis was performed at 75V by loading the mixer of 1 µL of 6X loading dye and 4 µL of PCR products with 0.5X TBE as electrophoresis buffer for about 1-2 h till the bromophenol blue front had migrated nearly to the bottom of the gel. A 2-log DNA ladder (NEB) was used as molecular standard. Gel documentation system (Bio-Rad) captured the image of the gels under UV light using UV transilluminator (Genei).

3.7.6. ExoSAP-IT Treatment

ExoSAP-IT (USB) is a specifically prepared buffer containing two hydrolytic enzymes, Exonuclease I and Shrimp Alkaline Phosphatase (SAP), for the elimination of undesired primers and dNTPs from a PCR product combination with no interference in downstream applications.

Five micro litres of PCR product were mixed with 0.5 µL of ExoSAP-IT and incubated at 37°C for 15 minutes followed by 5 minutes of enzyme inactivation at 85°C.

3.7.7. Sequencing Reaction

In PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) sequencing reaction was carried out by using the BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA).

The Sequencing PCR mix consisted of 6.6 µL distilled water, 1.9µL 5X Sequencing Buffer, 0.3 µL Forward Primer, 0.3 µL Reverse Primer, 0.2µL Sequencing Mix and 1 µL Exosap treated PCR product.

3.7.8. Sequencing PCR Amplification Profile

96°C	-	2 minutes	
96°C	-	30 seconds	30 cycles
50°C	-	40 seconds	
60°C	-	4 minutes	
4°C	-	∞	

3.7.9. Post Sequencing PCR Clean-up

A mixer of distilled water, 125mM EDTA, 3M sodium acetate (p^H 4.6) and ethanol were prepared. In each well of the sequencing plate containing sequencing PCR product, 50 µL was added and mixed by vortex mixer and incubated at room temperature for 30 minutes. After incubation, the mixer was centrifuged at 3700 rpm for 30 minutes. The supernatant was decanted and 50 µL of 70 per cent ethanol was added to each well and spun at 3700 rpm for 30 minutes. The ethanol wash was repeated twice by decanting the supernatant and adding 70 per cent ethanol. After final wash, the supernatant was allowed to air dry. The cleaned up and air-dried product was sequenced in ABI 3500 DNA Analyzer (Applied Biosystems) using Sanger DNA sequencing method.

3.7.10. Sequence Analysis

Sequence Scanner Software v1 (Applied Biosystems) was used to check the sequence quality. Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.1 (Drummond *et al.*, 2010). The consensus sequence was created using the software BioEdit 7.2 (Hall *et al.*, 2011).

NCBI-BLAST module was used to compare the sequence similarity and the best match in Genbank database of obtained sequence database of unkown culture (Altschul *et al.*, 1990).

3.8. *IN-VITRO* PLANT GROWTH PROMOTION STUDY

The selected isolates were subjected to roll towel assay. Seeds of ragi (*Eleusine coracana*) was surface sterilized with 1 per cent sodium hypochlorite for 2-3 minutes and soaked for four h in liquid culture of the selected isolates with 10^8 CFU mL^{-1}. Twelve bacterized seeds were arranged in a row on roll towel moistened with sterile water. The rolled paper towels were kept at 28°C, and the saturated moisture condition was maintained by watering the roll towel every day. For each treatment, four replications were kept. The plant growth parameters were observed after ten days.

3.8.1. Observations

3.8.1.1. Percentage Seed Germination

The percentage of seed germination was calculated using the following formula.

$$\text{Seed germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100$$

3.8.1.2. Length of Root

The root length was measured after 10 days from the base of the stem to the tip of the root. It was expressed in centimetres.

3.8.1.3. Length of Shoot

The shoot length was measured after 10 days from the base of the stem to the tip of the longest leaf and expressed in centimetres.

3.8.1.4. Seedling Vigour Index

The seedling vigour index was calculated using the following formula (Abdul-Baki and Anderson, 1973),

Seedling Vigour Index = Germination Percentage × (Shoot length + Root length)

3.8.1.5. Fresh Weight of Root

The root fresh weight was measured and expressed in mg.

3.8.1.6. Fresh Weight of Shoot

The shoot fresh weight was measured and expressed in mg.

3.8.1.7. Dry Weight of Root

The plant samples were dried by keeping in hot air oven at 55°C for 24 h. The root dry weight was measured in mg.

3.8.1.8. Dry Weight of Shoot

The shoot dry weight was measured and expressed in mg.

3.8.1.9. Root shoot ratio

The root shoot ratio was calculated using the dry weight of root and shoot using the formula,

$$\text{Root shoot ratio} = \frac{\text{Dry weight of root}}{\text{Dry weight of shoot}}$$

3.9. ASSESSMENT OF ANTI-FUNGAL ACTIVITY OF THE SELECTED BACTERIAL ISOLATES

Dual culture plate technique (Dennis and Webster, 1971) was performed to assess the anti-fungal activity of the bacterial isolates. The fungal pathogens, *Rhizoctonia solani, Sclerotium rolfsii, Helminthosporium* sp. and *Fusarium* sp. available at the Department of Agricultural Microbiology, College of Agriculture were used for the assay. The fungal pathogens were grown on PDA plates for 3-5 days and mycelial discs were punched out from the actively growing region of fungal colony using a 5mm sterile corkborer. This was inoculated at the centre of PDA plate prepared for dual culture plate assay and bacterial streaks of 1.5 cm were made on both sides of mycelial disc at a distance of 3.5 cm from the centre. The plates were incubated at 28±2°C. In test plates of *Rhizoctonia solani* and *Sclerotium rolfsii*, inhibition was checked and zone was recorded (in mm) after 48 h. In *Fusarium* sp. and *Helminthosporium* sp. dual culture plates the inhibition zone was recorded (in mm) after 96 h.

3.7. SIDEROPHORE PRODUCTION

The siderophore production was qualitatively assessed using the modified Chrome Azurol Sulfonate (CAS) agar plate as described by Milagres *et al.* (1999) by combining two solutions. The two solutions were autoclaved separately and mixed together slowly. This finally obtained solution of 100ml volume was added to 900ml of Succinate agar medium (pH 7) and poured to sterile Petri dishes and allowed to solidify. Twenty-four h old bacterial isolates were spot inoculated and incubated at 28±2°C for 24 to 72 h. The zone of colouration indicated the presence of siderophore.

3.8. POTASSIUM SOLUBILISATION BY THE BACTERIAL ISOLATES

The potassium solubilization potential of selected bacterial isolates was assessed using Aleksandrov medium as per the method described by Lu and Huang (2010). Aleksandrov medium was prepared with insoluble form of potassium aluminosilicate and sterilized by autoclaving. This was melted and cooled to 45°C and poured into sterilized Petri plates to a thickness of 3 mm. The plates were spot inoculated with bacterial isolates and incubated for 7 days at 28±2°C. The zone of potassium solubilization was recorded. For each isolate, three replication plates were maintained.

3.9. SILICON SOLUBILIZATION BY THE BACTERIAL ISOLATES

The silicon solubilization potential of bacterial isolates was assessed by plate assay as described by Vasanthi *et al.* (2013). Agar plates of 3mm thickness were prepared using Bunt and Rovira agar medium amended with 0.25 per cent magnesium trisilicate. Spot inoculation of the bacterial isolates were done and three replications were maintained. The plates were incubated for about 7 days and observed for clear zone of solubilization.

3.10. PHOSPHORUS SOLUBILIZATION BY THE BACERIAL ISOLATES

The phosphate solubilizing ability of the selected isolates were evaluated by spot inoculating and incubating at 28±2°C for 5 days. The clear zone of solubilization was observed and measured if any.

3.13. STATISTICAL ANALYSIS

The collected data were subjected to statistical analysis using Analysis of Variance Technique (ANOVA) under Completely Randomized Design (CRD). The data on mean, Standard Error (SE) and Critical Difference (CD) were obtained using GRAPES (Gopinath *et al.*, 2021).

RESULTS

4. RESULTS

Nitrogen fixing and phosphate solubilizing bacteria were isolated from rhizosphere soils of ragi plants from Attappady region. The succeeding results were obtained through the statistical analysis of data from the experiments carried-out during the course of study.

4.1. ISOLATION OF NITROGEN FIXING BACTERIA (NFB) AND PHOSPHATE SOLUBILIZING BACTERIA (PSB)

4.1.1. Isolation of NFB Associated with Ragi Rhizosphere Soil

Thirty-six bacterial isolates capable of nitrogen fixation were obtained from the collected soil and root samples. The isolates were given code numbers from ATY1-ATY36 as shown in Table 1.

4.1.2. Isolation of PSB Associated with Ragi Rhizosphere Soil

Eight isolates of bacteria capable of phosphate solubilization showing clear zone of phosphate solubilization were obtained from different regions of Attappady hill tract. Code numbers, PSB1-PSB8, were given for these isolates as shown in Table 2.

4.2. MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF THE ISOLATES

4.2.1. Morphological Characters of the Selected Isolates

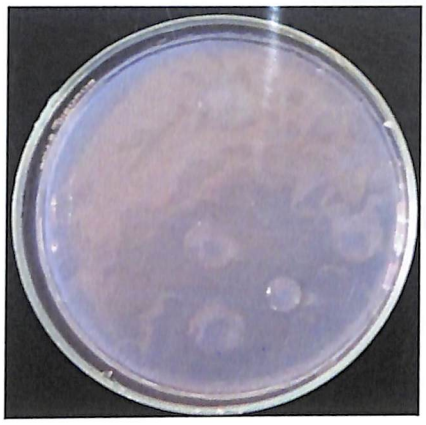
Morphological characters of colonies of pure cultured bacterial isolates are presented in Table 3. The colony characters such as colony shape, colony size, colony colour, elevation, margin, opacity and texture were visually noted (Plate 1-5).

The morphological characters of bacterial cells are mentioned in Table 4. All the twenty isolates were studied using both Gram staining and Anthony's capsule staining method and results are given in Table 5. The cell characters such as cell shape, cell size, cell arrangement and motility were observed.

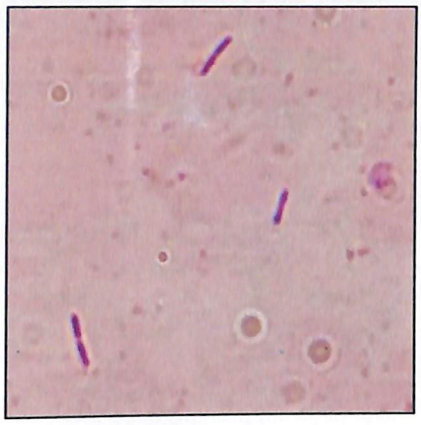
Table 1. List of nitrogen fixing bacterial isolates obtained from different regions of Attappady

Sl. No.	Isolates	Location	Medium	Sl. No	Isolates	Location	Medium
1	ATY1	Pudur	JM	19	ATY19	Sholayur	BM
2	ATY2	Goolikadavu	JM	20	ATY20	Narasimukku1	BM
3	ATY3	Sholayur	JM	21	ATY21	Agali	BM
4	ATY4	Narasimukku1	JM	22	ATY22	Kavundikkal	BM
5	ATY5	Kavundikkal	JM	23	ATY23	Narasimukku1	BM
6	ATY6	Agali	JM	24	ATY24	Narasimukku2	BM
7	ATY7	Narasimukku1	JM	25	ATY25	Pudur	NFB
8	ATY8	Narasimukku2	JM	26	ATY26	Goolikadavu	NFB
9	ATY9	Agali	WB77	27	ATY27	Sholayur	NFB
10	ATY10	Kavundikkal	WB77	28	ATY28	Sholayur	NFB
11	ATY11	Narasimukku1	WB77	29	ATY29	Pudur	NFB
12	ATY12	Narasimukku2	WB77	30	ATY30	Goolikadavu	NFB
13	ATY13	Pudur	WB77	31	ATY31	Sholayur	NFB
14	ATY14	Goolikadavu	WB77	32	ATY32	Narasimukku1	NFB
15	ATY15	Sholayur	WB77	33	ATY33	Agali	NFB
16	ATY16	Narasimukku1	WB77	34	ATY34	Kavundikkal	NFB
17	ATY17	Pudur	BM	35	ATY35	Narasimukku1	NFB
18	ATY18	Goolikadavu	BM	36	ATY36	Narasimukku2	NFB

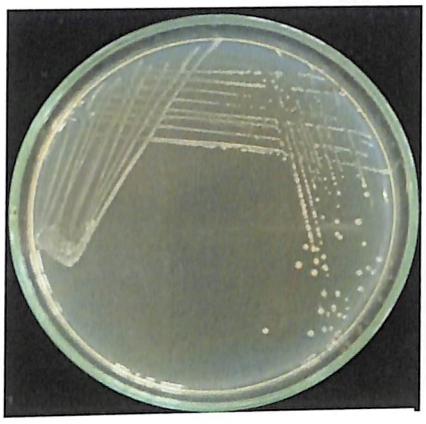
JM-Jensen's Medium; WB77-Waksman Base No.77; BM-Burks Medium; NFB-N-Free bromothymol blue



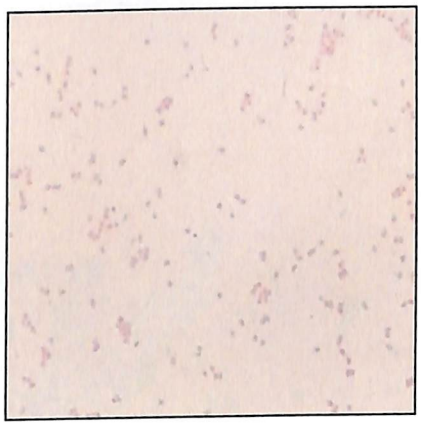
ATY1



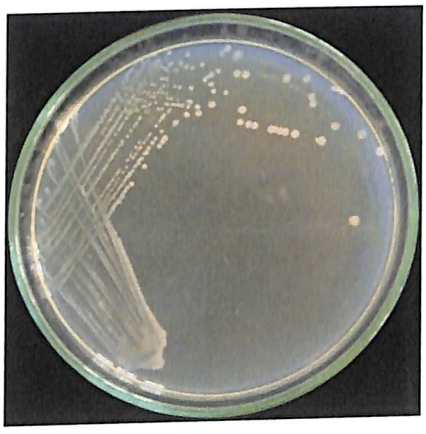
Gram positive



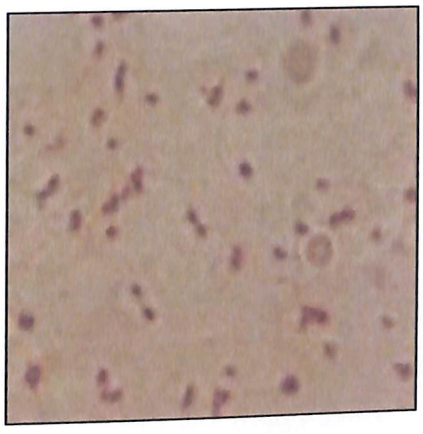
ATY4



Gram negative



ATY6



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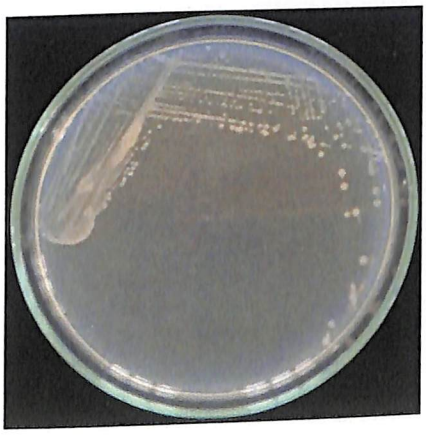
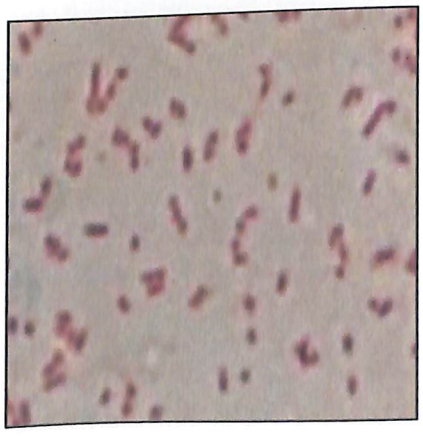
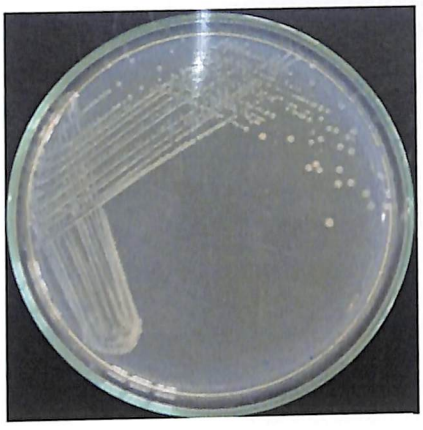


ATY10

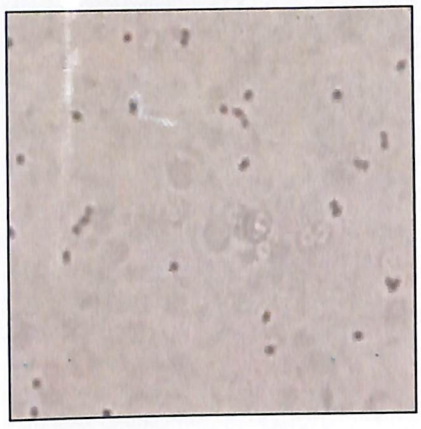


Gram negative

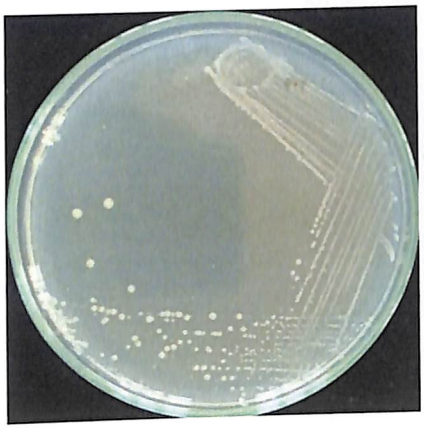
Plate 1. Colony characters and Gram staining of isolates-ATY1, ATY4, ATY6, ATY10



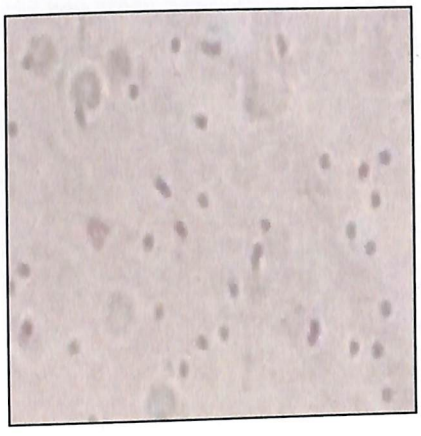
ATY17



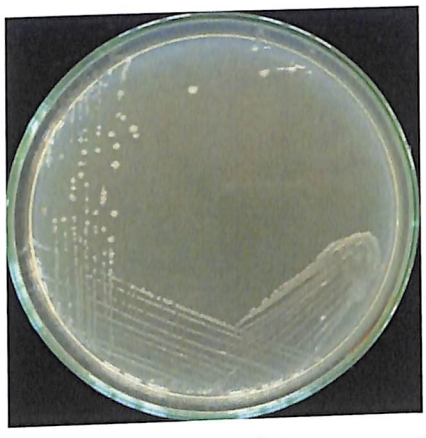
Gram positive



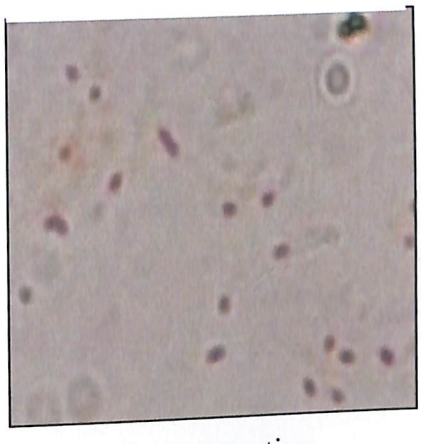
ATY23



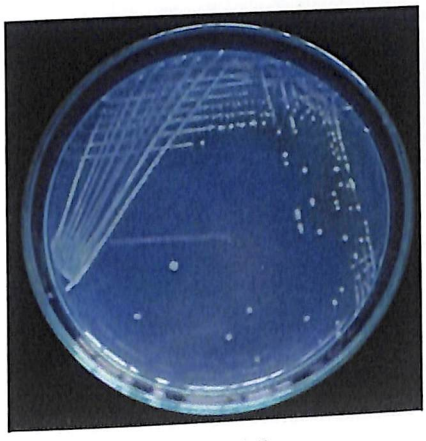
Gram positive



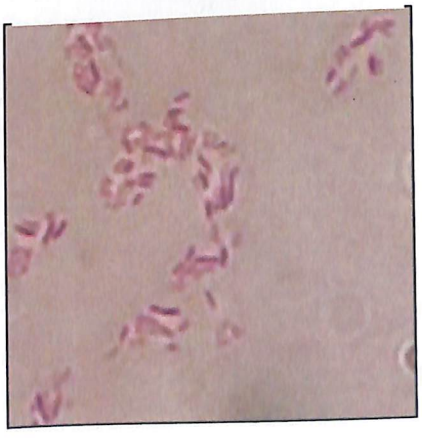
ATY24



Gram negative

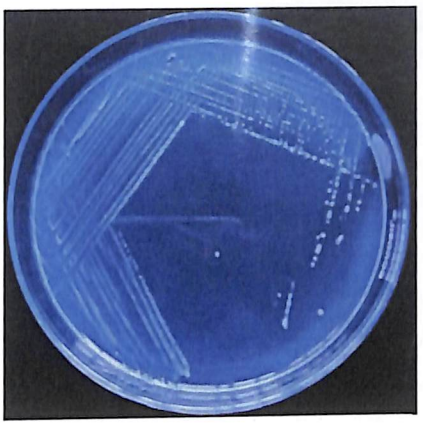


ATY25

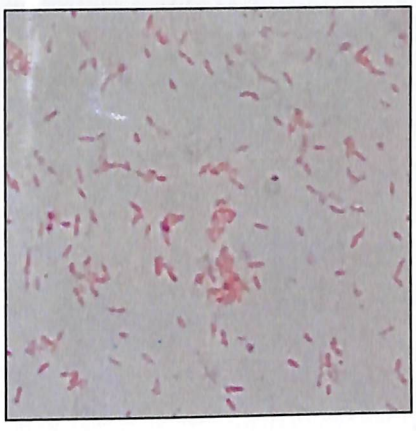


Gram negative

Plate 2. Colony characters and Gram staining of isolates-ATY17, ATY23, ATY24, ATY25



ATY28



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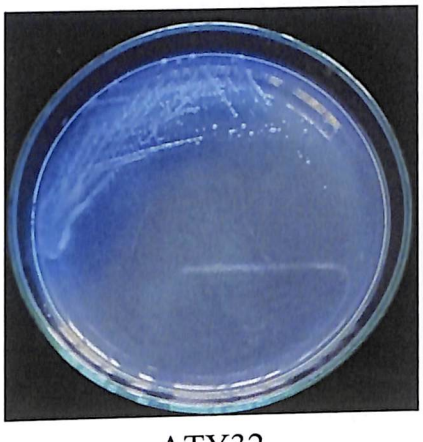
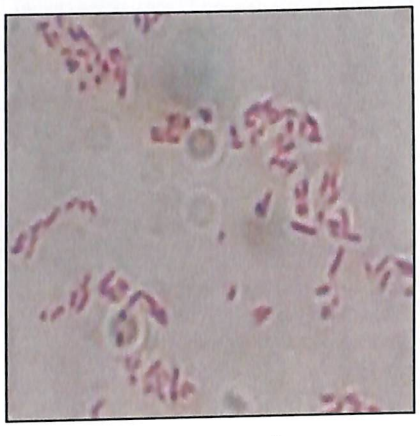


ATY32



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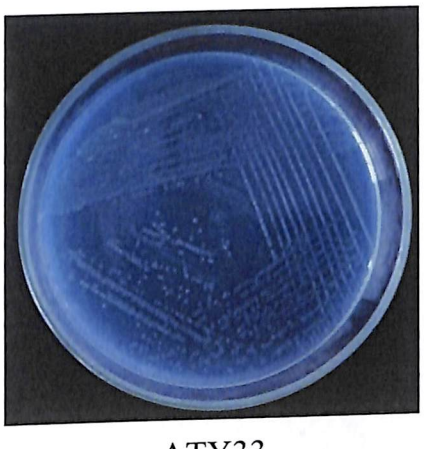

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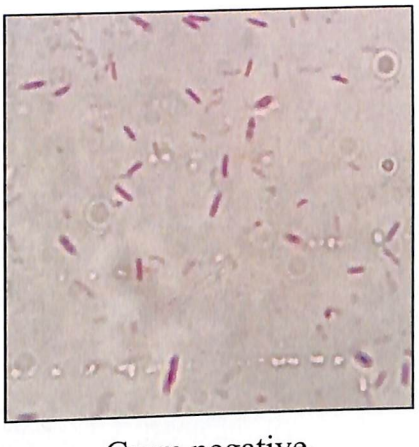

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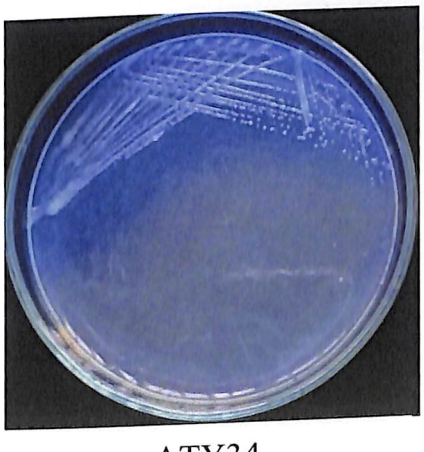
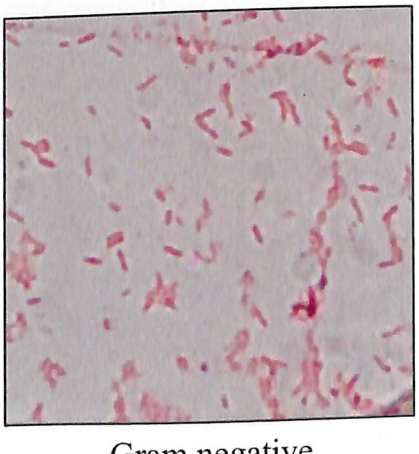
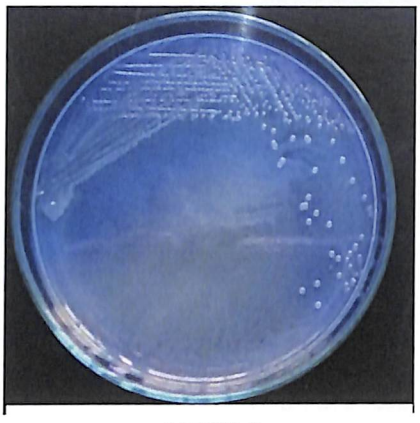


ATY34

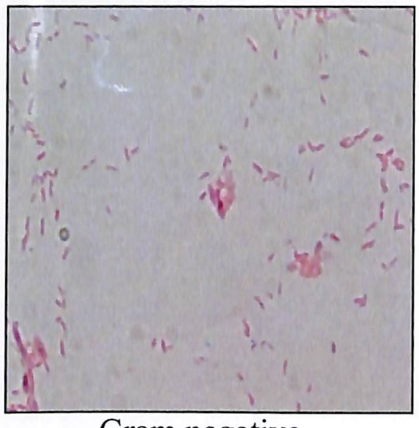


Gram negative

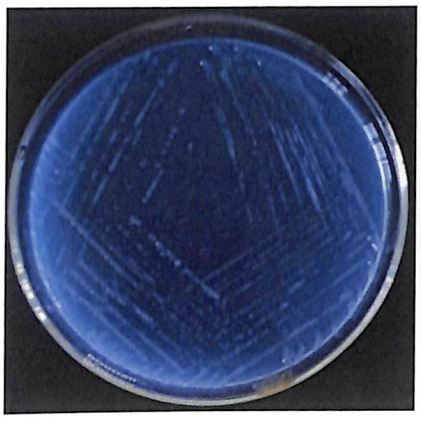
Plate 3. Colony characters and Gram staining of isolates-ATY28, ATY32, ATY33, ATY34



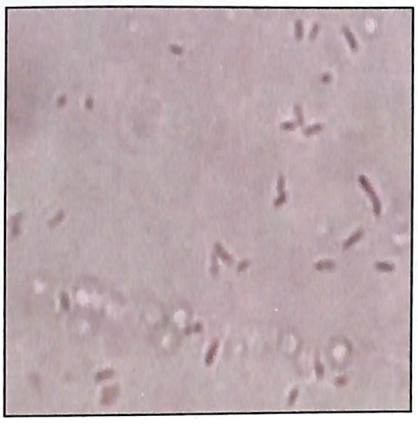
ATY35



Gram negative

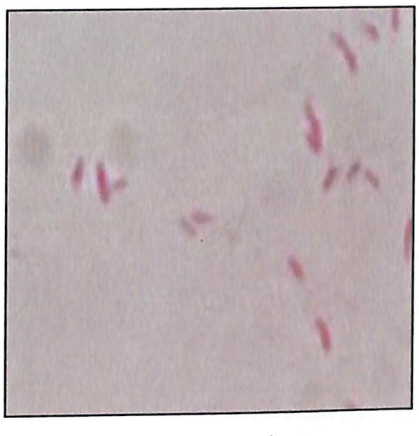


ATY36



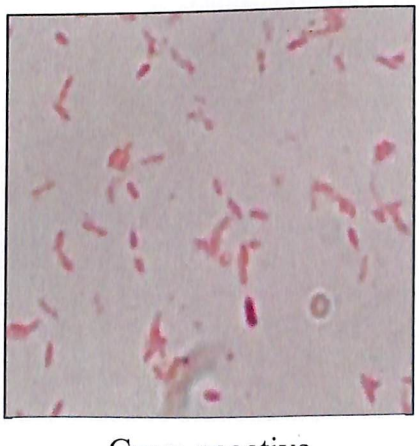
Gram positive

PSB1



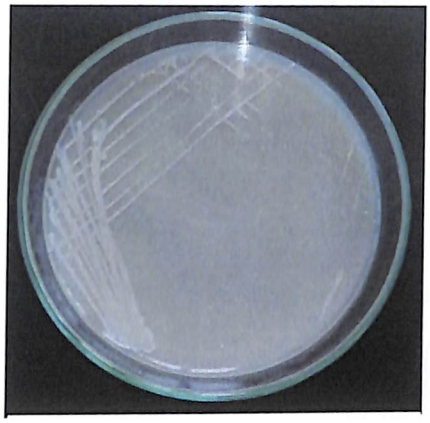
Gram negative

PSB3

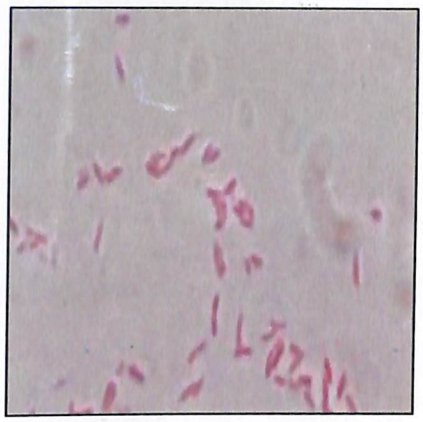


Gram negative

Plate 4. Colony characters and Gram staining of isolates-ATY35, ATY36, PSB1, PSB4

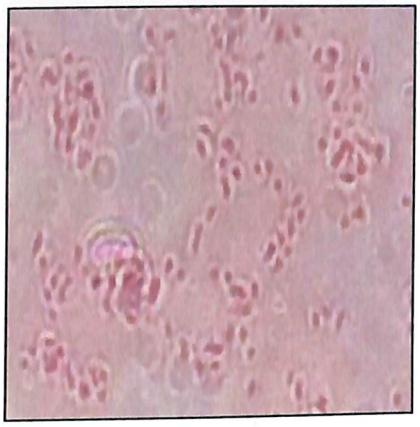


PSB4



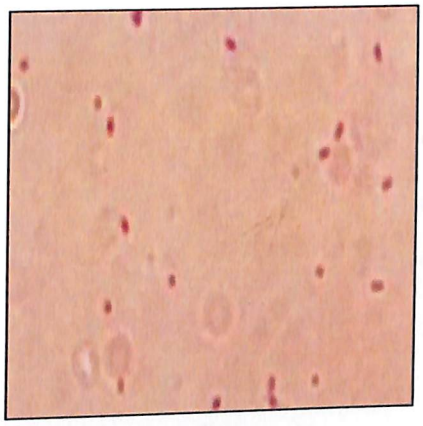
Gram negative

PSB5

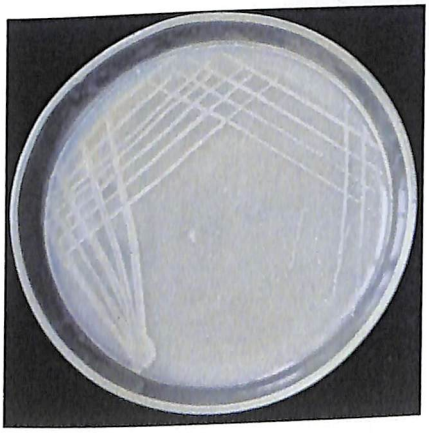


Gram negative

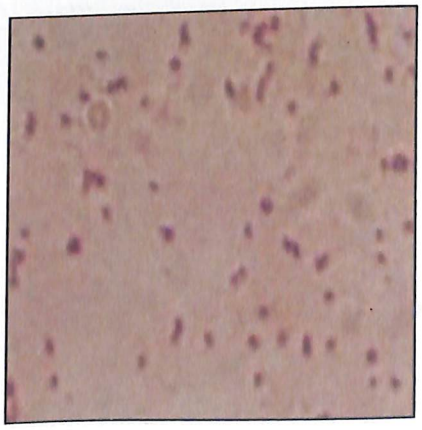
PSB6



Gram negative



PSB7



Gram negative

Plate 5. Colony characters and Gram staining of isolates-PSB4, PSB5, PSB6 and PSB7

Table 2. List of phosphate solubilizing bacterial isolates obtained from different regions of Attappady

Sl. No.	Isolates	Location	Medium
1	PSB1	Pudur	PKM
2	PSB2	Goolikadavu	PKM
3	PSB3	Sholayur	PKM
4	PSB4	Narasimukku1	PKM
5	PSB5	Agali	PKM
6	PSB6	Kavundikkal	PKM
7	PSB7	Narasimukku2	PKM
8	PSB8	Narasimukku1	PKM

PKM-Pikovskaya Medium

Majority of the isolates had raised round shape mucoidal colonies with white colour and entire margin. The colony size ranged from 0.5–4 mm. The colonies of PSB1 and PSB3 were off white in colour and ATY33 was light green and in ATY10 the colonies were 5 mm diameter with undulated margin.

The isolate ATY1 was diplobacilli and ATY4, ATY17, ATY23, ATY24 and PSB7 were spherical in shape. ATY33 cells showed large cells of size 2.49 µm X 5.81 µm. All isolates except PSB4 and PSB5 were motility in nature.

Gram staining of the cells revealed that sixteen isolates were Gram negative. ATY1, ATY17, ATY23 and ATY36 were Gram positive in nature (Plate 1-5). Eleven isolates had capsule.

4.2.2. Biochemical characters of selected isolates

Various biochemical tests were carried out for all the twenty isolates. Most of the isolates showed negative reaction for oxidase test (Plate 6), H_2S production, Voges Proskauer test and cellulose degradation activity. Also, the isolates showed positive reaction towards catalase test, indole production, citrate utilization (Plate 7), methyl red test, gelatin hydrolysis and polysaccharide production (Plate 9) as mentioned in Table 6. Only the two isolates PSB1 and PSB3 showed positive reaction for H_2S production. Cellulose degrading activity was observed only in ATY33 (Plate 10). Starch hydrolysis activity was observed in ATY10, ATY17, ATY23, ATY28, ATY33 and PSB6 (Plate 8).

4.3. ESTIMATION OF PLANT GROWTH PROMOTION ACTIVITY

4.3.1. Quantitative Estimation of Indole Acetic Acid Produced by the Bacterial Isolates

The IAA production by all the selected isolates ranged between 101.22±0.77 µg mL^{-1} and 3.26±1.30 µg mL^{-1}. The maximum IAA production was recorded by the isolate ATY10 (101.22±0.77 µg mL^{-1}), followed by the isolate ATY36 (59.08±1.12 µg mL^{-1}) and ATY35 (52.04±3.61 µg mL^{-1}) and the minimum IAA production was recorded by

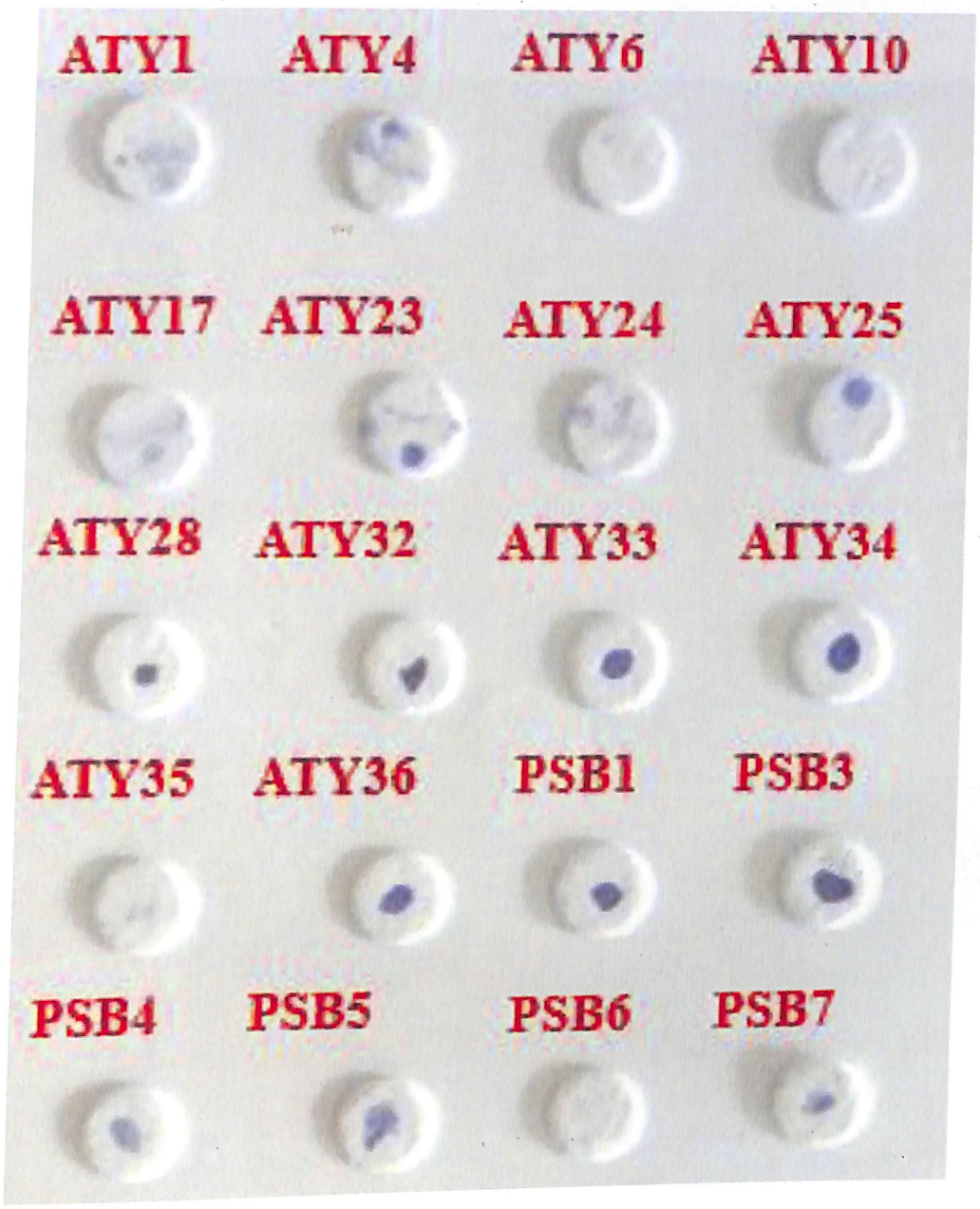


Plate 6. Oxidase test of selected NFB and PSB isolates

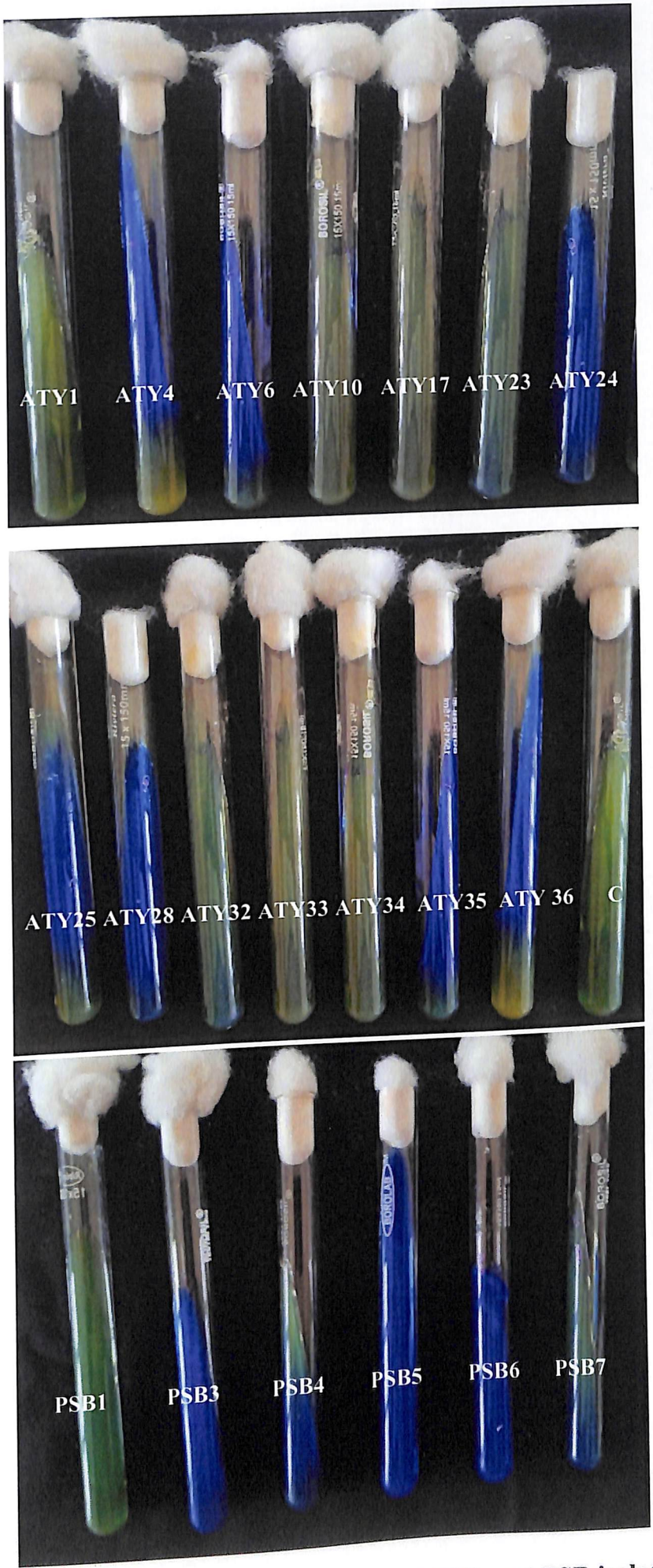


Plate 7. Citrate utilization test of selected NFB and PSB isolates

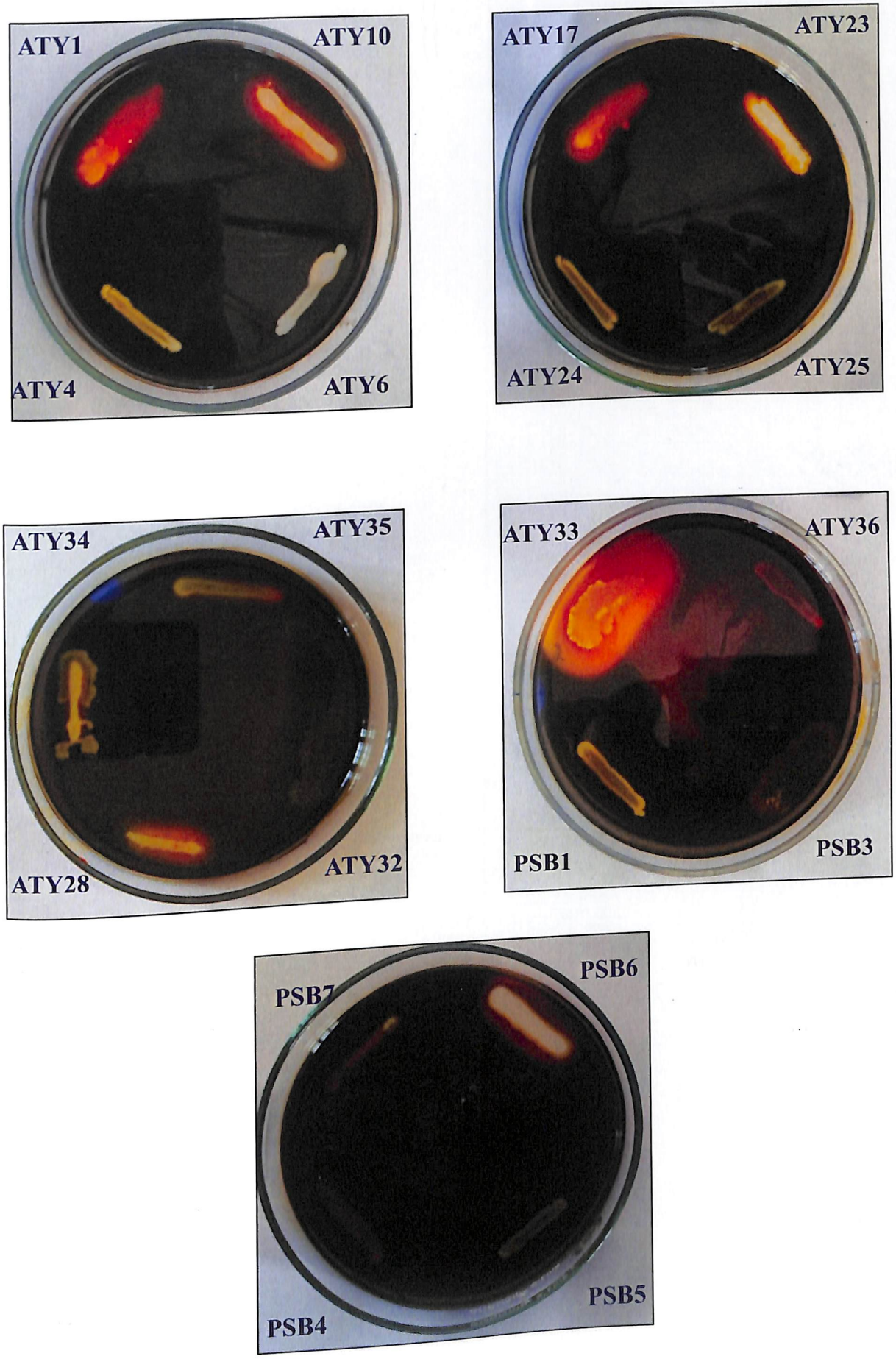


Plate 8. Starch hydrolysis test of selected NFB and PSB isolates

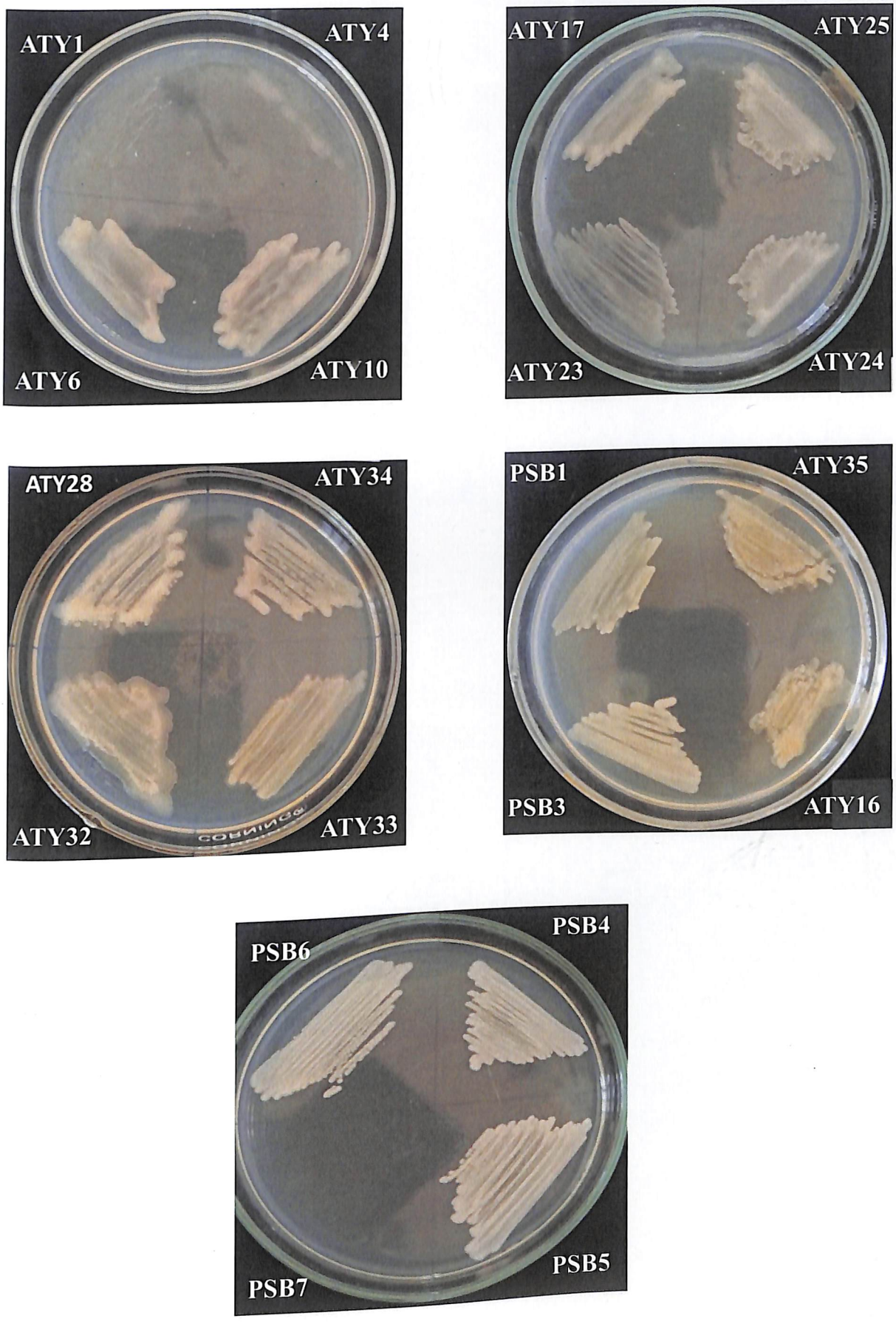


Plate 9. Polysaccharide production by selected NFB and PSB isolates

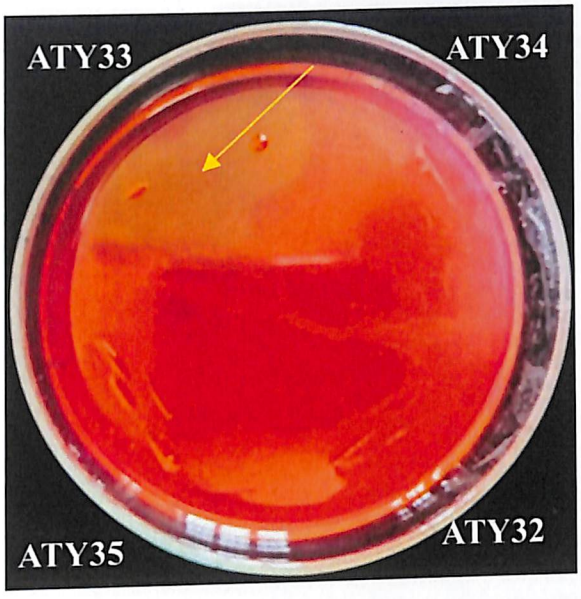
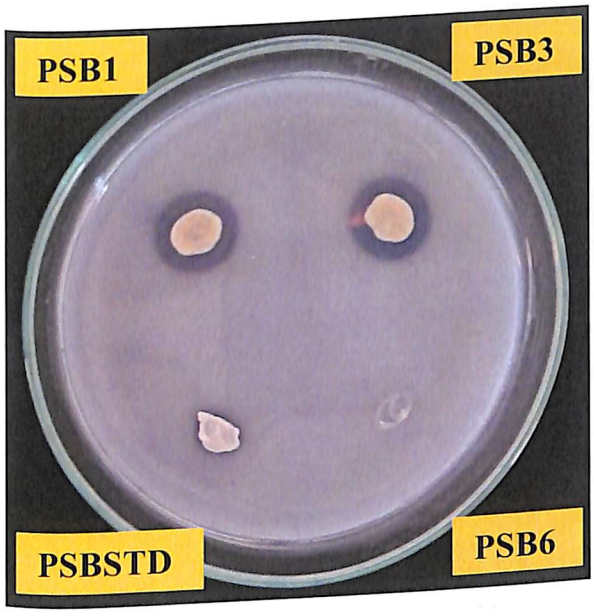


Plate 10. Cellulose degradation activity of ATY33 on CMC agar



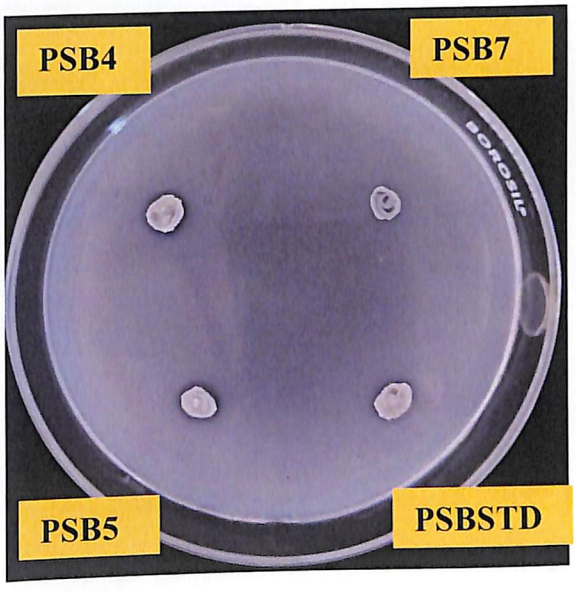


Plate 11. Phosphate solubilization by selected PSB isolates and standard culture (PSBSTD) on Pikovskaya's agar

Table 3. Colony characteristics of the NFB and PSB isolates from different regions of Attappady

Sl. No.	Isolates	Colony Shape	Colony Size (mm dia)	Colony Margin	Elevation	Colony Colour	Opacity	Texture
1	ATY1	Round	4	Entire	Raised	White	Translucent	Viscid
2	ATY4	Round	3	Entire	Raised	White	Translucent	Mucoid
3	ATY6	Round	2	Entire	Raised	White	Translucent	Mucoid
4	ATY10	Irregular	5	Undulated	Raised	White	Translucent	Viscid
5	ATY17	Round	1	Entire	Raised	White	Opaque	Mucoid
6	ATY23	Round	1.5	Entire	Flat	White	Opaque	Mucoid
7	ATY24	Round	1	Entire	Raised	White	Translucent	Mucoid
8	ATY25	Round	2	Entire	Raised	White	Opaque	Mucoid
9	ATY28	Round	1.5	Entire	Raised	White	Translucent	Mucoid
10	ATY32	Round	1	Entire	Raised	White	Opaque	Mucoid
11	ATY33	Round	0.5	Entire	Raised	Light green	Translucent	Smooth
12	ATY34	Round	2	Entire	Raised	White	Translucent	Mucoid
13	ATY35	Round	0.7	Entire	Raised	White	Translucent	Mucoid
14	ATY36	Round	1	Entire	Raised	White	Translucent	Mucoid
15	PSB1	Round	1	Entire	Raised	Half white	Opaque	Smooth
16	PSB3	Round	1	Entire	Raised	Half white	Opaque	Smooth
17	PSB4	Round	1.5	Entire	Raised	White	Opaque	Smooth
18	PSB5	Round	1	Entire	Raised	White	Opaque	Smooth
19	PSB6	Round	1.5	Entire	Raised	White	Opaque	Smooth
20	PSB7	Round	1	Entire	Raised	White	Opaque	Mucoid

Table 4. Morphological characteristics of the NFB and PSB isolates from different regions of Attappady

Sl. No.	Isolates	Cell Shape	Cell Size (µm)		Arrangement	Motility
			Diameter	Length		
1	ATY1	Rod	1.66	4.98	Diplobacilli	Motile
2	ATY4	Spherical	0.83	0.83	Coccus	Motile
3	ATY6	Rod	0.83	1.66	Single rod	Motile
4	ATY10	Rod	0.83	1.66	Single rod	Motile
5	ATY17	Spherical	1.66	1.66	Coccus	Motile
6	ATY23	Spherical	0.83	0.83	Coccus	Motile
7	ATY24	Spherical	0.83	0.83	Coccus	Motile
8	ATY25	Rod	1.66	4.98	Single rod	Motile
9	ATY28	Rod	0.83	1.32	Single rod	Motile
10	ATY32	Rod	0.83	3.32	Single rod	Motile
11	ATY33	Rod	2.49	5.81	Single rod	Motile
12	ATY34	Rod	0.83	1.66	Single rod	Motile
13	ATY35	Rod	0.83	1.99	Single rod	Motile
14	ATY36	Rod	0.83	3.32	Single rod	Motile
15	PSB1	Rod	0.83	4.98	Single rod	Motile
16	PSB3	Rod	0.83	3.32	Single rod	Motile
17	PSB4	Rod	0.83	1.66	Single rod	Non-motile
18	PSB5	Rod	0.83	3.32	Single rod	Non-motile
19	PSB6	Rod	1.66	3.32	Single rod	Motile
20	PSB7	Spherical	0.83	0.83	Coccus	Motile

Table 5. Staining characteristics of the NFB and PSB isolates from different regions of Attappady

Sl. No.	Isolates	Gram Staining	Capsule Staining
1	ATY1	Positive	Present
2	ATY4	Negative	Absent
3	ATY6	Negative	Present
4	ATY10	Negative	Present
5	ATY17	Positive	Present
6	ATY23	Positive	Absent
7	ATY24	Negative	Absent
8	ATY25	Negative	Present
9	ATY28	Negative	Absent
10	ATY32	Negative	Present
11	ATY33	Negative	Absent
12	ATY34	Negative	Absent
13	ATY35	Negative	Present
14	ATY36	Positive	Present
15	PSB1	Negative	Present
16	PSB3	Negative	Present
17	PSB4	Negative	Present
18	PSB5	Negative	Absent
19	PSB6	Negative	Absent
20	PSB7	Negative	Absent

Table 6. Biochemical characteristics of the NFB and PSB isolates from different regions of Attappady

Sl. No.	Isolates	Oxidase	Catalase	Nitrate Reductase	H_2S Production	Indole Production	Citrate Utilization
1	ATY1	-	+	+	-	+	-
2	ATY4	-	-	-	-	+	+
3	ATY6	-	+	-	-	+	+
4	ATY10	-	+	-	-	+	-
5	ATY17	-	+	+	-	+	-
6	ATY23	-	+	-	-	+	-
7	ATY24	-	+	-	-	+	+
8	ATY25	+	+	-	-	+	+
9	ATY28	+	+	-	-	+	+
10	ATY32	+	-	-	-	+	-
11	ATY33	-	+	+	-	+	+
12	ATY34	+	+	-	-	+	+
13	ATY35	+	+	-	-	+	-
14	ATY36	+	+	-	-	+	+
15	PSB1	-	+	-	+	+	+
16	PSB3	-	+	-	+	+	+
17	PSB4	-	+	-	-	+	+
18	PSB5	+	+	-	-	+	+
19	PSB6	-	+	-	-	+	+
20	PSB7	-	+	-	-	+	+

Table 6. Biochemical characteristics of the NFB and PSB isolates from different regions of Attappady (continued)

Sl. No.	Isolates	MR	VP	Gelatin Hydrolysis	Starch Hydrolysis	Cellulose Degradation	Polysacchride Production
1	ATY1	+	-	-	-	-	+
2	ATY4	+	-	-	-	-	+
3	ATY6	+	-	+	-	-	+
4	ATY10	+	-	+	+	-	+
5	ATY17	+	-	+	+	-	+
6	ATY23	+	-	+	+	-	+
7	ATY24	+	-	+	-	-	+
8	ATY25	+	-	-	-	-	+
9	ATY28	+	-	+	+	-	+
10	ATY32	+	-	+	-	-	+
11	ATY33	+	-	+	+	+	-
12	ATY34	+	-	+	-	-	+
13	ATY35	+	-	+	-	-	+
14	ATY36	+	-	-	-	-	+
15	PSB1	+	-	-	-	-	+
16	PSB3	+	-	-	-	-	+
17	PSB4	+	-	-	-	-	-
18	PSB5	+	-	-	-	-	-
19	PSB6	+	-	-	+	-	+
20	PSB7	+	-	+	-	-	+

the isolate PSB1 (3.26±1.30 μg mL^{-1}). The IAA production of isolate ATY10 was on par with ASPSTD (88.66±12.38 μg mL^{-1}) and better than AZOSTD (47.89±1.27 μg mL^{-1}). The IAA production was lower in PSB isolates when compared to NFB isolates. All PSB isolates and PSBSTD showed low IAA production compared to NFB isolates (Table 7).

4.3.2. Quantitative Estimation of Gibberellic Acid Produced by the Bacterial Isolates

The highest gibberellic acid (GA) content was recorded by the isolate ATY10 with 10.07±2.41 μg mL^{-1} which was on par with the isolate ATY35 (9.41±0.87 μg mL^{-1}) and the lowest value of GA was recorded by the isolate ATY17 (2.18±0.44 μg mL^{-1}). The isolates ATY10 showed better GA production than the standard cultures used in the study AZOSTD (3.65±0.68 μg mL^{-1}) and ASPSTD (3.48±0.14 μg mL^{-1}). GA production of PSB isolates were low compared to most of the NFB isolates. The isolate PSB1 recorded maximum GA production (3.43±0.28 μg mL^{-1}) among the PSB isolates but it was on par with other PSB isolates and standard culture (Table 8).

4.3.3. Quantitative Estimation of the Extracellular Ammonia Production by the Bacterial Isolates

The maximum extracellular ammonia production was recorded by the bacterial isolate ATY17 (176.53±5.07 μmol mL^{-1}) which was on par with the isolates ATY25 (176.51±5.09 μmol mL^{-1}), ATY6 (174.50±7.93 μmol mL^{-1}) and ATY35 (170.19±14.03 μmol mL^{-1}). It was on par with the standard cultures. The highest amount of extracellular ammonia production by PSB isolates was recorded by the isolate PSB4 (175.33±6.76 μmol mL^{-1}) and the lowest value among all the twenty isolates were recorded by ATY10 (75.59±10.85 μmol mL^{-1}) (Table 9).

Table 7. Indole Acetic Acid production by the NFB and PSB isolates from different regions of Attappady

Sl. No.	Isolates	IAA production ($\mu g\ mL^{-1}$)*
1	ATY1	18.67 ± 7.73^{efg}
2	ATY4	33.52 ± 10.56^{cdef}
3	ATY6	49.50 ± 6.25^{bcd}
4	ATY10	101.22 ± 0.77^{a}
5	ATY17	49.12 ± 18.10^{bcd}
6	ATY23	32.15 ± 0.38^{cdef}
7	ATY24	51.55 ± 3.28^{bc}
8	ATY25	26.01 ± 1.98^{defg}
9	ATY28	43.07 ± 8.95^{bcd}
10	ATY32	42.42 ± 2.35^{bcde}
11	ATY33	6.72 ± 1.22^{g}
12	ATY34	47.69 ± 2.95^{bcd}
13	ATY35	52.04 ± 3.61^{bc}
14	ATY36	59.08 ± 1.12^{b}
15	AZOSTD	47.89 ± 1.27^{bcd}
16	AZPSTD	88.66 ± 12.38^{a}
17	PSB1	3.26 ± 1.30^{g}
18	PSB3	3.54 ± 1.04^{g}
19	PSB4	11.99 ± 8.37^{fg}
20	PSB5	3.37 ± 0.47^{g}
21	PSB6	11.66 ± 0.36^{fg}
22	PSB7	7.37 ± 7.78^{g}
23	PSBSTD	14.41 ± 6.19^{fg}

* Mean (±SD) of 3 replications. Values in a column followed by same letter do not differ significantly according to Duncan's Multiple Range Test ($p \leq 0.05$)

Table 8. Gibberellic Acid production by the NFB and PSB isolates from different regions of Attappady

Sl. No.	Isolates	GA production (μg mL^{-1})*
1	ATY1	3.27 ± 0.04^{cd}
2	ATY4	3.28 ± 1.21^{cd}
3	ATY6	2.70 ± 0.18^{d}
4	ATY10	10.07 ± 2.41^{a}
5	ATY17	2.18 ± 0.44^{d}
6	ATY23	3.05 ± 2.02^{d}
7	ATY24	3.09 ± 0.20^{d}
8	ATY25	5.26 ± 1.07^{bc}
9	ATY28	2.77 ± 0.20^{d}
10	ATY32	6.19 ± 2.23^{b}
11	ATY33	3.52 ± 1.26^{cd}
12	ATY34	4.09 ± 0.12^{cd}
13	ATY35	9.41 ± 0.87^{a}
14	ATY36	4.06 ± 0.59^{cd}
15	AZOSTD	3.65 ± 0.68^{cd}
16	AZPSTD	3.48 ± 0.14^{cd}
17	PSB1	3.43 ± 0.28^{cd}
18	PSB3	3.22 ± 0.14^{d}
19	PSB4	2.71 ± 0.09^{d}
20	PSB5	2.95 ± 0.08^{d}
21	PSB6	2.64 ± 0.14^{d}
22	PSB7	2.70 ± 0.60^{d}
23	PSBSTD	3.87 ± 0.11^{cd}

* Mean (±SD) of 3 replications. Values in a column followed by same letter do not differ significantly according to Duncan's Multiple Range Test ($p \leq 0.05$)

Table 9. Extracellular ammonia production by the NFB and PSB isolates from different regions of Attappady

Sl. No.	Isolates	Ammonia production (μmol mL^{-1})*
1	ATY1	89.58 ± 4.29^{fg}
2	ATY4	149.39 ± 5.81^{abcde}
3	ATY6	174.50 ± 7.93^{a}
4	ATY10	75.59 ± 5.85^{g}
5	ATY17	176.53 ± 5.07^{a}
6	ATY23	164.92 ± 10.91^{ab}
7	ATY24	168.31 ± 6.68^{ab}
8	ATY25	176.51 ± 5.09^{a}
9	ATY28	157.07 ± 5.59^{ab}
10	ATY32	114.91 ± 9.02^{cdefg}
11	ATY33	89.76 ± 1.26^{fg}
12	ATY34	159.01 ± 7.98^{ab}
13	ATY35	170.19 ± 14.03^{a}
14	ATY36	153.34 ± 2.06^{abc}
15	AZOSTD	152.00 ± 19.00^{abcd}
16	AZPSTD	171.15 ± 2.32^{a}
17	PSB1	149.10 ± 3.85^{abcde}
18	PSB3	112.25 ± 5.30^{defg}
19	PSB4	175.33 ± 6.76^{a}
20	PSB5	148.69 ± 9.18^{abcde}
21	PSB6	148.02 ± 5.39^{abcde}
22	PSB7	109.12 ± 18.19^{efg}
23	PSBSTD	128.95 ± 8.76^{bcdef}

* Mean (±SD) of 3 replications. Values in a column followed by same letter do not differ significantly according to Duncan's Multiple Range Test ($p \leq 0.05$)

4.4. ESTIMATION OF NITROGEN CONTENT OF THE ISOLATES

The highest nitrogen content was found in the isolate ATY33 with 30.75±3.88 μg mL^{-1} followed by the isolate ATY32 (30.75±3.88 μg mL^{-1}) and ATY35 (21.00±5.65 μg mL^{-1}) and the lowest nitrogen content was recorded by the isolate ATY4 (9±2.82 μg mL^{-1}) (Table 10). The nitrogen content of ATY33, ATY28 was recorded to be higher than the standard cultures of AZOSTD (12.50±2.12 μg mL^{-1}) and ASPSTD (15.50±2.12 μg mL^{-1}).

4.5. ASSESSING THE PHOSPHATE SOLUBILIZATION POTENTIAL OF THE BACTERIAL ISOLATES UNDER *IN VITRO* CONDITIONS

4.5.1. Plate Assay for Phosphate Solubilization

The isolate PSB1 was superior to all other PSB isolates in phosphate solubilization with 18.23±0.25 mm of zone of solubilization in diameter. This was followed by the isolate PSB3 (17.23±0.20 mm) and PSB4 (10.33±0.57 mm) and the lowest phosphate solubilizing capacity was recorded by the isolate PSB7 7.06±0.11 mm (Table 11). The isolates PSB1, PSB3 and PSB4 showed more P solubilization than the standard culture (8.16±0.15 mm) on plate assay (Plate 11).

The Phosphate Solubilizing Index (PSI) was recorded maximum in the isolate PSB1 (2.82±0.025) followed by the isolates PSB3 (2.72±0.021) and PSB4 (2.42±0.096). The lowest PSI was shown by the isolate PSB6 (2.11±0.019) (Table 12). The PSI of PSB1 was observed to be greater than the standard culture (2.36±0.025).

4.5.2. Broth Assay for Quantification of Phosphate Solubilization by the PSB Isolates

Among six phosphate solubilizing bacteria the isolate PSB1 showed maximum capacity in solubilizing insoluble tricalcium phosphate to soluble form of phosphorus (53.41±1.72 mg L^{-1}). This was followed by the isolate PSB3 with a capacity of solubilizing 49.94±0.17 mg L^{-1} and PSB4 with 16.85±0.57 mg L^{-1}. The lowest amount of phosphorus solubilization was quantified as 4.54±0.21 mg L^{-1} by the isolate PSB7

Table 10. Nitrogen content of the NFB and PSB isolates from different regions of Attappady

Sl. No.	Isolates	Nitrogen ($\mu g\ mL^{-1}$)*
1	ATY1	11.75 ± 1.06^{cd}
2	ATY4	9 ± 2.82^{d}
3	ATY6	19.50 ± 7.77^{bc}
4	ATY10	18.00 ± 0.00^{bc}
5	ATY17	11.90 ± 2.97^{cd}
6	ATY23	16.75 ± 1.76^{bcd}
7	ATY24	17.25 ± 1.06^{bc}
8	ATY25	12.65 ± 4.03^{cd}
9	ATY28	21.50 ± 6.36^{b}
10	ATY32	12.65 ± 4.03^{cd}
11	ATY33	30.75 ± 3.88^{a}
12	ATY34	21.00 ± 5.65^{b}
13	ATY35	16.25 ± 1.06^{bcd}
14	ATY36	12.50 ± 2.12^{cd}
15	AZOSTD	16.25 ± 3.18^{bcd}
16	AZPSTD	15.50 ± 2.12^{bcd}

* Mean (±SD) of 3 replications. Values in a column followed by same letter do not differ significantly according to Duncan's Multiple Range Test ($p \leq 0.05$)

(Table 13). The obtained quantification results of phosphorus showed that PSB1 was found to solubilize P greater than standard culture (15.12±0.24 mg L^{-1}).

4.6. SCREENING OF THE BEST BACTERIAL ISOLATES

Three bacterial isolates each of nitrogen fixers and phosphate solubilizers were selected for further analysis based on the data obtained from the assessment done (4.3 to 4.5) with all the twenty isolates.

4.6.1. Weighted average ranking

The isolates ATY10, ATY34 and ATY35 were selected as the best three NFB isolates from the fourteen nitrogen fixing bacterial isolates (Table 14). Also, the isolates PSB1, PSB3 and PSB4 were selected as the best three phosphate solubilizers among six PSB isolates based on the weighted average ranking (Table 15).

4.7. MOLECULAR CHARACTERIZATION OF THE SELECTED ISOLATES

The selected isolates were further subjected to molecular characterization by the agarose gel electrophoresis of the PCR amplified product showed bands of approximately 1.5 kbp size (Plate no.12). The results obtained by 16s rRNA gene sequencing is presented in the (Table 16) and query cover, percent identity, best match in GenBank database of the isolates is given (Table 17).

4.8. *IN VITRO* PLANT GROWTH PROMOTION STUDY USING ROLL TOWEL ASSAY

The plant growth parameters such as germination percentage, number of days taken for germination, plant height, fresh weight, dry weight, seedling vigour index and Root shoot ratio are presented (Table 18-21.) (Plate 13).

Under roll towel assay the germination percentage of ragi was observed to be superior in the treatment *Aeromonas hydrophila* PSB4 with 81.24±4.16 per cent followed by *Pantoea agglomerans* ATY10 treated seeds with 79.16±4.80 per cent

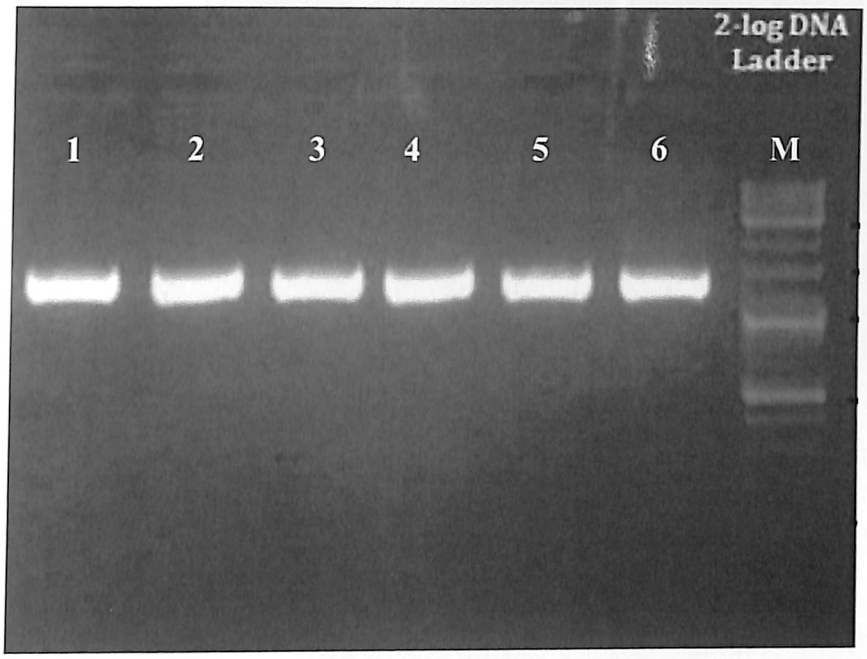
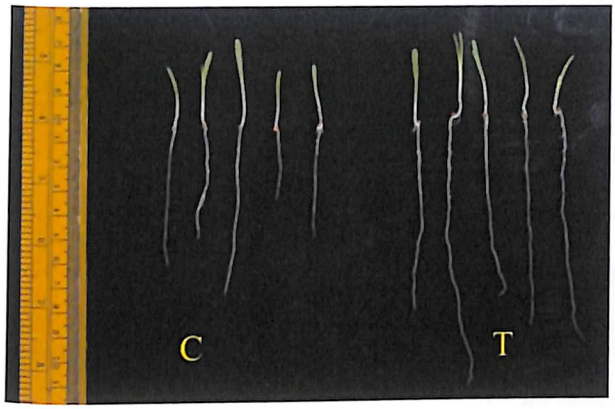
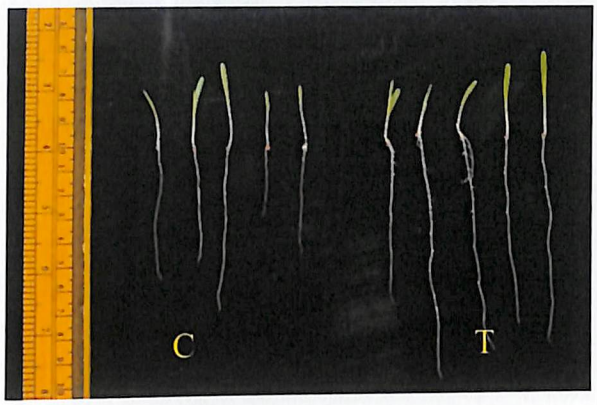


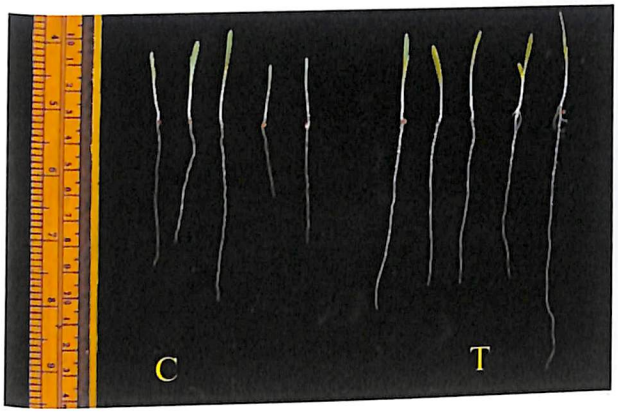
Plate 12. Agarose gel electrophoresis of PCR amplified products of 16S rRNA genes from six bacterial isolates. Lane M - 1 kbp DNA ladder. Lane 1-ATY10, 2-ATY34, 3-ATY35, 4-PSB1, 5-PSB3, 6-PSB4.



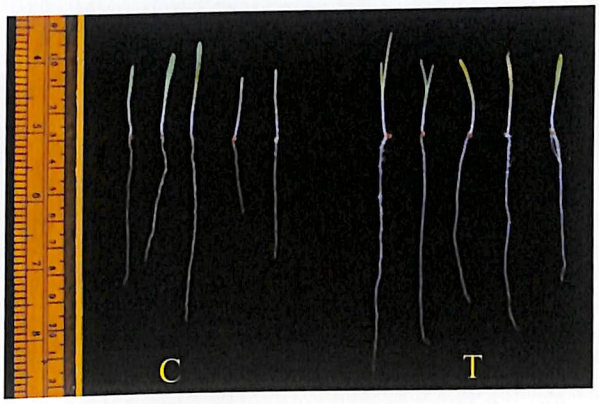
Pantoea agglomerans ATY10



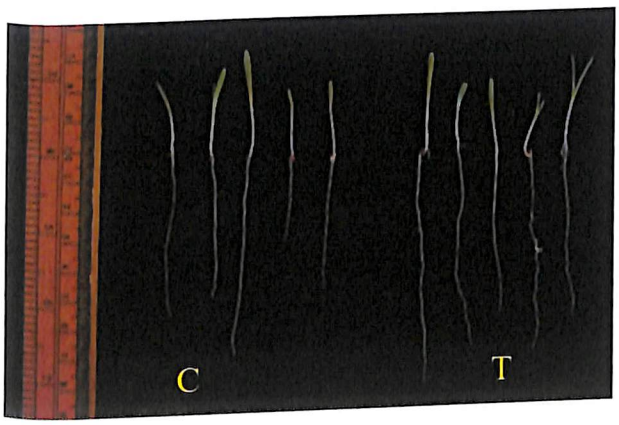
Rhizobium sp ATY34



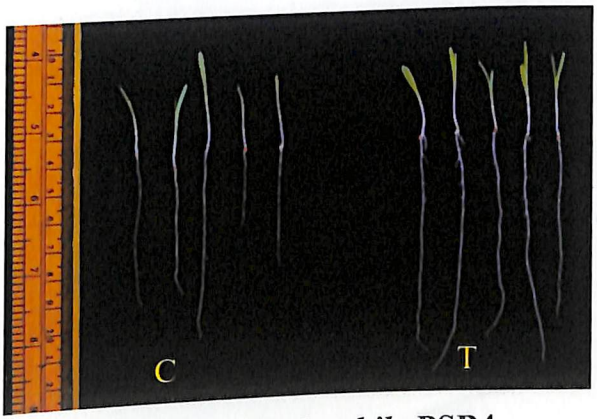
Ensifer adhaerens ATY35



Burkholderia territorii PSB1



Burkholderia cepacia PSB3



Aeromonas hydrophila PSB4

Plate 13. Effect of biopriming of ragi seeds with selected isolates on seedling growth at 10 DAT

Table 11. Phosphate solubilization zone of selected PSB isolates

Sl. No.	Isolates	Diameter of zone (mm)*
1	PSB1	18.23 ± 0.25^{a}
2	PSB3	17.23 ± 0.20^{b}
3	PSB4	10.33 ± 0.57^{c}
4	PSB5	8.10 ± 0.10^{d}
5	PSB6	8.13 ± 0.11^{d}
6	PSB7	7.06 ± 0.11^{e}
7	PSBSTD	8.16 ± 0.15^{d}

* Mean (±SD) of 3 replications. Values in a column followed by same letter do not differ significantly according to Duncan's Multiple Range Test ($p \leq 0.05$)

Table 12. Phosphate solubilizing Index of selected PSB isolates

Sl. No.	Isolates	PSI*
1	PSB1	2.82 ± 0.025^{a}
2	PSB3	2.72 ± 0.021^{b}
3	PSB4	2.42 ± 0.096^{c}
4	PSB5	2.35 ± 0.017^{c}
5	PSB6	2.17 ± 0.019^{d}
6	PSB7	2.16 ± 0.016^{d}
7	PSBSTD	2.36 ± 0.025^{c}

* Mean (±SD) of 3 replications. Values in a column followed by same letter do not differ significantly according to Duncan's Multiple Range Test ($p \leq 0.05$)

Table 13. Quantification of phosphorus solubilization by selected PSB isolates

Sl. No.	Isolates	Phosphorus (mg L^{-1})*
1	PSB1	53.41 ± 1.72^{a}
2	PSB3	49.94 ± 0.17^{b}
3	PSB4	16.85 ± 0.57^{c}
4	PSB5	16.57 ± 0.51^{cd}
5	PSB6	7.48 ± 0.03^{e}
6	PSB7	4.54 ± 0.21^{f}
7	PSBSTD	15.12 ± 0.24^{d}

* Mean (±SD) of 3 replications. Values in a column followed by same letter do not differ significantly according to Duncan's Multiple Range Test ($p \leq 0.05$)

Table 14. Weighted average ranking of NFB isolates from different regions of Attappady

Sl. No.	Isolates	Weighted average ranking
1	ATY1	13
2	ATY4	4
3	ATY6	8
4	ATY10	1
5	ATY17	11
6	ATY23	13
7	ATY24	6
8	ATY25	11
9	ATY28	6
10	ATY32	10
11	ATY33	8
12	ATY34	3
13	ATY35	2
14	ATY36	5

Table 15. Weighted average ranking of PSB isolates from different regions of Attappady

Sl. No.	Isolates	Weighted average ranking
1	PSB1	1
2	PSB3	2
3	PSB4	3
4	PSB5	4
5	PSB6	5
6	PSB7	6

Table 16. 16S rRNA gene sequence of selected NFB and PSB isolates

Isolates	16S rRNA gene sequence
ATY10	GAGGCCAGGGGGAGTGGCAGACGGGTGAGTAACGCGTGGGAACATACCCTTTCCTGCGGAATAGCTCCGGGAAACTGGAATTAATACCG CATACGCCCTACGGGGGAAAGATTTATCGGGGAAGGATTGGCCCGCGTTGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACG ATCCATAGCTGGTCTGAGAGGATGATCAGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTGGGGAATATTGGA CAATGGGCGCAAGCCTGATCCAGCCATGYMGCAGTGAGACTGATRAAGGCCTTAACGGGTTCGTAAAAGCTCTTTCACYGRAGAGAKATA ATGACCGGWWTCCCGGAGGAATGAAAGCCCCGGCTAACTTCGTGCCAGCAGGCCGCGGTAATACGAAAGGGGGCTAGCGTTGGTTCGGA ATTACTGGGCGTAAAGCGCACGTAGGGCGGGATATTTAAGTCAGGGGTGAAATCCCAGAGCTCAACTCTGGAACTGCCTTTGATACTGGG TATCTTGAGTATGGAAGAGGTAAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAGATATTCGGAGGAACACCAGTGGCGAAGGCGGCTT ACTGGTCCATTACTGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATGTT AGCCGTCGGGCAGTATACTGTTCGGTGGCGCAGCTAACGCATTTAAACATTCCGCCTGGGGAGTACGGTCGCAAGATTWAAACTCAAAGG AATTGACCGGGGGCCCGCACAASCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCCGCAGAACCTTACCAAGCTCTTGACATTCKGGGT ATGGGCATTGGGAGACGATGGTCCATTCCARTTATGGCCTTGGCCCCCAARAACAKGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGA TGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGCCCTTAGTTGCCAGCATTTAGTTGGGCACTCTAAGGGGACTGCCGGTGATAAGCCG AGAGGAAGGTGGGGATGACGTCAAGTCCTCATGGCCCTTACGGGCTGGGCTACACACGTGCTACAATGGTGGTGACAGTGGGCAGCGAG ACAGCGATGTCGAGCTAATCTCCAAAAGCCATCTCAGTTCGGATTGCACTCTGCAACTCGAGTGCATGAAGTTGGAATCGCTAGTAATCGC AGAACAGGATTGTGTCCTAATTCGTC
ATY34	AAAATTAAGAGAGCTTGCTCTCGGGTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGA AACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCAGATGTGCCCAGATGGGATTAGCTA GTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACT CCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTA AAGTACTTTCAGCGGGGAGGAAGGTGTTGTGGTTAATAACCACAGCGATTGACGTTACCCCGGCAGAAGAAGGCATCSGGCTAACTTCCG TSCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGT GAAATCCCCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAA ATGCGTAGAGATCTGGAGGAATACCCGGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAAC AGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAG TCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGA TGCAACGCGAAGAACCTTACCTACTCTTGACATCCAGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCTGAGACAGGTGCTGCA TGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTTAGGCCGGGAA CTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTA CAATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACT CCATGAAGTCGGAATCGCTAGTAATCGTAGATCAGGATGGTTTCCCCACCTTCGT

Table 16. Cont.

Isolates	16S rRNA gene sequence
ATY35	GGCCCCAGGGGGGGCGGGCAGACGGGTGAGTAACGCGTGGGAATCTACCCTTTTCTACGGAATAACGCAGGGAAACTTGTGCTAATACCG
	TATAAGCCCTTCGGGGGAAAGATTTATCGGGAAAGGATGAGCCCGCGTTGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACG
	ATCCATAGCTGGTCTGAGAGGATGATCAGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTGGGGAATATTGGA
	CAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGAAGTGTGACTGAAGGTCYYTASGGTTGTTAAAGGCTCTTTCACCGGTGAAGATWA
	TGACSGTAAMCSGAGAWGAAGCCCCCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAARGGGGCTAGCGTTGTTCGGAATTACTGGG
	CGTAAAGCGCACGTAGGCGGACATTTAAGTCAGGGGTGAAATCCCGGGGCTCAACCCCGGAACTGCCTTTGATACTGGGTGTCTAGAGTA
	TGGAAGAGGTGAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAGATATTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGTCCATT
	ACTGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAATGTTAGCCGTCGGGC
	AGTTTACTGTTCGGTGGCGCAGCTAAACGCAATTAAACATTCCGCCTGGGGAGTACSGTCGCAAGATTAAAACTCAAAGGAATTGAACGG
	GGGCCCCGCACAAGCGGGTGGAGCATGTGGTTTAATTTCKAAGCAACGCGCAGAAACCTTAACCASCCCTTGGACATCCCGATTCGCRRAT
	TACAGAGAATGATTTTTTCYTTCAGTATCGGCTGGGGATCGGAGACARGTGMTGCATGGCWGTCGTCAGACTCGTGYCGTGASATGCYTS
	KGKTRAGTMYCGCAACGAGCGCAACCCTCGCCCTTAGTTGCCAGCATTTAGTTGGGCACTCTAAGGGGACTGCCGGTGATAAGCCGAGAG
	GAAGGTGGGGATGACGTCAAGTCCTCATGGCCCTTACGGGCTGGGCTACACACGTGCTACAATGGTGGTGACAGTGGGCAGCGAGACCGC
	GAGGTCGAGCTAATCTCCAAAAGCCATCTCAGTTCGGATTGCACTCTGCAACTCGAGTGCATGAAGTTGGAATCGCTAGTAATCGCAGAA
	CAGCAAGCTGCCGATTGCTCC
PSB1	TACCACGGGGGTTCCTTGCACCTGGTGGCGAGTGGCGAACGGGTGAGTAATACATCGGAACATGTCCTGTAGTGGGGGATAGCCCGGCGA
	AAGCCGGATTAATACCGCATACGATCTACGGATGAAAGCGGGGGACCTTCGGGCCTCGCGCTATAGGGTTGGCCGATGGCTGATTAGCTA
	GTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCAGTAGCTGGTCTGAGAGGACGACCAGCCACACTGGGACTGAGACACGGCCCAGAC
	TCCTACGGGAGGCAGCAGTGGGGAATTTTGGACAATGGGCGAAAGCCTGATCCAGCAATGCCGCGTGTGTGAAGAAGGCCTTCGGGWTR
	WAAMGGCATCCTTTTGTTCMGGGAAAAGGAAAATTCCCTTGSCTCTTAGATACAGTCGGGGGATGACCGGTACCCGGAAAGAATAAGGC
	ACSGGCTAACTACGTGCCAGCAGCCGGCGGTAATACGTAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTT
	TGCTAAGACCGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTGGTGACTGGCAGGCTAGAGTATGGCAGAGGGGGGTAGAATTCC
	ACGTGTAGCAGTGAAATGCGTAGAGATGTGGAGGAATACCGATGGCGAAGGCAGCCCCCTGGGCCAATACTGACGCTCATGCACGAAAG
	CGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCAACTAGTTGTTGGGGATTCATTTCCTTAGTAACGTAG
	CTAACGCGTGAAGTTGACCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGATGAT
	GTGGATTAATTCGATGCAACGCGAAAAACCTTACCTACCCTTGACATGGTCGGAATCCTGCTGAGAGGCGGGAGTGCTCGAAAGAGAACC
	GGCGCACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCTTAGTTGCT
	ACGCAAGAGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCCTCATGGCCCTTATGGGTAGGGCTT
	CACACGTCATACAATGGTCGGAACAGAGGGTTGCCAACCCGCGAGGGGGAGCTAATCCCAGAAAACCGATCGTAGTCCGGATTGCACTCT
	GCAACTCGAGTGCATGAAGCTGGAATCGCTAGTAATCGCGGATCAGGATGCCCGCGATTATCGC

Table 16. Cont.

Isolates	16S rRNA gene sequence
ATY35	CGAAACCGGGGGGGGCTTGCACCTGGTGGCGAGTGGCGAACGGGTGAGTAATACATCGGAACATGTCCTGTAGTGGGGGATAGCCCGGC GAAAGCCGGATTAATACCGCATACGATCTACGGATGAAAGCGGGGGACCTTCGGGCCTCGCGCTATAGGGTTGGCCGATGGCTGATTAGC TAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCAGTAGCTGGTCTGAGAGGACGACCAGCCACACTGGGACTGAGACACGGCCCAG ACTCCTACGGGAGGCAGCAGTGGGGAATTTTGGACAATGGGCGAAAGCCTGATCCAGCAATGCCGCGTGTGTGAAGAAGGCCTTCGKSTT GTTAAATGCACAYTTTGTTCCAGGAAAGRAATYCKTGSCTMTAWTACAGTYSGGGGATGACGGTACSGRAAGAATAAGCACSGGCTAAAC TACGTGCCAGCAGCCCGCGGTAATACGTAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTTTGCTAAGACC GATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTGGTGACTGGCAGGCTARAGTATGGCAGAGGGGGGTAGAATTCCACGTGTAGCA GTGAAAATGCGTAAGAGATGTGGGAGGAATACCGATGGCGAAGGCAGCCCCCTGGGCCAATACTGACGCTCATGCACGAAAGCGTGGGG AGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCAACTAGTTGTTGGGGATTCATTTCCTTAGTAACGTAGCTAACGC GTGAAGTTGACCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGATGATGTGGATT AATTCGATGCAACGCGAAAAACCTTACCTACCCTTGACATGGTCGGAATCCTGCTGAGAGGCGGGAGTGCTCGAAAGAGAACCGGCGCAC AGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCTTAGTTGCTACGCAAG AGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCCTCATGGCCCTTATGGGTAGGGCTTCACACGT CATACAATGGTCGGAACAGAGGGTTGCCAACCCGCGAGGGGGAGCTAATCCCAGAAAACCGATCGTAGTCCGGATTGCACTCTGCAACTC GAGTGCATGAAGCTGGAATCGCTAGTAATCGCGGATCAGGATGCCGCCCGATTTTGGG
PSB1	CGGCGGACGGGTGAGTAATGCCTGGGAAATTGCCCAGTCGAGGGGGATAACAGTTGGAAACGACTGCTAATACCGCATACGCCCTACGG GGGAAAGCAGGGGACCTTCGGGCCTTGCGCGATTGGATATGCCCAGGTGGGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACG ATCCCTAGCTGGTCTGAGAGGATGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCA CAATGGGGGAAACCCTGATGCAGCCATGCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGAGGAGGAAAGGTTGATG CCTAATACGTATCAACTGTGACGTTACTCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGT TAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTGGATAAGTTAGATGTGAAAGCCCCGGGCTCAACCTGGGAATTGCATTTAAA ACTGTCCAGCTAGAGTCTTGTAGAGGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATYTGGAGGAATACCGGTGGCGAA GGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCSTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACG ATGTYGATTTGGAGGCTGTGTCCTTGAGACGTGGCTTCCGGAGCTAACGCGTTWAATTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAA ACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTTGATGCAACGCGAAGAACCTTACCTGGCCTTGACATG TCTGGAATCCTGCAGAGATGCGGGAGTGCCTTTGGGAATCAGAACACAGGTGCTGCATGGCCGTTGTCAGCTTGTGTTGTGAGATGTTGGG TTAAGTCCCGCAACGAGCGCAACCCCTGTCCTTTGCTGCCAGCACGTAATGGTGGGAACTCAAGGGAGACTGCCGGTGATAAACCGGAGG AAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCAGGGCTACACAC

Table 17. Details of the bacterial sequence obtained through BLAST search and its best match in GenBank database

Sl. No.	Sample code	Max. Score	Query cover (%)	Total. Score	Per cent identity (%)	Best match in GenBank database	Accession no.
1.	ATY10	2333	98	2333	99.23	*Pantoea agglomerans*	HM161866.1
2.	ATY 34	2074	98	2777	96.36	*Rhizobium* sp. *FSBRD3*	KJ184987.1
3.	ATY35	2032	99	2032	95.28	*Ensifer adhaerens*	KX507144.1
4.	PSB1	2250	99	2250	99	*Burkholderia territorii*	MK418836.1
5.	PSB3	2259	98	2259	97.92	*Burkholderia cepacia*	MT565309.1
6.	PSB4	2028	100	2028	98.4	*Aeromonas hydrophila*	MZ378784.1

All the treated plants recorded significantly higher germination percentage than uninoculated control (68.74±4.17) (Table 18).

Days taken for germination was comparatively lower in *Pantoea agglomerans* ATY10 (3.08±0.14 days) and *Rhizobium* sp. ATY34 (3.25±0.00) bioprimed seeds than other treatments (Table 19).

Treatment effect on shoot, root and seedling length of ragi seeds were observed. The maximum shoot length and root length among nitrogen fixers were recorded by the isolate *Pantoea agglomerans* ATY10 bio-primed seeds with shoot length of 3.31±0.11 cm, root length of 7.76±0.70 cm, and seedling length of 11.08±0.65 cm and the minimum were recorded by control with shoot length (2.54±0.03 cm), root length (2.54±0.03 cm) and seedling length (7.19±0.40 cm). In case of phosphate solubilizers, the isolate *Aeromonas hydrophila* PSB4 treated seeds recorded maximum shoot length of 3.19±0.12 cm, root length of 7.00±0.65 cm and seedling length of 10.20±0.77 cm (Table 20).

Pantoea agglomerans ATY10 treated seeds showed highest seedling vigour index of 876.62±61.92 followed by the isolate *Aeromonas hydrophila* PSB4 treated seeds with 820.418±28.38 and the lowest value was recorded by control (493.50±5.63) (Table 20).

Effect of ragi seeds bioprimed with selected isolates on shoot and root biomass was observed and the maximum fresh weight of shoot was recorded in seeds treated with *Pantoea agglomerans* ATY10 (14.000±0.359 mg), which was followed by *Rhizobium* sp. ATY34 treatment (13.781±0.290 mg). The minimum fresh weight was observed in control (13.781±0.290 mg). The maximum fresh weight of root was recorded by *Burkholderia territorii* PSB1 treated plants with 3.762±0.118 mg, which was on par with treatments *Rhizobium* sp. ATY34 (3.734±0.160 mg), *Aeromonas hydrophila* PSB4 (3.513±0.272 mg), and *Pantoea agglomerans* ATY10 (3.475±0.196 mg). The minimum root fresh weight was recorded by hydroprimed seeds with 2.048±0.265 mg (Table 21).

The maximum dry weight of shoot was observed in *Burkholderia territorii* PSB1 treated plant with 0.877±0.013 mg which was on par with *Pantoea agglomerans*

ATY10 (0.870±0.008) treatment, and the minimum shoot dry weight was recorded by hydroprimed seeds (0.552±0.010 mg). In case of root dry weight, superior root dry weight was recorded by *Pantoea agglomerans* ATY10 with 0.443±0.017 mg followed by *Rhizobium* sp. ATY34 (0.398±0.010 mg). The lowest root dry weight was recorded by hydroprimed seeds (0.215±0.006 mg) which is on par with control (0.225±0.013 mg) (Table 21).

Root shoot ratio was obtained by the ratio between the dry root mass and dry shoot mass, the higher root shoot ratio of *Pantoea agglomerans* ATY10 (0.50) showed to have greater root density, which was on par with *Rhizobium* sp. ATY34 (0.49) and *Aeromonas hydrophila* PSB4 (0.49). The lower root shoot ratio was recorded by control (0.36) (Table 20).

4.9. ASSESMENT OF ANTI-FUNGAL ACTIVITY OF THE SELECTED BACTERIAL ISOLATES

4.9.1. Antagonistic Activity of Selected Isolates Against *Rhizoctonia solani*

Three isolates inhibited the mycelial growth of *R. solani* with maximum zone of inhibition of 3.16±0.11 mm recorded by the bacterial isolate *Burkholderia territorii* PSB1 followed by *Burkholderia cepacia* PSB3 (1.5±0.00 mm) and *Pantoea agglomerans* ATY10 (0.73±0.05 mm) (Table 22). Three isolates *Rhizobium* sp. ATY34, *Ensifer adhaerens* ATY35 and *Aeromonas hydrophila* PSB4 were not found to inhibit the growth of *R. solani* (Plate 14).

4.9.2. Antagonistic Activity of Selected Isolates Against *Sclerotium rolfsii*

The selected isolates were subjected to the assessment of antagonistic activity against *Sclerotium rolfsii.* Two isolates *Burkholderia territorii* PSB1 and *Burkholderia cepacia* PSB3 recorded inhibition with 0.58±0.05 mm and 0.55±0.00 mm of zone of inhibition against *Sclerotium rolfsii* respectively (Table 23) (Plate 15).

Table 18. Effect of biopriming of ragi seeds with selected NFB and PSB isolates on seed germination percentage and number of days taken for germination

Sl. No.	Isolates	Seed germination*	
		Percentage (%)	Days taken
1.	*Pantoea agglomerans* ATY10	79.16±4.80^{a}	3.08±0.14^{a}
2.	*Rhizobium* sp. ATY34	75.00±6.80^{abc}	3.25±0.00^{ab}
3.	*Ensifer adhaerens* ATY35	74.99±6.80^{abc}	3.50±0.00^{cd}
4.	*Burkholderia territorii* PSB1	77.08±4.16^{ab}	3.33±0.14^{bc}
5.	*Burkholderia cepacia* PSB3	74.99±0.00^{abc}	3.41±0.14^{bc}
6.	PSB4	81.24±4.16^{a}	3.41±0.14^{bc}
7.	Hydropriming	70.83±4.81^{bc}	3.50±0.00^{cd}
8.	Control	68.74±4.17^{c}	3.66±0.14^{d}

* Mean (±SD) of 4 replications. Values in a column followed by same letter do not differ significantly according to Duncan's Multiple Range Test ($p \leq 0.05$)

Table 19. Effect of biopriming of ragi seeds with selected NFB and PSB isolates on shoot, root and seedling length

Sl. No.	Isolates	Shoot length (cm)*	Root length (cm)*	Seedling length (cm)*
1.	*Pantoea agglomerans* ATY10	3.31±0.11^{a}	7.76±0.70^{a}	11.08±0.65^{a}
2.	*Rhizobium* sp. ATY34	3.06±0.23^{b}	7.30±0.74^{ab}	10.37±0.97^{ab}
3.	*Ensifer adhaerens* ATY35	3.22±0.02^{ab}	6.89±0.17^{b}	10.12±0.19^{b}
4.	*Burkholderia territorii* PSB1	3.12±0.12^{ab}	6.80±0.21^{b}	9.92±0.11^{b}
5.	*Burkholderia cepacia* PSB3	3.19±0.20^{ab}	7.00±0.03^{b}	10.20±0.23^{b}
6.	*Aeromonas hydrophila* PSB4	3.15±0.12^{ab}	6.97±0.65^{b}	10.12±0.77^{b}
7.	Hydropriming	2.75±0.19^{c}	4.79±0.11^{c}	7.54±0.23^{c}
8.	Control	2.54±0.03^{c}	4.65±0.43^{c}	7.19±0.40^{c}

* Mean (±SD) of 3 replications. Values in a column followed by same letter do not differ significantly according to Duncan's Multiple Range Test ($p \leq 0.05$)

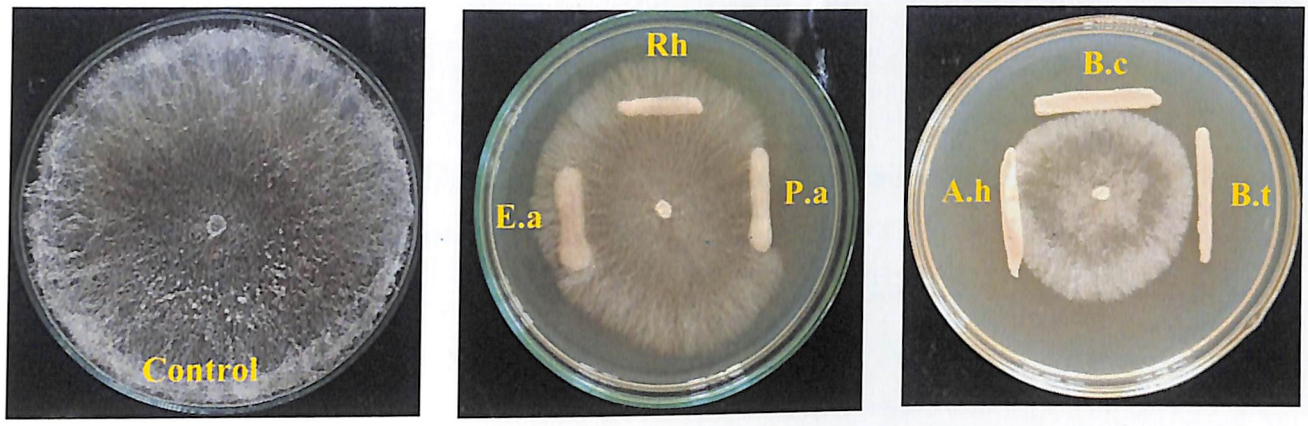


Plate 14. Antagonistic activity of selected isolates against *Rhizoctonia solani*

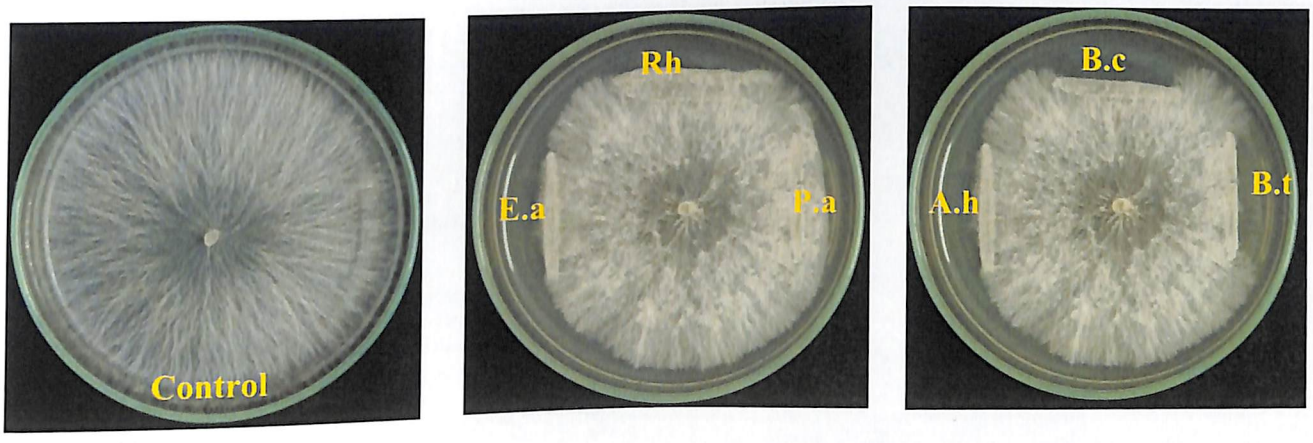


Plate 15. Antagonistic activity of selected isolates against *Sclerotium rolfsii*

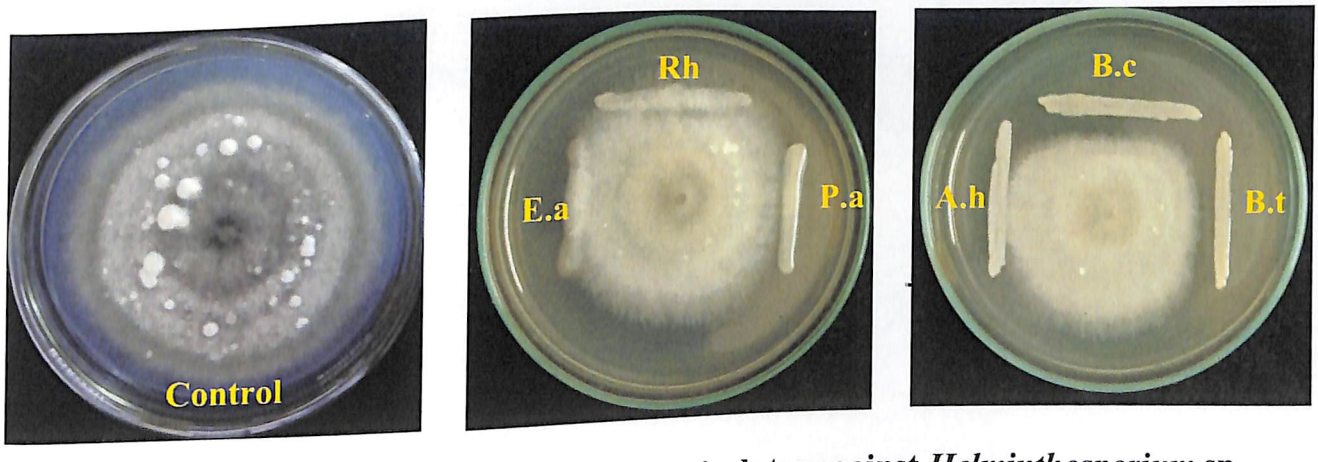


Plate 16. Antagonistic activity of selected isolates against *Helminthosporium* sp.

Note: P.a-*Pantoea agglomerans* ATY10, Rh-*Rhizobium* sp. ATY34, E.a-*Ensifer adhaerens* ATY35, B.t-*Burkholderia territorii* PSB1, B.c-*Burkholderia cepacia* PSB3 and A.h-*Aeromonas hydrophila* PSB4

Table 20. Effect of biopriming of ragi seeds with selected NFB and PSB isolates on root shoot ratio and seedling vigour index

Sl. No.	Isolates	Root shoot ratio	Seedling vigour index*
1.	*Pantoea agglomerans* ATY10	0.50	876.62±61.92[a]
2.	*Rhizobium* sp. ATY34	0.49	778.48±107.17[b]
3.	*Ensifer adhaerens* ATY35	0.382	758.62±63.09[b]
4.	*Burkholderia territorii* PSB1	0.40	765.56±48.87[b]
5.	*Burkholderia cepacia* PSB3	0.39	765.00±17.27[b]
6.	*Aeromonas hydrophila* PSB4	0.49	820.41±28.38[ab]
7.	Hydropriming	0.41	534.42±37.52[c]
8.	Control	0.32	493.50±5.63[c]

* Mean (±SD) of 4 replications. Values in a column followed by same letter do not differ significantly according to Duncan's Multiple Range Test ($p \leq 0.05$)

Table 21. Effect of biopriming of ragi seeds with selected NFB and PSB isolates on seedling growth on 10th DAT*

Sl. No.	Isolates	Fresh weight (mg)*		Dry weight (mg)*	
		Shoot	Root	Shoot	Root
1.	*Pantoea agglomerans* ATY10	14.000±0.359[a]	3.475±0.196[a]	0.870±0.008[a]	0.443±0.017[a]
2.	*Rhizobium* sp ATY34	13.781±0.290[a]	3.734±0.160[a]	0.809±0.009[b]	0.398±0.010[b]
3.	*Ensifer adhaerens* ATY35	12.325±0.395[bc]	3.125±0.359[b]	0.815±0.006[b]	0.312±0.005[d]
4.	*Burkholderia territorii* PSB1	12.694±0.266[b]	3.762±0.118[a]	0.877±0.013[a]	0.357±0.010[c]
5.	*Burkholderia cepacia* PSB3	11.838±0.493[c]	2.438±0.262[c]	0.712±0.013[c]	0.278±0.010[e]
6.	*Aeromonas hydrophila* PSB4	12.281±0.806[bc]	3.513±0.272[a]	0.820±0.008[b]	0.407±0.010[b]
7.	Hydropriming	8.922±0.639[d]	2.048±0.265[d]	0.552±0.010[e]	0.215±0.006[f]
8.	Control	8.762±0.083[d]	2.126±0.169c[d]	0.698±0.010[d]	0.225±0.013[f]

*DAT-Days After Treatment

* Mean (±SD) of 4 replications. Values in a column followed by same letter do not differ significantly according to Duncan's Multiple Range Test ($p \leq 0.05$)

4.9.3. Antagonistic Activity of Selected Isolates Against *Helminthosporium* sp.

Among six isolates, only two isolates were recorded to have antagonistic activity against *Helminthosporium* sp. The *Burkholderia cepacia* PSB3 recorded 5.66±0.28 mm of zone of inhibition against *Helminthosporium* sp. and the *Burkholderia territorii* PSB1 recorded 4.17±0.28 mm (Table 25) (Plate 16).

4.9.4. Antagonistic Activity of Selected Isolates Against *Fusarium* sp.

Burkholderia cepacia PSB3 showed maximum antagonistic activity against *Fusarium* sp. with 4.13±0.05 mm zone of inhibition. This was followed by the *Pantoea agglomerans* ATY10 (3.13±0.05 mm) and *Ensifer adhaerens* ATY35 (3.00±0.10 mm) and the minimum was recorded by *Burkholderia territorii* PSB1 (2.76±0.05 mm) (Table 24) (Plate 17). Other two isolates *Rhizobium* sp. ATY34 and *Aeromonas hydrophila* PSB4 did not show any inhibition against *Fusarium* sp.

4.10. SIDEROPHORE PRODUCTION

The presence of siderophore production in bacterial isolates were confirmed by spot inoculating the isolates on to Chrome Azurol S (CAS) agar medium plates. Only the isolate *Aeromonas hydrophila* PSB4 showed change in colour of the media from dark blue to orange zone around the bacterial colony (Plate 19) indicating the presence of siderophore production (Table 26).

4.11. POTASSIUM SOLUBILISATION BY THE BACTERIAL ISOLATES

The selected isolates were subjected to plate assay for assessing the potassium solubilizing capacity. *Burkholderia territorii* PSB1 (11.00±0.50 mm) and *Burkholderia cepacia* PSB3 (12.66±0.28 mm) formed a clear zone of solubilization around their colony (Plate 18) by solubilizing potassium alumino silicate present in Alexsandrov media (Table 27).

Table 22. Antagonistic activity of selected isolates against *Rhizoctonia solani*

Sl. No.	Isolates	Zone of inhibition (mm)*
1	*Pantoea agglomerans* ATY10	0.73 ± 0.05^{c}
2	*Rhizobium* sp. ATY34	0.00 ± 0.00^{d}
3	*Ensifer adhaerens* ATY35	0.00 ± 0.00^{d}
4	*Burkholderia territorii* PSB1	3.16 ± 0.11^{a}
5	*Burkholderia cepacia* PSB3	1.5 ± 0.00^{b}
6	*Aeromonas hydrophila* PSB4	0.00 ± 0.00^{d}

* Mean (±SD) of 4 replications. Values in a column followed by same letter do not differ significantly according to Duncan's Multiple Range Test ($p \leq 0.05$)

Table 23. Antagonistic activity of selected isolates against *Sclerotium rolfsii*

Sl. No.	Isolates	Zone of inhibition (mm)*
1	*Pantoea agglomerans* ATY10	0.00 ± 0.00^{c}
2	*Rhizobium* sp. ATY34	0.00 ± 0.00^{c}
3	*Ensifer adhaerens* ATY35	0.00 ± 0.00^{c}
4	*Burkholderia territorii* PSB1	0.58 ± 0.05^{a}
5	*Burkholderia cepacia* PSB3	0.55 ± 0.00^{b}
6	*Aeromonas hydrophila* PSB4	0.00 ± 0.00^{c}

* Mean (±SD) of 4 replications. Values in a column followed by same letter do not differ significantly according to Duncan's Multiple Range Test ($p \leq 0.05$)

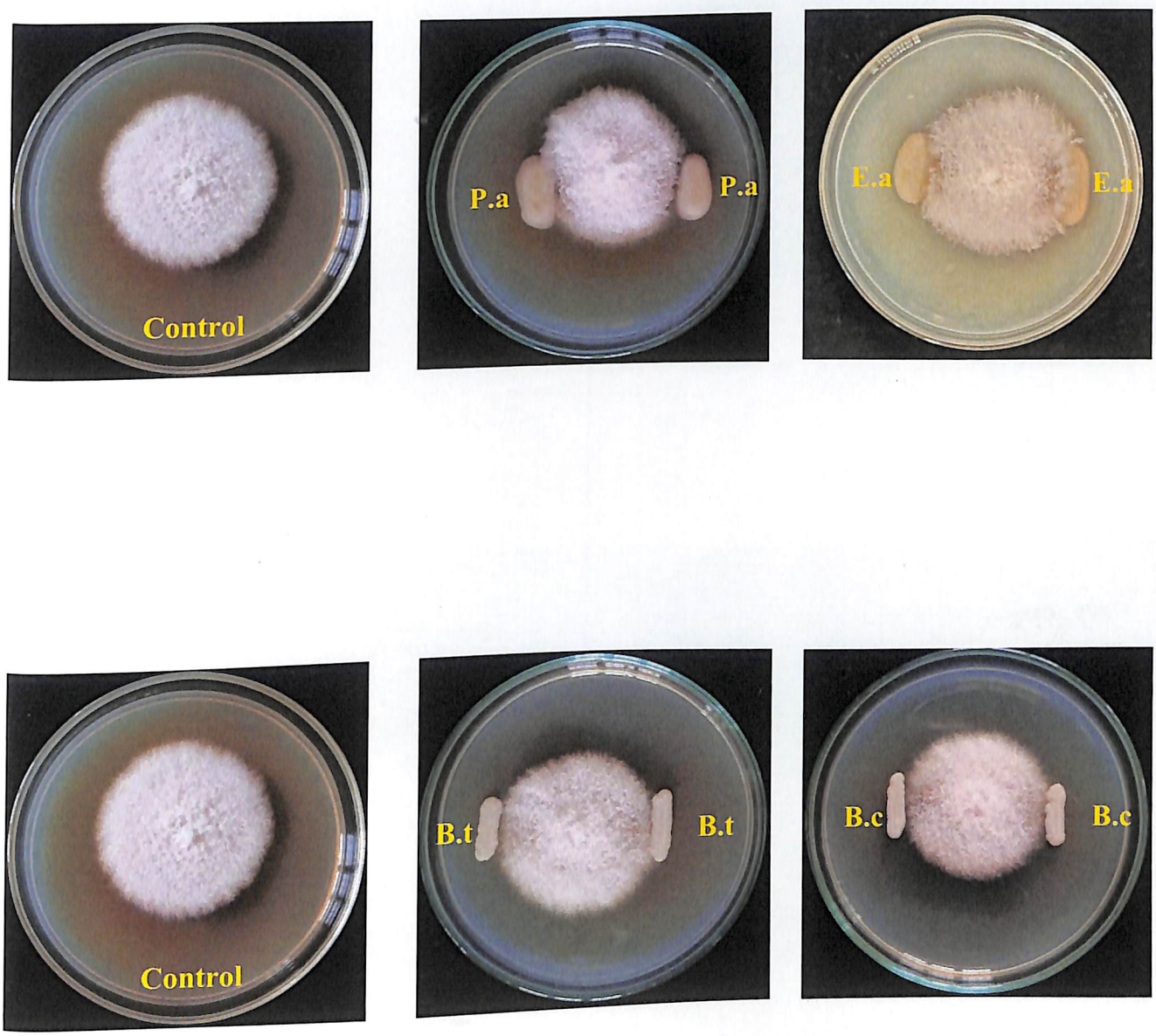


Plate 17. Antagonistic activity of selected isolates against *Fusarium* sp.

Note: P.a-*Pantoea agglomerans* ATY10, Rh-*Rhizobium* sp. ATY34, E.a-*Ensifer adhaerens* ATY35, B.t-*Burkholderia territorii* PSB1, B.c-*Burkholderia cepacia* PSB3 and A.h-*Aeromonas hydrophila* PSB4

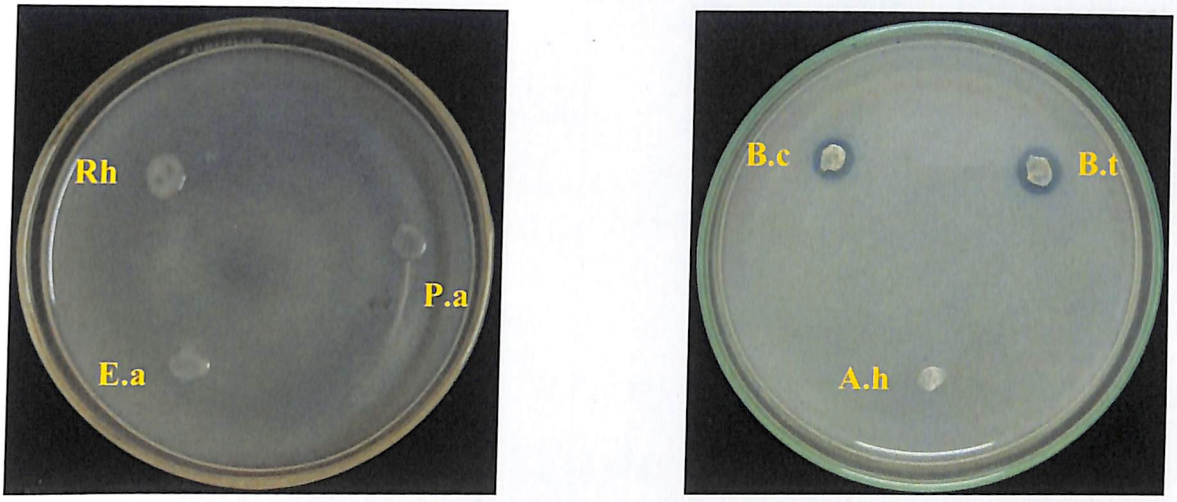


Plate 18. Potassium solubilizing ability of the selected isolates on Aleksandrov agar

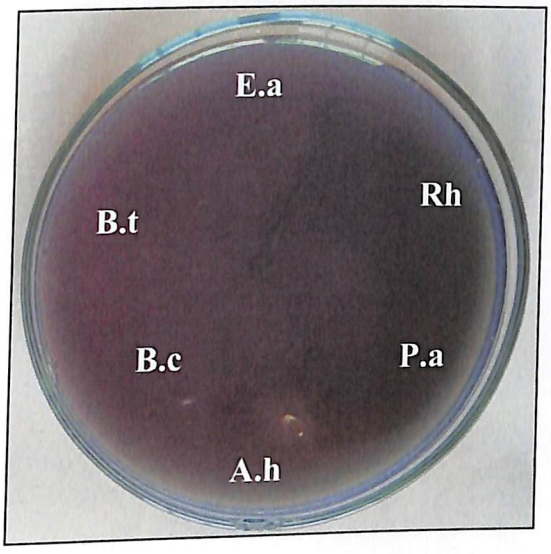


Plate 19. Siderophore production by the selected isolates on CAS agar

Note: P.a-*Pantoea agglomerans* ATY10, Rh-*Rhizobium* sp. ATY34, E.a-*Ensifer adhaerens* ATY35, B.t-*Burkholderia territorii* PSB1, B.c-*Burkholderia cepacia* PSB3 and A.h-*Aeromonas hydrophila* PSB4

Table 24. Antagonistic activity of the selected isolates against *Helminthosporium* sp.

Sl. No.	Isolates	Zone Of Inhibition (mm)*
1	*Pantoea agglomerans* ATY10	0.00±0.00^{c}
2	*Rhizobium* sp. ATY34	0.00±0.00^{c}
3	*Ensifer adhaerens* ATY35	0.00±0.00^{c}
4	*Burkholderia territorii* PSB1	4.17±0.28^{b}
5	*Burkholderia cepacia* PSB3	5.66±0.28^{a}
6	*Aeromonas hydrophila* PSB4	0.00±0.00^{c}

* Mean (±SD) of 4 replications. Values in a column followed by same letter do not differ significantly according to Duncan's Multiple Range Test ($p \leq 0.05$)

Table 25. Antagonistic activity of selected isolates against *Fusarium* sp.

Sl. No.	Isolates	Zone of inhibition (mm)*
1	*Pantoea agglomerans* ATY10	3.13±0.05^{b}
2	*Rhizobium* sp. ATY34	0.00±0.00^{e}
3	*Ensifer adhaerens* ATY35	3.00±0.10^{c}
4	*Burkholderia territorii* PSB1	2.76±0.05^{d}
5	*Burkholderia cepacia* PSB3	4.13±0.05^{a}
6	*Aeromonas hydrophila* PSB4	0.00±0.00^{e}

* Mean (±SD) of 4 replications. Values in a column followed by same letter do not differ significantly according to Duncan's Multiple Range Test ($p \leq 0.05$)

Table 26. Qualitative analysis of siderophore production by selected isolates

Sl. No.	Isolates	Siderophore production
1	*Pantoea agglomerans* ATY10	-
2	*Rhizobium* sp. ATY34	-
3	*Ensifer adhaerens* ATY35	-
4	*Burkholderia territorii* PSB1	-
5	*Burkholderia cepacia* PSB3	-
6	*Aeromonas hydrophila* PSB4	+

(+) Presence of siderophore production; (-) Absence of siderophore production

Table 27. Potassium solubilising ability of selected isolates

Sl. No.	Isolates	Diameter of zone (mm)*
1	*Pantoea agglomerans* ATY10	0.00 ± 0.00^{c}
2	*Rhizobium* sp. ATY34	0.00 ± 0.00^{c}
3	*Ensifer adhaerens* ATY35	0.00 ± 0.00^{c}
4	*Burkholderia territorii* PSB1	11.00 ± 0.50^{b}
5	*Burkholderia cepacia* PSB3	12.66 ± 0.28^{a}
6	*Aeromonas hydrophila* PSB4	0.00 ± 0.00^{c}

* Mean (±SD) of 4 replications. Values in a column followed by same letter do not differ significantly according to Duncan's Multiple Range Test ($p \leq 0.05$)

4.12. SILICON SOLUBILISATION BY THE BACTERIAL ISOLATES

Silicon solubilizing ability of selected isolates were assessed using Bunt and Rovira media. None of the selected isolates was known to solubilize silicon. Zone of silicate solubilization was not recorded.

4.13. PHOSPHORUS SOLUBILIZATION BY THE NFB ISOLATES

The phosphate solubilizing capacity of all the selected NFB isolates were evaluated by spot inoculating in Pikovskaya's agar media. None of the NFB isolates were recorded to solubilize insoluble tricalcium phosphate in the media.

DISCUSSION

5. DISCUSSION

Attappady is a tribal development block situated on the eastern slope of the Western Ghats, in Palakkad district, Kerala (Haseena, 2015). Attappady is one among the largest tribal settlements in Kerala with considerable livestock population. People here depend mostly on subsistence farming with low inputs for their livelihood. Millet cultivation is being promoted in Attappady by implementing the Millet Village Project so as to rejuvenate the tribal agriculture and improve the nutritional status of the tribal population in the area. The project also helps in improving the standard of living of tribal farmers and generate a better return by the sale of their products under the 'Attappady Organic' brand.

Millets are hardy, photo-insensitive, climate resilient crops with low carbon and water footprint and require low or no external inputs. They are adapted to harsh environment of the semi-arid tropics and thus are the backbone of dry land agriculture.

Finger millet or ragi (*Eleusine coracana* (L.) Gaertn. subsp. *coracana*) is an essential food crop in areas where subsistence farming is practiced. It is one among the major crops grown in semi-arid, hilly tribal areas of India and Africa with low input cost (Mincyte, 2011). Ragi contains high amount of Ca, Fe and Mg and amino acid methionine with higher dietary fibre and mineral, which are not provided by rice. It offers better opportunities for food and nutrition security among tribal people (Pradhan *et al.*, 2019).

The tribal farmers of Attappady are reluctant in using chemical fertilizers and pesticides in their cultivation and hence the fertility of soil is mainly based on the organic inputs given and microbial population present in these soils. The possibilities of encountering unique prospective biofertilizer microorganisms is high in these soils. The current study was conducted by collecting samples from the ragi fields of Attappady region and isolating the beneficial rhizosphere microorganisms.

Forty-four bacterial isolates were obtained from the rhizosphere soils of Attappady region using different medium out of which, thirty-six isolates were from N-free media (Waksman Base No. 77, N-free malate bromothymol blue, Jensen's, Burk's

agar media) and the remaining eight from tricalcium phosphate amended Pikovskaya's agar medium.

Cereals and millets form associative or mutualistic relationships with bacteria and fungi, including nitrogen-fixing bacteria known as diazotrophs. Various reports are available on the isolation of nitrogen fixing bacteria using N-free media from the rhizosphere soils of cereals and millets (Raffi and Charyulu, 2012; Majeed *et al.*, 2015; Abbasi *et al.*, 2011). Biological nitrogen fixation (BNF) is a promising alternative source of nitrogen for grain production, accounting for 30-50 percent of nitrogen (Beatty and Good, 2011; Ormeno-Orrillo *et al.*, 2013; Rogers and Oldroyd, 2014; Bloch *et al.*, 2020). The isolation of phosphate solubilizing bacteria was reported in cereals and millet crops using insoluble phosphate amended medium and many were isolated and selected based on the zone of clearance shown by the potential P solubilizing bacteria (Kour *et al.*, 2020a; Kour *et al.*, 2020b; Tahir *et al.*, 2013; Suleman *et al.*, 2018).

From forty-four isolates, twenty isolates were further selected based upon the similarities in colony morphology, in order to eliminate repetition. Kalam *et al.* (2020) isolated 60 bacterial colonies from tomato rhizosphere and selected seven based on distinct colony morphology.

In the present study the colony pattern of selected isolates was mostly raised with irregular shape, undulated margin, mucoidal appearance possessing white colour and of size ranging from 0.5–4 mm. The colony colour was off white in PSB1 and PSB3, and in ATY33 it was light green. ATY10 had undulated margin. Tho *et al.* (2015) isolated *Pantoea agglomerans* having circular to irregular shaped colonies with raised elevation, smooth margin, and mucoid texture. The strain ATY10 was observed to have similar colony characters which was later identified as *Pantoea agglomerans.*

Based on the staining character of the cells, most of the isolates were Gram negative except ATY1, ATY17, ATY23 and ATY36 which were Gram positive in nature and eleven isolates had capsule. The cell shape of ATY1 was diplobacilli and all others were rod shaped except ATY4, ATY17, ATY23, ATY24 and PSB7 which were spherical. The cell size ranging between diameter 0.83-2.49 µm and length (0.83-5.81

μm). Motility check revealed that all isolates were motile in nature except PSB4 and PSB5. The cell characters of PSB3, which was later identified as *Burkholderia cepacia* PSB3 was similar to the studies conducted by Quan *et al.* (2006). They isolated *Burkholderia cepacia* which were Gram-negative, motile and approximately 0.5 to 1.0 μm wide by 1.5 to 3.0 μm long in size.

Even though morphological and biochemical characterization of the isolates cannot precisely reveal the identity of the isolates it will give an insight into the nature of the isolates and the nutritional requirements which can in turn help in selecting an efficient isolate suitable for our purpose.

A plethora of reviews exhaustively documents almost all aspects of PGPR (Lugtenberg and Kamilova, 2009; Etesami *et al.,* 2015; Parray *et al.,* 2016; Backer *et al.,* 2018). PGPR, through their direct and indirect effects, can bring about substantial plant growth promotion (PGP). They act directly by facilitating nitrogen absorption and assimilation, mineral solubilization, production of phytohormones (Ahmad *et al.,* 2008; Shaikh *et al.,* 2018; Kalam *et al.,* 2020b). Indirectly, PGPR, through their biocontrol mechanisms, lytic enzymes (Jadhav *et al.,* 2020), antibiotics (Vinay *et al.,* 2016; Reshma *et al.,* 2018), siderophores (Shaikh *et al.,* 2016) 1-aminocyclopropane-1-carboxylic acid (ACC) (Glick, 2014; Sagar *et al.,* 2020), guarding host plants against pathogens (Shaikh *et al.,* 2018).

Different screening techniques are effectively employed to select bacterial strains with potential PGP activity. Each method has its own advantage and disadvantage. The 'field first strategy' proposed by Baliyan *et al.* (2018) emphasise the importance of selecting PGPR based on their survivability prior to *in vitro* screening. Even if this strategy is desirable for selecting field competent PGPR, it is more expensive, laborious and time consuming. *In vitro* screening techniques are usually employed to select efficient bacterial strains with multiple plant growth-promotions (Ahmad *et al.,* 2008; Cakmakci *et al.,* 2007). Even though there are reports of inconsistency in correlation between *in vitro* PGP activity and *in vivo* growth promotion (Khalid *et al.,* 2004; Yobo *et al.,* 2004), these techniques are simple and efficient for screening large numbers of isolates (Campbell, 1989). Combination of *in*

vitro and *in vivo* screening can help in identification of effective strains for sustainable agriculture.

In the present study, all the twenty isolates were subjected to analysis of plant growth promotion activity such as IAA, GA and ammonia production under *in vitro* conditions considering the advantages of such screening techniques. Secondary metabolites such as auxin and gibberellin, as natural phytohormones involve in mitigating abiotic stress, dormancy and stunted growth. Phytohormone production by bacterial isolate promotes plant growth (Restu *et al.*, 2019). Many researchers have reported the presence of plant growth promoting traits in bacteria isolated from the rhizosphere soils, which enhanced shoot length, root length and plant biomass. Some plant growth promoting bacteria have also been attributed to exhibit biocontrol activity against plant pathogens (Navarro-Noya *et al.*, 2012; Kumar *et al.*, 2014; Abdallah *et al.*, 2019).

The quantitative estimation of IAA production by the bacterial isolates can be assessed using the L-tryptophan amended specific broth. IAA is the most important auxins synthesized using tryptophan as precursor. It promotes plant cell division, differentiation, lateral and adventitious roots elongation. Microorganisms are able to synthesis IAA through IPA, indole-3-acetamide, tryptamine and indole-3-acetaldehyde and the final pathway involves an oxidase oxidising the side chain of tryptophan. Thus, tryptophan amended media could promote faster IAA production by microorganisms capable of producing IAA. Many nitrogen fixing bacteria are able to synthesis IAA (Fett *et al.*, 1987; Libbert *et al.*, 1966; Ivanova *et al.*, 2001; Raheem *et al.*, 2018).

In this study the highest IAA production was reported by *Pantoea agglomerans* ATY10 with 101.22±0.77 µg mL^{-1} followed by *Ensifer adhaerens* ATY35 with 52.04±3.61 µg mL^{-1} of IAA production (Fig. 1). This was 94.5 percent higher than *Ensifer adhaerens* ATY35 and 14.17 percent higher than the standard culture of *Azospirillum lipoferum* used in the study. A reasonably higher IAA production of 2.191 g L^{-1} by *Pantoea agglomerans* strain PVM was reported by Apine and Jadhav (2011). Sergeeva *et al.* (2007) also reported higher IAA production of *Pantoea agglomerans* in tryptophan added medium. A study conducted on *Ensifer adhaerens* for heavy metal

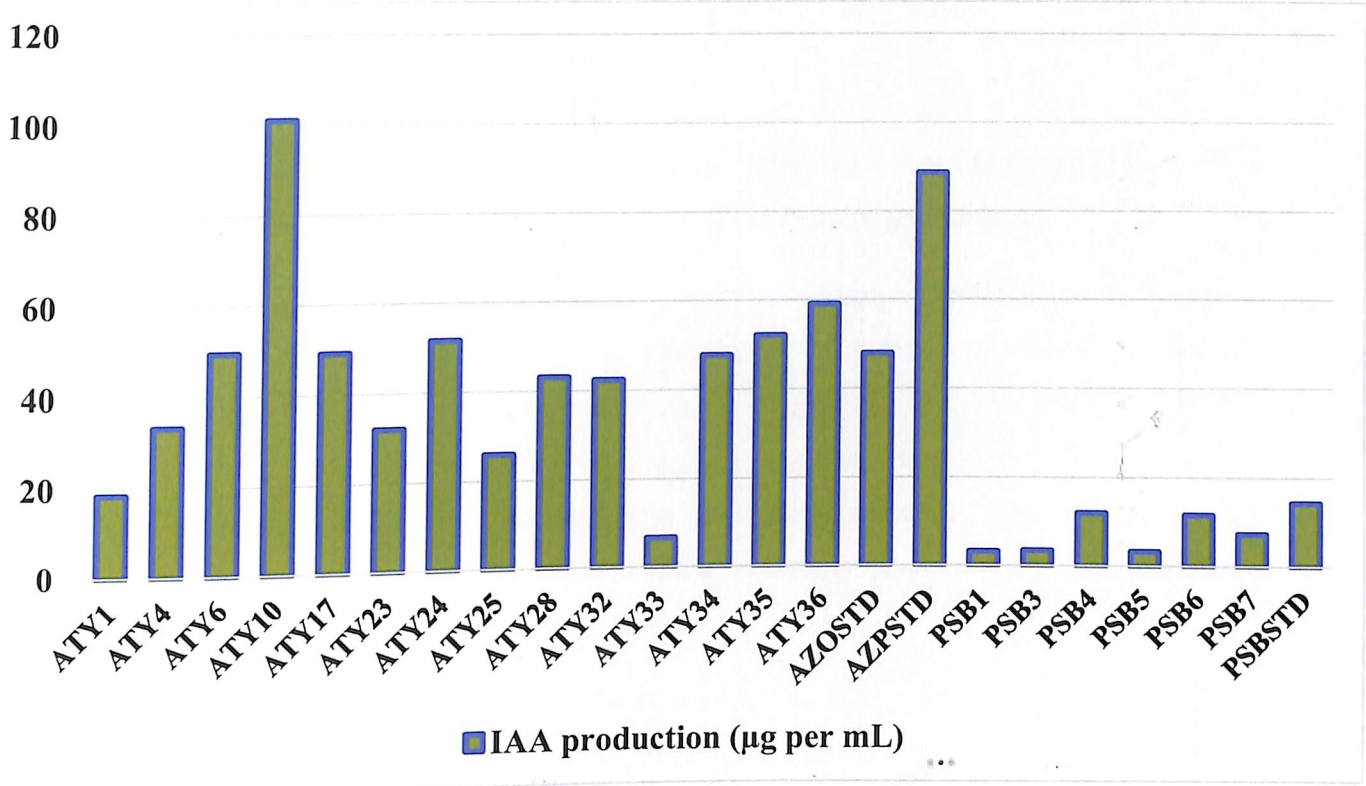


Figure 1. Indole Acetic Acid production by the selected bacterial isolates

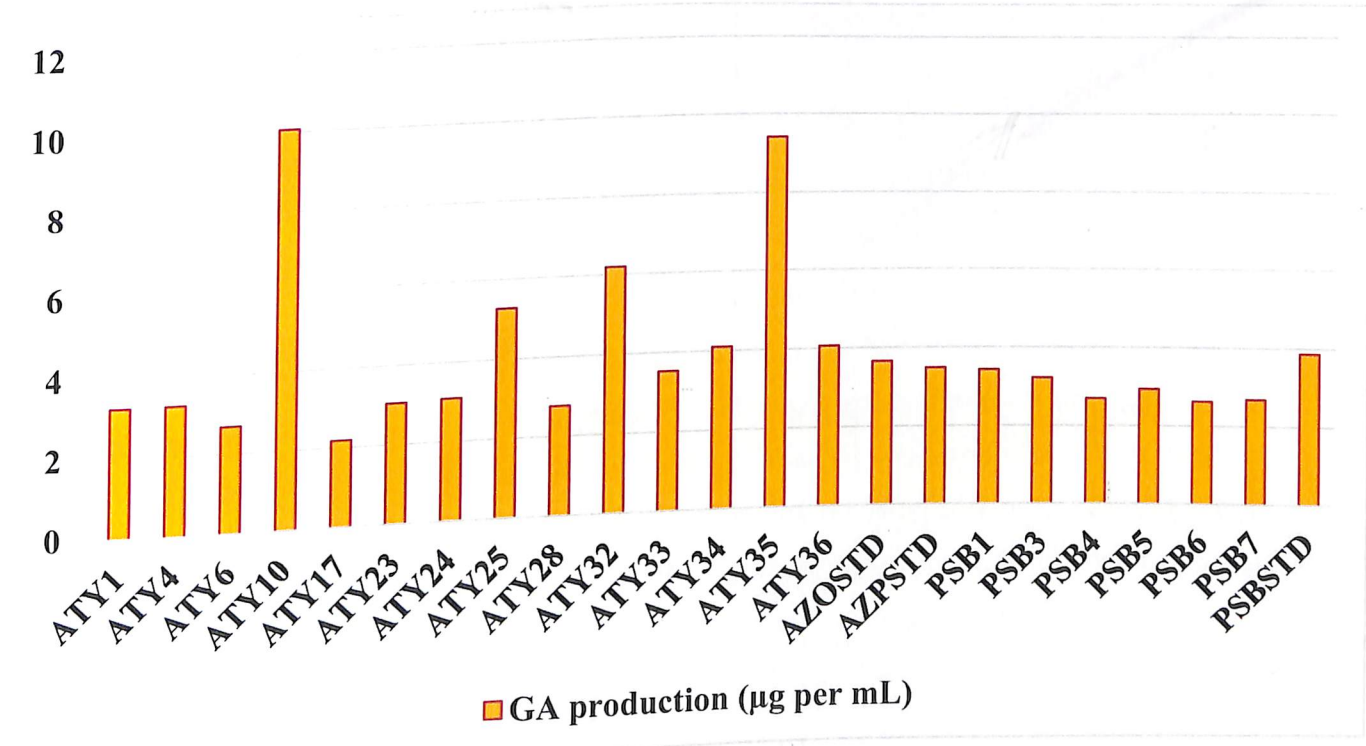


Figure 2. Gibberellic Acid production by the selected bacterial isolates

bioaccumulation by Oves *et al.* (2017) showed that the isolate has the potential of IAA production of range 27-31 μg mL^{-1} in heavy metal amended medium.

Gibberellins act by modifying plant morphology and are involved in elongation of plant tissue particularly stem, also induces the plant uptake of minerals, sugars and proteins (Atzorn *et al.,* 1988). Faster shoot elongation and better plant growth was promoted by the induction of active hairy root zone (Hamida and Elkomy, 1998). Gibberellic acid plays an important role in plants for switching from vegetative stage to reproductive stage. It also aids in seed germination, flowering and fruit production in plant (Dong *et al.,* 2017; Ma *et al.,* 2018).

The quantitative estimation of gibberellic acid was carried out for all the selected twenty isolates and the superior GA production was reported by the strain *Pantoea agglomerans* ATY10 with 10.07±2.41 μg mL^{-1} of GA production which was 7.01 percent higher compared to the next best isolate (Fig. 2). A similar GA production of (114 ng mL^{-1}) by the strain *Pantoea agglomerans* YS19 was reported by Feng *et al.* (2006) and it was also interpreted that application of *Pantoea agglomerans* with accountable production of phytohormones could improve plant growth. The bacterial strain *Ensifer adhaerens* ATY35 produced 9.41±0.87 μg mL^{-1} of GA and the results were similar to the findings of Rathore *et al.* (2016). He reported multiple plant growth promoting traits by *Ensifer adhaerens* in *Brassica napus.* The PSB isolates were having low IAA production compared to the nitrogen fixing bacterial isolates. Similar result was observed by Baig *et. al.,* (2014) in *Bacillus* isolates having phosphate solubilization. They observed that when phosphate solubilization potential increased IAA production by the isolates decreased. The observation of the current study is also in line with this, there was a stoichiometric decrease in IAA production with increase in P-solubilization. In an *in vitro* study conducted by Wuryanto *et al.,* (2018) it was revealed that IAA production by a bacterial isolate decreased with increase in acidity of the medium and IAA production was highest at pH 7 (Fig. 3.). The general mechanism of phosphate solubilization by bacterial isolates is through organic acid production, this explains the reason for decrease in IAA production by PSB isolates.

The plant growth promoting bacteria assisted host plant by producing ammonia, which accumulates and supplies nitrogen to their host plants. This aided plants in

improving root and shoot elongation and biomass of the plant (Singh *et al.*, 2019). The bacterial isolates ATY17 (176.53±5.07 µmol mL^{-1}), ATY25 (176.51±5.09 µmol mL^{-1}), PSB4 (175.33±6.76 µmol mL^{-1}) and ATY35(170.19±14.03 µmol mL^{-1}) recorded highest amount of extracellular ammonia production on quantification with Nessler's reagent (Fig. 4). *Ensifer adhaerens* strain TMX-23 was reported to produce ammonia which was confirmed by the addition of Nessler's reagent (Zhou *et al.*, 2013). Sandhya *et al.* (2017) reported that the plant growth promoting endophytes from maize included *Pseudomonas* spp., *Sinorhizobium* sp., and *Enterobacter* sp. which were also involved in the production of ammonia.

The nitrogen content of bacterial isolates was estimated by internationally recognized micro-Kjeldahl method to assay soils, water, fertilizer and other material. Micro-Kjeldahl method can be used for a comparative evaluation of Nitrogen fixation by different bacterial strains in N-free broth (Henderson and Wilson, 1970). On micro-kjeldahl estimation, a superior nitrogen fixing capacity was shown by the isolate ATY33 with 30.75±3.88 µg mL^{-1} of N and ATY34 recorded high N content of 21.00±5.65 µg mL^{-1} (Fig. 5). Mazumdar and Deka (2013) assessed the nitrogen content of nitrogen fixing bacteria from crude oil contaminated soil and reported 17.45±0.66 mg L^{-1} with AM02 and 9.47±0.34 mg L^{-1} with AM07. Study conducted by Andrade *et al.* (2014) to assess the plant growth activity by endophytic bacteria in banana root using Kjeldahl method revealed that EB-04 (*Bacillus subtilis*), EB-64 (*Bacillus pumilus*), and EB-169 (*B. pumilus*) exhibited modest rates of N fixation, generating 15, 9 and 15 µg mL^{-1} of nitrogen, respectively.

Qualitative and quantitative assessment were carried for phosphate solubilizing bacteria using plate assay and broth assay. Different phosphorous sources like tricalcium phosphate, rock phosphate, aluminium phosphate etc. are used for qualitative assessment of phosphorous solubilization. Tricalcium phosphate is easily solubilized by many PSB isolates, so can be used effectively for detection of P-solubilization by bacterial isolates (Bashan *et al.*, 2013).

The PSB isolates showed clear zone of solubilization on Pikovskaya's agar medium. The zone of solubilization was recorded maximum by PSB1 (18.23±0.25 mm), PSB3 (17.23±0.20 mm) and PSB4 (10.33±0.57 mm) and the Phosphate

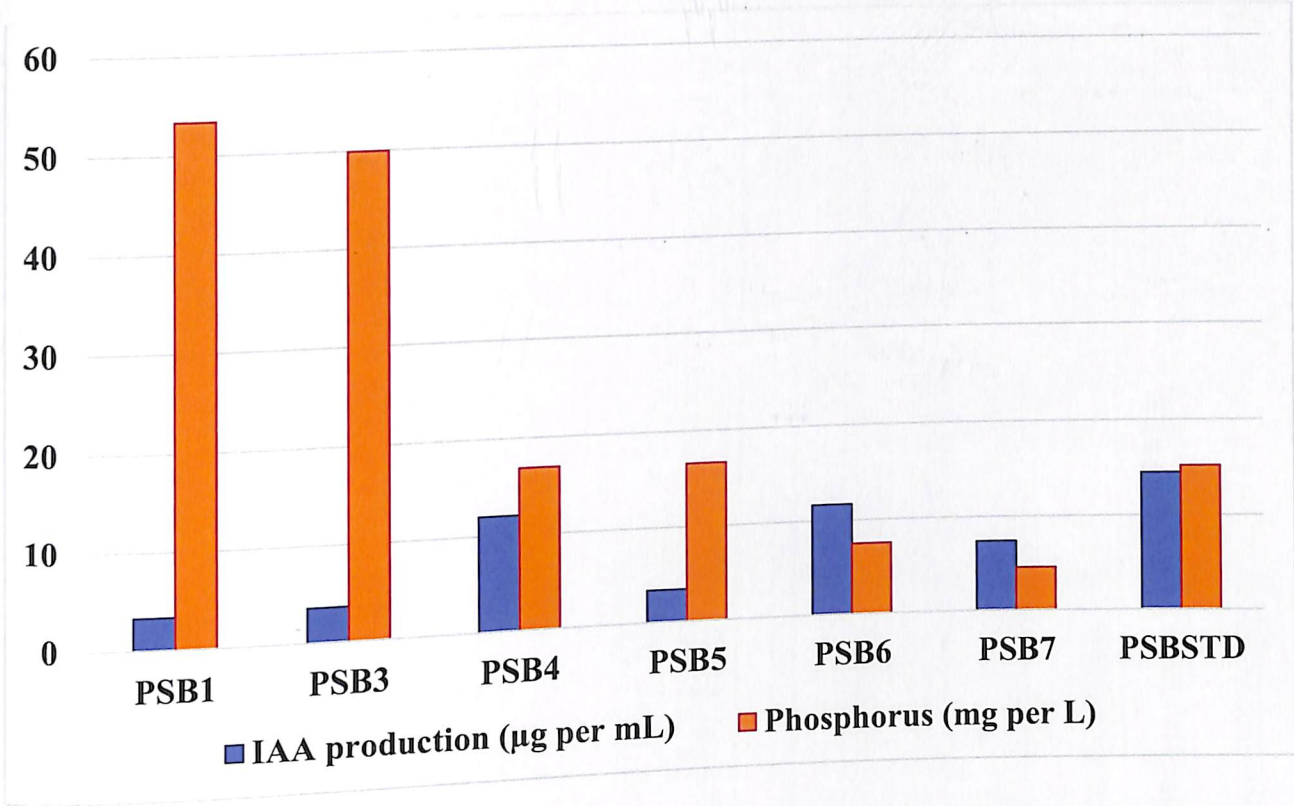


Figure 3. Comparison of IAA production with phosphorus solubilization by PSB isolates

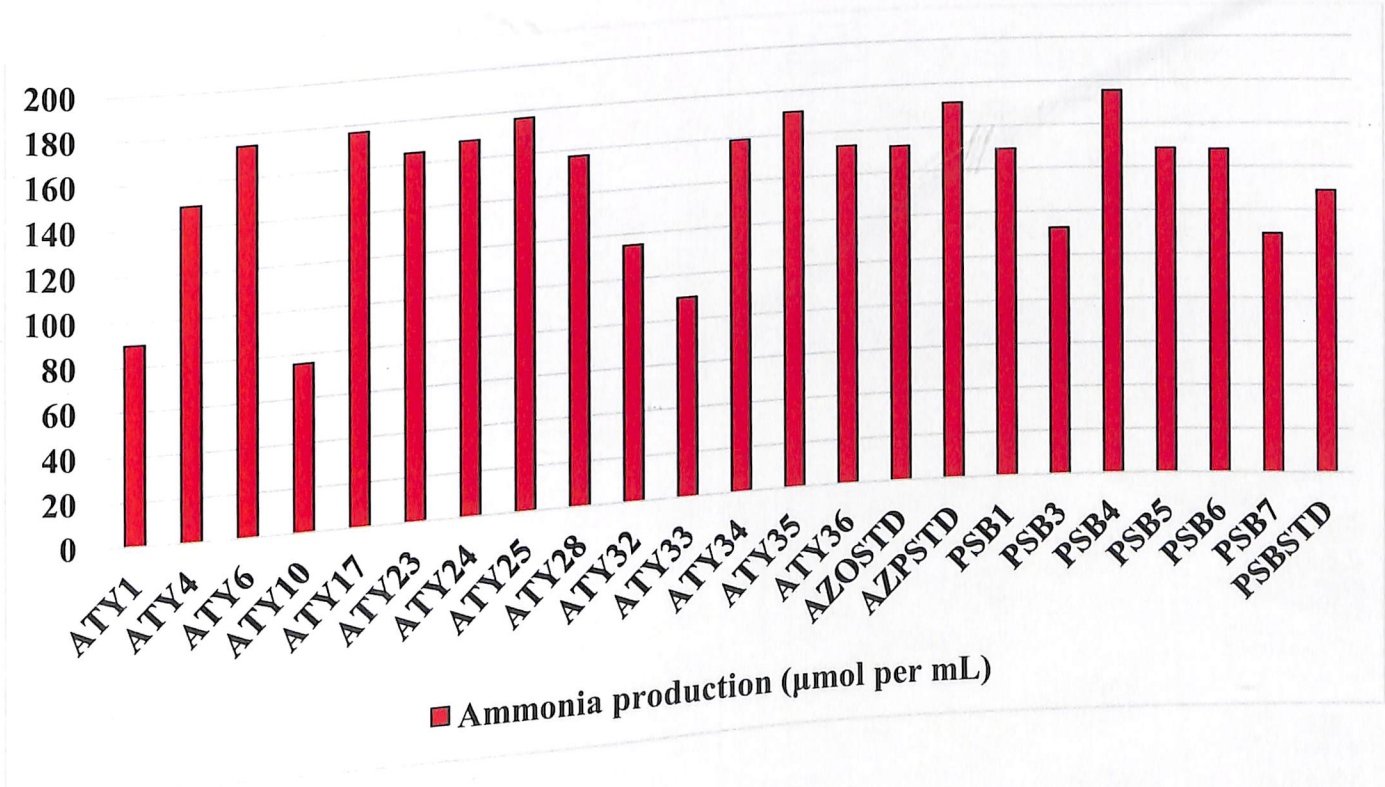


Figure 4. Extracellular ammonia production by the selected bacterial isolates

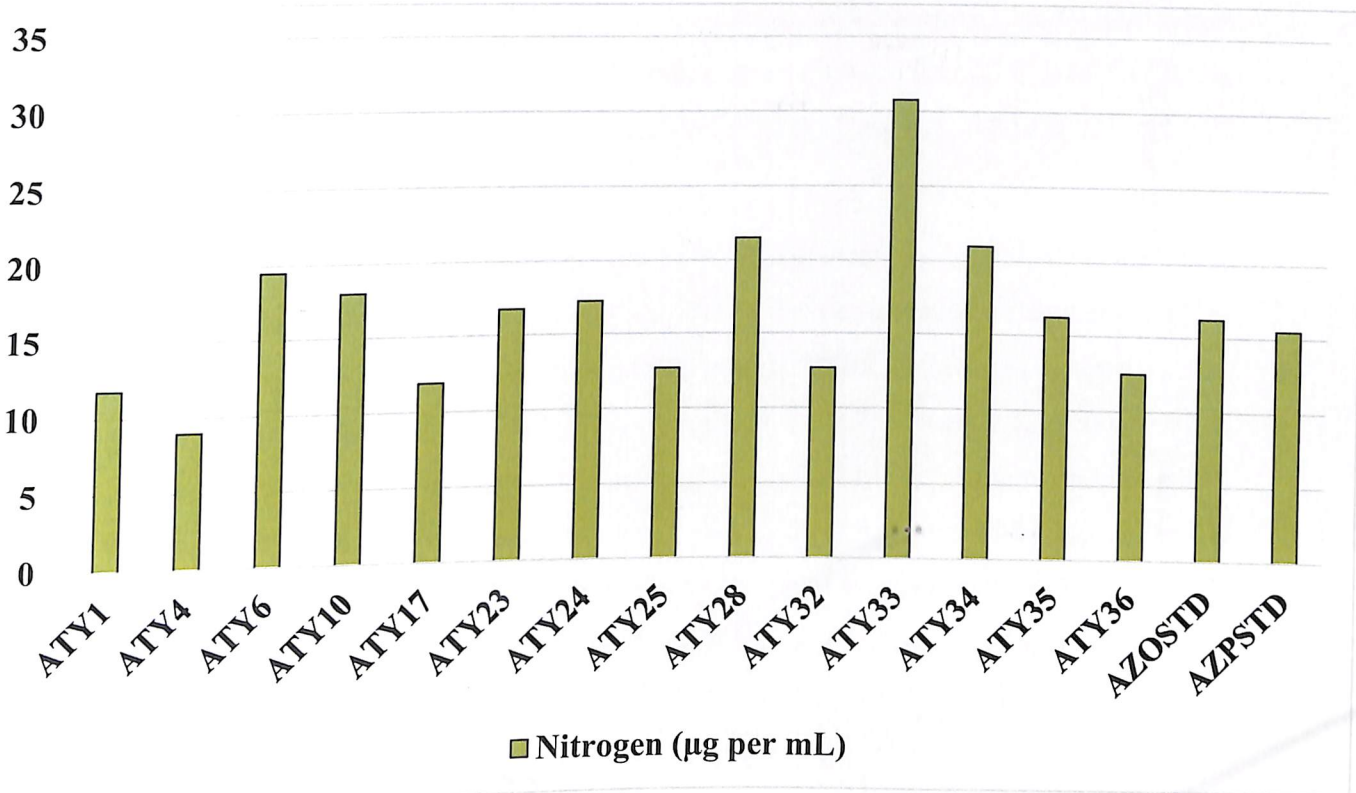


Figure 5. Estimation of nitrogen content of broth cultures of NFB isolates by micro-Kjeldahl method

Solubilizing Index of the strains were 2.82±0.025, 2.72±0.021 and 2.72±0.096, respectively (Fig. 6). Similar results were obtained by Karpagam and Nagalakshmi (2014) with three strains of phosphorus solubilizing bacteria psm1, psm2, psm6 which showed maximum Phosphate Solubilization Index (PSI) of 2.23, 2.15 and 2.11 in agar plates along with soluble phosphate production of 0.37 mg L^{-1}, 0.30 mg L^{-1} and 0.28 mg L^{-1} respectively in broth assay.

The broth assay of phosphate solubilizing bacteria exhibited quantitative measure of phosphorus solubilization on 10 days incubated broth culture and highest amount was recorded by *Burkholderia territorii* PSB1 (49.94±0.17 mg L^{-1}) and *Burkholderia cepacia* PSB3 (49.94±0.17 mg L^{-1}) (Fig. 7). Saddick (2020) identified *Burkholderia* sp. with maximum concentration of soluble P (84.8 mg of soluble P L^{-1}) isolated from rice while the lowest concentration was reported as 10.85 mg of soluble P L^{-1}. Similarly, Lin *et al.*, 2006 studied the P solubilization mechanism of *Burkholderia cepacia* CC-Al74 with high ability for solubilizing tricalcium phosphate and also, demonstrated that the P-solubilization increased from 1 g mL^{-1} to 200 g mL^{-1} during exponential growth, when the pH decreased from 8 to 3.

The best six bacterial isolates of NFB and PSB isolates were selected based on the weighted average ranking due to variations in activity of plant growth promoting traits by the bacterial isolates. Based on this, the isolates ATY10, ATY34, ATY35, PSB1, PSB3 and PSB4 were selected and molecular characterization of these isolates were done at Rajiv Gandhi Centre for Biotechnology (RGCB), Thiruvananthapuram. The isolates were identified as *Pantoea agglomerans* ATY10, *Rhizobium* sp. ATY34, *Ensifer adhaerens* ATY35, *Burkholderia territorii* PSB1, *Burkholderia cepacia* PSB3 and *Aeromonas hydrophila* PSB4 based on 16S rRNA sequence homology. Sergeeva *et al.*, reported IAA producing *Pantoea agglomerans* from the rhizosphere soil of canola. Jaya *et al.* (2019) identified ACC deaminase gene encoding rhizobacteria *Burkholderia territorii* from pineapple rhizosphere. Also, *Ensifer adhaerens* was isolated from rhizosphere of tobacco by Lei *et al.* (2009).

Seed germination and seed vigour are considered as two major factors that influence crop yield both by direct and indirect effects. The direct effects include seed quality that affects the plant performance. The indirect effects include germination

percentage and the time taken from sowing to emergence which ultimately affects the plant population density (Ellis, 1992). Roll towel assay was performed to assess the effect of the selected bacterial isolates on seed germination and seedling vigour of ragi seeds variety CO15. Roll towel assay helped in maintaining optimum level of moisture necessary to assess the germination of seeds and to evaluate the effect of bacterial biopriming in ragi seeds. According to the formula given by Abdul-Baki and Anderson (1973) seedling vigour index was calculated. In roll towel assay the seed bacterization with the selected isolates showed significant improvement in the plant parameters such as plant height, weight and biomass over control. The effect of bioprimed seeds with plant growth promoting bacteria was reported by many researchers (Negi *et al.*, 2019; Roslan *et al.*, 2020) which showed a comparably positive result over seeds maintained as control.

The current study revealed that the maximum germination was recorded in *Pantoea agglomerans* ATY10 and *Aeromonas hydrophila* PSB4 treated seeds and the minimum in control (Fig. 8) and the maximum germination percentage by *Pantoea agglomerans* ATY10 might be due to the highest production of gibberellins which is similar to the study conducted by Feng *et al.* (2006) who reported that rice endophyte *Pantoea agglomerans* YS19 promoted the growth on pre-milk stage in rice by promoting the transportation of photosynthetically assimilated products from source to sink and are due to the enhanced production of phytohormones and enhanced nitrogen fixing activity by bacteria. Quecine *et al.* (2012) reported that sugarcane plants were observed to produce more dry mass 30 days after inoculation of *Pantoea agglomerans* 33.1.

Besides, seed germination and seedling vigour index, significant difference was observed between treatments in plant parameters such as shoot length, root length, fresh weight, dry weight and root shoot ratio. Nitrogen fixing bacteria *Pantoea agglomerans* ATY10 bioprimed ragi seeds variety CO15 recorded higher 30.3 per cent increase in shoot length, 66.8 percent in root length, and 54.1 percent increase in seedling length (Fig. 9). Similar results were observed by Xie *et al.* (2017) in mulberry seedlings treated with *Pantoea agglomerans* SWg2. Significant increase in seedling development with increase in length of plumule and radicle of mulberry seedlings was observed. The

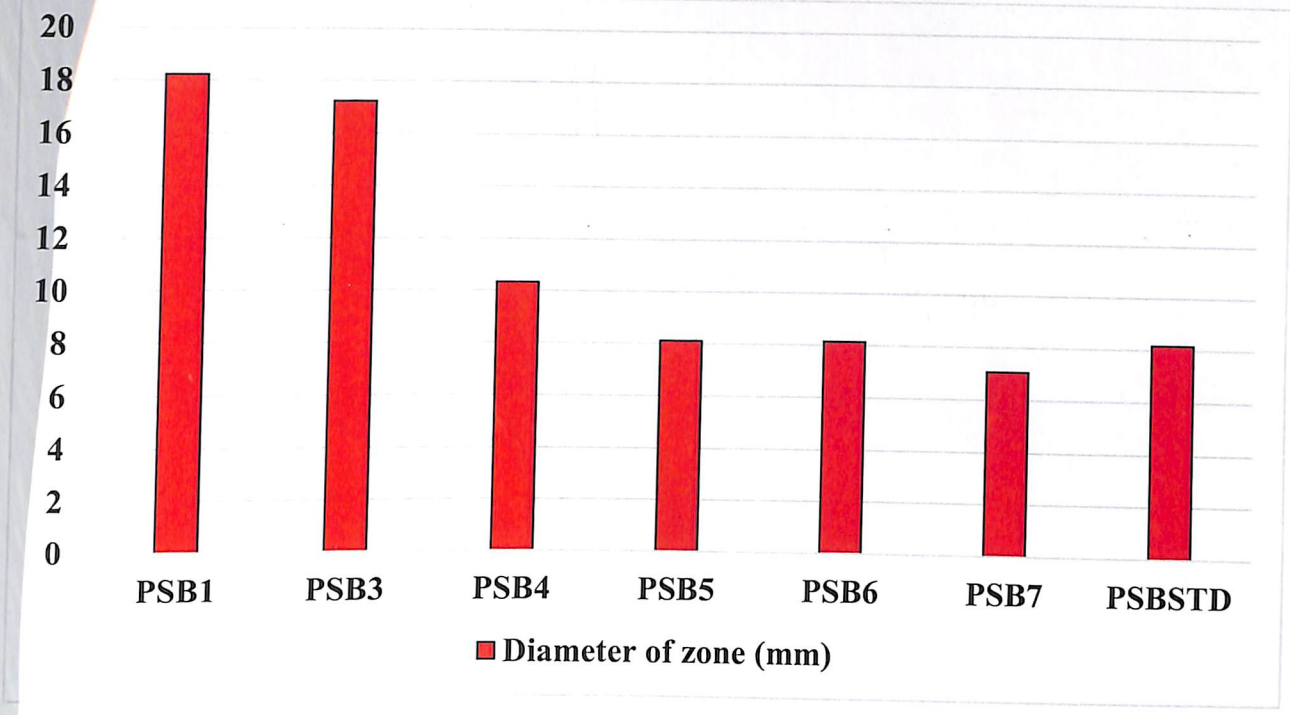


Figure 6. Qualitative analysis of phosphate solubilization by PSB isolates on Pikovskaya's agar

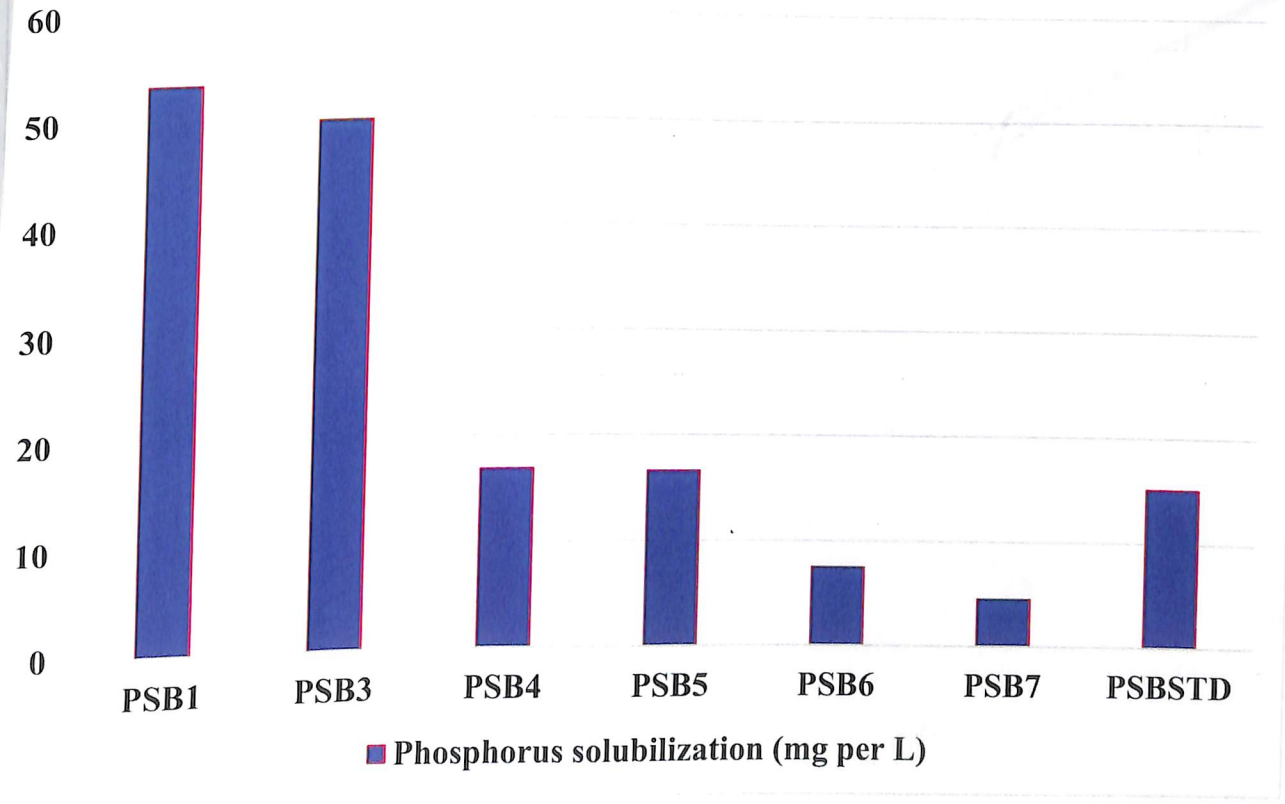


Figure 7. Estimation of phosphate solubilization by PSB isolates in Pikovskaya's broth

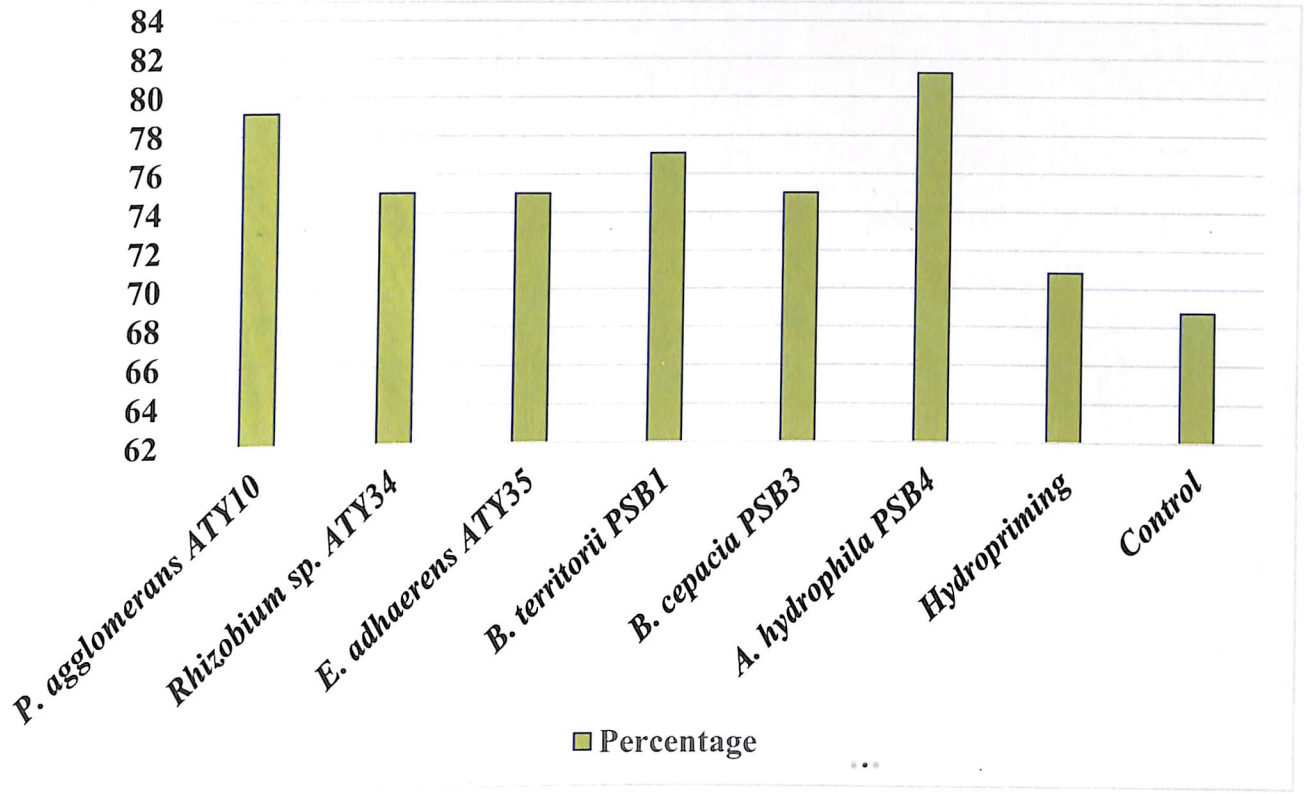


Figure 8. Effect of biopriming of ragi seeds with selected NFB and PSB isolates on germination percentage

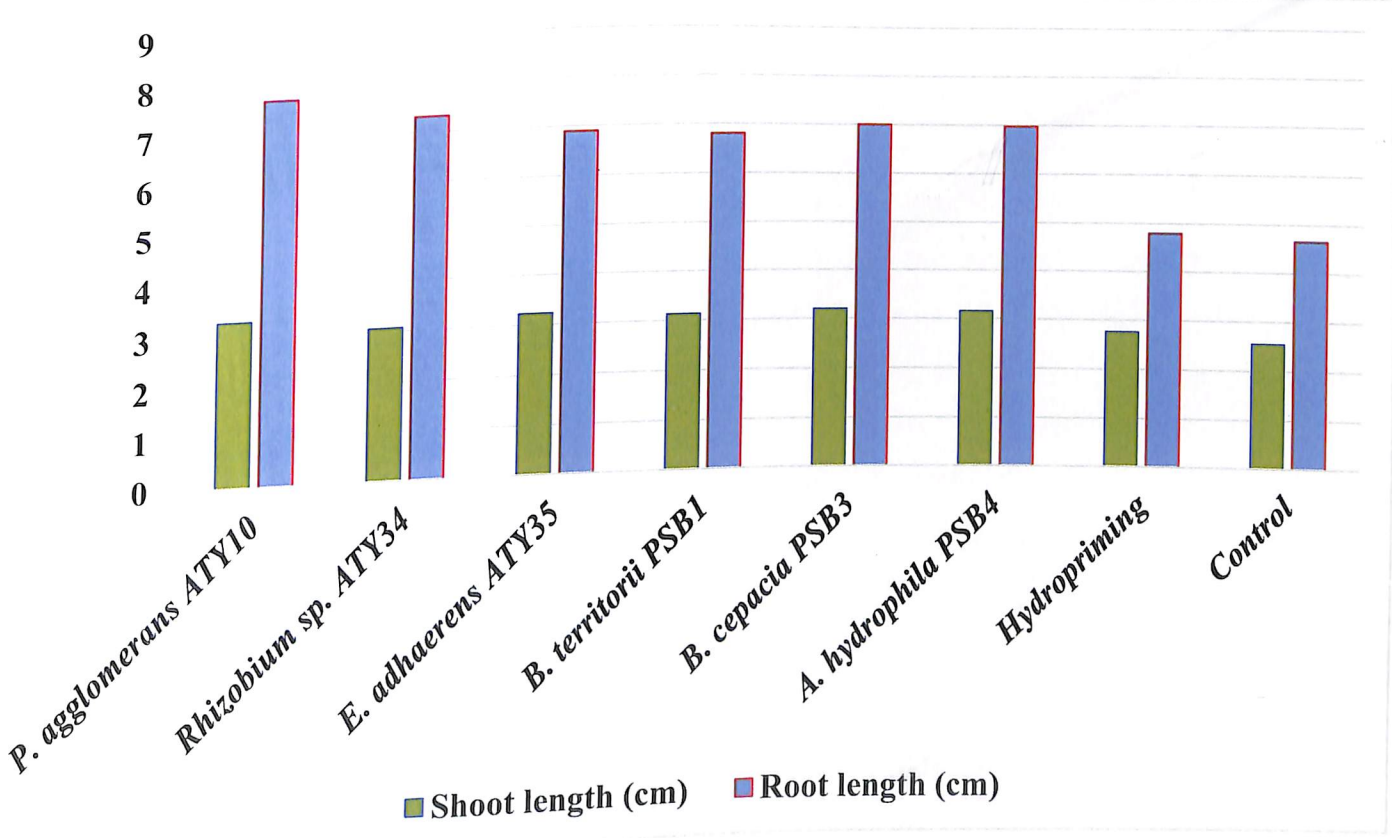


Figure 9. Effect of biopriming of ragi seeds with selected NFB and PSB isolates on shoot and root length of seedlings

increase in shoot and root length of ragi seedling by *Pantoea agglomerans* ATY10 was mainly due to the highesr production of IAA. The auxin IAA is known to strongly affect root growth and architecture. Exogenous IAA of rhizobacterial origin can increase root length and biomass and enhance plant growth by regulating the expression of host genes related to auxin response, defense, hormone and cell wall synthesis (Ruzzi and Aroca, 2015; Backer *et al.*,2018).

Seedling vigour index was calculated using seed germination percentage, shoot length and root length. Seedling vigour index of the treated seeds were significantly high. The highest seedling vigour index was observed in *Pantoea agglomerans* ATY10 (876.62±61.92) treated ragi seeds and followed by the isolate *Aeromonas hydrophila* PSB4 (820.418±28.38). The fresh weight of shoot increased by 60.9 per cent and 57.4 per cent in *Pantoea agglomerans* ATY10 and *Rhizobium* sp. treated seeds respectively over control. Root fresh weight of *Burkholderia territorii* PSB1 and *Rhizobium* sp. ATY34 treated plants increased by 177.3 per cent and 175.9 per cent over control (Fig.10). Mei *et al.* (2021) has reported that *Pantoea agglomerans* treatment on tall fescue seedlings significantly increased plant growth.

The efficiency of different treatment was evaluated by analysing the data on ragi seedling dry weight, as fresh weight which affected by various factors of environmental and technical parameters. Therefore, dry weight determination was used rather than fresh weight determination for plant growth promotion studies (Bashan *et al.*, 2017; Huang *et al.*, 2017). *Burkholderia territorii* PSB1 and *Pantoea agglomerans* ATY10 treated seedlings recorded higher shoot dry weight and it was 25.6 and 24.6 percent more than control respectively. Ragi seedling root dry weight was increased by 96.8 per cent by *Pantoea agglomerans* ATY10 treated plant over control (Fig. 11). This is in accordance with the results obtained by Sharon *et al.* (2016) who reported increased root and shoot dry weight on treatment with *Pantoea* sp. Pot1 in tomato seedlings under greenhouse conditions.

Root shoot ratio was obtained from the ratio between the dry root mass and dry shoot mass, the higher root shoot ratio of *Pantoea agglomerans* ATY10 (0.59) exhibited greater root density, which was on par with *Rhizobium* sp. ATY34 (0.49) and *Aeromonas hydrophila* PSB4 (0.49). The lower root shoot ratio was reported by control

(0.36). Saghafi *et al.* (2018) evaluated the influence of *Rhizobium* treatment on canola. The study recorded increased plant height by 35 per cent and dry weight of shoot by 26 per cent over control.

The six selected isolates of Nitrogen fixing bacteria and phosphate solubilizing bacteria were assessed for multiple traits such as antifungal activity against plant pathogens Of the six isolates tested for biocontrol potential *Burkholderia territorii* PSB1 and *Burkholderia cepacia* PSB3 showed antagonistic activity against all the four test pathogens, siderophore production, phosphorus, potassium and silicon solubilizing capacity.

Ragi is susceptible to various plant pathogenic fungal disease which includes foot rot by *Sclerotium rolfsi*, Brown spot by *Helminthosporium* sp., Banded blight by *Rhizoctonia solani* and head mold by *Fusarium* sp. (Madhuri *et al.*, 2009; Sawant *et al.*, 2020; Nagaraja and Reddy, 2010; Chala *et al.*, 2019; Mohammadi and Darvishnia, 2014). Antifungal activity of the six selected isolates was assessed by dual culture plate technique against plant pathogenic fungi *viz.*, *Rhizoctonia solani, Sclerotium rolfsii, Fusarium* sp. and *Helminthosporium* sp.

Three isolates were found to inhibit the mycelial growth of *R. solani.* The maximum zone of inhibition of 3.16±0.11 mm was exhibited by *Burkholderia territorii* PSB1 followed by *Burkholderia cepacia* PSB3 (1.5±0.00 mm) and *Pantoea agglomerans* ATY10 (0.73±0.05 mm). Barnett *et al.* (2006) reported that *R. solani* induced disease in wheat was suppressed on inoculation with *Pantoea, Exiguobacterium,* and Microbacteria bacterial groups. Shabanamol *et al.* (2018) reported the biocontrol activity of nitrogen fixing endophytic bacteria *Lysinibacillus sphaericus* against *R. solani* in rice.

The antagonistic activity of the selected isolates against *Sclerotium rolfsii* was assessed and two isolates *Burkholderia territorii* PSB1 and *Burkholderia cepacia* PSB3 recorded 0.58±0.05 mm and 0.55±0.00 mm of zone of inhibition respectively. The effectiveness of *Burkholderia cepacia* T1A-2B, and *Pseudomonas* sp. T4B-2A were studied by De Curtis *et al.* (2010) in tomato against *S. rolfsii* and *R. solani* and reported

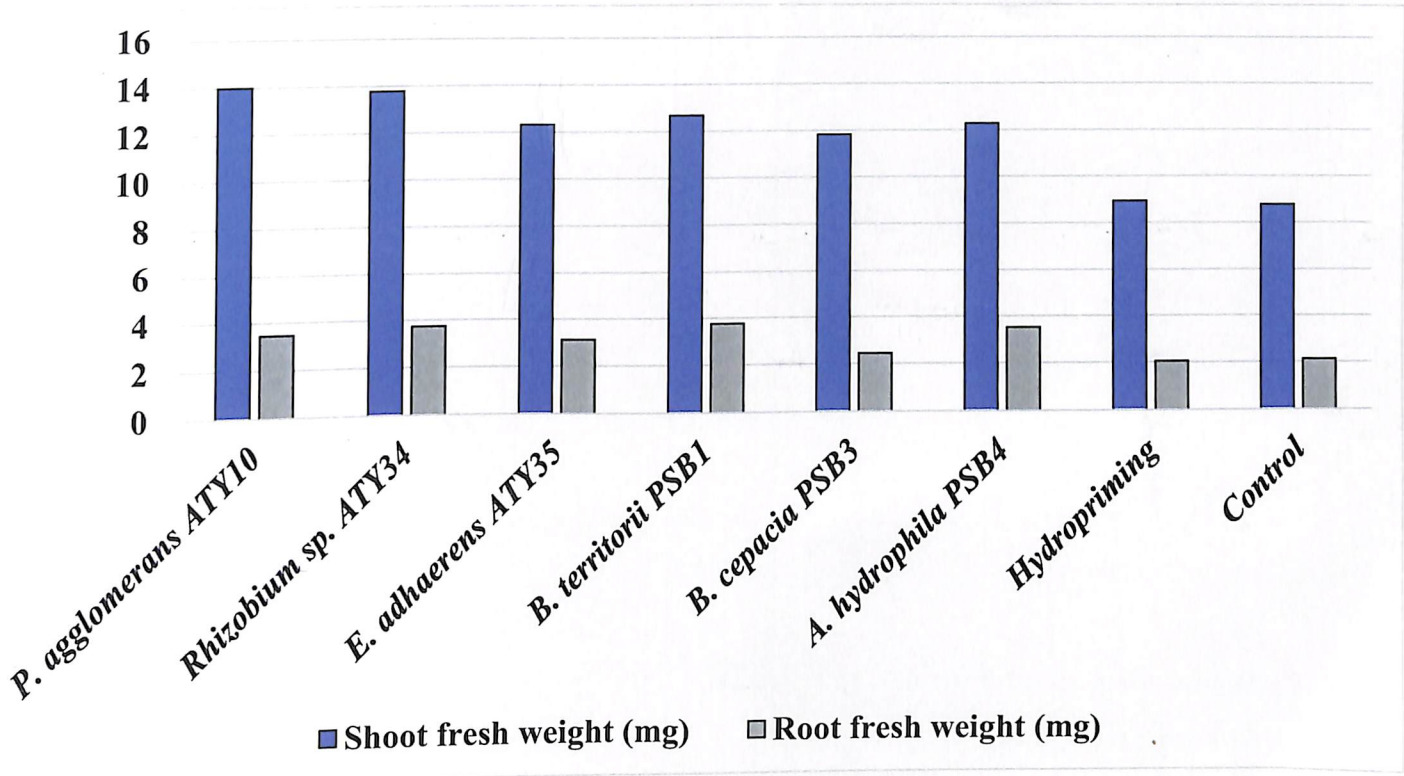


Figure 10. Effect of biopriming of ragi seeds with selected NFB and PSB isolates on plant fresh weight

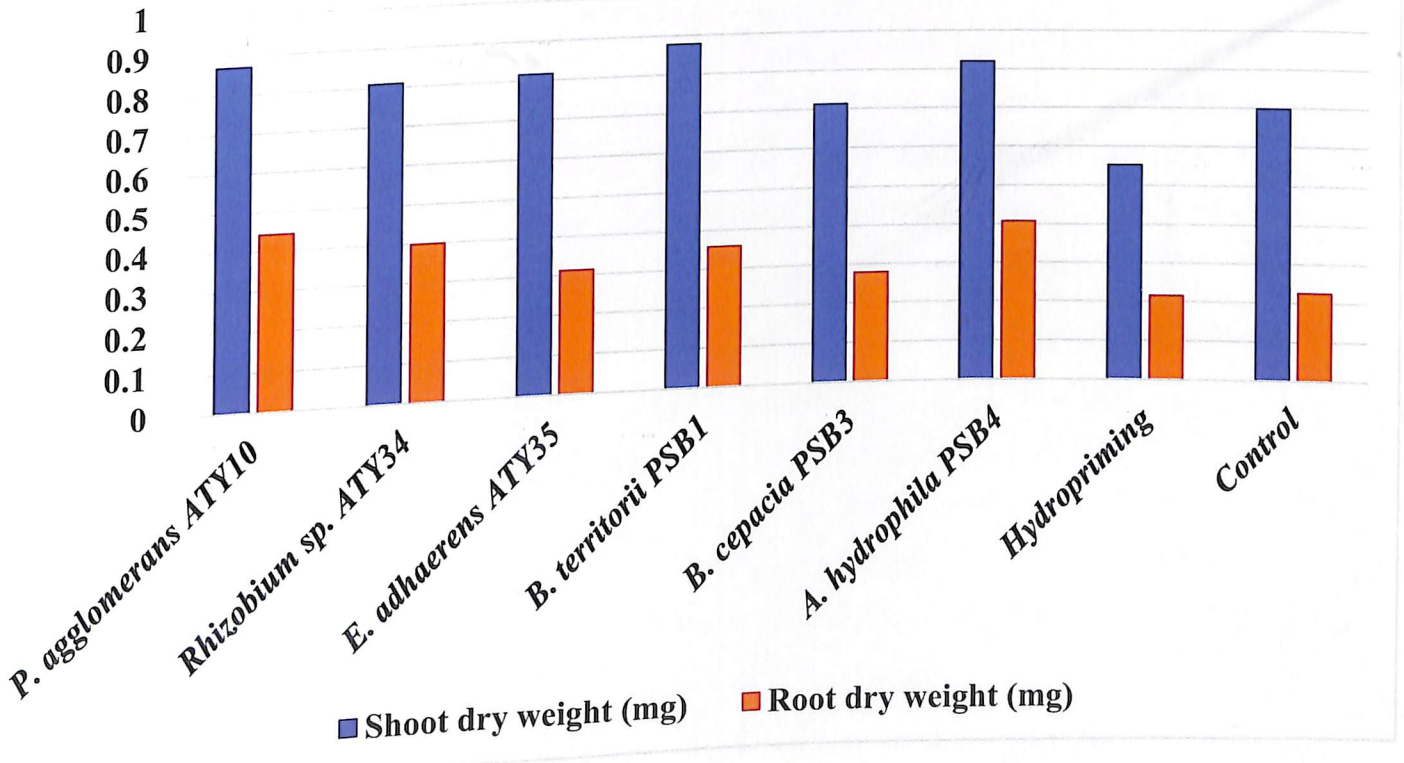


Figure 11. Effect of biopriming of ragi seeds with selected NFB and PSB isolates on plant dry weight

that the strain *Burkholderia cepacia* T1A-2B had reported 58.33 percent and 63.8 per cent disease suppression against *S. rolfsii* and *R. solani* respectively.

The maximum antagonistic activity against *Fusarium* sp. was recorded by *Burkholderia cepacia* PSB3 with 4.13±0.05 mm of zone of inhibition followed by the isolates *Pantoea agglomerans* ATY10 (3.13±0.05 mm) and the minimum was reported by *Burkholderia territorii* PSB1 (2.76±0.05 mm). Similar results for the antifungal activity against Fusarium was reported by other scientists as well. Zhizhou *et al.* (2020) identified an antifungal phenazine-1-carboxylic acid (PCA) produced by the strain *Burkholderia* sp. HQB-1 effective against *Fusarium oxysporum* with lowest minimum inhibitory compound of 1.56 $\mu g\ mL^{-1}$. Yu *et al.* (2010) reported *Pantoea agglomerans* strain 59-4 to suppress green and blue mold on mandrins. *Pantoea* strains reported to produce natural products with antibiotic activity, such as pantocins, herbicolins, microcins and phenazines against pathogenic fungi and bacteria (Walterson and Stavrinides, 2015).

Among six isolates two had antagonistic activity against *Helminthosporium* sp. The bacterial isolate *Burkholderia cepacia* PSB3 reported 5.66±0.28 mm zone of inhibition against *Helminthosporium* sp. and *Burkholderia territorii* PSB1 reported 4.17±0.28 mm. Zhao *et al.* (2014) reported maximum antifungal activity of plant growth promoting phosphate solubilizing *Burkholderia cepacia* SCAUK0330 isolated from maize rhizosphere against *Helminthosporium maydis* SCAU3321.

Of the six isolates tested for biocontrol potential *Burkholderia territorii* PSB1 and *Burkholderia cepacia* PSB3 showed antagonistic activity against all the four test pathogens. This is in accordance with results obtained by Zhao *et al.* (2014) which showed that the isolate *Burkholderia cepacia* SCAUK0330 had broad antimicrobial activity against a wide range of plant pathogens including *Rhizoctonia solani, Sclerotium rolfsii, Fusarium* sp. and *Helminthosporium* sp. that usually cause serious loss in agricultural production.

Siderophores are low-molecular-weight compounds that chelate the ferric ion (Fe^{3+}) with high specific activity and function as transporters of Fe (III) into microbial cells under iron-limiting condition. The presence of siderophore production assessed

by spot inoculating the isolates on to chrome azurol S (CAS) agar plates and the results showed that only *Aeromonas hydrophila* PSB4 produced siderophore among the six bacterial isolates. Attappady soils are acidic (pH 5.5 to 5.7) where the siderophore producing bacteria could ensure iron chelation to some extent. Similar reports were obtained by Stintzi *et al.* (2000) in *Aeromonas hydrophila* strain 495A2 which was capable of producing four bis-catecholate siderophores, collectively named amonabactins. Also, Gupta and Gopal (2008) conducted research on the siderophore producing ability by the plant growth promoting bacteria and the research revealed that *Pseudomonas fluorescens, Azospirillum brasilense, Enterobacter* sp. were capable of producing siderophore under iron-limiting conditions.

Potassium has a key role in plant growth and metabolism. It is involved in drought tolerance and disease resistance (Heidari and Jamshid 2010). Potassium is required for activation of different enzymes stimulating physiological processes such as respiration, stomatal regulation, photosynthesis, osmoregulation etc. About 98 per cent of total potassium in soils is in unavailable and slowly available forms. Several bacteria are known to solubilize these large reserves of potassium in soil (Sharma *et al.*, 2016). Potassium solubilizing capacity of the selected isolates were evaluated through plate assay using Aleksandrov agar plates. In plate assay *Burkholderia territorii* PSB1 and *Burkholderia cepacia* PSB3 showed clear zone of potassium solubilization with diameter of zone 11.00±0.50 mm and 12.66±0.28 mm, respectively. *Burkholderia cepacia* was isolated by Zhang and Kong (2014) as potassium solubilizing bacteria found to solubilize k-feldspar powder in solid and liquid medium. Meena *et al.* (2014) reported that the potassium solubilizing mechanism of bacteria was by the release of certain organic acids.

None of the NFB isolates solubilized insoluble tricalcium phosphate in Pikovoskaya's media. None of the selected isolates solubilized silicates contained in media in the form of magnesium trisilicate, by forming clear zone of solubilization around the bacterial colonies on agar plates. The general mechanism of silicate solubilization is by acidification. The lack of silicon solubilizing ability might be due to the absence of specific organic acid (citric, malic, tartaric and gluconic acid) which help in solubilization of magnesium trisilicate. Study conducted by Vasanthi *et. al.*,

(2016) revealed that bacterial isolates which did not solubilize silicate in plate assay were capable of solubilizing silicates in broth assay and the extent of solubilization varied with the species, silicate mineral used and the incubation period. In their study they identified the production of a variety of organic acids including citric, malic, tartaric, hydroxypropionic acid, gluconic, oxalic acid by different *Bacillus* spp.

The current study revealed that *Pantoea agglomerans* ATY10 could be a promising nitrogen fixing bacteria with plant growth promotion activity. The increase in plant growth promotion might be due to the higher production of IAA and GA by *Pantoea agglomerans* ATY10. *Burkholderia territorii* PSB1and *Burkholderia cepacia* PSB3 are observed to be efficient phosphate solubilizers with strong biocontrol activity against plant pathogenic fungi. Nitrogen fixing and phosphate solubilizing isolates with potential to be applied as biofertilizer and biocontrol agents for sustainable agriculture has been identified in this study. They can be exploited for commercial production after further evaluations.

SUMMARY

6. SUMMARY

Biofertilizers are an important source of essential nutrients for plant growth and development and plays a major role in maintaining soil fertility and improves soil health. Biofertilizers are eco-friendly, cost effective, non-toxic and is an alternative for chemical fertilizers. They involve in plant growth promotion by atmospheric nitrogen fixation, solubilizing essential elements such as phosphorus, potassium, silicon, zinc etc. and also, by producing phytohormones such as auxin, gibberellin, cytokinin, ACC. Some are involved in biocontrol activity against plant pathogens.

The economy, livelihood and nutritional status of tribal people of Attappady hill tract depend on millet cultivation. The tribal farming system followed for millet cultivation resort to very less use of chemical fertilizers, which ensures a huge microbial biomass in soil. This helps to maintain soil fertility and promotes crop growth. Therefore, isolating and analyzing the beneficial microorganisms present in these regions have more significance. The present study entitled "Isolation and characterization of beneficial rhizosphere microorganisms from ragi grown in Attappady hill tract of Kerala", was conducted in the Department of Agricultural Microbiology, College of Agriculture, Vellayani, Thiruvananthapuram.

The main objective of the current study was to isolate and screen putative NFB and PSB isolates based on the analysis of plant growth promotion activity and *in vitro* plant growth promotion study using roll towel assay. Further, the selected isolates were identified by molecular characterization and tested for their multiple traits such as bio control activity, siderophore production and potentiality in solubilizing essential element such as P, K and Si. The salient findings of the current study are summarized as below.

A total of forty-four bacteria were isolated from root and soil samples collected from ragi fields in Attappady region using different N-free media and Pikovskaya's agar medium. In these, thirty-six were nitrogen fixers (NFB) and eight were phosphate solubilizers (PSB). The isolates were designated as ATY1-ATY36 for NFB isolates and PSB1-PSB8 for PSB isolates. After screening based on colony morphological characters twenty isolates were, selected in order to avoid duplication of isolates. All twenty isolates were characterized by morphology and biochemical tests.

The twenty selected isolates were subjected to plant growth promotion study. Further, nitrogen content estimation was done in NFB isolates. Plate assay and broth assay was carried out for PSB isolates using Pikovskaya's medium.

The Indole Acetic Acid (IAA) production by all the twenty isolates showed a wide variation ranging from 101.22 μg mL^{-1} to 3.26 μg mL^{-1}. The isolate ATY10 showed maximum IAA production of 101.22 μg mL^{-1}. Gibberellic Acid (GA) production of all the twenty isolates were done and the results ranged between 10.07±2.41 μg mL^{-1} and 2.18±0.44 μg mL^{-1}. The maximum GA production was recorded by the isolate ATY10 with 10.07±2.41 μg mL^{-1}.

All the twenty isolates were subjected to the estimation of extracellular ammonia production. The extracellular ammonia of the bacterial isolates ranged between 176.53±5.07 μmol mL^{-1} and 75.59±10.85 μmol mL^{-1}. The nitrogen content of selected NFB isolates ranged between 30.75±3.88 μg mL^{-1} and 9±2.82 μg mL^{-1}. The bacterial isolate ATY33 produced maximum nitrogen content of 11.75±1.06 μg mL^{-1}

The six phosphate solubilizing bacteria obtained through isolation process were subjected to plate assay and broth assay using Pikovskaya's medium supplemented with tricalcium phosphate. The clear zone of phosphate solubilization ranged between 18.23±0.25 mm and 7.06±0.11 mm. The maximum zone of solubilization was recorded by PSB1 with 18.23±0.25 mm. The phosphate solubilizing index was calculated using diameter of colony and zone of solubilization. The phosphate solubilizing index ranged from 2.72±0.021 to 2.11±0.019. The maximum phosphate solubilizing index was recorded by PSB1 (2.82±0.025).

The quantification of phosphate solubilization of selected six isolates ranged between 53.41±1.72 mg L^{-1} and 4.54±0.21 mg L^{-1}. The isolate PSB1 recorded maximum value of phosphate solubilization of 53.41±1.72 mg L^{-1}.

Three bacterial isolates each of nitrogen fixers and phosphate solubilizers were screened based on weighted average ranking. The isolates ATY10, ATY34 and ATY35 were selected as the best three nitrogen fixing bacterial isolates and the isolates PSB1, PSB3 and PSB4 were selected as the best three phosphate solubilizers based on plant

growth promotion activity, nitrogen content, zone of phosphate solubilization and quantification of phosphorus solubilization.

The selected isolates were subjected to molecular characterization and were identified as *Pantoea agglomerans* ATY10, Rhizobium sp. ATY34, *Ensifer adhaerens* ATY35, *Burkholderia territorii* PSB1, *Burkholderia cepacia* PSB3 and *Aeromonas hydrophila* PSB4 as per 16s rRNA sequence.

Effect of biopriming of selected NFB and PSB isolates were assessed by *in vitro* plant growth promotion study in ragi seeds using roll towel assay. The study showed a significant increase in germination percentage, days taken for germination, plant height, plant biomass, seedling vigour index and root shoot ratio over control. The maximum germination percentage was recorded by the treatment *Aeromonas hydrophila* PSB4 with 81.24±4.16 percent. The number of days taken for germination was recorded lower by *Pantoea agglomerans* ATY10 treated seeds.

Under roll towel assay the isolate *Pantoea agglomerans* ATY10 treated seeds recorded maximum shoot length (3.31±0.11 cm), root length (7.76±0.70 cm) and seedling length (11.08±0.65 cm). Also, *Pantoea agglomerans* ATY10 treated seeds showed highest seedling vigour index of 876.62±61.92. Highest shoot fresh weight was recorded in seedling treated with *Pantoea agglomerans* ATY10 (14.000±0.359 mg) and the highest root fresh weight was recorded by seedlings treated with *Burkholderia territorii* PSB1 (3.762±0.118 mg). The maximum dry weight of shoot was observed in the *Burkholderia territorii* PSB1 treated plant with 0.877±0.013 mg and the maximum root dry weight was recorded by the treatment *Pantoea agglomerans* ATY10 with 0.443±0.017 mg. Root shoot ratio was obtained by dividing the dry root mass and the dry shoot mass, the higher root shoot ratio of *Pantoea agglomerans* ATY10 (0.50) showed to have greater root density.

The selected NFB and PSB isolates were further subjected to dual culture plate assay, siderophore production, potentiality of solubilization of P, K and Si for assessing their multiple traits towards plant growth promotion. *Burkholderia territorii* PSB1 showed maximum zone of inhibition of 3.16±0.11 mm against *Rhizoctonia solani* and 0.58±0.05 mm against *Sclerotium rolfsii.* The maximum antagonistic activity against

Fusarium sp. was recorded by the PSB3 with 4.13±0.05 mm of zone of inhibition. Also, the isolate *Burkholderia cepacia* PSB3 recorded 5.66±0.28 mm of zone of inhibition against *Helminthosporium* sp.

Among the selected isolates, only *Aeromonas hydrophila* PSB4 showed siderophore production in Chrome Azurol S (CAS) agar medium plates. The isolate *Burkholderia territorii* PSB1 and *Burkholderia cepacia* PSB3 were observed to solubilize potassium alumino silicate present in Alexsandrov medium with 11.00±0.50 mm and 12.66±0.28 mm diameter of zone of inhibition. None of the selected isolates solubilized silicate in magnesium trisilicate amended Bunt and Rovira medium. Also, none of the NFB isolates solubilized tricalcium phosphate supplemented in Pikovskaya's agar medium. Only three PSB isolates *Burkholderia territorii* PSB1 (17.83±0.28 mm), *Burkholderia cepacia* PSB3 (16.5±0.00 mm) and *Aeromonas hydrophila* PSB4 (9.21±0.05 mm) were found to produce clear zone of solubilization around the colonies in Pikovskaya's agar medium.

In the present study, *Pantoea agglomerans* ATY10 treated plants showed increased plant growth parameters in roll towel assay which was due to the production of phytohormones such as IAA and GA in a significantly higher concentration compared to other isolates. Also, the isolates *Burkholderia territorii* PSB1 and *Burkholderia cepacia* PSB3 was effective against *Rhizoctonia solani, Sclerotium rolfsii, Fusarium* sp. and *Helminthosporium* sp. under *in vitro* study and also exhibited higher potential in solubilizing tricalcium phosphate and potassium alumino silicate.

Further studies on the selected NFB and PSB isolates may be done to assess the response of these isolates under *in vivo* conditions in plant growth promotion and disease suppression activity.

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APPENDICES

APPENDIX-I

COMPOSITION OF MEDIA

1. Jensen's Media

Sucrose	– 20.0g
K_2HPO_4	– 1.0g
$MgSO_4.7H_2O$	– 0.5g
Na_2MoO_4	– 0.001g
$FeSO_4.7H_2O$	– 0.01g
$CaCO_3$	– 2.0g
Agar	– 20.0 g
Distilled water	– 1000 mL
pH	– 7.0-7.2

Sucrose, K_2HPO_4, $MgSO_4.7H_2O$, Na_2MoO_4, $FeSO_4.7H_2O$ and $CaCO_3$ were dissolved in 500 mL distilled water and volume made upto 1000 mL. It was then distributed to 250 mL flask of 100 mL each each and pH was adjusted. Two grams of agar-agar was added to each flask and autoclaved at 15 lbs pressure and 121°C for 15 minutes.

2. Waksman No. 77 Media

$(NH_4)_2SO_4$	- 0.2g
KH_2PO_4	- 3.0g
$MgSO_4.7H_2O$	- 0.5g
$CaCl_2.6H_2O$	- 0.025g
$FeSO_4.7H_2O$	- trace
Agar	- 20.0g
S^0	- 10
Distilled water	- 1000 mL

$(NH_4)_2SO_4$, KH_2PO_4, $MgSO_4.7H_2O$, $CaCl_2.6H_2O$ and $FeSO_4.7H_2O$ were dissolved in 500 mL distilled water and volume made upto 1000 mL. It was then distributed to 250 mL flask of 100 mL each. Two grams of agar-agar was added to each flask and autoclaved at 15 lbs pressure and 121°C for 15 minutes.

3. Burk's Meida

$MgSO_4$	- 0.2g
K_2HPO_4	- 0.8g
KH_2PO_4	- 0.2g
$CaSO_4$	- 0.13g
$FeCl_3$	- 0.0014g
Sodium molybdate	- 0.00025g
Sucrose	- 20.0g
Agar	- 20.0g
Distilled water	- 1000 mL

$MgSO_4$, K_2HPO_4, KH_2PO_4, $CaSO_4$, $FeCl_3$, Sodium molybdate and sucrose were dissolved in 500 mL distilled water and volume made upto 1000 mL. It was then distributed to 250 mL flask of 100 mL each. Two grams of agar-agar was added to each flask and autoclaved at 15 lbs pressure and 121°C for 15 minutes.

4. N-Free Malate Bromothymol Blue Media

L-Malic	-5g
K2HPO4	-0.5g
MgSO4. 7H2O	-0.2g
NaCl	-0.02g
Trace element solution	-2mL
Bromothymol blue	-2mL
Fe EDTA	-4mL
KOH	-4g
Agar	-20.0g
Distilled water	-1000 mL
pH	-6.8

L-Malic, K2HPO4, MgSO4. 7H2O, NaCl, Trace element solution, Bromothymol blue and Fe EDTA were dissolved in 500 mL distilled water and volume made upto 1000 mL and pH was adjusted with KOH solution. It was then distributed

to 250 mL flask of 100 mL each. Two grams of agar-agar was added to each flask and autoclaved at 15 lbs pressure and 121°C for 15 minutes.

5. Pikovskaya's Media

Glucose	- 10.0g
Ca3(PO4)2	- 5.0 g
(NH4)2SO4	- 0.5 g
Yeast extract	- 0.5 g
KCl	- 0.2 g
MgSO4	- trace
FeSO4	- trace
Agar-agar	- 20.0 g
Distilled water	- 1000 mL
pH	- 7.0

Glucose, Ca3(PO4)2, (NH4)2SO4, Yeast extract, KCl, MgSO4, and FeSO4 were dissolved in 500 mL distilled water and volume made up to 1000 mL. Twenty grams agar-agar was added into this mixture and autoclaved at 15 lbs pressure and 121 °C for 15 minutes.

6. Aleksandrov Media

Aleksandrov agar	- 29.6 g
Distilled water	- 1000 mL
Agar-agar	- 5.0 g
pH	- 7.2 ± 0.2

Ready-made (Hi-media) Aleksandrov agar was dissolved in 500 mL distilled water and volume made up to 1000 mL and pH was adjusted. Five grams agar-agar was added into this mixture and autoclaved at 15 lbs pressure and 121 °C for 15 min.

7. Potato Dextrose Agar

Peeled and sliced potatoes	- 200.0 g
Dextrose	- 20.0 g
Agar-agar	- 20.0 g
Distilled water	- 1000 mL

Potatoes were boiled in 500 mL of distilled water and the extracts was collected by filtering through a muslin cloth. Agar-agar was dissolved separately in 500 mL of distilled water. The potato extract was mixed in the molten agar and 20 g of dextrose was dissolved in the mixture. The volume was made up to 1000 mL with distilled water and medium was sterilized at 15 lbs pressure and 121°C for 15 minutes.

8. Simmon's citrate agar

Sodium citrate	- 0.2 g
Magnesium sulphate	- 0.02 g
Ammonium dihydrogen	- 0.1 g
Phosphate	- 15g
Agar	- 20.0g
pH	- 6.8

Sodium citrate, Magnesium sulphate, Ammonium dihydrogen, Phosphate and agar were dissolved in 500 mL distilled water and volume made upto 1000 mL. It was then distributed to test tubes and autoclaved at 15 lbs pressure and 121°C for 15 minutes.

9. Nutrient Gelatin

Beef extract	- 3.0g
Peptone	- 5.0g
Gelatin	- 120g
Agar	- 20.0g
DW	– 1000 mL
pH	- 6.6-7.0

Beef extract, peptone, gelatin and agar were dissolved in 500 mL distilled water and volume made upto 1000 mL. It was then distributed to test tubes and autoclaved at 15 lbs pressure and 121°C for 15 minutes.

10. SIM medium

Casein digest	- 20g
Peptic digest	- 6.1g
Ferrous ammonium sulphate	- 0.2g
Sodium thiosulfate	- 0.2g
Agar	- 3.5g
DW	- 1000 mL

Casein digest, Peptic digest, Ferrous ammonium sulphate, Sodium thiosulfate and agar were dissolved in 500 mL distilled water and volume made upto 1000 mL. It was then distributed to test tubes and autoclaved at 15 lbs pressure and 121°C for 15 minutes.

11. Starch agar

Beef extract	- 3.0g
Soluble starch	- 10g
Agar	- 12g
DW	- 1000 mL

Beef extract and soluble starch were dissolved in 500 mL distilled water and volume made up to 1000 mL. Twenty grams agar-agar was added into this mixture and autoclaved at 15 lbs pressure and 121 °C for 15 minutes.

12. CMC (Carboxymethylcellulose) agar medium

NaCl	- 6.0g
$(NH_4)SO_4$	- 1.0g
KH_2PO_4	- 0.5g
K_2HPO_4	- 0.5g
$MgSO_4$	- 0.1g
$CaCl_2$	- 0.1g
CMC	- 0.1%
Agar	- 20.0g
Distilled water	- 1000 mL

$NaCl$, $(NH_4)SO_4$, KH_2PO_4, K_2HPO_4, $MgSO_4$, $CaCl_2$ and CMC were dissolved in 500 mL distilled water and volume made up to 1000 mL. Twenty grams agar-agar was added into this mixture and autoclaved at 15 lbs pressure and 121 °C for 15 minutes.

13. Peptone broth

Peptone	-	10.0g
NaCl	-	5.0

Peptone and NaCl were dissolved in 500 mL distilled water and volume made up to 1000 mL. It was then distributed to test tubes of 10 mL each and autoclaved at 15 lbs pressure and 121 °C for 15 minutes.

14. MRVP broth

Peptone	-7.0g
K_2HPO_4	– 5.0g
Glucose	–5.0g
Distilled Water	-1000 mL
pH	- 6.7

Peptone, K_2HPO_4 and glucose were dissolved in 500 mL distilled water and volume made up to 1000 mL. It was then distributed to test tubes of 10 mL each and autoclaved at 15 lbs pressure and 121 °C for 15 minutes.

15. Reagent for the spectrophotometric estimation of Gibberellic acid

1. Zinc acetate solution: 21.9 g of zinc acetate was dissolved in 80 ml of distilled water. One ml of glacial acetate acid was added and the volume made upto 100 ml with distilled water.

2. Potassium ferrocyanide solution: 1.6 g of potassium ferrocyanide solution in 100ml of distilled water.

16. Reagent for siderophore production

Solution 1: 60.5 mg CAS in 50 ml de-ionized water and mixing it with 10 ml Fe^{3+} solution (containing 1 mmol/lit FeCl3 .6H2O in 10 mmol L^{-1} HCl).

Solution 2: 72.9 mg of hexa - decyl trimethyl ammonium bromide (HDTMA) in 40 ml de-ionized water, yielding a dark blue solution.

APPENDIX-II

COMPOSITION OF STAINS USED

1. Crystal violet

   One volume saturated alcohol solution of crystal violet in four volumes of one per cent aqueous ammonium oxalate.2.

2. Gram's iodine

   Iodine crystals - 1.0 g

   Potassium iodide - 2.0 g

   Distilled water - 300 mL

3. Safranin

   Ten ml saturated solution of safranin in 100 ml distilled water

4. Copper sulphate

   20% of copper sulphate was prepared by dissolving in distilled water

APPENDIX-III

BLASTN analysis data of 16S rRNA gene of bacterial isolates

a) *Pantoea agglomerans* ATY10

Sequences producing significant alignments — Download — Select columns — Show 10

select all 1 sequences selected — GenBank — Graphics — Distance tree of results — MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Pantoea agglomerans strain 3BQN7 16S ribosomal RNA gene, partial sequence	Pantoea agglomerans	2333	2333	98%	0.0	99.23%	1465	HM161866.1
Pantoea agglomerans strain BB7 16S ribosomal RNA gene, partial sequence	Pantoea agglomerans	2331	2331	98%	0.0	99.23%	1386	MT071499.1
Enterobacter sp. T2 chromosome, complete genome	Enterobacter sp. T2	2331	18458	98%	0.0	99.23%	4854376	CP048736.1
Enterobacter sichuanensis strain SGAir0282 chromosome, complete genome	Enterobacter sichuanensis	2331	18479	98%	0.0	99.23%	4711389	CP027986.1
Pantoea sp. strain HSTU-ASh65 16S ribosomal RNA gene, partial sequence	Pantoea sp.	2331	2331	98%	0.0	99.23%	1438	MN538300.1
Enterobacter kobei strain WCHEK045523 chromosome, complete genome	Enterobacter kobei	2331	18558	98%	0.0	99.23%	4953590	CP032897.1
Gamma proteobacterium BIWA50 gene for 16S ribosomal RNA, partial sequence	gamma proteobacterium BIWA50	2331	2331	98%	0.0	99.23%	1470	LC217437.1
Enterobacter sp. strain P18 16S ribosomal RNA gene, partial sequence	Enterobacter sp.	2331	2331	98%	0.0	99.23%	1404	KY084459.1
Leclercia adecarboxylata strain CIP 82.92 16S ribosomal RNA gene, partial sequence	Leclercia adecarboxylata	2331	2331	98%	0.0	99.23%	1454	KT825517.1
Leclercia adecarboxylata strain I1_5 16S ribosomal RNA gene, partial sequence	Leclercia adecarboxylata	2331	2331	98%	0.0	99.23%	1457	KT799662.1

b) *Rhizobium* sp. ATY34

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Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rhizobium sp. FSBRD3 16S ribosomal RNA gene, partial sequence	Rhizobium sp. FSBRD3	2074	2777	98%	0.0	96.36%	1792	KJ184987.1
Agrobacterium tumefaciens strain R6-364 16S ribosomal RNA gene, partial sequence	Agrobacterium tumefaciens	2073	2073	98%	0.0	96.35%	1412	JQ659820.1
Agrobacterium sp. SCAUS157 16S ribosomal RNA gene, partial sequence	Agrobacterium sp. SCAUS157	2071	2071	97%	0.0	96.42%	1399	KF836042.1
Uncultured bacterium clone MF9Y-B93 16S ribosomal RNA gene, partial sequence	uncultured bacterium	2067	2067	98%	0.0	96.28%	1448	KY609421.1
Agrobacterium tumefaciens strain R6-409 16S ribosomal RNA gene, partial sequence	Agrobacterium tumefaciens	2067	2067	98%	0.0	96.21%	1422	JQ659845.1
Agrobacterium tumefaciens strain R1-133 16S ribosomal RNA gene, partial sequence	Agrobacterium tumefaciens	2067	2067	98%	0.0	96.28%	1425	JQ659544.1
Beijerinckia fluminensis strain LTL4 16S ribosomal RNA gene, partial sequence	Beijerinckia fluminensis	2067	2067	98%	0.0	96.28%	1340	MT846027.1
Agrobacterium tumefaciens strain LKRO5 16S ribosomal RNA gene, partial sequence	Agrobacterium tumefaciens	2067	2067	98%	0.0	96.28%	1367	HQ331128.1
Agrobacterium tumefaciens strain XI 16S ribosomal RNA gene, partial sequence	Agrobacterium tumefaciens	2067	2067	98%	0.0	96.28%	1414	HM582868.1
Uncultured Agrobacterium sp. gene for 16S rRNA, partial sequence, clone: DHUP36	uncultured Agrobacterium sp.	2067	2067	98%	0.0	96.28%	1408	AB451540.1

c) *Ensifer adhaerens* ATY35

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Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Ensifer adhaerens strain JS1020 Soil 9_F3Ptero 16S ribosomal RNA gene, partial sequence	Ensifer adhaerens	2032	2032	99%	0.0	95.28%	1359	KX507144.1
Ensifer sp. strain PZS_S05 16S ribosomal RNA gene, partial sequence	Ensifer sp.	2032	2032	99%	0.0	95.28%	1366	KY992904.1
Ensifer adhaerens strain PZG_S19 16S ribosomal RNA gene, partial sequence	Ensifer adhaerens	2032	2032	99%	0.0	95.28%	1394	KY660614.1
Ensifer adhaerens strain PZG_S11 16S ribosomal RNA gene, partial sequence	Ensifer adhaerens	2032	2032	99%	0.0	95.28%	1410	KY660602.1
Ensifer adhaerens strain PZS_S05 16S ribosomal RNA gene, partial sequence	Ensifer adhaerens	2032	2032	99%	0.0	95.28%	1410	KY660583.1
Ensifer sp. A6(2012) 16S ribosomal RNA gene, partial sequence	Ensifer sp. A6(2012)	2032	2032	99%	0.0	95.20%	1356	JX941528.1
Ensifer sp. LC471 16S ribosomal RNA gene, partial sequence	Ensifer sp. LC471	2032	2032	99%	0.0	95.20%	1280	JQ014374.1
Sinorhizobium adhaerens 16S rRNA gene, strain HAMBI 1631	Ensifer adhaerens	2032	2032	99%	0.0	95.28%	1426	AJ420774.1
Ensifer sp. strain LXo18a 16S ribosomal RNA gene, partial sequence	Ensifer sp.	2030	2030	98%	0.0	95.41%	1302	MN830067.1
Ensifer sp. strain BR 14462 16S ribosomal RNA gene, partial sequence	Ensifer sp.	2025	2025	98%	0.0	95.33%	1271	MT904913.1

d) *Burkholderia territorii* PSB1

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Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Burkholderia territorii strain SH117 16S ribosomal RNA gene, partial sequence	Burkholderia territorii	2250	2250	99%	0.0	97.71%	1371	MK418836.1
Burkholderia cepacia strain HB21 16S ribosomal RNA gene, partial sequence	Burkholderia cepacia	2248	2248	98%	0.0	98.00%	1481	MT565309.1
Burkholderia sp. strain lsyb85 16S ribosomal RNA gene, partial sequence	Burkholderia sp.	2248	2248	98%	0.0	98.00%	1370	KY689937.1
Burkholderia sp. strain lsyb42 16S ribosomal RNA gene, partial sequence	Burkholderia sp.	2248	2248	98%	0.0	98.00%	1417	KY678897.1
Burkholderia cepacia SUPP2119 gene for 16S ribosomal RNA, partial sequence	Burkholderia cepacia	2248	2248	98%	0.0	98.00%	1323	LC507271.1
Burkholderia cepacia 17JN01 gene for 16S ribosomal RNA, partial sequence	Burkholderia cepacia	2248	2248	98%	0.0	98.00%	1323	LC507261.1
Burkholderia cepacia 17AO07 gene for 16S ribosomal RNA, partial sequence	Burkholderia cepacia	2248	2248	98%	0.0	98.00%	1323	LC507258.1
Burkholderia cepacia 17AK24 gene for 16S ribosomal RNA, partial sequence	Burkholderia cepacia	2248	2248	98%	0.0	98.00%	1323	LC507256.1
Burkholderia cepacia 16YM34 gene for 16S ribosomal RNA, partial sequence	Burkholderia cepacia	2248	2248	98%	0.0	98.00%	1323	LC507252.1
Burkholderia cepacia 16JN03 gene for 16S ribosomal RNA, partial sequence	Burkholderia cepacia	2248	2248	98%	0.0	98.00%	1323	LC507246.1

e) *Burkholderia cepacian* PSB3

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Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Burkholderia cepacia strain HB21 16S ribosomal RNA gene, partial sequence	Burkholderia cepacia	2259	2259	98%	0.0	97.92%	1481	MT565309.1
Burkholderia sp. strain Isyb85 16S ribosomal RNA gene, partial sequence	Burkholderia sp.	2259	2259	98%	0.0	97.92%	1370	KY689937.1
Burkholderia cepacia SUPP2119 gene for 16S ribosomal RNA, partial sequence	Burkholderia cepacia	2259	2259	98%	0.0	97.92%	1323	LC507271.1
Burkholderia cepacia 17JN01 gene for 16S ribosomal RNA, partial sequence	Burkholderia cepacia	2259	2259	98%	0.0	97.92%	1323	LC507261.1
Burkholderia cepacia 17AO07 gene for 16S ribosomal RNA, partial sequence	Burkholderia cepacia	2259	2259	98%	0.0	97.92%	1323	LC507258.1
Burkholderia cepacia 17AK24 gene for 16S ribosomal RNA, partial sequence	Burkholderia cepacia	2259	2259	98%	0.0	97.92%	1323	LC507256.1
Burkholderia cepacia 16YM34 gene for 16S ribosomal RNA, partial sequence	Burkholderia cepacia	2259	2259	98%	0.0	97.92%	1323	LC507252.1
Burkholderia cepacia 16JN03 gene for 16S ribosomal RNA, partial sequence	Burkholderia cepacia	2259	2259	98%	0.0	97.92%	1323	LC507246.1
Burkholderia cepacia strain QEH47 16S ribosomal RNA gene, partial sequence	Burkholderia cepacia	2259	2259	98%	0.0	97.92%	1488	MN508423.1
Burkholderia cepacia strain QEH9 16S ribosomal RNA gene, partial sequence	Burkholderia cepacia	2259	2259	98%	0.0	97.92%	1488	MN508422.1

f) *Aeromonas hydrophila* PSB4

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Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Aeromonas hydrophila strain DeswrAH 16S ribosomal RNA gene, partial sequence	Aeromonas hydrophila	2028	2028	100%	0.0	98.94%	1410	MZ378734.1
Aeromonas hydrophila strain TGDY 16S ribosomal RNA gene, partial sequence	Aeromonas hydrophila	2028	2028	100%	0.0	98.94%	1405	JQ599381.1
Aeromonas hydrophila strain 265.12.3 16S ribosomal RNA gene, partial sequence	Aeromonas hydrophila	2023	2023	100%	0.0	98.85%	1386	KY742769.1
Aeromonas hydrophila subsp. hydrophila partial 16S rRNA gene, strain HE1	Aeromonas hydrophila subsp. hydrophila	2023	2023	100%	0.0	98.85%	1395	LN624790.2
Aeromonas sp. 1805 chromosome, complete genome	Aeromonas sp. 1805	2021	20124	100%	0.0	98.85%	4834781	CP038515.1
Aeromonas hydrophila strain TI1 16S ribosomal RNA gene, partial sequence	Aeromonas hydrophila	2021	2021	100%	0.0	98.85%	1537	MT384379.1
Aeromonas hydrophila strain HX-3 chromosome, complete genome	Aeromonas hydrophila	2021	20158	100%	0.0	98.85%	4941513	CP046954.1
Aeromonas hydrophila strain 8_SK_CIFE 16S ribosomal RNA gene, partial sequence	Aeromonas hydrophila	2021	2021	100%	0.0	98.85%	1392	MN784473.1
Aeromonas hydrophila strain TL7 16S ribosomal RNA gene, partial sequence	Aeromonas hydrophila	2021	2021	100%	0.0	98.85%	1537	MN493074.1
Aeromonas sp. strain PI-25 16S ribosomal RNA gene, partial sequence	Aeromonas sp.	2021	2021	100%	0.0	98.85%	1424	MK165127.1

ISOLATION AND CHARACTERIZATION OF BENEFICIAL RHIZOSPHERE MICROORGANISMS FROM RAGI GROWN IN ATTAPPADY HILL TRACT OF KERALA

by

GAYATHRI M

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Abstract of the thesis

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VELLAYANI, THIRUVANANTHAPURAM-695 522

KERALA, INDIA

2021

ABSTRACT

The study entitled "Isolation and characterization of beneficial rhizosphere microorganisms from ragi grown in Attappady hill tract of Kerala" was conducted during the year 2019-2021 in the Department of Agricultural Microbiology, College of Agriculture, Vellayani, Thiruvananthapuram with the objective to isolate nitrogen fixing and phosphate solubilizing bacteria from soil and root samples collected from Attapady and select efficient isolates through *in vitro* screening process and assess the plant growth promotion activity by roll towel assay.

Thirty six nitrogen fixing bacteria and eight phosphate solubilizing bacteria were isolated from root and soil samples from ragi grown in Attappady region. The isolates were designated as ATY1-ATY36 for NFB isolates and PSB1-PSB8 for PSB isolates. Twenty isolates were selected after eliminating isolates with similarities in colony morphological characters, in order to avoid repetition among the isolates obtained from the same sample. Both NFB and PSB isolates were characterized by morphology and biochemical tests.

All selected isolates were subjected to plant growth promotion activity studies. The Indole Acetic Acid (IAA) production showed a wide range of variation from 101.22 $\mu g\ mL^{-1}$ to 3.26 $\mu g\ mL^{-1}$. Gibberellic Acid (GA) production of all the twenty isolates were done and the results ranged between 10.07 $\mu g\ mL^{-1}$ and 2.18 $\mu g\ mL^{-1}$. Maximum IAA and GA production was recorded in isolate ATY10. Extracellular ammonia production of the bacterial isolates ranged between 176.53±5.07 $\mu mol\ mL^{-1}$ and 75.59 $\mu mol\ mL^{-1}$. The nitrogen content of NFB isolates ranged between 30.75 $\mu g\ mL^{-1}$ and 9 $\mu g\ mL^{-1}$. The isolate ATY33 had maximum nitrogen content. The selected PSB isolates were subjected to plate assay and broth assay using Pikovskaya's medium. The isolate PSB1 recorded maximum zone of solubilization (18.23 mm), phosphate solubilizing index (2.82) and quantification of phosphate solubilization (53.41 $mg\ L^{-1}$).

The bacterial isolates were screened based on weighted average ranking. In NFB, ATY10, ATY34 and ATY35 were selected for further studies and in PSB, the isolates PSB1, PSB3 and PSB4 were selected. The selected isolates were identified as *Pantoea agglomerans* ATY10, *Rhizobium* sp. ATY34, *Ensifer adhaerens* ATY35,

Burkholderia territorii PSB1, *Burkholderia cepacia* PSB3 and *Aeromonas hydrophila* PSB4 by 16s rRNA sequencing. Effect of these isolates were assessed for plant growth promotion *in vitro* in ragi seeds using roll towel assay. Maximum germination percentage was recorded by *Aeromonas hydrophila* PSB4 (81.24 per cent) and the minimum number of days taken for germinationwas recorded by *Pantoea agglomerans* ATY10 treated seeds. Also, the treatment *Pantoea agglomerans* ATY10 recorded maximum shoot length (3.31 cm), root length (7.76 cm), seedling length (11.08 cm), seedling vigour index (876.62), root dry weight (0.443 mg) and root shoot ratio (0.50). The treatment *Burkholderia territorii* PSB1 recorded maximum root fresh weight (3.762 mg) and shoot dry weight (0.877 mg).

The six isolates were subjected to assess the multiple traits such as phosphorus, potassium and silicate solubilizing capacity, siderophore production and antifungal activity. *Burkholderia territorii* PSB1 showed maximum zone of inhibition against *Rhizoctonia solani* (3.16 mm) and *Sclerotium rolfsii* (0.58 mm). The maximum antagonistic activity against *Fusarium* sp. and *Helminthosporium* sp. was recorded by *Burkholderia cepacia* PSB3 with 4.13 mm and 5.66 mm of zone of inhibition respectively. *Burkholderia territorii* PSB1 and *Burkholderia cepacia* PSB3 solubilized potassium alumino silicate in agar also, with clearance zone of 11.00 mm and 12.66 mm (in diameter), respectively. None of the isolates solubilized magnesium trisilicate in Bunt and Rovira medium. The siderophore production was observed in *Aeromonas hydrophila* PSB4 isolate in Chrome Azurol S (CAS) agar medium plates.

Based on the results of the present study, it can be concluded that *Pantoea agglomerans* ATY10 is the superior isolate among NFB isolates for plant growth promotion. Also, the isolate *Burkholderia territorii* PSB1 is best among PSB isolates for plant growth promotion and biocontrol activity.

സംഗ്രഹം

"കേരളത്തിലെ അട്ടപ്പാടി മലയോരപ്രദേശത്തെ കൂവരക് കൃഷിയിൽനിന്നും റൈസോസ്ഫിയർ സൂക്ഷ്മജീവികളെ വേർതിരിച്ചെടുത്തു അവയുടെ സ്വഭാവ പഠനം നടത്തുക" എന്ന തലക്കെട്ടുള്ള ഗവേഷണ പഠനം 2019-2021 വർഷങ്ങളിൽ വെള്ളായണി കാർഷിക കോളേജിലെ അഗ്രികൾച്ചറൽ മൈക്രോബയോളജി വിഭാഗത്തിൽ നടത്തി. നൈട്രജൻ ഫിക്സ് ചെയ്യുന്നതും ഫോസ്ഫറസ് ലയിപ്പിക്കുന്നതുമായ ബാക്ടീരിയകളെ അട്ടപ്പാടിയിൽ നിന്നു ശേഘരിച്ച മണ്ണ്-വേര് സാംപിളുകളിൽ നിന്നും വേർതിരിച്ചെടുക്കുകയും അവയുടെ ഇൻവിട്രോ സ്ക്രീനിങ്ഗിലൂടെയും സസ്യങ്ങളുടെ വളർച്ച ത്വരിതപ്പെടുത്താനുള്ള കഴിവു പഠിച്ച് മെച്ചപ്പെട്ടവയെ തെരഞ്ഞെടുക്കുക എന്ന ലക്ഷ്യത്തോടെയാണ് പഠനം നടത്തിയത്. ...

മുപ്പത്താറു നൈട്രജൻ ഫിക്സിങ് ബാക്ടീരിയകളെയും എട്ട് ഫോസ്ഫറസ് ലയന ശേഷിയുള്ള ബാക്ടീരിയകളെയും അട്ടപ്പാടിയിൽ നിന്നു ശേഘരിച്ച സാംപിളുകളിൽ നിന്നു വേർതിരിച്ചെടുക്കാൻ സാധിച്ചു. ഇവയിലെ നൈട്രജൻ ഫിക്സിങ് ബാക്ടീരിയകൾക്ക് ATY1-ATY36 എന്നും ഫോസ്ഫറസ് ലയിപ്പിക്കുന്ന ബാക്ടീരിയകൾക്ക് PSB1-PSB8 എന്നും നാമകരണം ചെയ്തു. ഇവയിൽനിന്ന് ഒരേപോലെ കോളനി സാദൃശ്യമുള്ളവയെ ഒഴിവാക്കി ഇരുപതു ഐസൊലേറ്റുകളെ തെരഞ്ഞെടുത്തു. ഇവയെ കോശത്തിന്റെയും കൊളനിയുടെയും രൂപം ജൈവരാസപ്രവർത്തനങ്ങൾ എന്നിവയുടെ അടിസ്ഥാനത്തിൽ തരംതിരിച്ചു.

തെരഞ്ഞെടുത്ത ഇരുപതു ഐസൊലേറ്റുകളുടെയും സസ്യങ്ങളുടെ വളർച്ച ത്വരിതപ്പെടുത്താനുള്ള കഴിവു പഠിച്ചു. ഐസൊലേറ്റുകളിലെ ഇൻഡോൾ അസെറ്റിക് ആസിഡ്ഡിന്റെ ഉൽപാദനം 3.26 $\mu g\ mL^{-1}$ മുതൽ 101.22 $\mu g\ mL^{-1}$ വരെ ആയിരുന്നു. ഗിബെറില്ലിക് ആസിഡ് ഉൽപാദനം 10.07±2.41 $\mu g\ mL^{-1}$ മുതൽ 2.18±0.44 $\mu g\ mL^{-1}$വരെ ആയിരുന്നു. പരമാവധി ഇൻഡോൾ അസെറ്റിക് ആസിഡ് ഉൽപാദനവും ഗിബെറില്ലിക് ആസിഡ് ഉൽപാദനവും ATY10 ഇൽ ആണ് കാണപ്പെട്ടത്. ബാഹ്യകോശ അമോണിയ ഉൽപാദനം 75.59±10.85 $\mu mol\ mL^{-1}$ മുതൽ 176.53±5.07 $\mu mol\ mL^{-1}$ വരെ ആയിരുന്നു. നൈട്രജൻ ഫിക്സിങ് ബാക്ടീരിയകളുടെ നൈട്രജൻന്റെ അളവു 9±2.82 $\mu g\ mL^{-1}$ മുതൽ 30.75±3.88 $\mu g\ mL^{-1}$ ആയിരുന്നു. ഇതിൽ ATY33 എന്ന ബാക്ടീരിയയാണ് പരമാവധി നൈട്രജൻ ഉൽപാദിപ്പിച്ചത്. ഫോസ്ഫറസ് ലയന ശേഷിയുള്ള എട്ട്

ബാക്ടീരിയകളുടെയും ലയനശേഷി പിക്കോവ്സ്കയ അഗാർ പ്ലെയ്റ്റുകളിലും അതുപോലെ തന്നെ ബ്രോത്ത് കൾച്ചറിലും വിലയിരുത്തി. ഇതിൽ ഐസൊലേറ്റു PSB1 ഏറ്റവുമധികം ലയനശേഷിയും (18.23±0.25 mm അഗാർ പ്ലെയ്റ്റുകളിൽ, 53.41±1.72 mg L^{-1} ബ്രോത്ത് കൾച്ചറിൽ) ഫോസ്ഫറസ് ലയന ഇൻഡെക്സും (2.82±0.025) കാണിച്ചു.

ബാക്ടീരിയൽ ഐസൊലേറ്റുകൾ വെയ്റ്റഡ് ആവറേജ് റാങ്കിംഗിനെ അടിസ്ഥാനമാക്കി പരിശോധിച്ചു. ഇതിനെ ആസ്പദമാക്കി മൂന്ന് നൈട്രജൻ ഫിക്സിങ് ബാക്ടീരിയകളെയും (ATY10, ATY34, ATY35) മൂന്ന് ഫോസ്ഫറസ് ലയന ശേഷിയുള്ള ബാക്ടീരിയകളെയും (PSB1, PSB3, PSB4) തുടർ പഠനത്തിനായി തിരഞ്ഞെടുത്തു. തിരഞ്ഞെടുത്ത ഐസൊലേറ്റുകളെ 16s rRNA സീക്വൻസിങ് ആധാരമാക്കി *Pantoea agglomerans* ATY10, *Rhizobium* sp. ATY34, *Ensifer adhaerens* ATY35, *Burkholderia territorii* PSB1, *Burkholderia cepacia* PSB3, *Aeromonas hydrophila* PSB4 എന്ന് തിരിച്ചറിഞ്ഞു.

ഈ ഐസൊലേറ്റുകളുടെ സസ്യങ്ങളുടെ വളർച്ച ത്വരിതപ്പെടുത്താനുള്ള കഴിവു കൂവരകു വിത്തുകളിൽ റോൾ ടവൽ മെത്തേഡ് ഉപയോഗിച്ച് വിലയിരുത്തി. *Aeromonas hydrophila* PSB4 ഉപയോഗിച്ച് പരിചരിച്ച വിത്തുകൾ കൂടുതൽ മുളച്ചതായി (81.24±4.16 %) കാണപ്പെട്ടു എന്നാൽ ഏറ്റവും കുറവ് സമയത്തിൽ വിത്തുകൾ മുളച്ചതു *Pantoea agglomerans* ATY10 ചേർത്ത വിത്തുകളിൽ ആയിരുന്നു. *Pantoea agglomerans* ATY10 ചേർത്ത വിത്തുകളിൽ തണ്ടിന്റെയും (3.31±0.11 cm) വേരിന്റെയും (7.76±0.70 cm) ചെടിയുടെയും നീളം (11.08±0.65 cm) ഏറ്റവും കൂടുതലായി കാണപ്പെട്ടു. സീഡ്ലിങ്ങ് വിഗർ ഇൻഡെക്സും (876.62±61.92), വേരിന്റെ ഈർപ്പരഹിത ഭാരവും (0.443±0.017 mg), വേര് തണ്ട് അനുപാതവും *Pantoea agglomerans* ATY10 ചേർത്ത വിത്തുകളിൽ തന്നെയായിരുന്നു കൂടുതൽ. എന്നാൽ *Burkholderia territorii* PSB1 ചേർത്ത വിത്തുകളുടെ തൈകൾക്കായിരുന്നു വേരിന്റെ ഭാരവും (3.762±0.118 mg), തണ്ടിന്റെ ഈർപ്പരഹിത ഭാരവും (0.877±0.013 mg) കൂടുതലായി കണ്ടത്.

സസ്യങ്ങളുടെ വളർച്ചക്കു ആവശ്യമായ പൊട്ടാസിയം, സിലിക്കേറ്റ് മുതലായ മറ്റു ധാതുക്കൾ ലയിപ്പിക്കാനുള്ള ഈ ഐസൊലേറ്റുകളുടെ കഴിവും, സിഡെറോഫോർ ഉല്പാദനശേഷിയും, രോഗകാരികളായ കുമിളുകളെ പ്രതിരോധിക്കാനുള്ള കഴിവും പരിശോധിച്ചു. *Burkholderia*

territorii PSB1 എന്ന ഐസൊലേറ്റു *Rhizoctonia solani* (3.16±0.11 mm), *Sclerotium rolfsii* (0.58±0.05 mm) എന്നീ കുമിളുകൾക്കെതിരെ പരമാവധി നിരോധന ശേഷി പ്രകടിപ്പിച്ചു. *Burkholderia cepacia* PSB3 ഐസൊലേറ്റ് *Fusarium* sp. (4.13±0.05 mm), *Helminthosporium* sp. (5.66±0.28 mm) എന്നീ കുമിളുകളുടെ വളർച്ച നിയന്ത്രിച്ചു. *Burkholderia territorii* PSB1 യും *Burkholderia cepacia* PSB3 യും പൊട്ടാസിയം ലയിപ്പിക്കാനുള്ള കഴിവു പ്രകടിപ്പിച്ചു എന്നാൽ സിലികേറ്റ് ലയിപ്പിക്കാനുള്ള കഴിവു ഒരു ഐസൊലേറ്റിനും ഇല്ലായിരുന്നു. *Aeromonas hydrophila* PSB4 സിഡെറോഫോർ ഉലപാദന ശേഷിയും പ്രകടിപ്പിച്ചു.

ഈ പഠനത്തിനെ ആസ്പദമാക്കി നൈട്രജൻ ഫിക്സിങ് ബാക്ടീരിയകളിൽ *Pantoea agglomerans* ATY10 ഉം ഫോസ്ഫറസ് ലയന ശേഷിയുള്ള ബാക്ടീരിയകളിൽ *Burkholderia territorii* PSB1 ഉം കൂവരകു ചെടികളിൽ വളർച്ച ത്വരിതപ്പെടുത്താൻ ഏറ്റവും ഫലപ്രദമാണെന്നു കണ്ടെത്തി. *Burkholderia territorii* PSB1 രോഗഹേതുക്കളായ കുമിളുകളെ നിയന്ത്രിക്കാൻ സഹായിക്കുന്നതായും കണ്ടെത്തി.