CHARACTERIZATION AND EVALUATION OF PLANT GROWTH PROMOTING RHIZOBACTERIA FROM RICE SOILS OF WAYANAD

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(2017-21-037)

DEPARTMENT OF AGRICULTURAL MICROBIOLOGY

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by

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(2017-21-037)

THESIS

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DEPARTMENT OF AGRICULTURAL MICROBIOLOGY
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2021

DECLARATION

W. R. K. D. W. K. V. Wickramasinghe (2017-21-037) hereby declare that this thesis entitled "**Characterization and evaluation of plant growth promoting rhizobacteria from rice soils of Wayanad**" is a bonafide record of research work done by me during the course of research and that the thesis has not been previously formed for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

Vellanikkara

Date: 18.05.2021

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CERTIFICATE

Certified that this thesis entitled "**Characterization and evaluation of plant growth promoting rhizobacteria from rice soils of Wayanad"** is a record of research work done independently by **Mrs. W. R. K. D. W. K. V. Wickramasinghe** (2017-21-037) under my guidance and supervision and that it has not been previously formed the basis for the award of any degree, diploma, associateship or fellowship to her.

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We, the undersigned members of the advisory committee of **Mrs. W. R. K. D. W. K. V. Wickramasinghe**, a candidate for the degree of **Doctor of Philosophy in Agriculture**, with major field in **Agricultural Microbiology**, agree that this thesis entitled **"Characterization and evaluation of plant growth promoting rhizobacteria from rice soils of Wayanad"** may be submitted by **Mrs. W. R. K. D. W. K. V. Wickramasinghe** (2017-21-037) in partial fulfilment of the requirement for the degree.

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W. R. K. D. W. K. V. Wickramasinghe

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LIST OF ABBREVIATIONS

Cfu	Colony forming unit
G	Grams
Mg	Milligram
H	Hours
Ha	Hectare
Mm	Millimeter
Nm	Nano meter
µg	Micro gram
Ml	Milliliter
µl	Micro liter
N	Normality
DNA	Deoxy ribonucleic acid
RNA	Ribonucleic acid
PCR	Polymerase Chain Reaction
TAE	Tris acetate EDTA buffer
Rpm	Revolution per minute
Bp	Base pair
Kb	Kilo base
Min	Minutes
Ppm	Parts per million
HCN	Hydrogen cyanide
CAS	Chrome azurol S
IAA	Indole acetic acids
RDF	Recommended dose of fertilizers
Kg	Kilo gram
Cm	Centimeter
dS	deciSeimens
M	Meter
EC	Electrical conductivity
T	Tons

Introduction

1. INTRODUCTION

Rice (*Oryza sativa* L,) is the second most widely grown crop and food for 70% of the world population. Rice consumption has been increasing over the years due to ever increasing population. To satisfy the growing demand, it requires intensive application of nutrients such as nitrogen (N), phosphorus (P) and potassium (K). Non-judicious application of chemical fertilizers and manures to enhance soil fertility and crop productivity has affected the complex systems of the biogeochemical cycles negatively. Therefore, it is time to explore and identify alternative strategies that can ensure competitive crop yield and environment health while maintaining agro-eco system sustainability (Jiao *et al*., 2012).

Microbial inoculants are promising components of integrated nutrient management systems and they may be categorized into three major groups: (1) plant growth promoting rhizobacteria (PGPR), (2) arbuscular mycorrhizal fungi (AMF), and (3) the nitrogen-fixing rhizobia, which are usually not considered as PGPR (Adesemoye and Kleopper, 2009).

Plant growth promoting rhizobacteria (PGPR) are beneficial bacteria that colonize host's rhizosphere and exert beneficial effects on plant growth. They stimulate plant growth through solubilizing nutrients in soil, biological nitrogen fixation, producing numerous plant growth regulators, protecting plants from phytopathogens by controlling or inhibiting them, improving soil structure and bioremediation of polluted soils by sequestering toxic heavy metals (Ahemad and Kibret, 2014; Lugtenberg and Kamilova, 2009).

A new revolution in agriculture perhaps the "Bio-Revolution" needs to be based on fewer intensive inputs with reduced environmental impact. A Bio-Revolution could be based on biological inputs (microbial inoculants or microbially produced compounds) and improved crops by manipulation of the phytomicrobiome community structure (Timmusk *et al*., 2017; Backer *et al*., 2018)

One of the possible ways to increase crop productivity as well as food quality without creating environmental issues is by the use of PGPR. There are several studies where PGPR were proved as good alternative of chemicals for increasing plant growth

and yield and help reduction in the use of hazardous agro-chemicals. The use of microbial based agricultural inputs had started in the early 20th century beginning with broad-scale rhizobial inoculants (Desbrosses and Stougaard, 2011) and more recently, strains of *Bacillus, Pseudomonas, Glomus*, and others have been commercialized. The use of bacterial inoculants *Bacillus*, *Pseudomonas*, *Actinobacteria*, *Lactobacillus*, *Acetobacter*, *Azospirillum*, *Paenibacillus*, *Serratia*, *Burkholderia*, *Herbaspirillum* and *Rhodococcus* on the improvement of crop production have been reviewed by many authors (Backer *et al*., 2018).

Recently, there has been great interest in the application of PGPR strains as multi-strain inoculum for crops to benefit from their different beneficial characteristics. Utilization of bacterial consortia has inconsistent effects on crop yield. Raja *et al*. (2006), stated that microbial consortium (*Azospirillum lipoferum* Az-204, *Bacillus megaterium var. phosphaticum*, and *Pseudomonas fluorescens* Pf 1) improved colonization potential, sustainability within the inoculants and crop growth of rice plants instead of using individual strains. Combination of PGPR strains (*Pseudomonas fluorescens*, *Bacillus subtilis, Azospirillum brasiliense*) with 75% nitrogen fertilizer significantly increased yield of promising Egyptian rice lines and reduced the usage of 25% inorganic nitrogen fertilizer (Elekhtyar, 2016).

It is hypothesized that this multi-strain biofertilizer inoculum can help promote plant growth and rice grain yield possibly through biological nitrogen fixation, solubilization of inorganic and organic P, K and Zn complexes in soil, siderophore production, phytohormone synthesis and controlling plant pathogens. It ensures reduction of agrochemical usage and environmental issues and promotion of a green and sustainable agriculture.

Despite the vast positive outcomes, there are inconsistent issues when it comes to application of PGPR on plants. Plant growth promotion and biocontrol efficacy of PGPR often depend upon rhizosphere competence of the microbial inoculants (Lugtenberg and Kamilova, 2009). Some PGPR strains exhibiting promising activities *in vitro* have variable performances in the rhizosphere in a given crop under a given set of conditions, because of that some inconsistence issues may arise when applying PGPR in fields. Therefore, isolation and characterization of native PGPR from rice

rhizosphere from specific locations are much needed to develop effective consortium of PGPR for rice cultivation. It is important to explore and identify region specific microbial strains which can be used as potential plant growth promoters to achieve higher yields under specific ecological and environmental conditions (Fischer *et al*., 2007). The use of indigenous PGPR can be an added advantage since they can easily acclimatize to the natural conditions and enhance the plant–microbe interactions (Verma *et al*., 2013).

Wayanad district is classified under the agro-ecological zone "High Hills" which has a salubrious climate and is situated on high altitudes ranging from 700 to 2100m in north-east of Kerala state. The name "Wayanad" is derived from two local words 'Vayal' meaning swamps and 'Nadu" meaning place. The climate of the plateau is quite different and temperature ranges from 13^0C to 33^0C. From November to January mist is common and following light showers from April and May, the south east monsoon brings 75% of annual rainfall during June to August. The soil in this area, in general, is acidic and poor in available nitrogen, phosphorus, potash and organic matter, it is well drained and responds to management practices. Though Kerala Agricultural University has developed biofertilizers, no attempt has been so far made to evaluate native strains from rice fields in Wayanad, where rice is being cultivated in an area of 22,772 ha.

Therefore, the present study was conducted to isolate and characterize PGPR strains from rice fields of Wayanad and formulate consortium of PGPR to improve growth and yield of rice.

Review of Literature

2. REVIEW OF LITERATURE

During the last two decades, exploitation of microorganisms in agriculture has increased tremendously due to their potential for nitrogen (N) fixation and solubilization of nutrients like phosphorus (P), potassium (K) and zinc (Zn), thus increasing nutrient uptake by plants and therefore yields (Vessey, 2003). However, soil-plant- microbe interactions are complex phenomena and detailed reports are available on how this interaction influences plant health and productivity.

To maintain plant health and achieve the desired crop yield, one possibility is to use soil microorganisms (bacteria, fungi, algae, etc.) that increase the nutrient uptake capacity and water use efficiency (Armada *et al*., 2014). Among these potential soil microorganisms, bacteria known as plant growth promoting rhizobacteria (PGPR) are the most promising. In this sense, PGPR may be used to enhance plant health and promote plant growth, without environmental contamination (Calvo *et al*., 2014).

Studies have shown that PGPR strains vary widely and their growth promoting ability may be highly specific to certain plant species, cultivar, soil, and genotype (Lucy *et al*., 2004; Fischer *et al*., 2007). Under such conditions, knowledge of native bacterial population and their identification is important for understanding their distribution and diversity.

Understanding diversity of native bacterial population, their characterization and identification in relation to the specific crops and regions are required for the development of efficient biofertilizers. Exploration of region-specific microbial strains which can be used as a potential plant growth promoters is much important to achieve desired crop growth with less environmental problems (Zahid *et al*., 2015).

2.1. Plant growth promoting rhizobacteria

Plant growth promoting rhizobacteria (PGPR) is a group of bacteria that can be found in the rhizosphere (Ahmad *et al*., 2008). Hiltner, in year 1904 discovered that rhizosphere which is the layer of soil influenced by the root, is much richer in bacteria than surrounding bulk soil (Lugtenberg and Kamilova, 2009). This rhizosphere effect

is caused by the fact that a substantial amount of the carbon fixed by the plant is secreted mainly as root exudates that provide nutrition for the rhizosphere organisms.

The term "plant growth promoting rhizobacteria" refers to bacteria that colonize roots of plants (rhizosphere) and enhance plant growth. The term "plant growth promoting rhizobacteria (PGPR)" for these beneficial microbes was introduced by Kloepper and Schroth (1978). These are non-pathogenic, strongly root colonizing bacteria which increase plant yield by one or more mechanisms (Babalola, 2010).

Weller and Thomashow (1994) stated that the narrow rhizosphere zone is rich in nutrients compared to the bulk soil; this is shown by the quantity of bacteria that are present surrounding the roots of the plants, generally 10 to 100 times higher than in the bulk soil. The microbes colonizing rhizosphere include bacteria, fungi, actinomycetes, protozoa, and algae, among which, bacteria are the most abundant (Kaymak, 2010). The enhancement of plant growth by the application of these microbial populations is well known and proven.

Plant growth promoting bacteria are characterized by three features: (i) they must be proficient to colonize the root surface, (ii) they must survive, multiply and compete with other microbiota at least for the time needed to express their plant growth promotion/protection activities, and (iii) they must promote plant growth (Kloepper, 1994).

The working mechanisms of PGPR are divided mainly into direct and indirect. The direct mechanisms include biofertilization, stimulation of root growth, rhizo-remediation, and plant stress control. Plant growth promoting rhizobacteria indirectly reduce the impact of diseases by induction of systemic resistance, competition for nutrition and niches with pathogen, and by producing siderophore, antibiotics and other volatile organic compounds such as hydrogen cyanide (HCN) and ammonia (Figueiredo *et al*., 2016).

In general, PGPR can be separated into extracellular (ePGPR), existing in the rhizosphere, on the rhizoplane, or in the space between cells of root cortex, and intracellular (iPGPR), which exist inside root cells, generally in specialized nodular structures (Figueiredo *et al*., 2010). Most rhizobacteria belong to Gram negative rods

with a lower proportion being Gram positive rods, cocci or pleomorphic (Bhattacharyya and Jha, 2012).

Plant growth promoting bacteria are widely distributed in the bacterial domain, mainly in the phyla Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes, and Proteobacteria (Figueiredo *et al*., 2010). Some examples of well-known ePGPR are *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Caulobacter*, *Chromobacterium*, *Erwinia*, *Flavobacterium*, *Micrococcus*, *Pseudomonas* and *Serratia* (Bhattacharya and Jha, 2012).

2.2. Mechanisms of plant growth promotion

According to Kloepper and Schroth (1981), PGPR mediated plant growth promotion occurs through the production of various substances by the whole microbial community in rhizosphere niche. PGPR can affect plant growth either directly or indirectly. Direct promotion of plant growth by PGPR involves either providing plants with a compound synthesized by the bacterium or facilitating the uptake of certain essential nutrients from the environment and indirectly the inhibitory effect of various pathogen on plant growth and development in the forms of biocontrol agents (Glick, 2012).

Direct stimulation includes biological nitrogen fixation, producing phytohormones like auxins, cytokinins and gibberellins, solubilizing minerals like phosphorus and iron, production of siderophores and enzymes and induction of systemic resistance, while indirect stimulation is basically related to biocontrol, including antibiotic production, chelation of available Fe in the rhizosphere, synthesis of extracellular enzymes to hydrolyse the fungal cell wall and competition for niches within the rhizosphere (Zahir *et al*., 2004).

Bacteria present in the soil are responsive to root exudates which consist of diverse group of compounds synthesized, accumulated and secreted by plant roots (Huang *et al*., 2014). The root exudates modify the chemical and physical properties of the soil and act as substrate and chemotactic signalling molecules and mediate the selection of microbial communities which interact with the plant (Chaparro *et al*., 2014). In addition to root exudates, quorum sensing molecules also help to regulate

bacterial community in the rhizosphere. Quorum sensing molecules are defined as signalling molecules responsible for bacterial cell to cell communication and allow bacteria to share information about their cell density (Badri *et al*., 2009). N -Acyl-homoserine lactone (AHL) is the most important quorum-sensing molecule and is generally found in Gram negative bacteria that live in association with plants (Babalola, 2010). According to Boyer *et al*. (2008), quorum sensing molecules are associated with rhizosphere competence and adaptation of *Azospirillum lipoferum* during the plant-host interactions.

2.2.1. Direct Mechanisms

2.2.1.1. Biological nitrogen fixation

Nitrogen (N) is the most vital nutrient for plant growth and productivity as well as being an integral part of proteins, nucleic acids and other essential biomolecules. Although there is about 78% N_2 in the atmosphere, it is unavailable to plants. Atmospheric N_2 is converted into plant utilizable forms by biological nitrogen fixation (BNF) which changes N_2 to ammonia, exclusively by prokaryotes, using complex enzyme system known as nitrogenase (Kim and Rees, 1994). In fact, BNF accounts for approximately two thirds of the nitrogen fixed globally and represents an economically sound alternative to chemical fertilizers (Ladha *et al*., 1997).

Nitrogen fixing organisms are generally categorized as symbiotic and non-symbiotic bacteria. PGPR that fix N_2 in non-leguminous plants, also called as diazotrophs, are capable of fixing non-obligate interactions with the host plants (Glick *et al*., 1999). Symbiotic N_2 fixing bacteria include members of the family Rhizobiaceae which forms symbiosis with leguminous plants (e.g. *Rhizobium*) and some others form symbiosis with non-leguminous trees (e.g. *Frankia*). Non -symbiotic (free living, associative and endophytes) nitrogen fixing microorgnisms include cyanobacteria (*Anabena*, *Nostoc, Aulosira, Calothrix*), *Azospirillum*, *Azotobacter*, *Gluconobacter diazotropicus* and *Azoarcus* (Bhattacharyya and Jha, 2012). However, non-symbiotic nitrogen fixing bacteria provide only a small amount of fixed nitrogen that the bacterially associated host plant requires (Glick, 2012).

PGPR belonging to the genera *Azospirillum*, *Azotobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Acinetobacter*, *Alcaligens*, *Arthrobacter*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Rhizobium*, *Mycobacterium* and *Serratia* are known to fix atmospheric N_2 through symbiotic and asymbiotic or associative nitrogen fixing process (Kumar *et al*., 2014). *Bacillus*, *Klebsiella*, *Pseudomonas* and *Enterobacter* recorded N_2-fixation efficiencies ranging from 2.1 (in *Enterobacter*) to 14.9 (*Bacillus*) mg N fixed per g of sucrose oxidized (Fayez, 1990). Kumar *et al*. (2014) reported nitrogen fixing ability of *B. megaterium*, *A. chlorophenolicus* and *Enterobacter* sp. as 11.8, 15.0 and 9.9 mg N fixed respectively, per gram of sucrose utilized.

Govindarajan *et al*. (2008) isolated thirteen bacterial strains from rice plants and identified all of them belong to the genus *Burkholderia*. Isolate MGK3 was consistently more active in reducing acetylene, showed nitrogen fixation ability and 16S rDNA sequence it confirmed as *Burkholderia vietnamiensis*. A total of 38 rice root-associated diazotrophs were isolated from the Tanjong Karang rice growing area of Malaysia and assessed for nitrogenase activity, production of indoleacetic acid (IAA). Results revealed that 10 diazotrophs were highly positive in the acetylene reduction assay (ARA) and these were identified as *Bacillus* sp. (9 diazotrophs) and *Burkholderia* sp. (Sb16) using the partial 16S rRNA gene sequence analysis (Nahar *et al*., 2013). Miao *et al*. (2015) stated that a bacterial isolate that presented nitrogenase activity, phosphate solubilizing ability; inhibited the growth of pathogens such as *Sclerotinia sclerotiorum*, *Gibberella zeae* and *Verticillium dahlia*, and produced small quantities of indole acetic acid (IAA) and was identified as *Burkholderia* sp.

Characterization of plant growth promoting traits of nitrogen fixers from rice rhizosphere revealed that all the diazotrophs were good plant growth promoting rhizobacteria and among them *Sphingomonas azotifigens* (K23), *Pseudomonas putida* (K4), *Herbaspirillum* sp (K16), *Stenotrophomonas maltophilia* (K6), *Pantoea agglomerans* (K15) and *Acinetobacter radioresistens* (K34) were better nitrogen fixers (Laskar *et al*., 2013).

Kumar and Gera (2014) isolated four strains of cultivable bacteria associated with the rhizosphere of sugarcane using N-free media and showed the presence of *nif*H gene (390 bp) in isolate MDB4, which also exhibited production of indole-3-acetic acid

(IAA) and ammonia. Isolate MDB4 identified as *Brevundimonas* sp. based on 16S rRNA gene sequencing data. Shaheena *et al*. (2016) studied multiphasic diazotrophs associated with black pepper in Wayanad district and identified efficient nitrogen fixer as *Microbacterium* (NKdS and NPPV), *Cellulosimicrobium* (NPS-1) and *Brevundimonas* (NKPV-2) based on 16S rDNA sequencing. Naqqash *et al*. (2020) isolated four nitrogen fixing strains TN37, TN39, TN40, and TN44 from potato rhizosphere and identified as *Brevundimonas* sp. based on 16S rRNA gene sequence. Further reported all strains contained *nif*H gene except TN39 and among them *Brevundimonas* sp. TN37 showed maximum nitrogen fixation and phosphate solubilization potential.

Akintokun *et al*. (2019) studied the nitrogen fixing ability of PGPR isolates from maize rhizosphere and revealed significant level of nitrogen fixing ability with *Alcaligenes faecalis* strain P156 fixing the highest amount of nitrogen (11.4 mg of N fixed g^{-1} of sucrose) and least by *Bacillus mojavensis* strain NBSL51 (6.3 mg N fixed g^{-1} of sucrose*)* and also reported N fixing ability of *Pseudomonas syringae pv. syringae* strain HS191, *Bacillus cereus* strain 20UPMNR, and *Pseudomonas aeruginosa* strain ZSL-2.

Based on nitrogenase activity Xie *et al*. (1998) reported that *Bacillus brevis*, *B. cereus*, *B. circulans*, *B. firmus*, *B. licheniformis*, *B. megaterium*, *B. pumilus*, and *B. subtilis* associated with rice have the capacity to fix nitrogen. Nitrogen fixing potential of diverse species of *Bacillus* has reported the presence of *nif*H gene and capability to fix atmospheric nitrogen (Saxena *et al*., 2020).

2.2.1.2 Solubilization of phosphate

Phosphorus (P) is the second most limiting plant nutrient after nitrogen. Soils may have large reservoir of total P but the amount available to plants is usually a tiny proportion of this total. This low availability of phosphorus to plants is because, in the vast majority of soils P is found in insoluble forms. Plants can only absorb it in two forms, the monobasic ($H_2PO_4^-$) and the dibasic (HPO_4^{2-}) ions (Bhattacharyya and Jha, 2012). The insoluble phosphorus present as an inorganic mineral such as apatite or

organic forms like inositol phosphate (soil phytate), phosphomonoester, phosphotriesters also remain unavailable to plants (Glick, 2012).

Phosphate solubilization takes place through various mechanisms such as acidification, secretion of organic acids or protons (Nahas, 1996; Richardson *et al*., 2009) and chelation and exchange reaction (Hameeda *et al*., 2008). Characteristically, inorganic phosphate solubilization occurs through low molecular weight organic acids which are synthesized by various bacteria. Mineralization of organic phosphorus occurs through the synthesis of phosphoric esters and phosphorus solubilization and mineralization coexist in the same bacterial strains (Ahemad and Kibret, 2014).

Chen *et al*. (2006) screened 36 bacterial strains with the capacity to solubilize mineral phosphate and found out the principal mechanism for their solubilization capacity as production of organic acids. Gluconic acid is the principal organic acid produced by many organisms, but other acids include 2-ketogluconic, acetic, citric, glycolic, isovaleric, isobutyric, lactic, malonic, oxalic, propionic, and succinic acids (Rodriguez and Fraga, 1999; Chen *et al*., 2006).

Bacteria are more effective in phosphate solubilization than fungi (Alam *et al*., 2002). Bacterial genera like *Azospirillum*, *Azotobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Microbacterium*, *Pseudomonas*, *Rhizobium* and *Serratia* are reported as the most significant phosphate solubilizing bacteria (Bhattacharyya and Jha, 2012).

Islam *et al*. (2007) isolated potential phosphate solubilizing bacteria from rice rhizosphere and identified P solubilizers based on their 16S rRNA gene sequencing. They were *Acinetobacter* sp. BR-12, *Klebsiella* sp. BR-15, *Acinetobacter* sp. BR-25, *Enterobacter* sp. BR-26, *Microbacterium* sp. BRS-1 and *Pseudomonas* sp. BRS-2 and among them highest phosphate solubilizing activity was exhibited by BR-25.

Estrada *et al*. (2013) studied 49 nitrogen fixing isolates for the ability to solubilize phosphate *in vitro* and reported seven isolates had ability to solubilize P*i* on agar plates. They belonged to the genera *Herbaspirillum* and *Burkholderia* and *Burkholderia* strain showed peak soluble P (around 200 mg P l^{-1}) in NBRIP liquid.

Miao *et al*. (2015) studied PGPR from soybean rhizosphere and reported strain coded 7016 which presented phosphate solubilizing, nitrogenase activity, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity and production of small quantity of IAA and identified as *Burkholderia* sp. based on 16S rDNA sequence analysis. Ghosh *et al*. (2016) studied potent phosphate solubilizing bacterial strain associated with *Lycopodium cernuum* L. rhizoids and identified as *Burkholderia tropica* (P4), *Burkholderia unamae* (P9) and *Burkholderia cepacia* (P10) based on 16S rRNA. Phosphate solubilization against $Ca_3(PO_4)_2$ suggested P4 (580.13.38 $\pm$ 13.38 µg ml^{-1}) as maximum and followed by the isolate P9 (517.12 $\pm$ 17.15 µg ml^{-1}) and P10 (485.18 $\pm$ 14.23 µg ml^{-1}). Their very good solubilizing efficacy against ferric phosphate, aluminum phosphate and four different complex rock phosphates were also reported.

Hussain *et al*. (2015) isolated two bacterial strains (KP-21 and KP-22) from rhizosphere of rapeseed and found promising in phosphate solubilization and auxin biosynthesis and identified as *Providencia vermicola* based on 16S rRNA gene sequencing analysis. Saranya (2016) studied native mineral phosphate solubilizing bacteria from Kerala soils and identified efficient P solubilizing bacteria as *Acinetobacter* sp., *Providencia* sp., *Achromobacter* sp., *Providencia alcalifaciens*, *Bacillus cereus*, *Pseudomonas* sp., and *Burkholderia* sp. Sharon *et al*., (2016) reported that phosphate solubilizing bacteria from potato rhizosphere had significantly high phosphate solubilization rate (956 mg l^{-1}) and it was designated as *Pantoea* sp. Pot1.

Panda *et al*. (2016) reported capacity of *Bacillus megaterium* M510 isolated from maize rhizosphere in the eastern Himalayan region to solubilize both aluminum phosphate and iron phosphate in addition to solubilization of tricalcium phosphate. Three species of *Bacillus viz*., *B. megaterium*, *B. subtilis* and *B. cereus* were tested for solubilization of phosphorus in the three different substrates such as poultry bones, fish bones and ash found all species produced organic acids and released available P from all the substrates (Saeid *et al*., 2018).

2.2.1.3 Solubilization of potassium

Potassium (K) is considered as an essential nutrient and a major constituent of all living cells. Potassium solubilization is carried out by a wide range of saprophytic bacteria, fungal strains and actinomycetes (Ahmad *et al*., 2016). There are strong evidences that soil bacteria are capable of transforming soil K to the forms available to plant effectively. Solubilization of K by potassium solubilizing bacteria (KSB) from insoluble and fixed forms (e.g., biotite, feldspar, illite, muscovite, orthoclase, and mica) is an important aspect regarding K availability in soils (Etesami *et al*., 2017).

It is generally believed that microorganisms contribute to the release of K^+ from potassium bearing minerals by several mechanisms. Mechanism of potassium solubilization are the methods by which the insoluble K and structurally unavailable form of K compounds conversion in to the soluble form (Uroz *et al*., 2009). As it occurs in the case of P solubilization, the major mechanism of K mineral solubilization is by production of organic and inorganic acids and production of protons (Meena *et al*., 2015). The most prominent acids released by the KSB are tartaric acid, citric acid, succinic acid, α-ketogluconic acid, and oxalic acid (Meena *et al*., 2014b). Uroz *et al*. (2009) stated that indigenous rhizospheric microorganisms are very effective in releasing K from structural K through solubilization and from exchangeable pools of total soil K by acidolysis, chelation and solubilization.

Solubilization of potassium by KSB from insoluble and fixed forms is an important aspect regarding K availability in soils. The ability to solubilize the silicate rocks by *B. mucilaginosus*, *B. circulanscan*, *B. edaphicus*, *Burkholderia*, *A. ferrooxidans*, *Arthrobacter* sp., *Enterobacter hormaechei*, *Paenibacillus mucilaginosus*, *P. frequentans*, *Cladosporium*, *Aminobacter*, *Sphingomonas*, *Burkholderia,* and *Paenibacillus glucanolyticus* has been reported and among these *B. mucilaginosus*, *B. edaphicus* and *B. circulanscan* were described as effective K solubilizers (Meena *et al*., 2016).

Setiawati and Mutmainnah (2016) isolated potassium solubilizing microorganisms (KSM) from sugarcane rhizosphere and evaluated their K solubilizing

ability using various minerals and results revealed that the amount of K released to the liquid broth was different and ranged from 0.16 to 6.34 mg l^{-1} (Trachyte); from 0.13 to 12.25 mg l^{-1} (Feldspar); from 1.24 to 15.57 mg l^{-1} (Leucite Pati) and from 1.01 to 4.59 mg l^{-1} (Leucite Situbondo).

Sugumaran and Janarthanam (2007) reported that *B. mucilaginosus* released 4.29 mg l^{-1} K in media supplemented with muscovite mica. Parmar *et al*. (2016) isolated K solubilizers from maize rhizosphere and reported 25 potassium solubilizers and among them two efficient isolates KSB-1 (46.25 µg ml^{-1}) and KSB-3 (42.37 µg ml^{-1}) were identified as *Bacillus licheniformis* and *Bacillus subtilis* respectively by Biolog system.

Three isolates of efficient K solubilizers from rice rhizosphere were identified as *Pantoea agglomerans*, *Rahnella aquatilis* and *Pseudomonas orientalis* based on 16S rRNA sequencing (Khanghahi *et al*. 2017). Amount of potassium released by the isolates from mica on 21st day of incubation was 35.36, 76.04 and 56.58 µg ml^{-1} respectively. Tan *et al*. (2017) reported that potassium solubilization potential of two indigenous isolates from rice rhizosphere UPMB 19 (*Lysinibacillus xylanilyticus*) and UPMP 20 (*Alcaligenes faecalis*) were 12.67 µg ml^{-1} and 11.45 µg ml^{-1} respectively.

Sun *et al*. (2020) isolated 18 strains of efficient potassium solubilizing rhizobacteria from *Mikania micrantha* and results revealed that the genus *Burkholderia* had the highest solubilizing ability (1.75 mg l^{-1}).

2.2.1.4. Solubilization of zinc

Zinc is one of the imperative micronutrients, required in small amounts for the proper growth and development of living organisms. In plants, it is involved in carbohydrate metabolism, auxin metabolism and acts as a significant anti-oxidant (Kamran *et al*., 2017). Plants uptake zinc as divalent cations and minor portion of the total zinc is present in soil solution as soluble form for plant uptake. Zinc deficiency is the one of the most widespread micronutrients due to the unavailability of zinc in soil.

The unavailable Zn compounds can be converted back to the available form through bioaugmentation of microbial inoculants having the ability to solubilize

insoluble Zn compounds (Saravanan *et al*., 2007). Among these inoculants, several plant growths promoting rhizobacteria (PGPR) have been documented for their ability to solubilize unavailable form of Zn in soil thus increasing plant growth (Hussain *et al*., 2015).

Various PGPR have shown zinc solubilization *in vitro i*ncluding *Pseudomonas aeruginosa*, *Gluconacetobacter diazotrophicus*, *Bacillus* sp., Pseudomonas *striata, Pseudomonas fluorescens, Burkholderia cenocepacia,* (Saravanan *et al*., 2007, Bapiri *et al*., 2012, Sharma *et al*., 2012; Pawar *et al*., 2015), *Acinetobacter* (Vaid *et al*., 2013) *Serratia liquefaciens, S. marcescens,* and *Bacillus thuringiensis* (Abaid-Ullah *et al*., 2015).

Saravanan *et al*. (2004) assessed zinc solubilization potential of *Bacillus* sp. and *Pseudomonas* sp. using zinc oxide, zinc sulphide and zinc carbonate and reported that solubilization potential varied with insoluble zinc source. Saravanan *et al*. (2007) reported that *Gluconacetobacter diazotrophicus* PAL5 solubilized zinc oxide more effectively than zinc carbonate and zinc phosphate.

Findings of Sharma *et al*. (2012) revealed that *Bacillus* isolates solubilized zinc at the rate of 3.45-4.65 µg ml^{-1} in broth supplemented with zinc phosphate and at the rate of 2.86-3.65 µg ml^{-1} broth supplemented with zinc carbonate. Vaid *et al*. (2013) isolated three bacteria from zinc deficient soils and showed their zinc solubilizing ability by lowering down the pH in the solution due to gluconic acid production. These isolates were identified as *Burkholderia* sp. SG1, *Acinetobacter* sp. SG2 and *Acinetobacter* sp. SG3 based on 16S rRNA gene sequencing analysis.

Mumtaz *et al*. (2017) studied zinc solubilization potential of *Bacillus* sp. and results of quantitative assay revealed that maximum solubilized concentration of insoluble ZnO was observed in rhizobacterial isolates S10 (*Bacillus aryabhattai*) and ZM63 (*Bacillus subtilis*) and quantity of released Zn in broth was 27.66 and 27.15 µg ml^{-1} respectively.

Abaid-Ullah *et al*. (2015) isolated Zn solubilizers FA-2, FA-4 and FA-3 and characterized as *Serratia liquefaciens*, *Serratia marcescens* and *Bacillus thuringiensis*

on the basis of 16S rRNA gene sequencing. Zinc solubilization was further quantified by atomic absorption spectrophotometer (AAS) and the strain FA-3 solubilized maximum Zn from ZnO ore, while FA-4 solubilized maximum Zn from $ZnCO_3$.

2.2.1.5. Production of IAA

One of the prominent mode of actions for growth promotion by PGPR is phyto-stimulation. Phytohormones that are not naturally synthesized by plants but are synthesized exogenously by natural and synthetic means are known as plant growth regulators. Auxins, abscisic acid, gibberellic acid, salicylic acid and ethylene are examples of phytohormones that are synthesized directly and indirectly by PGPR, which act as plant growth regulators (Egamberdieva *et al*., 2015). Auxins are important phytohormones, and the auxin indole-3-acetic acid (IAA) was shown to promote several growth and developmental events, such as cell division, elongation, and differentiation. It is reported that more than 80% rhizosphere bacteria possess the ability of producing auxin as secondary metabolite ((Pattern and Glick, 1996).

Bacterial IAA increases surface area and length of roots and provide plant greater access to the nutrient uptake; acts as a reciprocal signalling molecule affecting gene expression of certain microorganisms and plays a very important role in rhizobacteria- plant interactions (Ahemad and Kibret, 2014).

IAA production by PGPR can vary among different species and strains, and is influenced by culture conditions, growth stage and substrate availability (Mirza *et al*., 2001). Numerous studies demonstrated that IAA is the common product of tryptophan metabolism for several rhizobacteria (Ahmad *et al*., 2006; Joseph *et al*., 2007; Aarab *et al*., 2015).

Higher level of IAA production by *Pseudomonas* was recorded by Xie *et al*. (1996). Seventy two isolates were screened for IAA production and it was found that more than 80% of of the isolates of *Azotobacter*, fluorescent pseudomonads and *Mesorhizobium ciceri* were IAA producers, whereas only 20% of *Bacillus* isolates were IAA producers (Ahmad, 2008).

Rodrigues *et al*. (2016) reported that 57% of rhizobacteria associated with sugarcane were positive for IAA production and amount of IAA production varied from 5.09 to 83.24 µg ml^{-1} in 24h, 17.88-124.12 µg ml^{-1} in 48h and 21.05-139.21µg ml^{-1} in 72h. Akintokun *et al*. (2019) screened isolates which had multiple abilities (P and K solubilization, N fixation) for IAA production. IAA production ranged between 9 and 94 mg l^{-1}.

2.2.1.6. Production of Ammonia

Production of ammonia is one of the most important traits of PGPR which benefits the crop (Babalola, 2010). Ammonia production is indirectly influencing the plant growth and is considered as an important characteristic of PGPR (Yadav *et al*., 2010). Ammonia production of PGPR can help satisfy the N demand of the host plant and in excess reduces the colonization of pathogens (Rodrigues *et al*., 2016). The accumulation of ammonia creates alkaline conditions of soil and suppresses the growth of certain fungi and nitrobacteria due to it potent inhibitory effect.

Agbodjato *et al.* (2015) isolated PGPR from maize rhizosphere, which included five *Bacillus* species, three *Pseudomonas* species and one *Serratia* species (*B. polymyxa*, *B. pantothenticus*, *B. anthracis, B. thuringiensis*, *B. circulans*, *P. cichorii*, *P. putida, P. syringae* and *Serratia marcescens*). Screening them for production of ammonia revealed that all the *Serratia* strains followed by 80% of *Bacillus* and 77.77% *Pseudomonas* produced ammonia. Rodrigues *et al*. (2016) isolated 136 plant growth-promoting bacteria associated with sugarcane and reported 45% isolates were positive for ammonia production. Batool and Iqbal (2019) also screened phosphate solubilizing rhizobacteria for production of ammonia and stated that 83.33% strains had ability to produce ammonia.

2.2.1.7. Production of hydrogen cyanide (HCN)

HCN is a secondary metabolite of several microorganisms and it is produced directly from glycine and from cyanogenic glycosides and both of these have been demonstrated in root exudates (Bakker and Schippers, 1987). Plant growth promoting rhizobacteria produce chemical compounds with different benefits for the plant and

among them, HCN is recognized as a biocontrol agent, based on its recognized toxicity against plant pathogens (Rijavec and Lapanje, 2016). To date various bacterial genera have been shown to be capable of producing HCN, including *Alcaligenes*, *Aeromonas*, *Bacillus*, *Pseudomonas*, *Enterobacter* and *Rhizobium* species (Kumar *et al*., 2014). Studies have shown that about 50% of *Pseudomonas* isolated from potato and wheat rhizosphere were able to produce HCN *in vitro* (Schipper *et al*., 1990).

Studies on HCN producing pseudomonads concluded that HCN could be toxic for plant pathogens (Voisard *et al*., 1989). Studies on granite mineral weathering indicated bacterially produced cyanide plays a minor role through the formation of soluble hexacyanoferrate complexes (Wongfun *et al*., 2014). HCN producing rhizobacteria could also act in other ways such as ability to form complexes with transitional metals in the mineral substrate (Faramarzi and Brandl, 2006). Rijavec and Lapanje (2016) proposed new role for HCN production by rhizobacteria in the sequestration of metals and the consequential indirect increase of nutrient availability.

Kumar *et al*. (2012) reported three *Bacillus* strains out of eight had positive reaction for HCN production. Aarab *et al*. (2015) screened rhizobacteria from rice fields for PGP activities and reported that only three out of nine isolates were HCN positive and all the three were identified as *Pseudomonas* sp. Rodrigues *et al*. (2016) isolated 136 plant growth-promoting bacteria associated with sugarcane and reported only 0.7% were positive for HCN production. Sharma *et al*. (2019) reported 35 isolates out of 143 rice associated rhizobacteria were positive for the HCN production.

2.2.1.8. Production of siderophore

Siderophores are low molecular weight (between 500 to 1500 dalton) iron binding protein compounds involved in the process of chelating ferric iron (Fe^{3+}) from the environment (Ferreria *et al*., 2019). Siderophores are of three types namely catecholates, hydroxamates and hydroxy-carboxylates (Khan *et al*., 2006). Hydroxymate type siderophore is produced by both bacteria and fungi, catechol type of siderophores is produced only by bacteria, whereas hydroxy carboxylates type of siderophore is produced by only few species of bacteria (Grobelak and Hiller, 2017).

Siderophore production may offer bacteria competitive advantages to colonize plant tissues and to reject other microorganisms from the same ecological niche (Loaces *et al*., 2011). Iron is commonly present in nature in the form of Fe^{3+} which is highly insoluble and microbial siderophore provides plants with Fe and enhances their growth (Prasad *et al*., 2010; Grobelak *et al*., 2015).

Loaces *et al*. (2011) studied endophytic siderophore producing bacteria (SPB) in rice and stated that less than 10% of the heterotrophic bacteria were siderophore-producers in young plants, but most of the heterotrophic bacteria were siderophore-producers in mature plants. They further stated that siderophore producing bacteria (SPB) of the genus *Burkholderia* were good antagonists of pathogenic fungi. Kumar *et al*. (2012) screened *Bacillus* strains isolated from common bean and found that all the strains produced siderophore. Quantitative analysis of siderophore showed that maximum amount of 38 μg ml^{-1} was recorded in *Bacillus* sp. BPR7. Tan *et al.* (2014) screened PGPR from rice rhizosphere and found UPMB19 (*Lysinibacillus xylanilyticus*) and UPMB20 (*Alcaligenes faecalis*) showing a slightly higher production rate of siderophore, through the halo zone observation.

Grobelak and Hiller (2017) reported that bacterial siderophores exhibited their effect on the growth and development of plants under stress conditions, such as a high concentration of heavy metals in the soil and the alkaline soil.

2.2.2. Indirect mechanisms

Rhizobacteria can, through various mechanisms of action, suppress plant diseases. Synthesis of a range of different antibiotics is most often associated with PGPR and these help to prevent proliferation of plant pathogens specially fungi (Compant *et al.*, 2005). For example, production of antifungal antibiotics such as iturin by *B. subtilis* has been reported to cause antagonism to pathogens of soybean seed such as *Rhizoctonia solani, Colletotrichum truncatum, Sclerotinia sclerotiorum, Macrophomina phaseolina and Phomopsis* sp. (Araujo *et al*., 2009).

A number of antagonistic bacteria are responsible for producing lytic enzymes such as chitinase, cellulases, β -1,3- glucanases and proteases that can lyse a portion of

the cell wall of many pathogenic fungi (Glick, 2012). Some antagonists produce siderophores, which scavenge iron, making it unavailable to the fungal pathogen (Schippers *et al.*, 1987).

Recent studies have indicated that biofilm formation in the rhizosphere is of considerable importance in controlling plant pathogens. Biofilm results in the release of various metabolites such as HCN, ammonia, toxins and antibiotics which have inhibitory effect on phytopathogens (Figueiredo *et al.*, 2016). The biofilm of *Bacillus subtilis* is composed of compounds of a family of surfactins, i.e., cyclic molecules with amino acids and lipids, which act as powerful biosurfactants with antifungal and antibacterial activity (Kwon and Kim, 2014).

Competition between pathogen and PGPR in the rhizosphere can limit the disease incidence and severity. Abundant non-pathogenic soil microbes (PGPR) rapidly colonize rhizosphere and use most of the available nutrients making it difficult for pathogens to grow.

Several of the PGPRs trigger a phenomenon in plants, known as Induced Systemic Resistance (ISR) that is phenotypically similar to acquired systemic resistance (ASR). When plants are exposed to pathogens, activation of defence mechanisms occurs at the induction site and this induces resistance via signalling pathway involving salicylic acid and the accumulation of proteins related to pathogenesis (PRPs), which are the major mechanisms involved in plant defence (Moraes, 1998). ISR is triggered by non-pathogenic microorganisms in the rhizosphere and resistance is activated by signalling pathway of jasmonic acid and ethylene (Pieterse *et al*., 1998).

Montano *et al*. (2014) stated that *Bacillus* and *Pseudomonas* sp. suppress the diseases caused by rice pathogens i.e., *X. oryzae pv. oryzae*, *Rhizoctonia solani*, and *M. oryzae* up to 90% depending on the bacteria used, pathogen and the rice variety. Yasmin *et al*. (2017) stated that *Pseudomonas aeruginosa* (strain BRp3) showed antagonism *in vitro* against different phytopathogens including *X. oryzae pv. oryzae* (Xoo) and *Fusarium* spp. Further it was reported that strain BRp3 showed consistent pathogen suppression of different strains of BLB pathogen in rice.

2.3. PGPR based biofertilizers

Biofertilizers contain living microorganisms, which when applied to seed, plant surface, or soil colonize the rhizosphere or the interior of the plant and promote plant growth by increasing or supplying availability of nutrients to the plant (Vessey, 2003).

Biofertilizers act in different ways on the constituent microorganisms used in products such as nitrogen fixation, phosphate solubilization and mobilization, solubilization of potassium and other micro nutrients, plant growth promotion by producing plant growth hormones (Raja, 2013; Kumar *et al*., 2018).

The demand for biofertilizers has increased in the last decade due to their eco-friendly nature and worldwide trend to reduce chemical fertilizer usage. European and Latin American countries are the leading consumers of biofertilizers because of strict regulations for use of chemical fertilizers (Raja, 2013).

In India, first commercial biofertilizer was produced in 1956 and Indian Standards of Specification for *Rhizobium* was established in 1976, followed ISI marks in 1977 (Barman, 2017). In the Indian biofertilizer market, *Rhizobium* and blue green algae (BGA) were considered as established biofertilizers whereas *Azolla*, *Azospirillum* and *Azotobactor* are at intermediate stage (Panda, 2011). However, recently, the following types of biofertilizers are available to Indian farmers: nitrogen fixers (*Rhizobium, Bradyrhizobium, Azospirillum, Azotobacter*), phosphate solubilizers (*Bacillus, Pseudomonas, Aspergillus*), phosphate mobilizers (Arbscular Mycorrhizal Fungi like *Glomus*), potassium solubilizers (*Frateuria aurantia*), silicate solubilizers (*Thiobacillus thiooxidans*), plant growth promoting biofertilizers such as *Pseudomona*s sp. (Barman, 2017).

Development of commercial inoculants of free-living N-fixing bacteria such as *Azoarcus* sp., *Burkholderia* sp., *Gluconacetobacter* sp., *Diazotrophicus* sp., *Herbaspirillum* sp., *Azotobacter* sp., *Bacillus polymyxa*, and especially *Azospirillum* sp. was begun since early 21st century (Vessey, 2003). Free living diazotrophic inocula can be applied on a wider range of crop plants than rhizobia.

Commercial formulations of the phosphate solubilizing microorganisms (PSM) such as *Bacillus megaterium*, *Bacillus polymyxa*, strains of P-solubilizing *Pseudomonas striata* and Fe mobilizing bacteria, *Acidithiobacillus ferrooxidans* have been commercialized by Agri Life, India (Backer, 2018).

2.4. Importance of native isolates of microorganisms for improving plant growth and yield

Use of commercially available plant growth promoting rhizobacteria in order to reduce the use of chemical fertilizers, without compromising yield is an important component of research in the field of agriculture. During the last two decades, use of PGPR based biofertilizers has tremendously increased. Application of commercially available *Azospirillum, Azotobacter, Bacillus* and *Pseudomonas* formulations as single inoculants or as consortia has been reported to improve growth and yield of many crops including rice, wheat and other horticultural crops (Lavakush *et al*., 2014; Raja *et al*., 2006; Mehta *et al*., 2014; Elekhtyar, 2016). However, PGPR strains vary widely with crop and soil types and their growth performances and PGP activities depend on complex interaction of soil, plant and microbes. Many researchers studied native PGPR isolates on plant growth and yield and reported their colonization and plant growth improvement are comparatively higher.

Sahoo *et al*. (2013) evaluated biofertilizer of novel *Azotobacter vinelandii* isolate along with commercial *Azotobacter* strain and revealed *A. vinelandii* was highly efficient to improve growth and yield of rice crop than commercial strain. Based on P solubilization and plant growth activities of native bacterial isolates from Wayanad district, two PGPR (*Providencia* sp. and *Pseudomonas* sp.) were evaluated on growth on cowpea. Growth and yield parameters were highest with native *Pseudomonas* sp. (Saranya, 2016). Twenty-four indigenous PGPR isolates from different rice cultivars in Afghanistan soils were evaluated on rice growth with two commercial inoculants *Bacillus pumilus* and *Azospirillum brasilense* as positive control and results revealed that more than 70% inoculated isolates significantly increased root and shoot dry weight (Habibi *et al*., 2019). Results suggested that isolates AF74 and AF79 belonging to *Enterobacter ludwigii*, isolate AF46 (*Pseudomonas brassicacearum*), AF112

(*Psuedomonas plecoglossicida*), and AF30 (*Rhizobium daejeonense*) were potential candidates as biofertilizers for rice crops in Afghanistan.

Indigenous PGPR easily acclimatize to the natural conditions and enhance the plant growth and yield (Verma *et al.*, 2013). Therefore, use of indigenous isolates have added advantage over commercial inoculants.

2.5. Rice

Rice is the world's leading food crop, cultivated over an area of 166.08 million hectares with the production of 769.52 million tonnes (FAOSTAT, 2018). India is the second largest producer of rice in the world after China.

India is considered as one of the original centres of rice cultivation, covering 43.19 million hectares with annual production about 110.15 million tonnes in 2016 -17. Rice provides 43% calorific requirement for more than 70% of Indian population. Rice productivity of India (3.6 t/ha) is far below world average (4.5 t/ha). Low productivity of rice in India is due to poor soil fertility, nutrient imbalance and indiscriminate usage of inorganic fertilizer and improper management practices (Biswakarma *et al*., 2018). To achieve potential yield of rice, balanced and sufficient fertilizer supply with appropriate management practices are needed.

2.5.1. Fertilizer usage of rice production

Chemical fertilizer application plays a vital role in enhancing rice grain yield and is considered as an effective way to address the food safety issues due to an increasing population. The average per hectare use of fertilizer on paddy was 119.1 kg and comprises 81.7 kg/ha N, 24.3 kg/ha P_2O_5 and 13.1 kg/ha K_2O (FAO, 2005). Among major nutrients, nitrogen is considered as most important essential nutrient element and has profound effect on growth and yield of rice (Panda, 2019). In the year 2017- 2018, total nitrogen fertilizer consumption was 17.4 Mt in India and the share of rice crop was 37% (FAI, 2018).

Chemical fertilizers are costlier and excess application of chemical fertilizers leads to environmental pollution and health issues (Liu *et al*., 2019). Demand of the

food in world is expected to double by 2050, therefore remains a greater challenge to achieve higher rice production with environmental sustainability. Therefore, efficient management of fertilizer in rice is one of the major options to meet this challenge.

2.5.2. Effect of nitrogen fixing bacteria on growth and yield of rice

Rice production depends highly on nitrogen fertilization, since most of the rice soils are N deficient (Bashan and de Bashan, 2010). Non-judicious application of inorganic nitrogen adversely affects the soil and environment. Biological nitrogen fixation (BNF) by diazotrophic bacteria like *Azotobacter* spp is known to supplement 0.4 - 0.9 t/ha (7-20%) in rice (Choudhury and Kennedy, 2004). Tejera *et al*. (2005) reported *Azotobacter* spp are able to fix at least 10 mg N per g of carbohydrate. Nitrogen fixing activity is more in strains associated with cultivated rice than those of wild counterparts (Choudhury and Kennedy, 2004).

Biological nitrogen fixation of different strains is highly location specific and therefore, native strains would be better suited (Kannan and Ponmurugan, 2010). Sahoo *et al*. (2013) assessed the performance of biofertilizer formulation of novel isolate *of Aztobacter vinelandii* along with commercial *Azotobacter* formulation in rice (*Oriza sativa* L. var. Khandagiri) and *A. vinelandii* was reported to be highly efficient to improve growth and yield of rice, without the recommended dose of nitrogen fertilizer.

Herbaspirillum is a broad host range endophyte which colonises rice, sugarcane, wheat, sorghum and other cereals (Bhattacharjee, 2008). Eighty different strains of *H. seropedicae* originally isolated from rice, maize and sorghum were test-inoculated in rice and revealed that inoculation with 12% of the tested strains led to a 100% increase of rice fresh weight over the control (Baldani *et al*, 2000). *Burkholderia* spp, another nitrogen fixer studied in the field with different forms of the bacteria (rhizospheric and endophytic) increased rice grain yield by 0.5 - 0.8 t / ha and plant biomass by 22 mg per plant (Baldani *et al.*, 2000).

Laskar *et al*. (2013) reported eleven genera of free living diazotrophs which were isolated from paddy field and they were *Sphingomonas azotifigens*, *Pseudomonas putida*, *Stenotrophomonas maltophilia*, *Pantoea agglomerans*, *Acinetobacter*

radioresistans, *Herbaspirillum* spp, *Herbaspirillum rubrisubalbicans*, *Klebsiella pneumoniae*, *Alcaligenes faecalis*, *Achromobacter xyloxidans* and *Enterobacter cloacae* subsp. *dissolvens*. Among these, six isolates were selected as good nitrogen fixers (*Sphingomonas azotifigens*, *Pseudomonas putida*, *Herbaspirillum* sp, *Stenotrophomonas maltophilia*, *Pantoea agglomerans* and *Acinetobacter radioresistens*) based on their acetylene reduction activity (ARA) and evaluation of their effect on rice plants revealed significant growth and yield increment with *Sphingomonas azotifigens* (Laskar *et al*., 2013).

2.5.3. Effect of phosphate solubilizing bacteria on plant growth and yield of rice

Microbe-mediated phosphate management in rice cultivation gains practical attention because of its eco-friendly nature. Phosphate solubilizing microorganisms (PSM) are able to convert organic and inorganic soil phosphate into bioavailable forms (Rodriguez and Fraga, 1999).

Inoculation of phosphate solubilizing diazotrophic *Herbaspirillum* strains (H18, ZA15) and a *Burkholderia vietaminensis* strain (AR114) increased rice grain yield from 33 to 47 % with tricalcium phosphate and 18 to 44 % with simple super phosphate, respectively (Estrada *et al*., 2013).

Lavakush *et al*. (2014) evaluated combined *Pseudomonas* culture (CPC) with *Azotobacter chroococcum* and *Azospirillum brasiliense* on rice growth and yield using different phosphorus fertilizer levels. CPC included PGPR strains *P. aeruginosa BHUJY16, P. aeruginosa BHUJY20, P. aeruginosa BHUJY13, P. putida BHUJY23 and P. fluorescens BHUJY29.* Results suggested that CPC with *A. chroococcum* and *A. brasiliense* could reduce the use of phosphorus fertilizer from 60 Kg/ha P_2O_5 to 30 Kg/ha P_2O_5 without any reduction of rice yield.

Stephen *et al*. (2015) isolated two indigenous rhizospheric phosphate solubilizing bacteria *Gluconacetobacter* sp. (MTCC 8386) and *Burkholderia* sp. (MTCC 8369) and examined for their growth enhancement potential in rice. The results revealed that inoculation of these two organisms in combination with 60% rock phosphate had greater impact on growth and yield of the rice.

Evaluation of bacterial strains *Rahnella aquatillis* (KM977991), *Enterobacter* sp. (KM977992), *Pseudomonas fluorescens* and *Pseudomonas putida* on different rice cultivars clearly indicated that single phosphate solubilizing bacteria inoculations increased grain yield, biological yield, total number of stems hill^{-1}, number of panicles hill^{-1} and plant height (Bakhshandeh *et al*., 2015)

Khan *et al*. (2017) also suggested application of two efficient PSM isolates *Burkholderia* spp and *Pseudomonas aeruginosa* by root dipping of seedlings and spraying at the flowering stage significantly enhanced the growth and yield of rice variety BRRI dhan-20. Results revealed that application of both strains with 50% recommended dosage of N, P and K fertilizer produced equivalent or higher yield of rice compared to the application of full dose of N, P, K fertilizer without inoculants. They concluded that these strains may have the potential to be used as bioinoculants for sustainable rice production.

Phosphate solubilizing bacteria *Burkholderia* spp (strain P4), inoculated in rice variety MTU 1010 with recommended dosage of N, P, K fertilizer increased dry matter production by 13.27% than the recommended dose of N, P, K fertilizer application alone (Biswakarma *et al*., 2018).

2.5.4. Effect of potassium solubilizing bacteria on plant growth and yield of rice

Potassium is an essential macro nutrient for rice growth and yield (Bakhshandeh *et al*., 2017). Farmers use large amounts of potassium fertilizer in the form of potassium sulphate (K_2SO_4) and potassium chloride (KCl) to overcome deficiency of K in rice production (De Souza *et al*., 2015). Potassium is an essential nutrient for increasing rice growth and grain yield which is absorbed by rice plant in a greater amount than other macro-nutrients such as nitrogen and phosphorus (Khanghahi *et al*., 2017). The PGPRs such as K solubilizers are able to colonize the surface of root system and stimulate plant growth (Meena and Maurya, 2017).

Khanghahi *et al*. (2019) conducted experiment to assess the effect of three native potassium solubilizing bacteria (KSB) on rice growth. Results revealed that three native KSB (*Pantoea agglomerans*, *Rahnella aquatilis*, and *Pseudomonas orientalis*)

significantly increased grain yield in both pot and field experiments in the range of 20-38% and 20-52% respectively, especially when half of the potassium fertilizer was applied.

2.5.5. Effect of zinc solubilizing bacteria on plant growth and yield of rice

Chaudhary *et al.* (2007) reported Zn deficiency as a key factor in determining the rice production in several parts of India. According to Singh (2009), 48% soils in India are affected by Zn deficiency. PGPR increases bioavailability of Zn by solubilizing different zinc ores present in soils such as ZnO, $ZnCO_3$, and $Zn_3(PO_4)_2$ (Sirohi *et al*., 2015). Therefore, PGPR could be utilized as alternative for chemical zinc fertilizer.

Shakeel *et al.* (2015) tested root associated two *Bacillus* strains on growth, yield and Zn translocation of rice. Results revealed that inoculation of consortium of *Bacillus* sp. and *Bacillus cereus* enhances Zn translocation towards grains and increase yield of basmati-385 and super basmati rice varieties by 22-49% and 18-47% respectively.

Two strains of *Acinetobacter* sp. significantly enhanced growth and yield traits of rice genotype BPT 5204 over uninoculated rice plants under pot experiment (Ghandhi and Muralidharan, 2016).

Vaid *et al*. (2014) evaluated three zinc solubilizers identified as strains of *Burkholderia* sp. SG1, *Acinetobacter* sp. SG2 and *Acinetobacter* SG3 for the growth promotion and zinc uptake in rice plants. Results revealed that these three isolates when used individually and in combination effectively increased growth parameters and zinc content over the control and zinc fertilizer treatments.

2.5.6. Effect of bacterial consortia on growth and yield of rice

Average N, P_2O_5 and K_2O absorbed by rice plants is around 22.2, 7.1 and 32 kg per ton of grain yield (Krishna, 2014). Average global fertilizer use of NPK is 107 kgh^{-1}, which could lead to environmental pollution (Meena and Maurya, 2017). Application of PGPR consortium to reduce chemical fertilizer usage has attracted the attention of researchers in the last two decades.

Raja *et al*. (2006) studied effect of individual microbe and microbial consortium viz., *Azospirillum lipoferum* Az-024, *Bacillus megaterium* var. *phosphaticum* and *Pseudomonas fluorescens* Pf-1 on rice plant growth under hydroponic conditions and revealed that the consortium improved colonization and enhanced crop growth.

Govindarajan *et al*. (2008) studied effects of the inoculation of indigenous *Burkholderia vietnamensis* MGK3 on rice seedlings in a comparison with four other diazotrophs, viz., *Gluconacetobacter diazotrophicus* LMG7603, *Herbaspirillum seropedicae* LMG6513, *Azospirillum lipoferum* 4B LMG4348 and *B. vietnamiensis* LMG10929. MGK3 alone and combined with other diazotrophs enhanced yield of rice. Combination of inoculants significantly increase rice yield 9.5 -23.6% while MGK3 alone increased 5.6 -12.16% over the uninoculated control. Best performance was obtained with combined application of PGPR.

Assessment of plant growth promoting rhizobacteria and rhizobia as multi strain biofertilizer on growth and nitrogen uptake of rice plants was carried out. Native PGPR, UPMB 19 (*Lysinibacillus xylanilyticus*) and an indigenous rhizobia UPMR 30 (*Bradyrhizobium japonicum*) were used and the mixed inoculum significantly promoted plant and root growth, number of tillers, plant dry weight as well as nutrient accumulation in plants (Tan *et al*., 2015).

Kumar *et al*. (2016) demonstrated that the rhizobacterial consortium (UKA-24: *Rhizobium radiobacter,* UKA-72: *Bacillus pumilus,* UKA-27: *Stenotrophomonas maltophilia* and AKA-1: *Pseudomonas putida*) was more effective than single inoculant on growth promotion and nutrient uptake of Basmati rice cultivar Pusa Sugandha 4. The components in the consortium performed different functions such as IAA production, phosphate solubilisation, siderophore production and nitrogen fixation.

PGPR strains (*Pseudomonas fluorescens* + *Bacillus subtilis* + *Azospirillum brasiliense*) along with 75% of nitrogen fertilizer were able to improve rice promising lines GZ9461 – 4 – 2 – 3- 1 and reducing inorganic fertilizer by 25% and reducing environmental pollution (Elekhtyar, 2016).

Materials and Methods

3. MATERIAL AND METHODS

The study entitled "Characterization and evaluation of plant growth promoting rhizobacteria from rice soils of Wayanad" was carried out during the period 2018 to 2020. Experiments comprised isolation of microorganisms on selective media from rhizosphere soils of Wayanad region, laboratory screening for PGP activities and characterization of selected isolates, pot culture evaluation and field evaluation of potential PGPR on rice growth. Laboratory experiments were carried out at the Department of Agricultural Microbiology, College of Horticulture, Vellanikkara. Pot culture and field evaluation of consortia were carried out at Regional Agricultural Research Station, Ambalavayal, Wayanad district.

3.1. Material

3.1.1. Chemicals, Glassware and plasticware

The chemicals used for the experiments were Analytical Grade (AR) and purchased from the agencies like Sisco Research laboratory (SRL), HIMEDIA and Merck India Ltd. The glassware and plasticware were obtained from Borosil, Tarson India Ltd and Merck. The molecular biology reagents were purchased from Sigma-Aldrich Chemicals Private Limited, Bangalore and Vision Scientific, Ernakulam.

3.1.2. Equipment

The equipments available at the Department of Agricultural Microbiology were used for microbiological and molecular experiments. The soil and plant chemical analyses were conducted using facilities available at the Department of Agronomy. Glassware for molecular biology experiments and microbiological media were sterilized by using autoclave Equitron SLEFA of Eutech Instruments, Mumbai. Isolation, purification and inoculation of microorganisms were carried out under sterile conditions (Laminar air flow chamber, Rotek Laboratory Instruments, Kochi). Compound binocular microscope (Leica ICC50) was used for microscopic examination of cells and photomicrography. For measuring pH of culture media, reagents and buffers, pH meter (Eutech pH tutor, Singapore) was used. Total nitrogen content in

microbial cultures was analysed using KELPLUS VA DSL and DISTYL EMBA. Centrifugation was carried out either in table top Eppendorf 5804R or SPINWIN MC-02 centrifuges. Eppendorf Master Cycler was used for amplification of 16S rRNA gene and visualization of DNA in agarose gel was carried out using UV Benchtop Transilluminator from UVP, USA.

3.2. Methodology

3.2.1. Collection of rhizosphere soil samples

Soil samples were collected from rice fields in ten locations in Wayanad District. These locations were Thanivayal, Nellara, Kuppamudi, Edakkal, Pottankoli, Ambalavayal, Malavayal, Ambukuthi, Marathat and Kolagappara. About 500g of rhizosphere soil samples were collected by pulling out two to three-month-old rice plants, with intact root system. Soil samples were placed in polythene bags and transferred to the Department of Agricultural Microbiology, Vellanikkara. Three random soil samples were taken from one location and mixed well, shade-dried and stored under 4^0C in refrigerator for further isolation process and soil analysis. The Global Positioning System (GPS) coordinates of ten selected locations were also recorded using GPS application on a smartphone.

3.2.2. Isolation and enumeration of plant growth promoting bacteria

Enumeration of rhizosphere bacteria was carried out by using serial dilution followed by spread plating. Different functional groups of microorganisms present in the rhizosphere were isolated on seven media (Table 1). Jensen's agar and Ashby's agar were used for enumerating nitrogen fixing bacteria. Nitrogen free Jensen's agar is recommended for detection and cultivation of nitrogen fixing bacteria. Ashby's agar medium is used for isolation of Azotobacter. Pikovskaya's agar, Aleksandrov agar and mineral salts medium (MSM) amended with 0.1% ZnO were used for the enumeration of phosphorus, potassium and zinc solubilizers respectively. King's B agar medium was used for isolating fluorescent pseudomonads. Total number of bacteria was enumerated on nutrient agar medium. Fogg's nitrogen free medium was used for isolation and enumeration of cyanobacteria. The composition of the media is given in Appendix 1.

Table 1: Media and dilutions used for isolation and enumeration of microorganisms

Media	Target organism	Dilution
Nutrient agar	Total bacteria	10^{-5}
Jensen's agar	Nitrogen fixers	10^{-4}
Ashby's agar	Nitrogen fixers	10^{-4}
Pikovskaya's agar	Phosphate solubilizers	10^{-3} to 10^{-4}
Aleksandrov agar	Potassium solubilizers	10^{-2} to 10^{-4}
Mineral salts medium (MSM)	Zinc solubilizers	10^{-2} to 10^{-4}
King's B agar	Fluorescent pseudomonads	10^{-2} to 10^{-4}
Fogg's nitrogen free medium	Cyanobacteria	10^{-2}

Ten grams of soil was transferred into a 250 ml Erlenmeyer flask containing 90 ml sterilized water and mixed thoroughly for fifteen minutes. Then one ml aliquot from this was transferred to sterile nine ml water blank and serially diluted. Dilution rates used for each medium are shown in Table 1. From respective dilutions, 100 µl aliquot was pipetted out and transferred to the respective media aseptically and spread properly throughout the Petri-dish by using a sterilized glass rod. The inoculated Petri-dishes were incubated at room temperature in the inverted position. Observations were made after two to five days according to the media used for enumeration.

For the enumeration of total bacterial population, colonies which appeared on nutrient agar medium were counted. For calculation of the population of nitrogen fixers, all the colonies which appeared on nitrogen free medium were counted. After three to five days of observation, colonies producing clear zones on Pikovskaya's, Aleksandrov's and MSM amended with 0.1 % ZnO were counted as phosphate, K and

Zn solubilizers respectively. To study the population of fluorescent pseudomonads, colonies producing fluorescent pigment on King's B medium were considered.

Fogg's medium was used for isolating N fixing cyanobacteria and 10 g of soil was inoculated to the sterilized Fogg's medium contained in EM flask. Incubation was done at room temperature in presence of diffused sunlight for more than one month. After the growth of cyanobacteria was visible, a small aliquot was transferred to the Fogg's nitrogen free agar plates and incubated for two to three days. Cyanobacteria were observed under a light microscope for the presence and absence of heterocysts.

Isolates which were growing on nitrogen free medium (Jensen's agar and Ashby's agar) were considered as nitrogen fixing bacteria. The development of clear halo zones around the colonies on Pikovskaya's medim, Aleksandrov's medium and mineral salts medium amended with 0.1 % ZnO were considered as phosphate solubilizers, K solubilizers and Zn solubilizers respectively. For selection of fluorescent pseudomonads, colonies which showed fluorescent on King's B medium were selected. These isolates were designated after the location from where isolated (Table 2) and based on their function such as nitrogen fixers (NF), phosphate solubilizers (PS), potassium solubilizers (KS), zinc solubilizers (ZnS) and fluorescent pseudomonads (FP).

Selected isolates from preliminary screening *viz*, N fixers, phosphate solubilizers, K solubilizers, Zn solubilizers and fluorescent pseudomonads were purified by repeated streaking on respective media. Purified bacterial strains were stored in nutrient agar in the form of slant cultures at 4^0C and also stored as 20% glycerol solution at -70^0C for further studies.

Table 2: Details of coding of isolates based on location from where they have been isolated

Location	Code
Thanivayal	Th
Edakkal	Ed
Kolagappara	Kg
Nellara	Nr
Kuppamudi	Kp
Ambalavayal	Av
Marathat	Mt
Ambukuthi	Ak
Malavayal	Mv
Pottankoli	Pk

3.2.3. *In vitro* screening of isolates for plant growth promotion (PGP) and antagonistic activities

3.2.3.1. Screening of bacterial isolates for nitrogen fixation

Total isolates obtained from nitrogen free medium (Jensen's agar and Ashby's agar) were further selected by growing them on nitrogen-free Jensen's agar. Isolates that exhibited profuse/ good growth on Jensen's agar medium were selected as potential nitrogen fixers.

3.2.3.2. Screening of bacterial isolates for phosphate, potassium and zinc solubilization

Bacterial isolates selected from Pikovskaya's agar, Aleksandrov agar and mineral salts medium amended with 0.1 % ZnO were considered as P, K and Zn solubilizers respectively and they were assessed for P, K and Zn solubilization.

Phosphate, potassium and zinc solubilizers were spot inoculated on centre of Pikovskaya's agar, Aleksandrov's agar and mineral salts medium amended with 0.1 % ZnO contained in Petri plates respectively and incubated at room temperature for 7 days. The presence of the clear halo zone around the colony indicated the solubilization capacity of the isolates. After 7 days of incubation period, diameter of colony and halo zone were measured and per cent solubilization efficiency of P, K and Zn calculated using following formula (Panhwar *et al*., 2012),

$$\text{Solubilization efficiency (\%SE)} = \frac{\text{Diameter of clear zone}}{\text{Diameter of colony}} \times 100$$

Selected N fixers, P, K, Zn solubilizers and fluorescent pseudomonads were further screened for plant growth promoting activities such as production of IAA, ammonia, HCN and siderophore. Selected isolates were screened for antagonistic activities against *Xanthomonas oryzae* and *Rhizoctonia solani* by using standard protocols.

3.2.3.3. Production of IAA

To screen the isolates for IAA production, a colorimetric assay was performed using Salkowski method. The selected isolates were inoculated in 25 ml Luria-Bertani broth supplemented with tryptophan at the rate of one mg ml^{-1} contained in test tubes and incubated in the dark for seven days. After incubation, fully grown cultures were centrifuged 3000 rpm for 30 min. The supernatant was mixed with 4 ml Salkowski reagent. Development of pink colour in the solution indicated IAA production by the respective organisms. The quantity of IAA produced was determined at 530 nm with the help of Spectro-photometer (Ahmad *et al*., 2008).

3.2.3.4. Production of ammonia

Bacterial isolates were tested for the production of ammonia in 4% peptone water. Freshly grown cultures of isolates were inoculated in sterilized peptone contained in test tubes and incubated for 48 -72 h at 30 ± 2^0C. After incubation 0.5 ml of Nessler's reagent was added to each tube. Development of orange to brown colour was a positive test for ammonia production (Cappuccino and Sherman, 1992).

3.2.3.5. Production of hydrogen cyanide

All the isolates were screened for the production of microbial HCN, using standard protocols, as described by Bakker and Schippers (1987). Nutrient agar supplemented with glycine at the rate of 4.4 g l^{-1} was used to screen for HCN production. The isolates were inoculated by using inoculation loop on sterilized medium. A filter paper disc impregnated with 0.5% picric acid (yellow) and 2.0% sodium carbonate was placed in the lid of each Petri dish. The Petri dishes were sealed with parafilm and incubated at room temperature for three to four days. Colour change of the filter paper from yellow to orange-brown at the end of incubation period indicated microbial production of cyanide.

3.2.3.6. Production of siderophore

Bacterial isolates were subjected to Chrome Azurol Sulfonate assay (Schwyn and Neilands, 1987), to screen for the production of siderophore. The blue green solution containing Chrome Azurol Sulfonate (CAS), Iron (III) solution and Hexadecyltrimethyl Ammonium Bromide (HDTMA) mixed with 100 ml nutrient agar was used for screening for the production of siderophore. The assay medium was prepared by dissolving 60.5g CAS in 50ml distilled water, which was then mixed with 1mM $FeCl_3.6H_2O$ in 10cmM HCL This solution was mixed with 72.9 g HDTMA dissolved in 40 ml distilled water. Nutrient agar medium and blue green solution containing CAS, Iron (III) and HDTMA were autoclaved separately and mixed together aseptically which was used for the screening. The bacterial isolates to be screened were spot-inoculated on the media and incubated at 30^0C $\pm$2 for three to four days. The presence of yellow to orange colour halo around the colony indicated the siderophore production by microorganisms.

3.2.3.7. Antagonistic activity against selected plant pathogens

Two economically important rice pathogens *Rhizoctonia solani* and *Xanthomonas oryzae* were used as test strains to study the antagonistic potent of N fixers, phosphate solubilizers, K solubilizers, Zn solubilizers and fluorescent pseudomonads.

The antagonism of selected isolates was tested by dual culture assay (Skidmore and Dickinson, 1976). The fungal disc of *R. solani* was placed at the centre of potato dextrose agar (PDA) contained in Petri plate. The bacterial culture was streaked at a distance of 3 cm from fungal disc on three sides of Petri plate and incubated at 30ºC±2. Control plate was maintained with fungal disc of *R. solani* without inoculating any bacterial isolates and observations were recorded after 4-7 days. The diameter of the pathogen was measured in both control and treatment plates and the per cent inhibition on growth of the test pathogen was calculated (Rabindran and Vidyasekaran,1996).

$$I = \frac{C - T}{C} \times 100$$

Where, I = Per cent inhibition

C= Radial growth of the pathogen in control

T = radial growth of the pathogen in treatment

The isolates were screened *in vitro* for inhibition of *X. oryzae* using plate diffusion method (Yasmin *et al*., 2017). A fresh culture of (100µl) of *X. oryzae* grown in nutrient agar (NA) broth was spread on NA plates. The fresh culture of bacterial strain grown on NA to be tested for antagonism was spot inoculated on NA plates already spread with *X. oryzae*. These plates were incubated at 30º C±2 for 48 hours. The presence of inhibition zones around the isolates showed antagonism effect against *X. oryzae*.

3.2.4. Quantification of nitrogen fixation and nutrient solubilization of selected isolates

Nitrogen fixers, phosphate solubilizers, potassium solubilizers and zinc solubilizers were further subjected to the quantification of nitrogen fixation and phosphate, K and Zn solubilization respectively.

3.2.4.1. Quantification of nitrogen fixation by nitrogen fixers

Nitrogen fixers that had multiple PGP characters were further subjected to the quantification of nitrogen fixation by micro-Kjeldahl method (Bremner,1960 and

Jackson, 1973). Loopful of freshly grown cultures of N fixers were inoculated to the sterilized 50 ml Jensen's broth contained in 250 ml flask and incubated for 14 days at room temperature. From this, 10 ml was taken for nitrogen estimation of each sample and 3 g of digestion mixture (potassium sulphate: copper sulphate in the ratio 5:1) and 10 ml concentrated sulfuric acid were added and kept for digestion in block digester until solution became clear. After completion of digestion, it was allowed to cool and then transferred to the distillation unit. For distillation, 40% NaOH and 4% Boric acid containing mixed indicator solution (0.2% bromocresol green + 0.3% methyl red in alcohol with 5:1 ratio) was used to trap the condensed NH_3. In distillation, red colour boric acid was changed to the bluish green colour when NH_3 was trapped. After distillation, it was titrated with 0.01N HCl till the end point indicated by reddish pink colour. Blank was also titrated without sample and reading was recorded. Total nitrogen content of the sample was expressed as mg N fixed per gram of carbon source utilized.

$$\text{mg of N/g of C source} = \frac{TV - BV \times N \times 0.014 \times 1000}{Y}$$

Where, TV = Titre value

BV = Blank Value

N = Normality of HCL

Y = Weight of carbon source

3.2.4.2. Quantification of phosphate solubilization by phosphate solubilizers

Isolates that showed halo zones around the colonies in the qualitative assay were further subjected to the quantification of phosphate solubilization by using phospho-molybdic blue colour method (Olsen *et al*., 1954). Phosphate solubilizers were inoculated to the sterilized 50 ml Pikovskaya's broth and incubated at room temperature for fourteen days. Uninoculated control was incubated for fourteen days. Experiment was done with three replicates. After 14 days broth cultures were centrifuged at 10,000 rpm for 10 min. Five millilitre of supernatant was taken in a test tube and volume made up to 8.6 ml by using distilled water. To this solution, one ml of ammonium molybdate reagent and 0.4 ml ANSA reagent was added. The contents were kept for 10 minutes for blue colour development and intensity of blue colour was measured using

spectrophotometer at 660 nm. The available P content in the broth was calculated by using standard phosphorus graph. The pH of broth was also recorded after 14 days.

The standard P curve was prepared by adding five ml of stock solution (35.2 mg of KH_2PO_4 was dissolved in 10 ml of 10 N H_2SO_4 and made up to 100 ml with distilled water) to 50 ml with distilled water. One ml of working standard contains 8 µg of phosphorus. From this working solution 0, 1, 2, 3, 4 and 5 ml were transferred to test tubes to get 0, 8, 16, 24, 32 and 40 µg of P and volume made up to 8.6 ml with distilled water. To this solution one ml of ammonium molybdate reagent and 0.4 ml ANSA reagent was added, mixed thoroughly and allowed to stand for 10 min for colour development. The colour intensity was measured in a spectrophotometer at 660 nm.

3.2.4.3. Quantification of potassium solubilization by potassium solubilizers

The isolates that exhibited solubilization on Aleksandrov agar were further examined for their ability to release K in broth. Overnight culture of each isolate was inoculated in 50 ml of sterilized Aleksandrov broth and experiment was conducted with four replicates. All the inoculated flasks were incubated at room temperature and centrifuged at 10,000 rpm for ten minutes. The available K in the supernatant was determined by using flame photometry (Sugumaran and Janarthanam, 2007).

3.2.4.4. Quantification of zinc solubilization by zinc solubilizers

The selected isolates which showed clear zones on MSM amended with 0.1% ZnO were further subjected to the quantification of zinc solubilization.

The bacterial isolates were inoculated in 50 ml sterilized mineral salts broth (Dextrose: 10.0 g, ammonium sulphate: 1.0 g, potassium chloride: 0.2 g, dipotassium hydrogen phosphate: 0.1 g; magnesium sulphate: 0.2 g, distilled water: 1000 ml, pH: 7.0) amended with 0.1% insoluble ZnO. Three flasks were maintained for each isolate with an uninoculated control. After 14 days, culture solution was centrifuged at 10,000 rpm for ten minutes. Ten ml of this solution was fed to Atomic Absorption Spectrophotometry (AAS) to determine the available zinc content (Saravanan *et al*., 2004).

Zinc solubilizing bacteria which had the ability to solubilize $ZnCO_3$ were also assed for quantification of zinc solubilization in mineral salts broth amended with 0.1% $ZnCO_3$.

3.2.5. Characterization and identification of selected potential PGPR isolates

Twenty promising PGPR isolates were characterized by cultural, biochemical and molecular methods.

3.2.5.1. Cultural characters

Purified isolates were grown on nutrient agar for observing the cultural characters. The size, shape, colour, elevation and optical properties of the colonies were recorded (Somasegaran and Hoben, 1994).

3.2.5.1.1 Gram staining

Purified isolates were studied for their morphological characters by using Gram staining (Vincent and Humphrey, 1970). Thin bacterial smear was prepared on clean glass slide and heat fixed. Smear was flooded with crystal violet (primary stain) for one minute and washed with water. Then Gram's iodine was added for one minute and decolorized with 95% ethanol. After that it was washed with distilled water and counterstained with safranin for 30 seconds and then washed. Smear was observed under a compound light microscope. Violet colour cells indicated Gram- positive reaction and red colour cells indicated Gram- negative reaction. Shape of the bacterial cells was also observed and recorded.

3.2.5.1.2. Endospore staining

Gram-positive isolates were further subjected to the spore staining. More than two weeks old cultures were used for endospore staining. Thin smear on clean glass slide was flooded with 5 per cent malachite green. It was steamed over water bath for five minutes. While steaming, stain was added from time to time, to avoid drying of stain. Then smear was washed with running tap water and counterstained with safranin

for 30 seconds followed by washing and drying the slide. Green dotted particles within either pink or red coloured cells indicated presence of endospores.

3.2.5.2. Biochemical tests

The isolates were subjected to biochemical tests in order to characterize their metabolic activities (Cappuccino and Sherman, 1992).

3.2.5.2.1. Oxidase test

Loopful of inoculum of 24-48 days old culture were rubbed on oxidase disc with substrate 1% tetramethyl-P-phenylene diamine dihydrochloride. Bacteria that produced cytochrome C oxidase, oxidized the reagent tetramethyl – P- phenylene diamine to indophenols which are purple coloured. Development of purple colour indicated production of oxidase enzymes.

3.2.5.2.2. Catalase test

Three to four drops of 3% H_2O_2 were placed on a clean glass slide and loopful of inoculum of test isolates mixed with H_2O_2 solution. Appearance of air bubbles was identified as catalase positive isolates and absence of air bubble indicated as catalase negative (Hayward, 1960).

3.2.5.2.3. Oxidation of sugars

Fermentative degradation of various carbohydrates such as glucose, fructose, maltose, mannitol, dulcitol and sorbitol were carried out in a fermentation tube. A nutrient broth containing different carbohydrate sources supplemented with a pH indicator (phenol red) was prepared and poured into test tubes. Durham's tubes were carefully inserted in test tubes in an inverted position without formation of bubbles. Sterilized tubes were inoculated with loopful of respective isolates and kept for 24 h - 48 h with uninoculated control. Colour change from red to yellow indicated acid production and presence of bubbles indicated gas production.

3.2.5.3. Molecular characterization

The promising isolates were subjected to molecular characterization by 16S rRNA gene sequencing. Amplification of 16S rRNA was done by using colony PCR (Woodman, 2008). Single colony from 24–48 h purified culture plates was picked and mixed with 20 µl sterilized deionized water and kept at 98^0 C for two minutes. After denaturation, samples were subjected to the centrifugation at 10,000 rpm for one minute and took 4 µl supernatant was used as template for amplification of 16S rRNA gene. Forward primer 8F and reverse primer 1522 R were used (Table 3).

Table 3: Primers used in 16S rRNA gene amplification

Primer details	Sequence 5'- 3.	Base pair
8 F	AGAGTTTGATCCTGGCTCAG	20
1522 R	AAG GAG GTG ATC CAG CCG CA	20

Composition of PCR reaction mixture is given in Table 4. After adding DNA template to the reaction mixture and spin momentary and was kept Eppendorf Master Cycler (Emerald Amp GT PCR). The details of master cycler program are given in Table 5.

Table 4: Composition of PCR reaction mixture

Component	Per reaction volume required (µl)
Takara master mix	25
PCR grade water	15
Forward Primer	2
Reverse Primer	2
Template	6
Total	50

Table 5: Detail of master cycler programme

No.	Step	Temperature (^{0}C)	Time (min)
1.	Initial denaturation	94	4.0
2.	Denaturation	94	0.30
3.	Annealing	55.2	0.45
4.	Primer extension	72	0.45
5.	step 2-4	35 cycles	
6.	Final extension	72	8.0
7.	Final hold	4	∞

3.2.5.3.1. Agarose gel electrophoresis

Agarose gel electrophoresis was used for assessment of amplified DNA fragments (Sambrook *et al*., 1989). One gram of agarose was dissolved in 100 ml 1X TAE buffer and 5 µl ethidium bromide was added. This was allowed to settle in gel casting tray with comb. Five micro litres of I kb DNA ladder was loaded into first well and following wells loaded with five micro litres of respective PCR products. Gel casting tray was placed in electrophoresis tank and filled with 1X TAE buffer and run at constant voltage. After separation of amplified DNA, bands were visualized under UV transilluminator and observation was taken with comparison to the 1500 bp position of the 1 kb ladder. Samples which showed intense bands at 1500 bp were selected for sequencing.

3.2.5.3.2. Purification and sequencing of PCR products

The PCR products were purified using Qiagen quick PCR purification kit as per the manufacturer's protocol and sequenced at Agri Genome Labs Pvt. Ltd, Kochi with primer 8F and 1522R.

3.2.5.3.3. Nucleotide sequence analysis

The BLAST (Basic Local Alignment Search Tool) programme of NCBI (National Centre for Biotechnology) was used for sequence analysis and nucleotide homology alignment of the isolates. The accession sharing with maximum homology

with the query sequence was considered for the identification of the test organisms. The sequences of all the isolates were deposited in the GenBank of the NCBI by using BankIt tool and the accession numbers were obtained for each isolate.

3.2.5.3.4. Phylogenetic tree construction

A BLASTn (Basic Local Alignment Search Tool) similarity search was performed with 16S rRNA gene sequence of each strain, and the best ten hits were selected based on percentage identity, query coverage and evalue. The closely related sequences were selected and retried from the NCBI (National Centre for Biotechnology Information) database. These sequences were used to perform multiple sequence alignment using MAFFT version 7. Before phylogenetic analysis, the best-fit DNA evolutionary substitution model for constructing the Maximum likelihood (ML) phylogenetic tree were selected using the MEGA 7/Jmodel test. The ML phylogenetic trees were constructed with 1000 bootstrap replications under Hasegawa–Kishino–Yano nucleotide substitution model using MEGA 7.

3.2.6. Selection of potential isolates of nitrogen fixers, phosphate solubilizers, potassium solubilizers and zinc solubilizers

Selection of potential isolates from each functional group was done based on ranking, taking into consideration, all the PGP traits. Higher value of weightage three was allotted to the direct mechanism (nitrogen fixation, phosphate, potassium and zinc solubilization) of isolates. Then weightage value two was allotted o the indirect mechanism (IAA production) and one was allotted to other indirect mechanism (production of NH3, HCN and siderophores and antagonism against phytopathogens).

For ranking of twenty N-fixers, weightages of three and two were allotted to the values of quantity of N-fixation and IAA production respectively. A weightage of one was allotted to each of the other PGP traits (production of NH3, HCN and siderophores and antagonism against phytopathogens). The actual value was multiplied by weightage, to get the score for the particular isolate. The sum of scores for various isolates of N-fixers was considered for ranking from one to twenty (Table 35 under Results chapter).

For ranking of 16 phosphate solubilizers, quantity of P solubilized was allotted a weightage of three, IAA production, a weightage of two and for other PGP traits, one each. Scores were calculated by multiplying the value with the weightage and sum of scores was considered for ranking of phosphate solubilizers from one to sixteen (Table 36 in Results).

The same procedure was followed for ranking of four isolates of K-solubilizers and six isolates of Zn-solubilizers (Tables 37 and 38 in Results). Best three N-fixers, three P-solubilizers and two each of K and Zn-solubilizers were selected for pot culture and field experiments.

3.2.7. Compatibility studies of promising isolates

Ten efficient isolates were selected from different functional groups and tested for compatibility using cross streak techniques (Raja *et al*., 2006). Two different isolates were streaked on vertically and horizontally on nutrient agar medium containing Plates and incubated for 48 h at 30^0C $\pm$ 2. Any inhibition of microorganism growth at cross point of two organisms were observed after incubation. If there were no inhibition at the cross point, the two microorganisms were considered as compatible organisms.

Further these isolates were subjected to the dual culture method and confirmed their compatibility. All the selected isolates were inoculated in sterilized nutrient broth and incubated for two days. Aliquot of one isolate was transferred into the nutrient agar medium spread throughout the plate by a sterilized glass rod. Other isolates for testing compatibility were spot inoculated at the centre of same Petri plate and incubated for two to three days for observation on inhibition zone. Absence of inhibition zone indicated compatibility among tested two isolates.

3.2.8. Preparation of talc based PGPR consortium

Three different consortia were prepared from the most efficient ten isolates of PGPR from different functional groups. Each consortium included five selected isolates consisting of two nitrogen fixers, one phosphate solubilizer, one K solubilizer and one Zn solubilizer.

A loopful each of the ten selected isolates was inoculated in separate sterile tubes containing nutrient broth, from 72 h old log phase pure cultures. These were incubated at a temperature of 30 ± 2^0 C for 6 days to get a population of 10^8 cfu ml^{-1} of liquid culture. Then serial dilution and plating was carried out to measure population of each isolate on nutrient agar. After confirmation of the presence of a population of 10^8 cfu ml^{-1} in nutrient broth, five millilitres each of the five selected isolates were mixed with 100 g of sterilized talc and air dried. After 6 days of incubation, serial dilution and plating was carried out for the enumeration of N fixers, phosphate solubilizers, K solubilizer and zinc solubilizers in the talc-based consortia by using Jensen's agar, Pikovskaya's agar, Aleksandrov agar and mineral salt medium amended with 0.1% ZnO respectively.

3.2.8. Pot culture evaluation of consortia

Three different talc based PGPR consortia consisting of most efficient N fixers, P, K and Zn solubilizers were evaluated for growth and yield of local rice variety "Valichoori "as test crop. The pot culture experiment was conducted at Regional Agricultural Research Station, Kerala Agricultural University, Ambalavayal, Wayanad district during March 2019 to August 2019. Soil physico-chemical and biological parameters were determined at the start of the experiment, using standard protocols (Table 9).

The experimental details are given below and treatment combinations are detailed in Table 6.

Design: CRD

Crop: Rice

Variety: Valichoori (local variety)

Location: RARS, Ambalavayal

Replications: 3, Treatments: 12

Table 6: Treatments used in pot culture

Treatments	Details of treatments
T_1	Consortium 1 with 50% RDF of N, P, K
T_2	Consortium 2 with 50% RDF of N, P, K
T_3	Consortium 3 with 50% RDF of N, P, K
T_4	PGPR Mix-1 (KAU formulation) with 50% RDF of N, P & K
T_5	Consortium 1 with 75% RDF of N, P & K
T_6	Consortium 2 with 75% RDF of N, P & K
T_7	Consortium 3 with 75% RDF of N, P & K
T_8	PGPR Mix-1(KAU formulation) with 75% RDF of N, P & K
T_9	Organic package of practices (KAU, 2017)
T_{10}	RDF (Recommended dosage of fertilizers)
T_{11}	Package of practice (KAU, 2017)
T_{12}	Control (No inoculum, no fertilizers)

RDF: Recommended dosage of fertilizer
RDF (%100): N: 70 kg/ha, P_2O_5: 45 kgha and K_2O: 45 kg/ha
Package of practice (KAU, 2017): 100% RDF + Organic manure 5t/ha
Organic package of practice (KAU, 2017): Farm yard manure 5t/ha

Germinated seeds of rice were treated with bioinoculant at the rate of 500 g carrier-based consortium for 80 kg of paddy seeds. Five nursery trays were maintained as uninoculated, PGPR Mix-I treated and consortium 1, 2 and 3 treated separately. After 21 days, seedlings were dipped in 10% of respective biofertilizer for 10 minutes and transplanted in pots. Recommended dosage of fertilizer was applied as basal dose, after 45 days and 60 days of planting. Biofertilizers were applied every month after transplanting upto panicle initiation stage at the rate of 2.5 kg in 100 kg organic matter.

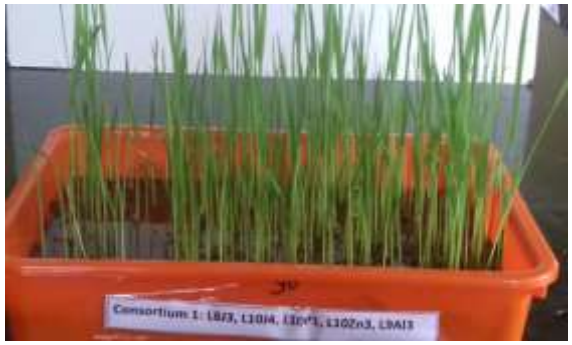


Fig a: Consortium 1 treated nursery

Fig b: Consortium 2 treated nursery

Fig c: Consortium 3 treated nursery

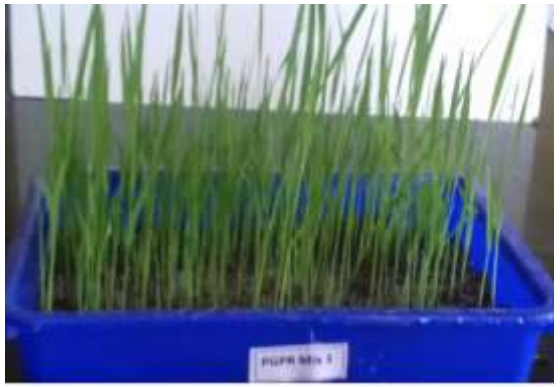


Fig d: PGPR Mix 1 treated nursery

Fig e: Uninoculted nursery

P1ate 1: Twenty one days old nurseries of rice for pot experiment

3.2.8.1 Enumeration of bacterial isolates in soil samples

Bacterial population of each treatment at monthly interval after transplanting upto flowering were enumerated by serial dilution and plating technique using selective media such as nutrient agar, Jensen's agar, Pikovskaya's agar, Aleksandrov agar and MSM amended with 0.1% ZnO.

3.2.8.2. Plant biometric characters

Plant height, number of tillers/plant, number of leaves/tiller were taken as growth parameters. Yield component like number of panicles/plants, filled grains/panicle were recorded. Final grain yield and straw yield were recorded.

3.2.9. Field evaluation of PGPR consortia

Best two treatments selected from pot culture were evaluated for rice growth and yield attributes in field conditions. The field trial was conducted at research field, Regional Agricultural Research Station, Kerala Agricultural University, Ambalavayal, Wayanad during July 2019 to December 2019.

Treatment combinations are detailed in Table 7 and experimental design as follows

Design: RBD

Crop: Rice

Variety: Valichoori (local variety)

Replications: 4

Treatments: 5

Season: Nanja

Location: RARS, Ambalavayal

Plot size: 5 m x 4 m

Table 7: Treatments used in field evaluation

Treatment No.	Details of treatments
T_1	Best consortium with 75% RDF of N, P & K
T_2	Second best consortium with 75% RDF of N, P & K
T_3	PGPR Mix-1 with 75% RDF of N, P & K
T_4	Recommended dosage of fertilizer (RDF) of N, P, K
T_5	Farmer's practice (control)

RDF: Recommended dosage of fertilizer
RDF (%100): N: 70 kg/ha, P_2O_5: 45 kgha and K_2O: 45 kg/ha
Farmer's practice: Farm yard manure 5t/ha

3.2.9.1. Nurseries for field transplanting

Seeds were soaked for overnight in wet gunny bags to hasten sprouting. The sprouted seed were sown by broadcasting. Separate nurseries were maintained for different bioinoculants treated seeds and uninoculated seeds. Pre germinated seeds of rice were treated with bioinoculants at the rate of 500 g for 80 kg of seeds.

3.2.9.2. Field preparation

The experimental field was ploughed twice followed by puddling in standing water. After land levelling, land was left for 2 days for the soil to settle. Plot was 5 m x 4 m in size with 50 cm width and 6 inches height bunds. Adequate number of bunds and channels were prepared for irrigation and drainage.

3.2.9.3. Transplanting of rice seedlings

Twenty-one days old seedlings were dipped with 10% respective bioinoculants for 30 minutes. Transplanting was done manually with 10 cm x 5 cm. Three to four plants were transplanted per hill.

3.2.9.4. Fertilizer application

After seven days of transplanting, basal application of fertilizer was done. Full dose of recommended nitrogen, phosphorus and potassium were applied in treatment T_4. Treatments T_1, T_2 and T_3 were combined application of bioinoculants with 75% of

recommended dosage of N, P and K respectively. Treatment T_5 was maintained as farmer's practice and only organic manure at the rate of 5t ha^{-1} farm yard manure. One third of recommended dose of fertilizer was applied as basal and two split doses after 45 days and 60 days of transplanting. Bio-inoculants were applied as soil application at every month up to panicle initiation stage at the rate of 2.5 kg biofertilizer in 100 kg organic matter. At least seven days gap was maintained between application of chemical fertilizer and bio- inoculant.

3.2.9.5. Cultural practices

Observations on pest and disease incidence in the field were taken at regular intervals and appropriate control measures were adopted. Field trial was maintained as weed free plot up to panicle initiation stage by hand weeding, whenever necessity.

3.2.9.6. Enumeration of bacterial isolates from rhizosphere soil samples

Bacterial population was enumerated by serial dilution and plating technique using array of media (nutrient agar, Jensen's agar, Pikovskaya's agar, Aleksandrov's agar and mineral salt medium amended with 0.1 % ZnO). Rhizosphere soil samples were taken during growth period at monthly interval after transplanting up to flowering stage.

3.2.9.7. Plant biometric characters

Plant height and number of tillers/m^2 were taken at maximum tillering stage. Number of panicles/m^2, number of grains/panicles, percentage grain filling, 1000 grain weight, and grain and straw yield/20 m^2 plot were recorded at harvesting stage. One square meter frame was placed in the middle of the plot and number of tillers were counted at maximum tillering stage and number of panicles were counted at harvesting stage.

3.2.10. Chemical analysis of rice plants at flowering stage

Plant samples were analysed to determine N, P, K and Zn content of plants in different treatments under both pot and field evaluation. Plant samples were taken at flowering stage, oven dried at 70^0C, powdered and sieved for the nutrient analysis. The methodologies used are depicted in Table 8.

Table 8: Methodologies employed for the chemical analysis of rice plant samples

Parameters	Method	Reference
Nitrogen	Micro-Kjeldahl method	Jackson, (1973)
Phosphorus	Vanado-molybdo phosphoric yellow colour method	Jackson, (1973)
Potassium	Diacid extract method using flame photometer	Jackson, (1973)
Zinc	Diacid method using atomic absorption spectrophotometer	Lindsay and Norwell, (1978)

3.2.11. Physico-chemical and microbial biomass carbon analysis of soil

The soil samples were analysed for pH, EC and nutrient availability such as nitrogen, phosphorus, potassium and zinc at harvesting in both pot and field experiments. After harvesting, soil samples were also analysed for microbial biomass carbon. Methodologies used for the soil analysis are detailed in Table 9.

Table 9: Methodologies employed for physico-chemical and microbial biomass carbon analysis of rhizosphere soil samples

Parameters	Method	Reference
Soil pH	Soil water suspension of 1:25 and read pH meter	Jackson, (1958)
Electrical conductivity	Soil water suspension of 1:25 read electrical conductivity meter	Jackson, (1958)
Organic carbon	Walkley and Black method	Walkley and Blak, (1934)
Microbial biomass carbon	Fumigation extraction method	Vance *et al*., (1987)
Available nitrogen	Alkaline permanganate method	Subbaiah and Asija, (1965)
Available phosphorus	Ascorbic acid reduced molybdophosphoric blue colour method	Watanabe and Olsen, (1965)
Available potassium	Neutral normal ammonium acetate using flame photometer	Jackson, (1958)
Available zinc	Extraction using 0.1 M HCl by atomic absorption spectrophotometer	Sims and Johnson, (1991)

3.3. Benefit cost ratio

Economics of cultivation was worked out after taking into account the cost of cultivation and the prevailing market price of rice. Benefit cost (B:C) ratio was calculated for the different treatments of field experiments. Benefit-cost ratio worked out using the following formula.

$$B:C = \frac{\text{Gross income per ha}}{\text{Cost of cultivation per ha}}$$

3.4. Statistical analysis

Statistical analysis was done on different characters using Web Based Agricultural Statistics Software Package (WASP). Analysis of variance studies was engaged to assess the variation among different parameters. In the cases where the effects were found to be significant, the critical difference was calculated at five or one percent probability level.

Fig a: Sowing of pre germinated seeds of rice nurseries

Fig b: Twenty-one days old rice seedlings for transplanting

Plate 2: Nurseries of rice for field experiment

Fig a: Field view after final land preparation

Fig b: Transplanting of rice seedlings

Plate 3: Field view at transplanting of rice seedlings

Results

4. RESULTS

The results of the research work entitled "Characterization and evaluation of plant growth promoting rhizobacteria from rice soils of Wayanad" carried out during 2018-2020 at the Department of Agricultural Microbiology, College of Horticulture, Vellanikkara are presented in this chapter.

4.1. Collection of rice rhizosphere soil samples

Rhizosphere soil samples were collected from ten different rice growing tracts in Wayanad district and the location of these rice fields along with Global positioning system (GPS) coordinates is presented in Table 10 and Plate 4.

Table 10: Details of rice fields selected for the collection of rhizosphere soil samples from Wayanad district of Kerala

Location name	GPS coordinates		Altitude (m)
	Latitude	Longitude	
Thanivayal (Th)	N 11.64799^0	E 76.21378^0	802
Edakkal (Ed)	N 11.61748^0	E 76.23915^0	887
Kolagappara (Kg)	N 11.64715^0	E 76.22716^0	808
Nellara (Nr)	N 11.59585^0	E 76.18624^0	760
Kuppamudi (Kp)	N 11.64741^0	E 76.21788^0	792
Ambalavayal (Av)	N 11.62193^0	E 76.22169^0	902
Marathat (Mt)	N 11.5946^0	E 76.20042^0	824
Ambukuthi (Ak)	N 11.60807^0	E 76.23969^0	910
Malavayal (Mv)	N 11.62515^0	E 76.24487^0	890
Pottankoli (Pk)	N 11.57399^0	E 76.19977^0	813

4.1.1. Physico-chemical properties of soil samples collected from rice fields in Wayanad

The rhizosphere soil samples collected from rice fields at ten different locations were analyzed for the physico-chemical parameters such as electrical conductivity, pH, organic carbon (%) and content of soil nutrients. The content of primary nutrients *viz.* total nitrogen (kg ha^{-1}), available phosphorus (kg ha^{-1}), available potassium (kg ha^{-1}) and micro nutrient zinc (mg kg^{-1}) are presented in Table 11.

All the soils were acidic and the pH values ranged from 4.16 (Ambukuthi and Edakkal) to 5.59 (Marathat). EC of soil was highly variable in different locations, ranging from 0.08 dS m^{-1} to 0.90 dS m^{-1}. The lowest EC was observed in Ambalavayal soil, whereas the highest value was observed in Thanivayal (Table 11).

Organic carbon content in soil samples varied from 0.32 to 1.17 per cent. Highest organic carbon content of 1.17 per cent was recorded in soil sample collected from Ambukuthi. Organic carbon content was low (less than 0.5 per cent) in three samples (Kolagappara, Ambalavayal and Pottankoli), medium (0.5 to 0.75 per cent) in one (Malavayal) and high (more than 0.75 per cent) in other samples.

The content of available nitrogen was low in all the soil samples, as the values recorded were less than 280 kg ha^{-1}. The values ranged between 137.98 kg ha^{-1} (Edakkal sample) to 275.96 kg ha^{-1} (Ambukuthi).

Available P content was low in two samples (Nellara and Kuppamudi), medium in four samples (Edakkal, Kolagappara, Ambalavayal and Malavayal). Highest available P content of 39.33 kg ha^{-1} was recorded in soil sample from Marathat and lowest value of 6.44 kg ha^{-1} in soil sample collected from Kuppamudi.

All the soil samples tested were low in available potassium content, since the values were less than 120 kg ha^{-1}. The values ranged from 21.28 kg ha^{-1} to 89.60 kg ha^{-1}. Highest K content (89.60 kg ha^{-1}) was observed in Pottankoli soil sample and it was lowest (21.28 kg ha^{-1}) in soil from Kolagappara.

Fig. a. Collection of rice rhizosphere soil samples from paddy fields

Fig. b. Map showing the locations of soil sample collection

Plate 4. Locations of rice fields in Wayanad for rhizosphere soil sample

The content of available zinc was low in all the soil samples tested, as the values were less than 0.6 mg kg^{-1}. Maximum content of zinc was observed in soil sample from Ambukuthi (0.50 mg kg^{-1}) and lowest in soil sample from Marathat (0.14 mg kg^{-1}).

4.2. Population of microorganisms in rice rhizosphere soil samples collected from different locations

The population of total bacteria, nitrogen fixers, phosphate solubilizers, potassium solubilizers, zinc solubilizers and fluorescent pseudomonads recorded in rhizosphere soil samples collected from ten locations in Wayanad, on suitable media and dilutions are detailed in Table 12 and Plate 5.

Total bacterial population varied in different locations, between 5.00 x 10^6 to 23.00 x 10^6 cfu g^{-1} of soil. Significantly higher of total bacteria was recorded in soil samples from Thanivayal, Kolagappara, Kuppamudi, Ambalavayal, Ambukuthi, Malavayal and Pottankoli, than the other locations (Table 12).

The population of free-living nitrogen fixing bacteria isolated using Jensen's nitrogen-free agar and Ashby's mannitol agar is given in Table 12. Population was higher on Jensen's nitrogen-free agar than on Ashby's mannitol agar medium, for all the soil samples tested. On Jensen's agar, significantly higher population of nitrogen fixers was recorded in Kolagappara, Ambukuthi and Pottankoli. Population was lowest on Jensen's agar in Edakkal (8.33 x 10^5 cfu g^{-1}). On Ashby's agar, significantly higher population of N-fixers was recorded in Pottankoli, Malavayal, Ambukuthi, Marathat and Kuppamudi soils. Lower populations were observed in Edakkal, Thanivayal, Kolagappara, Malavayal, Nellara and Ambalavayal. Population of cyanobacteria on N-free Fogg's medium was different in different samples and was significantly higher in soil samples from Kuppamudi (18.76 x 10^4 cfu g^{-1} of soil). Lowest population of cyanobacteria was recorded in soil sample from Nellara (1.83 x 10^4 cfu g^{-1} of soil).

Population of phosphate solubilizers varied with locations and it ranged from 2.66 x 10^5 cfu g^{-1} of soil (observed in Nellara sample) to 13.33 x 10^5 cfu g^{-1} (observed in Pottankoli). Population of phosphate solubilizers was significantly higher in soils from Pottankoli, Ambukuthi, Malavayal, Kuppamudi, Edakkal and Thanivayal.

Significantly lower population was observed in the locations Kolagappara, Nellara and Ambalavayal.

Population of potassium solubilizers ranged from 0.70 x 10^5 cfu g^{-1} of soil (Nellara) to 4.66 x 10^5 cfu g^{-1} (Kolagappara and Malavayal). Significantly higher population was observed in Thanivayal, Kolagappara, Ambalavayal, Ambukuthi and Malavayal, than other locations. Lowest population was observed in Marathat and Nellara locations (0.40 x 10^5 and 0.70 x 10^5 cfu g^{-1}).

Zn solubilizing microorganisms isolated on mineral salt medium containing ZnO also showed significantly different population in different locations. Significantly higher population of Zn solubilizers was observed in soil samples from Malavayal, Pottankoli, Ambukuthi, Kuppamudi and Thanivayal. Lowest population was observed in Nellara (0.43 x 10^5 cfu g^{-1} soil).

Fluorescent pseudomonads were detected at 10^{-5} dilution on King's B agar dilution, only in samples from two locations *viz*. Kuppamudi (1.33 x 10^5 cfu g^{-1} of soil) and Pottankoli (0.67 x 10^5 cfu g^{-1} of soil).

Table 11: Physico-chemical properties of the rice rhizosphere soil samples from Wayanad district

Location name	pH	EC (dSm^{-1})	Organic C %	Available N ($kg\ ha^{-1}$)	Available P ($kg\ ha^{-1}$)	Available K ($kg\ ha^{-1}$)	Available Zn ($mg\ kg^{-1}$)
Thanivayal (Th)	4.96	0.90	0.93	213.25	25.76	29.91	0.30
Edakkal (Ed)	4.16	0.36	0.77	137.98	24.28	35.84	0.21
Kolagappara (Kg)	5.01	0.23	0.32	150.52	20.56	21.28	0.24
Nellara (Nr)	4.34	0.27	1.15	225.79	7.43	45.92	0.41
Kuppamudi (Kp)	4.49	0.23	1.021	188.16	6.44	26.88	0.26
Ambalavayal (Av)	5.32	0.08	0.44	150.28	18.33	47.04	0.33
Marathat (Mt)	5.59	0.16	0.89	150.52	39.33	80.64	0.14
Ambukuthi (Ak)	4.16	0.83	1.17	275.96	25.02	47.04	0.50
Malavayal (Mv)	5.10	0.16	0.64	263.42	12.14	40.32	0.41
Pottankoli (Pk)	5.07	0.12	0.49	200.70	29.23	89.60	0.48
Critical limits as per Tandon (2004)		< 1.0	0.5 to 0.75	280 to 560	10 to 25	120 to 280	0.6

Table 12: Population of microorganisms in rice rhizosphere soil samples from different locations in Wayanad

Locations	Total bacteria cfu g^{-1} of soil	Nitrogen fixing microorganisms			Phosphate solubilizers cfu g^{-1} of soil	K solubilizers cfu g^{-1} of soil	Zn solubilizers cfu g^{-1} of soil	Fluorescent pseudomonads cfu g^{-1} of soil
		Jensen's Agar cfu g^{-1} of soil	Ashby's Agar cfu g^{-1} of soil	Cyanobacteria on Fogg's N-free agar cfu g^{-1} of soil				
Thanivayal (Th)	7.33×10^6 (6.805) ^{ab}	17.33×10^5 (6.218) ^{f}	8.33×10^5 (5.916) ^{bc}	4.76×10^4 (4.665)^{cd}	8.33×10^5 (5.874) ^{abcd}	1.66×10^5 (5.201) ^{ab}	3.66×10^5 (5.551) ^{abc}	ND
Edakkal (Ed)	5.00×10^6 (6.563) ^{b}	8.33×10^5 (5.916) ^{g}	4.66×10^5 (5.667) ^{c}	4.90×10^4 (4.670)^{cd}	9.66×10^5 (5.960) ^{abc}	1.00×10^5 (5.159) ^{bc}	1.33×10^5 (5.100) ^{d}	ND
Kolagappara (Kg)	15.33×10^6 (7.184) ^{a}	46.66×10^5 (6.669) ^{a}	9.33×10^5 (5.888) ^{bc}	3.96×10^4 (4.563)^{cd}	4.66×10^5 (5.619) ^{de}	4.66×10^5 (5.651) ^{a}	1.06×10^5 (5.201) ^{cd}	ND
Nellara (Nr)	6.66×10^6 (6.593) ^{b}	24.33×10^5 (6.385) ^{cde}	8.66×10^5 (5.884) ^{bc}	1.83×10^4 (4.244)^{e}	2.66×10^5 (5.003) ^{e}	0.70×10^5 (4.667) ^{cd}	0.43×10^5 (4.434) ^{e}	ND
Kuppamudi (Kp)	17.66×10^6 (7.244) ^{a}	23.33×10^5 (6.365) ^{def}	15.00×10^5 (6.162) ^{ab}	18.76×10^4 (5.273)^{a}	7.66×10^5 (5.874) ^{abcd}	1.33×10^5 (5.100) ^{bc}	3.00×10^5 (5.460) ^{abcd}	1.33×10^5 (5.100)
Ambalavayal (Av)	11.33×10^6 (7.009) ^{ab}	25.00×10^5 (6.376) ^{def}	11.33×10^5 (5.946) ^{bc}	4.93×10^4 (4.679)^{cd}	4.66×10^5 (5.651) ^{cde}	1.66×10^5 (5.201) ^{ab}	2.00×10^5 (5.259) ^{cd}	ND
Marathat (Mt)	15.00×10^6 (7.159) ^{a}	30.66×10^5 (6.486) ^{bcd}	16.00×10^5 (6.197) ^{ab}	7.66×10^4 (4.852)^{bc}	6.33×10^5 (5.793) ^{bcd}	0.40×10^5 (4.333) ^{d}	2.33×10^5 (5.318) ^{bcd}	ND
Ambukuthi (Ak)	19.66×10^6 (7.284) ^{a}	37.66×10^5 (6.578) ^{ab}	15.00×10^5 (6.159) ^{ab}	9.26×10^4 (4.963)^{b}	10.66×10^5 (6.053) ^{ab}	2.66×10^5 (5.418) ^{ab}	2.66×10^5 (5.401) ^{abcd}	ND
Malavayal (Mv)	20.00×10^6 (7.240) ^{a}	17.00×10^5 (6.227) ^{ef}	12.33×10^5 (6.082) ^{ab}	3.53×10^4 (4.54)^{d}	6.33×10^5 (5.874) ^{abcd}	4.66×10^5 (5.651) ^{a}	6.00×10^5 (5.761) ^{a}	ND
Pottankoli (Pk)	23.00×10^6 (7.294) ^{a}	36.33×10^5 (6.544) ^{abc}	25.33×10^5 (6.402) ^{a}	7.33×10^4 (4.861)^{bc}	13.33×10^5 (6.163) ^{a}	1.33×10^5 (5.100) ^{bc}	5.33×10^5 (5.678) ^{ab}	0.67×10^5 (4. 826)

Log transformation values are given in parenthesis, ND: not detected

4.3. Isolation, purification and maintenance of isolates

Predominant colonies of microorganisms on different media obtained from various locations were purified by repeated streaking on respective media and used for further screening. A total of 60 nitrogen fixers, 59 phosphate solubilizers, seven K solubilizers, 21 Zn solubilizers and two fluorescent pseudomonads were obtained in pure culture (Table 13). Based on the growth on N-free medium/ solubilization zone in case of phosphate, potassium and zinc solubilizers, potential isolates were selected through preliminary screening. These isolates included 32 nitrogen fixers, 16 phosphate solubilizers, four potassium solubilizers, six Zn solubilizers and two fluorescent pseudomonads (Table 13). These isolates were named after the location from where isolated, maintained as slant cultures in a refrigerator and used for further screening for plant growth promoting activities.

Table 13: Isolates obtained from each functional group

Locations	Number of total isolates obtained from each functional group				
	N-fixers	**Phosphate solubilizers**	**K-solubilizers**	**Zn-solubilizers**	**Fluorescent pseudomonads**
Thanivayal (Th)	6	7	1	2	-
Edakkal (Ed)	4	8	-	1	-
Kolagappara (Kg)	10	-	-	1	-
Nellara (Nr)	2	2	-	1	-
Kuppamudi (Kp)	8	6	1	2	1
Ambalavayal (Av)	6	6	-	4	-
Marathat (Mt)	9	8	-	-	-
Ambukuthi (Ak)	6	8	1	4	-
Malavayal (Mv)	5	8	3	3	-
Pottankoli (Pk)	4	6	1	3	1
Total	60	59	7	21	2
No. of isolates selected for screening	32	16	4	6	2

4.4. *In vitro* screening of selected isolates from different functional groups for plant growth promoting activities and antagonistic activities against plant pathogens

All the isolates obtained were screened under *in vitro* conditions for plant growth promotion activities including the production of ammonia, IAA, HCN and siderophore, using the standard procedure detailed under the chapter Materials and Methods. The results of these experiments are detailed below.

4.4.1. Plant growth promoting and antagonistic activities of nitrogen fixers under *in vitro* conditions

Thirty-two nitrogen fixing bacteria were screened for PGP activities such as production of ammonia, IAA, HCN and siderophore and antagonistic activity against two rice pathogens.

4.4.1.1 Plant growth promoting activities of nitrogen fixers

The results of experiments on plant growth promoting activities of nitrogen fixers are presented in Table 14.

The results revealed that, out of 32 isolates tested, 30 were positive for ammonia production. The isolate $KgNF_9$ recorded high production of ammonia. Thirteen isolates were medium producers of ammonia and 16 isolates recorded low reaction. Six isolates ($KgNF_1$, $KpNF_6$, $AvNF_2$ $AvNF_4$, $AkNF_3$ and $PkNF_4$) recorded high production of IAA and four isolates recorded medium. Eighteen isolates failed to produce any IAA. None of the isolates showed positive reaction for HCN production, whereas thirteen isolates were positive for siderophore production. Isolates $KpNF_5$, $AvNF_1$, $AvNF_2$, $AkNF_2$ and $AkNF_3$ were medium level producers of siderophore. Nineteen isolates did not produce siderophores.

4.4.1.2. Antagonistic activities of nitrogen fixers against plant pathogens under *in vitro* conditions

All the 32 isolates of nitrogen fixers were screened for antagonistic activities against two pathogens affecting rice (fungal pathogen *Rhizoctonia solani* and bacterial pathogen *Xanthomonas oryzae*) by dual culture method. Only two isolates ($KpNF_5$ and $MtNF_4$) exhibited antagonism against the pathogen *Rhizoctonia solani* and two isolates

($KgNF_1$ and $KpNF_5$) against *Xanthomonas oryzae* (Table 14 and Plate 9). Isolate $KpNF_5$ showed antagonistic activity against both the pathogens tested. None of the other isolates exhibited any antagonistic activity against either of the plant pathogens tested.

4.4.1.3 Quantification of IAA production by selected isolates

Quantification of IAA was carried out for all the 14 isolates found to be positive for IAA production in the qualitative assay. Results are presented in Table 15. The isolates were significantly different in the amount of IAA produced and the values ranged from 2.50 µg ml^{-1} (observed in the isolate $MtNF_3$) to 34.83 µg ml^{-1} (recorded in $KgNF_1$). $KgNF_1$ recorded the highest IAA production of 34.83 µg ml^{-1}, as compared to the other isolates.

Table 14: Plant growth promoting activities and antagonistic activities of selected nitrogen fixers under *in vitro* conditions

Isolates	IAA production	NH3 production	HCN production	Siderophore production	Antagonistic activity	
					R. solani	*X. oryzae*
$ThNF_1$	-	++	-	-	-	-
$EdNF_1$	-	+	-	-	-	-
$EdNF_2$	-	++	-	-	-	-
$KgNF_1$	+++	++	-	+	-	+
$KgNF_4$	-	+	-	-	-	-
$KgNF_5$	-	-	-	-	-	-
$KgNF_9$	+	+++	-	-	-	-
$NrNF_2$	-	+	-	-	-	-
$NrNF_6$	-	-	-	-	-	-
$KpNF_2$	++	+	-	-	-	-
$KpNF_4$	+	+	-	-	-	-
$KpNF_5$	-	++	-	++	+	+
$KpNF_6$	+++	+	-	-	-	-
$KpNF_7$	++	+	-	-	-	-
$AvNF_1$	-	+	-	++	-	-
$AvNF_2$	+++	+	-	++	-	-
$AvNF_3$	+	+	-	+	-	-
$AvNF_4$	+++	+	-	+	-	-
$MtNF_1$	-	+	-	-	-	-

$MtNF_2$	-	+	-	-	-	-
$MtNF_3$	+	++	-	-	-	-
$MtNF_4$	-	++	-	-	+	-
$AkNF_2$	-	++	-	++	-	-
$AkNF_3$	+++	++	-	++	-	-
$AkNF_4$	-	++	-	-	-	-
$AkNF_5$	-	++	-	+	-	-
$MvNF_1$	-	+	-	-	-	-
$MvNF_2$	-	++	-	-	-	-
$PkNF_1$	-	+	-	+	-	-
$PkNF_2$	++	++	-	+	-	-
$PkNF_3$	++	+	-	+	-	-
$PkNF_4$	+++	++	-	+	-	-

Positive reactions (High +++, Medium ++. Low +), Negative reactions (-)
NF: Nitrogen fixers,
Th: Thanivayal, Ed: Edakkal, Kg: Kolagappara, Nr: Nellara, Kp: Kuppamudi
Av: Ambalavayal, Mt: Marathat, Ak: Ambukuthi, Mv: Malavayal, Pk: Pottankoli

Table 15: Quantity of IAA production by nitrogen fixing isolates

Isolate	IAA Production (µg ml^{-1})
$KgNF_1$	34.83 ^{a}
$KgNF_9$	3.50 ^{g}
$KpNF_2$	14.83 ^{cd}
$KpNF_4$	5.00 ^{fg}
$KpNF_6$	19.33 ^{bc}
$KpNF_7$	15.83 ^{cd}
$AvNF_2$	18.83 ^{bc}
$AvNF_3$	6.16 ^{efg}
$AvNF_4$	25.00 ^{b}
$MtNF_3$	2.50 ^{g}
$AkNF_3$	23.16 ^{b}
$PkNF_2$	11.16 ^{def}
$PkNF_3$	12.33 ^{de}
$PkNF_4$	20.66 ^{bc}
CD (0.01)	8.44

NF: nitrogen fixers
Kg: Kolagappara, Kp: Kuppamudi, Av: Ambalavayal, Mt: Marathat Ak: Ambukuthi
Pk: Pottankoli

Fig.a. Total bacteria on Nutrient agar at 10^{-6} dilution

Fig.b. Nitrogen fixing bacteria on Jensen's agar at 10^{-5}

Fig. c. Phosphate solubilizing bacteria on Pikovskaya's agar at 10^{-5}

Fig.d. Zinc solubilizing bacteria on Mineral salt medium

Plate 5: Enumeration of rhizosphere bacteria on different array of media

Fig. a. Nitrogen fixing bacterial colonies on Jensen's agar

Fig. b. $ThNF_1$ on Jensen's agar

Fig. c. Phosphate solubilizing bacterial colonies on Pikovskaya's agar

Fig. d. $MtPS_6$ on Pikovskaya;s agar

Fig. e. Zinc solubilizing bacterial colonies on Mineral salts medium

Fig. f. $AvZnS_1$ on Mineral salts medium

Plate 6: Colonies of N fixers, phosphate and Zn solubilizers on respective media

Fig. a. $ThNF_1$

Fig. b. $KgNF_4$

Fig. c. $KgNF_9$

Fig. d. $KpNF_5$

Fig. e. $KpNF_6$

Fig. f. $MtNF_1$

Plate 7: Nitrogen fixing bacteria colonies on Jensen's agar

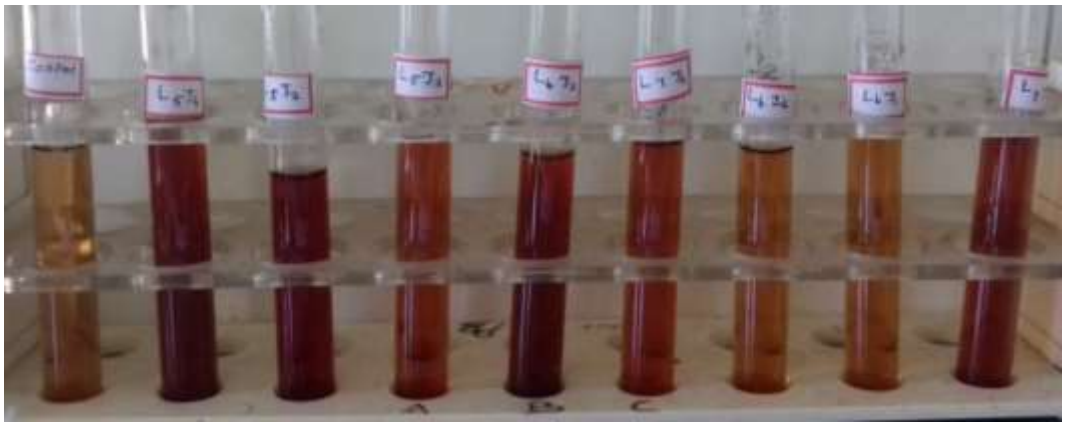


Fig. a. Indole acetic acid production by nitrogen fixers

Fig. b. Ammonia production by nitrogen fixers

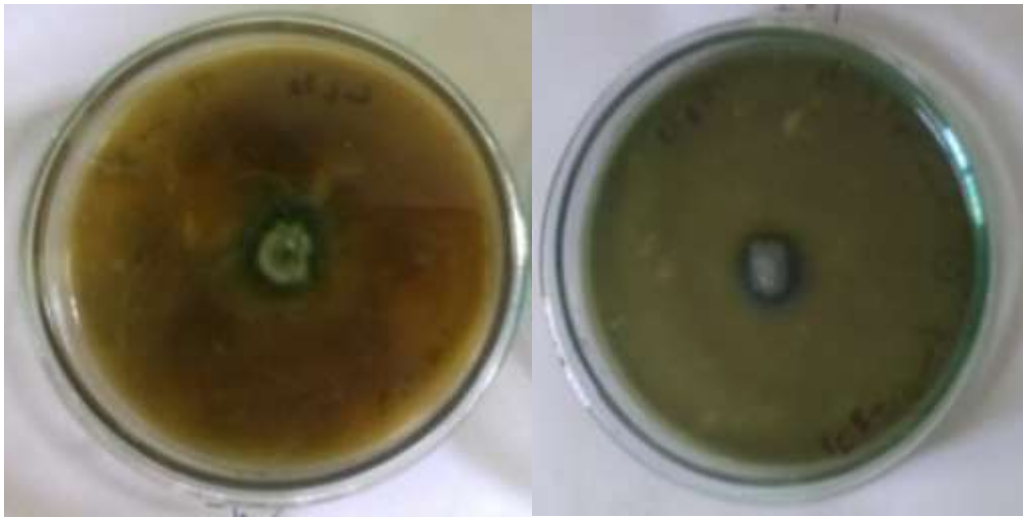


Fig. c. Siderophore production by nitrogen fixers

Plate 8: Plant growth promoting activities of nitrogen fixers

Fig. a. Antagonism of isolate $KpNF_5$ against *X. oryzae*

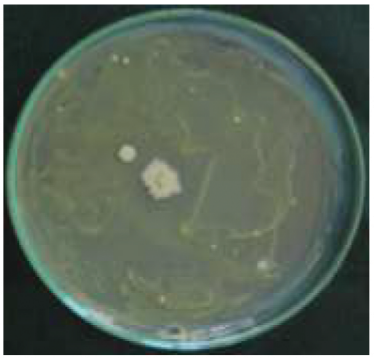


Fig. b. Antagonism of isolate $KgNF_1$ against X. *oryzae*

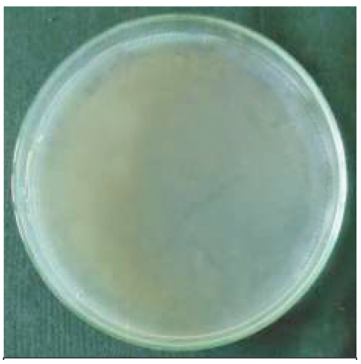


Fig. c. Control plate of *X. oryzae*

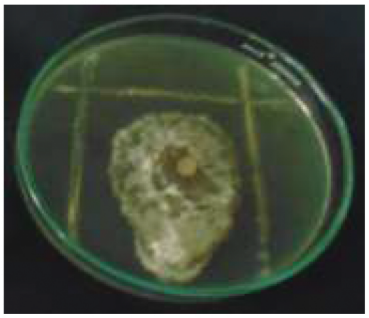


Fig. c. Antagonism of isolate $KpNF_5$ against *R. solani*

Fig. d. Antagonism of isolate $MtNF_4$ against *R. solani*

Fig. f. Control plate of *R. solani*

Plate 9: Antagonistic activities of nitrogen fixers

Based on the results of various tests conducted on PGP characters of N-fixers, a comparison of the isolates was carried out and 20 best isolates with multiple PGP traits were selected (Table 16) for further quantification of the nitrogen fixed.

Table 16. PGP activities of N-fixing bacteria selected for quantification of N-fixation

Isolates	N fixation	IAA production	NH3 production	HCN production	Siderophore production	Antagonistic activity	
						R. solani	*X. oryzae*
$KgNF_1$	+	+++	++	-	+	-	+
$KgNF_9$	+	+	+++	-	-	-	-
$KpNF_2$	+	++	+	-	-	-	-
$KpNF_4$	+	+	+	-	-	-	-
$KpNF_5$	+	-	++	-	++	+	+
$KpNF_6$	+	+++	+	-	-	-	-
$KpNF_7$	+	++	+	-	-	-	-
$AvNF_1$	+	-	+	-	++	-	-
$AvNF_2$	+	+++	+	-	++	-	-
$AvNF_3$	+	+	+	-	+	-	-
$AvNF_4$	+	+++	+	-	+	-	-
$MtNF_3$	+	+	++	-	-	-	-
$MtNF_4$	+	-	++	-	-	+	-
$AkNF_2$	+	-	++	-	++	-	-
$AkNF_3$	+	+++	++	-	++	-	-
$AkNF_5$	+	-	++	-	+	-	-
$PkNF_1$	+	-	+	-	+	-	-
$PkNF_2$	+	++	++	-	+	-	-
$PkNF_3$	+	++	+	-	+	-	-
$PkNF_4$	+	+++	++	-	+	-	-

Positive reactions (High +++, Medium ++. Low +), Negative reactions (-)
NF: Nitrogen fixers
Th: Thanivayal, Ed: Edakkal, Kg: Kolagappara, Nr: Nellara, Kp: Kuppamudi
Av: Ambalavayal, Mt: Marathat, Ak: Ambukuthi, Mv: Malavayal, Pk: Pottankoli

4.4.1.4. Quantification of nitrogen fixation by selected N fixers

Twenty nitrogen fixers which showed multiphasic PGP activities, as detailed in Table 16, were subjected to quantification of nitrogen fixation by micro-Kjeldahl method (Jackson, 1973 and Bremmer, 1960). Results showed that the isolates differed significantly with respect to the nitrogen fixation ability (Table 17). Amount of nitrogen fixed by the N fixers varied from 1.86 to 9.33 mg of N g^{-1} of sucrose utilized. Significantly higher amount of fixed nitrogen was observed in the isolates $AkNF_3$, $PkNF_4$, $KgNF_1$, $KpNF_5$, $KpNF_2$, $AkNF_2$ (Table 17).

Table 17: Nitrogen fixation by selected N fixers under *in vitro* conditions

Isolate	Amount of nitrogen fixed (mg of N g^{-1} of sucrose utilized)
$KgNF_1$	8.86 ^{ab}
$KgNF_9$	6.06 ^{defg}
$KpNF_2$	7.93 ^{abcd}
$KpNF_4$	4.66 ^{fgh}
$KpNF_5$	8.40 ^{abc}
$KpNF_6$	4.26 ^{gh}
$KpNF_7$	3.73 ^{hij}
$AvNF_1$	6.53 ^{cdef}
$AvNF_2$	2.33 ^{ij}
$AvNF_3$	5.60 ^{efgh}
$AvNF_4$	1.86 ^{j}
$MtNF_3$	4.20 ^{ghi}
$MtNF_4$	7.00 ^{bcde}
$AkNF_2$	7.93 ^{abcd}
$AkNF_3$	9.33 ^{a}
$AkNF_5$	4.16 ^{ghi}
$PkNF_1$	6.06 ^{defg}
$PkNF_2$	4.66 ^{gh}
$PkNF_3$	6.06 ^{defg}
$PkNF_4$	9.33 ^{a}
CD (0.01)	2.56

NF: nitrogen fixers
Kg: Kolagappara, Kp: Kuppamudi, Av: Ambalavayal, Mt: Marathat
Ak: Ambukuthi, Pk: Pottankoli

4.4.2. Plant growth promoting and antagonistic activities of phosphate solubilizing rhizosphere bacteria

Sixteen predominant bacterial isolates capable of phosphate solubilization obtained from the rice fields of Wayand (Table 13) were screened for PGP activities (production of IAA, NH_3, HCN and siderophores) and also antagonistic activities against two rice pathogens (*Rhizoctonia solani* and *Xanthomonas oryzae*).

4.4.2.1. Screening for plant growth promoting activities

The results of the experiments conducted on the plant growth promoting activities of phosphate solubilizers are detailed in Table 18.

Out of the sixteen P solubilizers tested, twelve isolates recorded IAA production. Two isolates *viz*. $EdPS_5$ and $MtPS_2$ showed high level of IAA production, based on the intensity of the pink color developed. Seven isolates recorded medium level of IAA production and three, low levels (Table 18).

Twelve isolates were positive for NH_3 production. Eleven isolates recorded low levels and the isolate $AvPS_1$ showed medium level of NH_3 production. Eight isolates were positive for siderophore production and among these, only one isolate ($MvPS_3$) recorded medium level of siderophore. All the other seven isolates recorded low levels of siderophore production. None of isolates were positive for the production of HCN.

4.4.2.2. Screening for antagonistic activities

All the 16 phosphate solubilizers were screened against two selected pathogens *R. solani* and *X. oryzae* and the isolate $MvPS_3$ exhibited antagonism against *X. oryzae* (Table 18 and Plate 12). None of the other isolates exhibited any antagonistic activity on either pathogen.

4.4.2.3. Quantification of IAA production by selected phosphate solubilizers

All the twelve isolates found to be positive for IAA production were subjected to the quantification of IAA produced. Production of IAA was significantly different in different isolates and it ranged between 2.66 µg ml^{-1} to 19.8 µg ml^{-1} (Table 19). Highest

production of IAA was recorded by the isolate $MtPS_2$ (19.8 µg ml^{-1}). This was followed by $EdPS_5$ (12.0 µg ml^{-1}), which was significantly lower than the production by $MtPS_2$.

Table 18: Plant growth promotion and antagonistic activities of phosphate solubilizers

Isolate	IAA production	NH3 production	HCN production	Siderophore production	Antagonistic activity	
					R. solani	*X. oryzae*
$ThPS_4$	-	+	-	-	-	-
$EdPS_4$	-	+	-	-	-	-
$EdPS_5$	+++	-	-	-	-	-
$AvPS_1$	++	++	-	+	-	-
$MtPS_2$	+++	+	-	+	-	-
$MtPS_5$	++	-	-	-	-	-
$MtPS_6$	++	-	-	-	-	-
$AkPS_4$	++	+	-	+	-	-
$AkPS_5$	-	+	-	-	-	-
$AkPS_7$	-	-	-	+	-	-
$MvPS_3$	++	+	-	++	-	+
$MvPS_4$	++	+	-	+	-	-
$PkPS_1$	++	+	-	+	-	-
$PkPS_2$	+	+	-	+	-	-
$PkPS_3$	+	+	-	-	-	-
$PkPS_5$	+	+	-	-	-	-

Positive reactions (High +++, Medium ++. Low +), Negative reactions (-)
PS: Phosphate solubilizers, Th: Thanivayal, Ed: Edakkal, Av: Ambalavayal
Mt: Marathat, Ak: Ambukuthi, Mv: Malavayal, Pk: Pottankoli

Table 19: Quantity of IAA production by selected P solubilizers

Isolates	IAA production (µg ml^{-1})
$EdPS_5$	12.00 [b]
$AvPS_1$	8.16 [bc]
$MtPS_2$	19.80 [a]
$MtPS_5$	4.83 [cd]
$MtPS_6$	5.33 [cd]
$AkPS_4$	4.66 [cd]
$MvPS_3$	7.00 [cd]
$MvPS_4$	3.66 [cd]
$PkPS_1$	4.66 [cd]
$PkPS_2$	4.83 [cd]
$PkPS_3$	2.66 [d]
$PkPS_5$	3.33 [d]
CD (0.01)	6.412

PS: Phosphate solubilizers
Ed: Edakkal, Av: Ambalavayal, Mt: Marathat, Ak: Ambukuthi
Mv: Malavayal, Pk: Pottankoli

4.4.2.4. Estimation of phosphate solubilisation by bacterial isolates

Phosphate solubilization efficiency of the phosphate solubilizing bacteria was estimated both qualitatively and quantitatively. Qualitative estimation was carried out based on the diameter of the clear zone produced on Pikovskaya's agar, around the bacterial colony, as detailed under Materials and Methods and the results are presented in Table 20. Phosphate solubilization efficiency of the isolates varied from 31.0 per cent to 127.7 per cent. Highest per cent solubilization was recorded by the isolate $PkPS_1$, followed by $PkPS_3$ and $AvPS_1$. Lowest phosphate solubilisation efficiency of 30.7 per cent was recorded by the isolate $AkPS_7$.

Quantification of the phosphate solubilized by the bacterial isolates was also determined by Mo-blue method (Olsen, 1954) and the results indicated that the efficiency of isolates varied significantly. Highest value of phosphate solubilization was recorded in the isolate $PkPS_1$ (134.88 µg ml^{-1}), and this was significantly superior to all other isolates. This was followed by $AkPS_4$ (121.33 µg ml^{-1}), $AvPS_1$ (113.83 µg ml^{-1}), $PkPS_2$ (108.3 µg ml^{-1}) and $PkPS_3$ (103.83 µg ml^{-1}). There was a drop in the pH of the broth in all the samples, which ranged from 4.10 to 6.54.

Table 20: Efficiency of P solubilisation by bacterial isolates

Isolates	P solubilization efficiency (%)	Quantity of phosphate solubilized (µg ml^{-1})	pH of broth after 14 days
$ThPS_4$	85.0	74.00 ^{f}	6.45
$EdPS_4$	96.5	82.33 ^{e}	6.12
$EdPS_5$	61.0	55.66 ^{h}	6.66
$AvPS_1$	122.5	113.33 ^{c}	4.10
$MtPS_2$	110.0	66.83 ^{g}	4.63
$MtPS_5$	77.7	82.00 ^{e}	5.12
$MtPS_6$	104.5	49.83 ^{h}	5.45
$AkPS_4$	85.1	121.33 ^{b}	5.32
$AkPS_5$	62.5	81.33 ^{e}	6.23
$AkPS_7$	30.7	72.5 ^{fg}	6.54
$MvPS_3$	110.5	77.66 ^{ef}	5.96
$MvPS_4$	31.0	81.66 ^{e}	4.85
$PkPS_1$	127.7	134.88 ^{a}	5.12
$PkPS_2$	70.0	108.33 ^{cd}	5.28
$PkPS_3$	123.8	103.83 ^{d}	5.42
$PkPS_5$	77.0	50.00 ^{h}	5.27

PS: Phosphate solubilizers,
Th: Thanivayal, Ed: Edakkal, Av: Ambalavayal, Mt: Marathat, Ak: Ambukuthi
Mv: Malavayal, Pk: Pottankoli

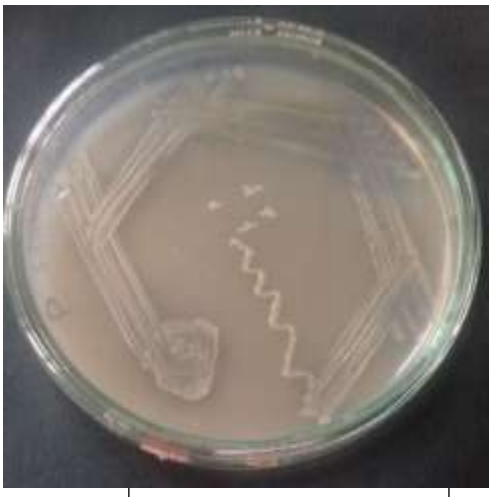


Fig. a. $AvPS_1$

Fig. b. $MtPS_4$

Fig. c. $AkPS_4$

Fig. d. $AkPS_5$

Plate 10: Phosphate solubilizing bacteria colonies on Pikovskaya's agar

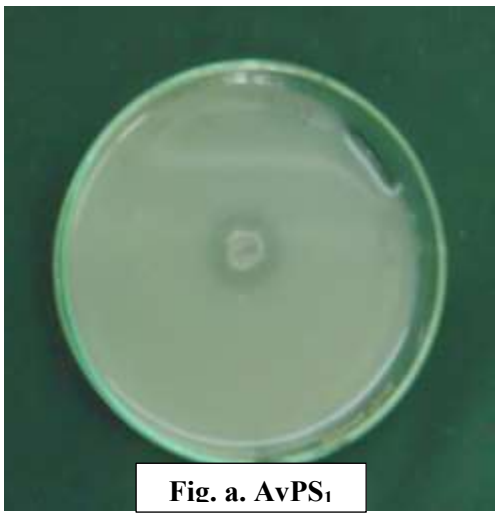


Fig. a. $AvPS_1$

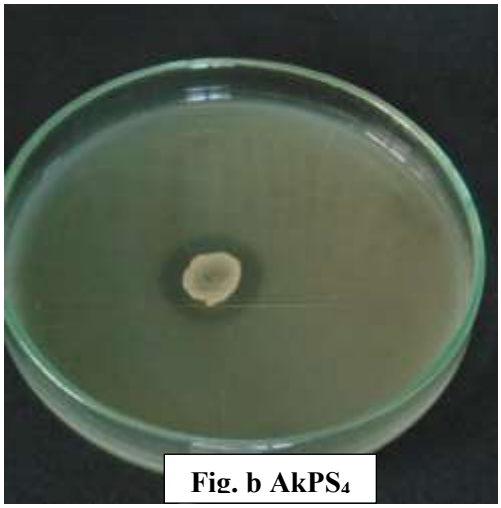
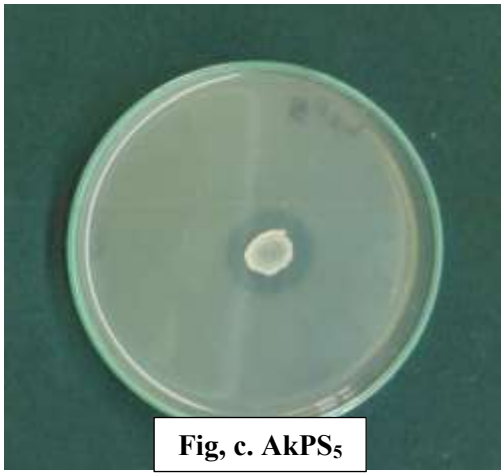


Fig. b $AkPS_4$



Fig, c. $AkPS_5$

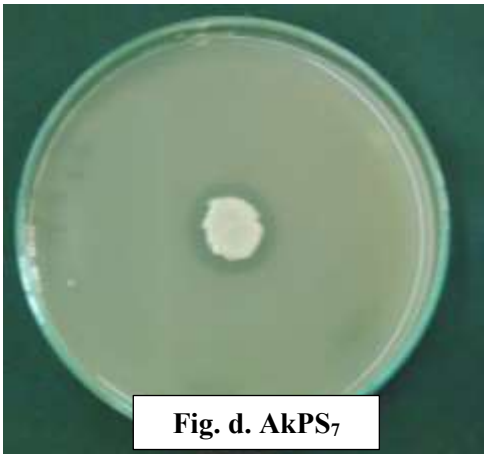


Fig. d. $AkPS_7$

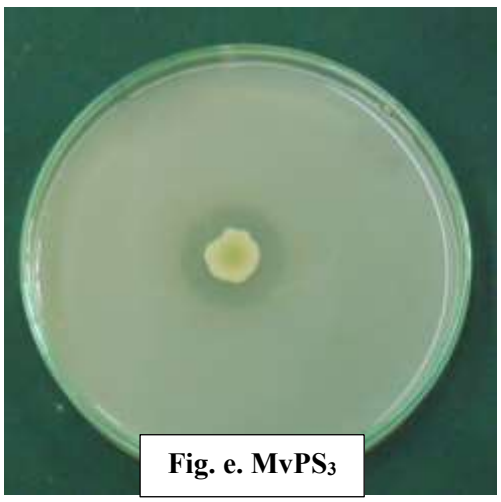


Fig. e. $MvPS_3$

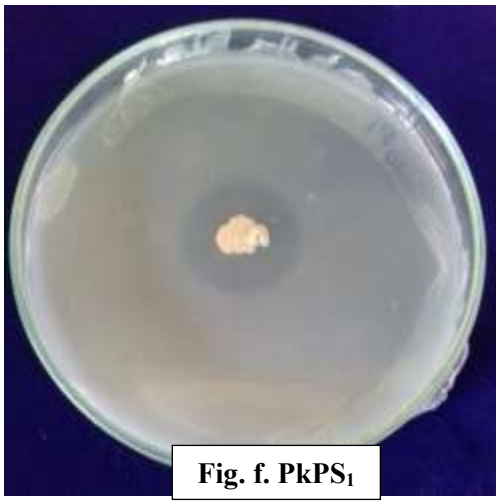


Fig. f. $PkPS_1$

Plate 11: Solubilization zones by phosphate solubilizers on Pikovskaya's agar

Fig. a. Indole acetic acid production by phosphate solubilizers

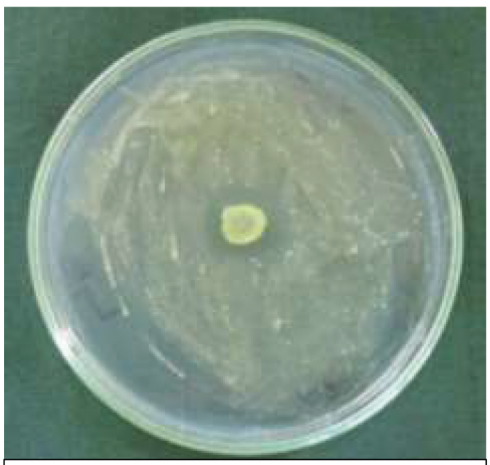


Fig. b. Antagonism of isolate MvPS$_3$ against *X. Oryzae*

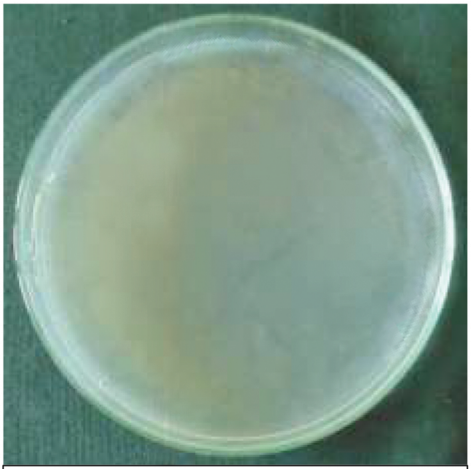


Fig. c. Control plate of *X. oryzae*

Plate 12: Plant growth promoting and antagonistic activities of P solubilizers

4.4.3. Plant growth promoting and antagonistic activities of potassium solubilizing isolates

All the four potassium solubilizing isolates were screened for PGP activities and antagonism against rice pathogens (*X. oryzae* and *R. solani*). The results are presented below.

4.4.3.1. Screening of K-solubilizers for PGP activities

The results of experiment on PGP activities of K-solubilizing bacteria are given in Table 21. Three isolates out of four test isolates recorded NH_3 production. None of the isolates produced IAA, HCN or siderophore.

4.4.3.2 Screening of K-solubilizers for antagonistic activities

All the four K-solubilizers were screened for antagonistic activity against *R. solani* and *X. oryzae* under *in vitro* conditions by the dual culture method. None of the isolates exhibited antagonism against either of the pathogens.

Table 21: Plant growth promotion and antagonistic activities of selected potassium solubilizers

Isolates	IAA production	NH3 production	HCN production	Siderophore production	Antagonistic activity	
					R. solani	*X. oryzae*
$AkKS_1$	-	+	-	-	-	-
$MvKS_1$	-	+	-	-	-	-
$MvKS_2$	-	-	-	-	-	-
$MvKS_3$	-	+	-	-	-	-

Positive reactions (High +++, Medium ++. Low +), Negative reactions (-)
KS: Potassium solubilizers
Ak: Ambukuthi, Mv: Malavayal

4.4.3.3. Efficiency of potassium solubilisation by bacterial isolates

Efficiency of potassium solubilization of the four selected isolates was determined both qualitatively and quantitatively. The results of the experiment are

presented in Table 22. Per cent solubilisation of K ranged from 51.14 (as recorded by $MvKS_2$) to 142.69 (as recorded by the isolate $MvKS_1$).

The results of the experiment on quantification of K solubilization by the isolates in Aleksandrov broth are presented in Table 22. The four selected isolates varied significantly in the amount of K solubilized. The isolate $MvKS_1$ recorded highest solubilisation of K (4.19 µg ml^{-1}) and was superior to the other three isolates. The K-solubilization efficiency was lowest for $MvKS_2$. All cultures showed a shift of pH in broth after 14 days incubation period. Initial pH of the broth was adjusted to 7.0 to 7.2. The pH of the broth at the end of incubation period ranged from 4.41 to 4.97.

Table 22: Efficiency of potassium solubilisation by bacterial isolates

Isolates	K solubilizing efficiency (%)	Amount of K solubilized (µg ml^{-1})	pH of the broth after 14 days
$AkKS_1$	79.34	1.81 ^{bc}	4.50
$MvKS_1$	142.69	4.19 ^{a}	4.40
$MvKS_2$	51.13	1.62 ^{c}	4.41
$MvKS_3$	111.18	3.02 ^{b}	4.97

KS: Potassium solubilizers
Ak: Ambukuthi, Mv: Malavayal

Fig. a. $MvKS_1$

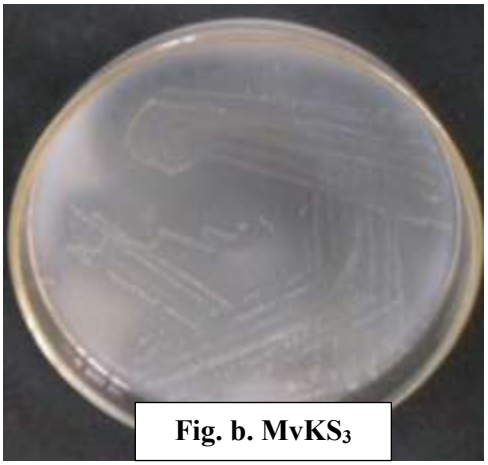


Fig. b. $MvKS_3$

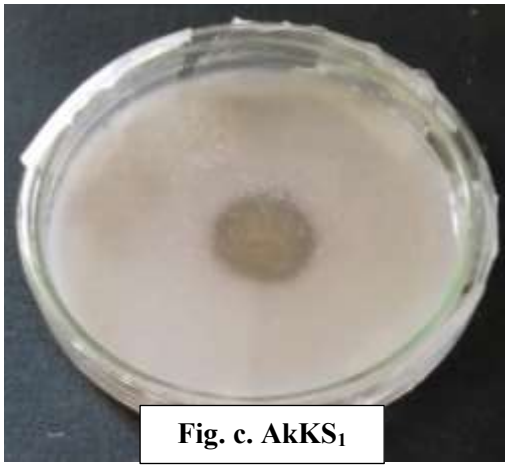


Fig. c. $AkKS_1$

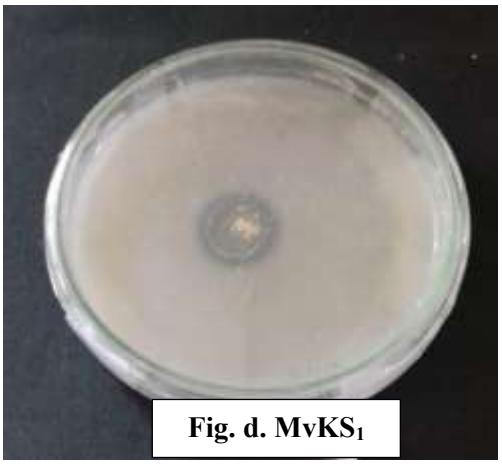


Fig. d. $MvKS_1$

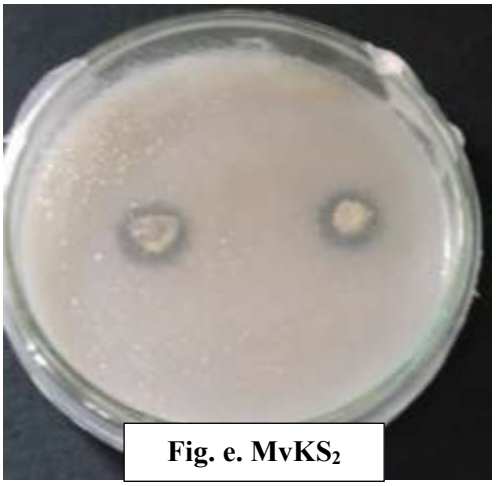


Fig. e. $MvKS_2$

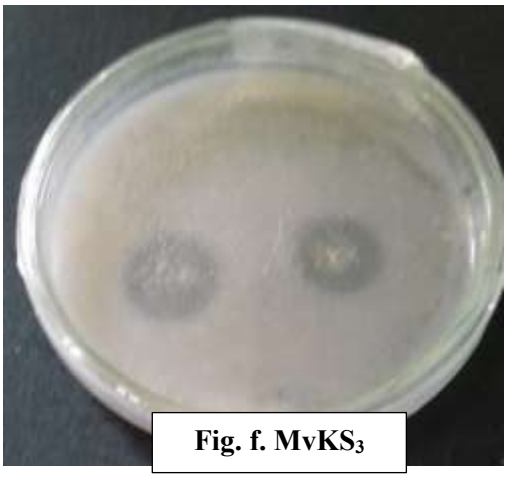


Fig. f. $MvKS_3$

Plate 13: Solubilization zones by potassium solubilizers on Aleksandrov's agar

4.4.4. Plant growth promoting and antagonistic activities of zinc solubilizing isolates

Six zinc solubilizers isolated from different locations were screened for PGP and antagonistic activities against *R. solani* and *X. oryzae* under *in vitro* conditions. The results of the experiment are given below.

4.4.4.1. Plant growth promoting activities of zinc solubilizing isolates

All the six selected isolates of zinc solubilizers obtained from the rice fields of Wayanad were screened for plant growth promoting activities including production of IAA, NH_3, HCN and siderophores. The results obtained from the experiments are presented in Table 23. None of the isolates recorded IAA production. Four isolates ($ThZnS_1$, $MvZnS_1$, $MvZnS_3$ and $PkZnS_3$ recorded low levels of NH_3 production. None of isolates were positive for HCN production. Two isolates *viz.* $MvZnS_1$, $MvZnS_3$ recorded medium level of siderophore production and the other isolates failed to produce siderophore.

4.4.4.2. Antagonistic activities of Zn solubilizers against plant pathogens

All the six isolates of Zn solubilizing bacteria were tested for antagonistic activities, by dual culture method, on two pathogens of rice *viz*. *R. solani* and *X. oryzae.* None of the isolates tested were found to possess antagonistic activities against either of the two pathogens (Table 23), as indicated by lack of inhibition zone.

4.4.4.3. Efficiency of isolates for zinc solubilization

Efficiency of all the six selected isolates for solubilisation of Zn was tested under *in vitro* conditions by qualitative and quantitative methods and the results are presented in Table 24. Qualitative estimation of solubilisation efficiency based on solubilisation zone produced on mineral salt medium with ZnO revealed that there was significant difference in the efficiency among the isolates. The efficiency ranged from 27.30 per cent (recorded by $AkZnS_4$) to 143.98 per cent (observed in the isolate $PkZnS_3$).

Zinc solubilisation efficiency was assessed for all the six isolates by quantification of zinc solubilized in mineral salts medium with ZnO as insoluble zinc

source in broth culture. The results are presented in Table 24. There was significant difference among the selected isolates in Zn solubilization efficiency. Significantly higher amount of Zn solubilization was observed in the isolates $PkZnS_3$ (18.57 µg ml-^{1}), $MvZnS_1$ (17.66 µg ml-^{1}) and $ThZnS_2$ (17.44 µg ml^{-1}). Significantly lower efficiency to solubilize ZnO was recorded in $AvZnS_1$ (14.67 µg ml^{-1}) and $AkZnS_4$ (14.05 µg ml^{-1}). All cultures reduced the pH of the broth after 14 days incubation period and it ranged from 6.12 to 6.80 (Table 24).

Ability of the isolates to solubilize zinc was also assessed using the insoluble source zinc carbonate in the mineral salts medium by plate assay. The results revealed that only two isolates ($PkZnS_3$ and $ThZnS_2$) possessed the ability to solubilize zinc carbonate. These two strains were further screened for quantification of zinc solubilization using $ZnCO_3$ as insoluble source in broth assay. Results showed that dissolution of zinc carbonate was 13.26 µg ml^{-1} ($PkZnS_3$) and 10.33 µg ml^{-1} ($ThZnS_2$) in broth (Table 25).

Table 23: Plant growth promotion and antagonistic activities of selected zinc solubilizers

Isolates	IAA production	NH3 production	HCN production	Siderophore production	Antagonistic activity	
					R. solani	*X. oryzae*
$ThZnS_2$	-	+	-	-	-	-
$AvZnS_1$	-	-	-	-	-	-
$AkZnS_4$	-	-	-	-	-	-
$MvZnS_1$	-	+	-	++	-	-
$MvZnS_3$	-	+	-	++	-	-
$PkZnS_3$	-	+	-	-	-	-

Positive reactions (High +++, Medium ++. Low +), Negative reactions (-)
ZnS: Zinc solubilizers
Th: Thanivayal, Av: Ambalavayal, Ak: Ambukuthi, Mv: Malavayal, Pk: Pottankoli

Table 24: Efficiency of bacterial isolates in Zn solubilization on mineral salt medium amended with ZnO

Isolates	Zn solubilization efficiency (%)	Zn quantification (µg ml $^{-1}$)	pH of the broth after 14 days
$ThZnS_2$	121.78	17.44 ^{ab}	6.78
$AvZnS_1$	56.87	14.67 ^{d}	6.80
$AkZnS_4$	27.30	14.05 ^{d}	6.72
$MvZnS_1$	119.43	17.66 ^{ab}	6.31
$MvZnS_3$	109.53	16.08 ^{bc}	6.45
$PkZnS_3$	143.98	18.57 ^{a}	6.12

ZnS: Zinc solubilizers
Th: Thanivayal, Ak: Ambukuthi, Mv: Malvayal, Pk: Pottankoli

Table 25: Efficiency of bacterial isolates in Zn solubilization on mineral salt medium amended $ZnCO_3$

Isolates	Ability to solubilize $ZnCO_3$	solubilization efficiency (%)	Zn quantification (µg ml^{-1})	pH of the broth after 14 days
$ThZnS_2$	+	42.85	10.33	6.22
$AvZnS_1$	-	-	NA	NA
$AkZnS_4$	-	-	NA	NA
$MvZnS_1$	-	-	NA	NA
$MvZnS_3$	-	-	NA	NA
$PkZnS_3$	+	77.77	13.26	6.85

Positive reactions (+), Negative reactions (-), NA: Not Applicable
ZnS: Zinc solubilizers
Th: Thanivayal, Ak: Ambukuthi, Mv: Malavayal, Pk: Pottankoli

4.4.5. Plant growth promoting and antagonistic activities of fluorescent pseudomonads isolated from the rice fields of Wayanad

Two fluorescent pseudomonads obtained in the study were screened for PGP and antagonistic activities against rice pathogens *R. solani* and *X. oryzae*.

4.4.5.1. Plant growth promoting activities of fluorescent pseudomonads

Two isolates of fluorescent pseudomonads ($KpFP_1$ and $PkFP_2$) were screened for plant growth promoting activities viz. production of IAA, NH_3, HCN and

siderophores. The results are presented in Table 26. Both isolates failed to produce IAA, but recorded positive reaction for NH_3 production. The isolate $KpFP_1$ exhibited production of HCN and siderophore, at low levels.

Table 26: Plant growth promotion and antagonistic activities of fluorescent pseudomonads

Isolates	IAA production	NH3 production	HCN production	Siderophore production	Antagonistic activity	
					R. solani	***X. oryzae***
$KpFP_1$	-	++	+	+	-	-
$PkFP_2$	-	+	-	-	-	-

Positive reactions (High +++, Medium ++. Low +), Negative reactions (-)
FP: Fluorescent pseudomonad
Kp: Kuppamudi, Pk: Pottankoli

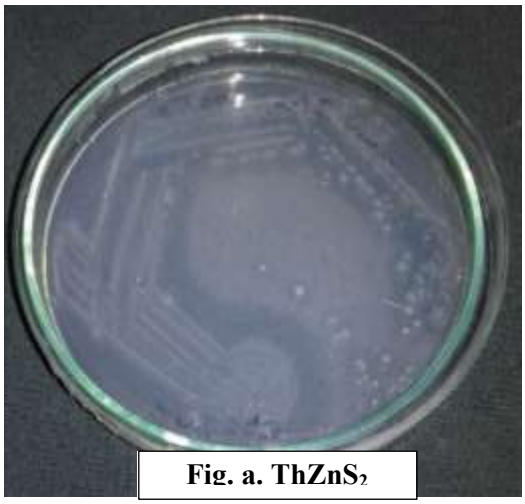


Fig. a. $ThZnS_2$

Fig. b. $AvZnS_1$

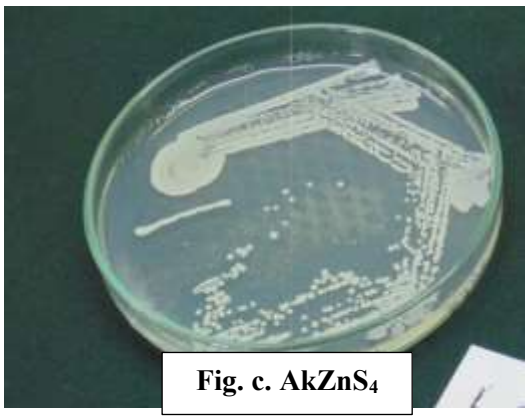


Fig. c. $AkZnS_4$

Fig. d. $MvZnS_1$

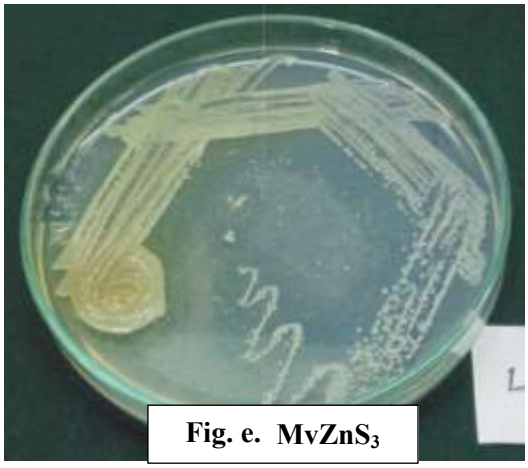


Fig. e. $MvZnS_3$

Fig. f. $PkZnS_3$

Plate 14: Zinc solubilizing bacteria colonies on Mineral salt medium amended with ZnO

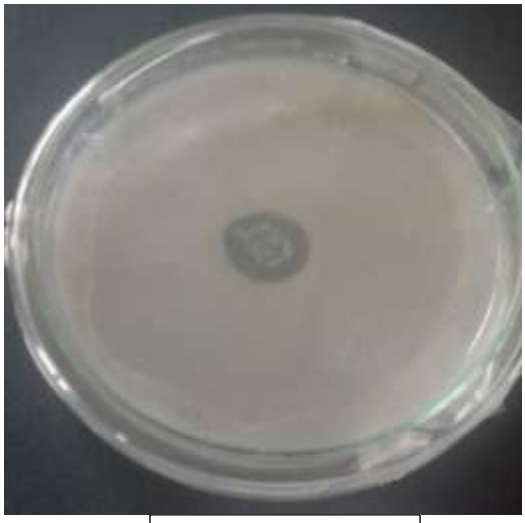


Fig. a. $ThZnS_2$

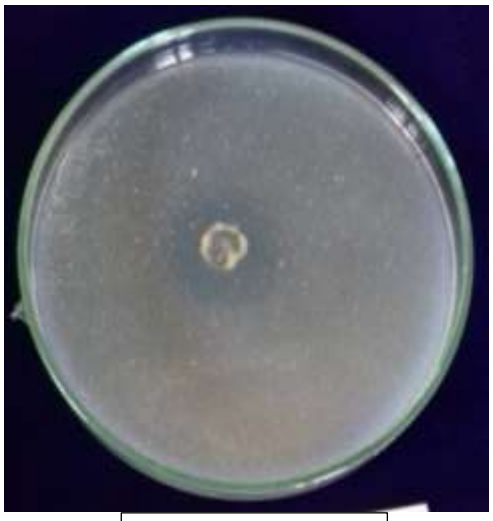


Fig. b. $MvZnS_1$

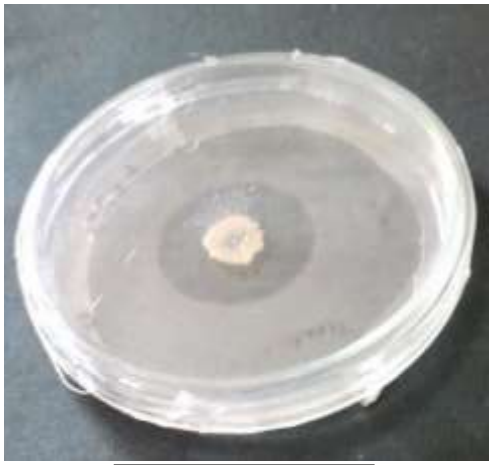


Fig. c. $MvZnS_3$

Fig. d. $PkZnS_3$

Plate 15: Solubilization zones by zinc solubilizers on Mineral salt medium amended with ZnO

Fig. a. *In vitro* screening for IAA production of K and Zn solubilizers

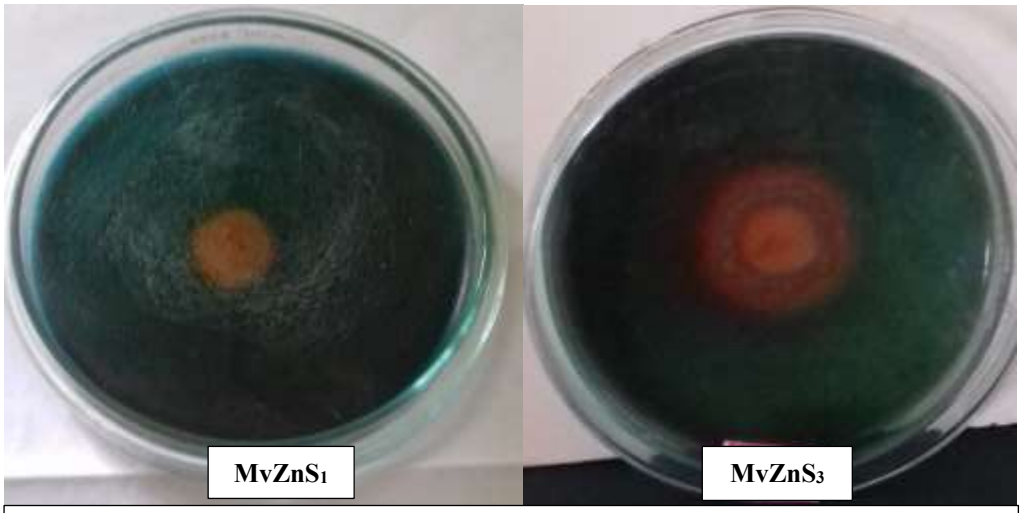


Fig. b. Siderophore production by zinc solubilizers

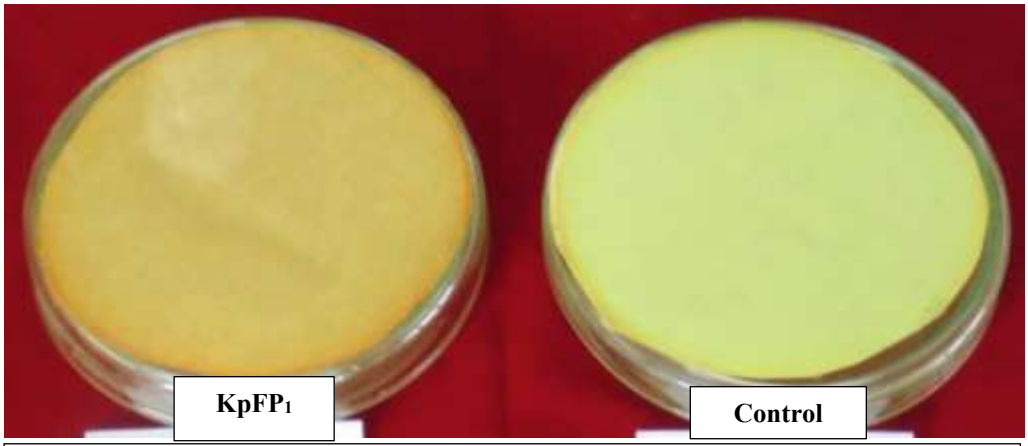


Fig. c. HCN production of fluorescent pseudomonads

Plate 16: Plant growth promoting activities of K solubilizers, Zn solubilizers and fluorescent pseudomonads

4.5. Selection of potential isolates of nitrogen fixers, phosphate solubilizers, potassium solubilizers and zinc solubilizers

Potential isolates under different functional categories were selected for further characterization and evaluation, based on their efficiency of N fixation and solubilization of P, K and Zn.

4.5.1. Selection of potential isolates of N-fixers

The PGP activities of all the N-fixers are consolidated in Table 27. Based on the efficiency of the isolates to fix N, in terms of N fixed in mg g^{-1} of sucrose utilized, seven potential isolates were selected for further characterization. These isolates included $AkNF_3$, $PkNF_4$, $KgNF_1$, $KpNF_5$, $KpNF_2$, $AkNF_2$ and $MtNF_4$.

Table 27. PGP characteristics of N-fixers

Isolate	N fixed (mg g^{-1} of sucrose)	Production of IAA µg ml^{-1}	Production of NH3	Production of HCN	Production of siderophore	Antagonistic activity	
						R.solani	*X.oryzae*
$AkNF_3$	9.33	23.16	++	-	++	-	-
$PkNF_4$	9.33	20.66	++	-	+	-	-
$KgNF_1$	8.86	34.83	++	-	+	-	+
$KpNF_5$	8.40	-	++	-	++	+	+
$KpNF_2$	7.93	14.83	+	-	-	-	-
$AkNF_2$	7.93	-	++	-	++	-	-
$MtNF_4$	7.00	-	++	-	-	+	-
$AvNF_1$	6.53	-	+	-	++	-	-
$KgNF_9$	6.06	3.50	+++	-	-	-	-
$PkNF_1$	6.06	-	+	-	+	-	-
$PkNF_3$	6.06	12.33	+	-	+	-	-
$AvNF_3$	5.60	6.16	+	-	+	-	-
$PkNF_2$	4.66	11.16	++	-	+	-	-
$KpNF_4$	4.66	5.00	+	-	-	-	-
$KpNF_6$	4.26	19.33	+	-	-	-	-
$MtNF_3$	4.20	2.50	++	-	-	-	-
$AkNF_5$	4.16	-	++	-	+	-	-

$KpNF_7$	3.73	15.83	+	-	-	-	-
$AvNF_2$	2.33	18.83	+	-	++	-	-
$AvNF_4$	1.86	25.00	+	-	+	-	-

NF: nitrogen fixers
Kg: Kolagappara, Kp: Kuppamudi, Av: Ambalavayal, Mt: Marathat, Ak: Ambukuthi
Pk: Pottankoli

4.5.2. Selection of potential isolates of phosphate solubilizers

The PGP activities of all the phosphate solubilizers tested are consolidated in Table 28. Based on the efficiency in terms of quantity of phosphate solubilized in µg ml^{-1}, five potential isolates ($PkPS_1$, $AkPS_4$, $AvPS_1$, $PkPS_2$ and $PkPS_3$) and antagonistic isolate against *X. oryzae* ($MvPS_3$) were selected for further characterization.

Table 28. PGP characteristics of phosphate solubilizers

Isolate	Quantity of phosphate solubilized µg ml^{-1}	Production of IAA µg ml^{-1}	Production of NH3	Production of HCN	Production of siderophore	Antagonistic activity	
						R. solani	*X. oryzae*
$PkPS_1$	134.88	4.66	+	-	+	-	-
$AkPS_4$	121.33	4.66	+	-	+	-	-
$AvPS_1$	113.33	8.16	++	-	+	-	-
$PkPS_2$	108.33	4.83	+	-	+	-	-
$PkPS_3$	103.83	2.66	+	-	-	-	-
$EdPS_4$	82.33	-	+	-	-	-	-
$MtPS_5$	82.00	4.83	-	-	-	-	-
$MvPS_4$	81.66	3.66	+	-	+	-	-
$AkPS_5$	81.33	-	+	-	-	-	-
$MvPS_3$	77.66	7.00	+	-	++	-	+
$ThPS_4$	74.00	-	+	-	-	-	-
$AkPS_7$	72.50	-	-	-	+	-	-
$MtPS_2$	66.83	19.80	+	-	+	-	-
$EdPS_5$	55.66	12.00	-	-	-	-	-
$PkPS_5$	50.00	3.33	+	-	-	-	-
$MtPS_6$	49.83	5.33	-	-	-	-	-

PS: phosphate solubilizers
Th: Thanivayal, Ed: Edakkal, Av: Ambalavayal, Mt: Marathat, Ak: Ambukuthi
Mv: Malavayal, Pk: Pottankoli

4.5.3. Selection of potential isolates of potassium solubilizers

The PGP activities of all the potassium solubilizers tested are consolidated in Table 29. Based on the efficiency in terms of quantity of K solubilized in µg ml^{-1}, three potential isolates were selected for further characterization. These included $MvKS_1$, $MvKS_3$ and $AkKS_1$.

Table 29. PGP characteristics of potassium solubilizers

Isolate	K quantification µg ml^{-1}	Production of IAA	Production of NH3	Production of HCN	Production of siderophore	Antagonistic activity	
						R. solani	*X. oryzae*
$MvKS_1$	4.19	-	+	-	-	-	-
$MvKS_3$	3.01	-	+	-	-	-	-
$AkKS_1$	1.81	-	+	-	-	-	-
$MvKS_2$	1.62	-	-	-	-	-	-

KS: potassium solubilizers
Ak: Ambukuthi, Mv: Malavayal

4.5.4. Selection of potential isolates of zinc solubilizers

The PGP activities of all the zinc solubilizers tested are consolidated in Table 30. Based on the efficiency in terms of quantity of Zn solubilized in µg ml^{-1}, three potential isolates were selected for further characterization. These included $PkZnS_3$, $MvZnS_1$ and $ThZnS_2$.

Table 30. PGP characteristics of zinc solubilizers

Isolate	Zn quantification µg ml^{-1}	Production of IAA	Production of NH3	Production of HCN	Production of siderophore	Ability to solubilize $ZnCO_3$
$PkZnS_3$	18.57	-	+	-	-	+
$MvZnS_1$	17.65	-	+	-	++	-
$ThZnS_2$	17.43	-	+	-	-	+
$MvZnS_3$	16.08	-	+	-	++	-
$AvZnS_1$	14.67	-	-	-	-	-
$AkZnS_4$	14.05	-	-	-	-	-

ZnS: zinc solubilizers
Th: Thanivayal, Av: Ambalavayal, Ak: Ambukuthi, Mv: Malavayal

Based on the functional efficiency of the isolates to fix N and solubilize phosphate, potassium and zinc, a total of twenty potential isolates were selected for further characterization and identification (Table 31).

Table 31: Potential isolates of PGPR selected for characterization

Nitrogen fixers	Phosphate solubilizers	Potassium solubilizers	Zinc solubilizers	Fluorescent pseudomonads
$PkNF_4$	$PkPS_1$	$MvKS_3$	$PkZnS_3$	$KpFP_1$
$AkNF_3$	$AkPS_4$	$MvKS_1$	$ThZnS_2$	
$KgNF_1$	$AvPS_1$	$AkKS_1$	$MvZnS_1$	
$KpNF_5$	$PkPS_2$			
$KpNF_2$	$PkPS_3$			
$AkNF_2$	$MvPS_3$			
$MtNF_4$				

NF: nitrogen fixers, PS: phosphate solubilizers, KS: potassium solubilizers
ZnS: zinc solubilizers, FP: fluorescent pseudomonads
Th: Thanivayal, Ed: Edakkal, Kg: Kolagappara, Kp: Kuppamudi, Av: Ambalavayal
Mt: Marathat, Ak: Ambukuthi, Mv: Malavayal, Pk: Pottankoli

4.6. Characterization and identification of selected potential PGPR isolates

Twenty potential isolates selected for further studies were subjected to characterization and identification. These isolates included seven N-fixers, six phosphate solubilizers, three K-solubilizers, three Zn-solubilizers and one fluorescent pseudomonad. These isolates were characterized by cultural, morphological and biochemical tests. Molecular characterization of isolates was carried out by using 16S rDNA sequences analysis.

4.6.1. Cultural and morphological characterization of potential PGPR isolates

Cultural and morphological characters of the selected PGPR isolates were assessed using standard microbiological procedures. The colony characteristics of twenty potential isolates were studied on nutrient agar. Size of the colonies ranged from small to large (Table 32). Three isolates ($AkNF_3$, $PkPS_1$ and $PkZnS_3$) produced large colonies, whereas seven isolates produced medium ($MvKS_1$, $KpNF_2$, $MtNF_4$, $PkPS_2$, $PkPS_3$, $MvPS_3$, and $AkKS_1$). All the other isolates produced small colonies. Colonies were circular in all the isolates.

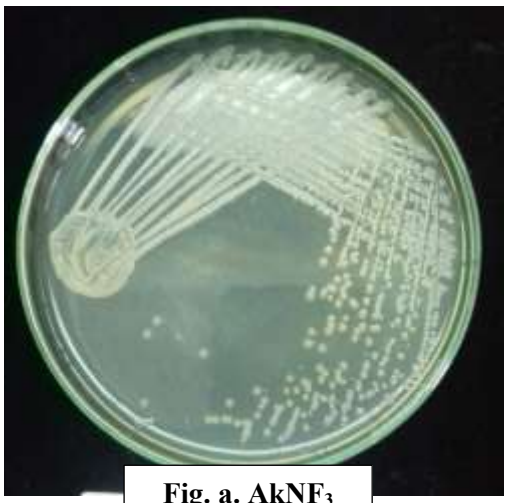


Fig. a. $AkNF_3$

Fig. b. $AvPS_1$

Fig. c. $MvPS_3$

Fig. d. $AkKS_1$

Fig. e. $AkPS_4$

Fig. f. $PkPS_1$

Plate 17: Promising PGPR isolates from Wayanad on nutrient agar

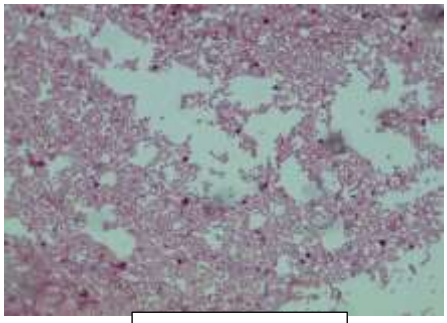


Fig. a. $KgNF_1$

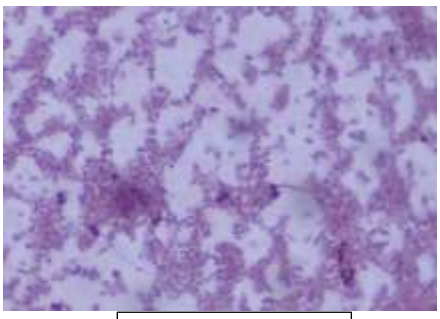


Fig. b. $AkNF_3$

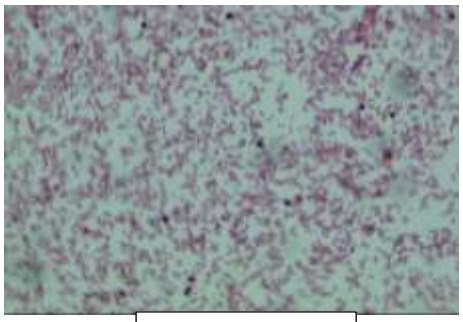


Fig. c. $AvPS_1$

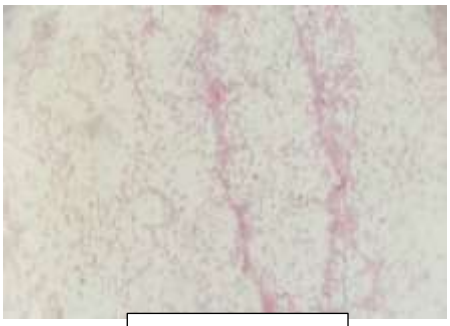


Fig. d. $AkPS_4$

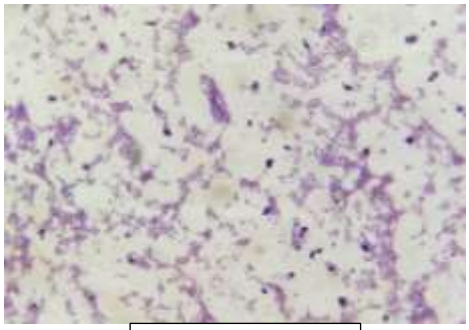


Fig. e. $PkPS_1$

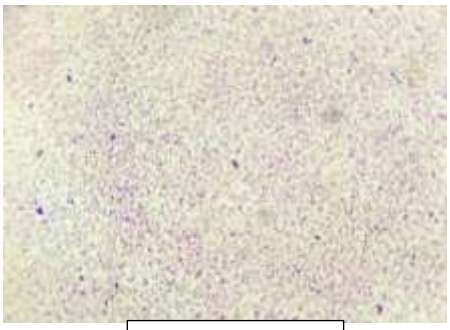


Fig. f. MvKS1

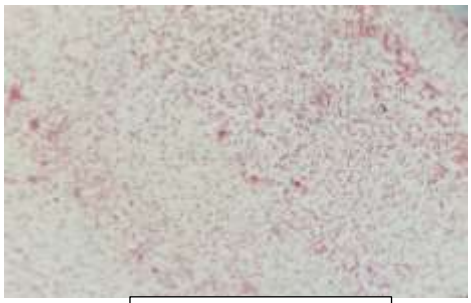


Fig. g. $ThZnS_2$

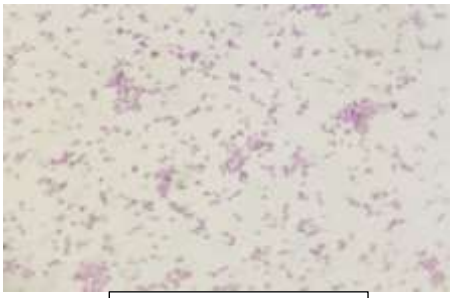


Fig. h. $MvZnS_3$

Plate 18: Gram staining reactions of promising PGPR isolates

Colonies of 13 isolates were cream colored. Two isolates ($AkKS_1$ and $MvPS_3$) produced yellow colonies, whereas the colonies of five isolates ($AkNF_3$, $AvPS_1$, $PkPS_1$, $ThZnS_2$ and $PkZnS_3$ were creamy white (Table 32). Sixteen isolates produced raised colonies and four isolates ($AkNF_3$, $PkPS_1$, $PkZnS_3$ and $KpNF_2$) flat colonies. Colonies were with entire margin in most of the isolates and three isolates ($AkNF_3$, $PkPS_1$ and $PkZnS_3$) produced colonies with undulate margin.

Colonies of all the isolates except two were dry. Two isolates ($MvKS_1$ and $KpNF_2$) produced mucoid colonies (Table 32).

Sixteen isolates were Gram negative, short rods except $AkNF_3$, $PkPS_1$, $MvKS_1$ and $PkZnS_3$ which were Gram positive, rods. Gram reaction of all the isolates was further confirmed by solubility in KOH, where Gram negative cultures formed viscous threads in the presence of KOH.

Gram positive isolates were tested for the presence of endospores and $AkNF_3$, $PkPS_1$ and $PkZnS_3$ showed the presence of endospores.

4.6.2. Biochemical tests of selected PGPR isolates

All the isolates were subjected to biochemical tests for the production of oxidase, catalase and ability to utilize different carbon sources. The results are presented in Table 33. All the isolates were oxidase positive, as indicated by the purple colour in presence of Kovac's reagent. All the isolates tested positive for catalase, indicated by the effervescence produced immediately on addition of 2-3 drops of hydrogen peroxide.

Utilization of carbohydrates by bacterial isolates was variable among the isolates. Acid production was indicated by a colour change from red to yellow production. Fifteen isolates utilized glucose and eight isolates utilized lactose. Maltose was utilized by nine isolates, mannitol utilized by fourteen and sorbitol utilized by three isolates. Most of the isolates utilized fructose and dulcitol. Two isolates ($AkNF_3$ and $PkZnS_3$) were very versatile, as they utilized all the carbohydrates tested.

Table 32: Colony morphology and staining reaction of selected PGPR isolates

Isolates	Colony morphology						Gram Reaction	Cell morphology	Spore staining
	Size	Shape	Color	Margin	Elevation	Texture			
$KgNF_1$	Small	Circular	Cream	Entire	Raised	Dry	-	Short rods	NA
$AkNF_3$	Large	Circular	Creamy white	Undulate	Flat	Dry	+	Rods	+
$PkNF_4$	Small	Circular	Cream	Entire	Raised	Dry	-	Short rods	NA
$AvPS_1$	Small	Circular	Creamy white	Entire	Raised	Dry	-	Short rods	NA
$AkPS_4$	Small	Circular	Cream	Entire	Raised	Dry	-	Short rods	NA
$PkPS_1$	Large	Circular	Creamy white	Undulate	Flat	Dry	+	Rods	+
$MvKS_1$	Medium	Circular	Cream	Entire	Raised	Mucoid	+	Short rods	-
$MvKS_3$	Small	Circular	Cream	Entire	Raised	Dry	-	Short rods	NA
$ThZnS_2$	Small	Circular	Creamy white	Entire	Raised	Dry	-	Short rods	NA
$PkZnS_3$	Large	Circular	Creamy white	Undulate	Flat	Dry	+	Rods	+
$KpNF_2$	Medium	Circular	Cream	Entire	Flat	Mucoid	-	Short rods	NA
$KpNF_5$	Small	Circular	Cream	Entire	Raised	Dry	-	Short rods	NA
$AkNF_2$	Small	Circular	Cream	Entire	Raised	Dry	-	Short rods	NA
$MtNF_4$	Medium	Circular	Cream	Entire	Raised	Dry	-	Short rods	NA
$PkPS_2$	Medium	Circular	Cream	Entire	Raised	Dry	-	Short rods	NA
$PkPS_3$	Medium	Circular	Cream	Entire	Raised	Dry	-	Short rods	NA

$MvPS_3$	Medium	Circular	Yellow	Entire	Raised	Dry	-	Short rods	NA
$AkKS_1$	Medium	Circular	Yellow	Entire	Raised	Dry	-	Short rods	NA
$MvZnS_1$	Small	Circular	Cream	Entire	Raised	Dry	-	Short rods	NA
$KpFP_1$	Small	Circular	Cream	Entire	Raised	Dry	-	Short rods	NA

Small: colony diameter < 0.2mm, Medium: colony diameter 0.2-0.5 mm, Large: colony diameter 0.5-0.7mm
Positive reactions (+), N Negative reactions (-), NA: Not Applicable
NF: nitrogen fixers, PS: phosphate solubilizers, KS: potassium solubilizers, ZnS: zinc solubilizers, FP: fluorescent pseudomonad
Th: Thanivayal, Ed: Edakkal, Kg: Kolagappara, Nr: Nellara, Kp: Kuppamudi, Av: Ambalavayal, Mt: Marathat, Ak: Ambukuthi
Mv: Malavayal, Pk: Pottankoli

Table 33: Biochemical characters of selected potential PGPR isolates

Isolates	Oxidase test	Catalase test	Oxidation of sugars						
			Glucose	Lactose	Maltose	Fructose	Mannitol	Dulcitol	Sorbitol
$KgNF_1$	+	+	-	-	-	+	+	+	-
$AkNF_3$	+	+	+	+	+	+	+	+	+
$PkNF_4$	+	+	-	-	-	+	+	+	-
$AvPS_1$	+	+	+	-	-	+	+	-	-
$AkPS_4$	+	+	+	-	+	-	-	+	-
$PkPS_1$	+	+	+	+	+	+	+	+	-
$MvKS_1$	+	+	+	+	+	+	+	-	-
$MvKS_3$	+	+	+	-	+	-	-	+	-
$ThZnS_2$	+	+	-	-	-	+	+	+	-
$PkZnS_3$	+	+	+	+	+	+	+	+	+
$KpNF_2$	+	+	+	-	+	+	-	-	-
$KpNF_5$	+	+	-	+	-	-	-	+	-
$AkNF_2$	+	+	+	+	+	+	+	+	-
$MtNF_4$	+	+	+	-	-	+	+	+	-
$PkPS_2$	+	+	+	+	-	+	+	+	-
$PkPS_3$	+	+	+	-	-	+	-	+	+

$MvPS_3$	+	+	+	-	-	+	+	+	-
$AkKS_1$	+	+	+	+	-	+	+	+	-
$MvZnS_1$	+	+	+	-	+	+	-	+	-
$KpFP_1$	+	+	-	-	-	+	+	+	-

Positive reactions (+), N negative reactions (-)
NF: nitrogen fixers, PS: phosphate solubiltizers, KS: potassium solubilizers, ZnS: zinc solubilizers, FP: fluorescent pseudomonads
Th: Thanivayal, Ed: Edakkal, Kg: Kolagappara, Nr: Nellara, Kp: Kuppamudi, Av: Ambalavayal, Mt: Marathat, Ak: Ambukuthi
Mv: Malavayal, Pk: Pottankoli

4.6.3. Molecular characterization of selected isolates

The twenty isolates were subjected to molecular characterization, however amplification of two isolate could not be obtained. Hence, eighteen isolates were identified by 16S rDNA sequence analysis. Homology search of nucleotide sequences obtained from the isolates are presented in Plates 19-36. The sequences of all the isolates were deposited in the GenBank of the NCBI and accession numbers received from GenBank for respective isolates are presented in Table 34.

Table 34: Details of identity and accessions numbers of selected isolates in GenBank of NCBI database

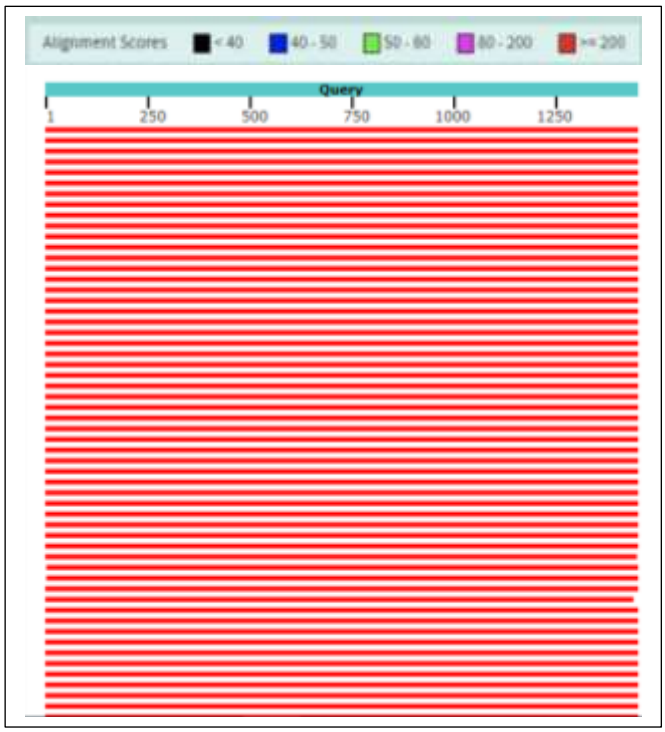
Isolate	Accession number in GenBank	Identity based on 16S rRNA gene sequence
$KgNF_1$	MW288152	*Pseudomonas putida* strain KgNF1
$AkNF_3$	MW288141	*Bacillus* sp. strain AkNF3
$PkNF_4$	MW269608	*Pseudomonas* sp. strain PkNF4
$AvPS_1$	MW290516	*Achromobacter* sp. strain AvPS1
$AkPS_4$	MW291534	*Acinetobacter schindleri* strain AkPS4
$PkPS_1$	MW290515	*Bacillus megaterium* strain PkPS1
$MvKS_1$	MW295415	*Microbacterium* sp. strain MvKS1
$MvKS_3$	MW295416	*Acinetobacter calcoaceticus strain* MvKS3
$ThZnS_2$	MW284891	*Achromobacter marplatensis* strain ThZnS2
$PkZnS_3$	MW295426	*Cytobacillus kochii* strain PkZnS3
$KpNF_5$	MW307349	*Alcaligenes faecalis* strain KpNF5
$MtNF_4$	MW307253	*Burkholderia* sp. strain MtNF4
$AkNF_2$	MW307254	*Brevundimonas naejangsanensis* strain AkNF2
$PkPS_2$	MW307345	*Burkholderia vietnamiensis* strain PkPS2
$PkPS_3$	MW356852	*Providencia vermicola* strain PkPS3
$MvPS_3$	MW307346	*Burkholderia cepacia* strain MvPS3
$AkKS_1$	MW307347	*Burkholderia cepacia* strain AkKS1
$MvZnS_1$	MW307349	*Acinetobacter* sp. strain MvZnS1

agagtttgat cctggctcag attgaacgct ggcggcaggc ctaacacatg caagtcgagc ggatgacggg agcttgctcc ttgattcagc ggcggacggg tgagtaatgc ctaggaatct gcctggtagt gggggacaac gtttcgaaag gaacgctaat accgcatacg tcctacggga gaaagcaggg gaccttcggg ccttgcgcta tcagatgagc ctaggtcgga ttagctagtt ggtgaggtaa tggctcacca atcgcgacga ccgtaactgg tctgagagga tgatcagtca cactggaact gagacacggt ccagactcct acgggaggca gcagtgattc atattggaca atgggcgaaa gcctgatcca gccatgccgc gtgtgtgaag aaggtcttcg gattgtaaag cactttaagt tgggaggaag ggcagtaagt taataccttg ctgttttgac gttaccgaca gaataagcac cggctaactc tgtgccagca gccgcggtaa tacagagggt gcaagcgtta atcggaatta ctgggcgtaa agcgcgcgta ggtggttcgt taagttggat gtgaaagccc cgggctcaac ctgggaactg catccaaaac tggcgggcta gagtacggta gagggtggtg tacgttaagc tgtagcggtg aaatgcgtag atcggctaat accagtgcgc ggcgaaggcg accacctgga ctgatactga cactgaggtg cgaaagcgtg gggagcaaac aggattagataccctggtag tccacgccgt aaacgatgtc aactagccgt tggaatcctt gagattttag tggcgcagct aacgcattaa gttgaccgcc tggggagtac ggccgcaagg ttaaaactca aatgaattga cacactggcg cacaagcggt ggagcatgtg gtttaattcg aagcaacgcgaagaacctta ccaggccttg acatgcagag aactttccag agatggattg gtgccttcgg gaactctgac acaggtgctg catggctgtc gtcagctcgt gtcgtgagat gttgggttaa gtcccgtaac gagcgcaacc cttgtcctta gttaccagca cgttatggtg ggcactctaa ggagactgcc ggtgacaaac cggaggaagg cttaagctca gtcaagtcat catggccctt acggcctggg ctacacacgt gctacaatgg tcggtacaga gggttgccaa gccgcgaggt ggagctaatc tcacaaaacc gatcgtagtc cggatcgcag tctgcaactc gactgcgtga agtcggaatc gctagtaatc gcgaatcaga atgtcgcggt gaatacgttc ccgggccttg tacacaccgc ccgtcacacc atgggagtcc gttgcaccag aagtagctag tctaaccttc tagaggacgg

Merged sequence

Description	Max. score	Query coverage (%)	Accession	Identity (%)	E value
Pseudomonas putida	2435	100	DQ458961.1	96.98	0.0
Pseudomonas sp. 3-CBA	2429	100	KU877215.1	96.91	0.0
Pseudomonas sp. 2-CBA	2429	100	KU877214.1	96.91	0.0
Pseudomonas sp. LQ26	2429	100	GU731675.1	96.91	0.0
Pseudomonas putida BASUP87	2429	100	GU396283.1	96.91	0.0
Pseudomonas sp. TSWCW20	2429	100	GQ284465.1	96.91	0.0
Pseudomonas sp. TIS1-127	2429	100	AB456678.1	96.91	0.0

Sequences showing homology



BLASTN output

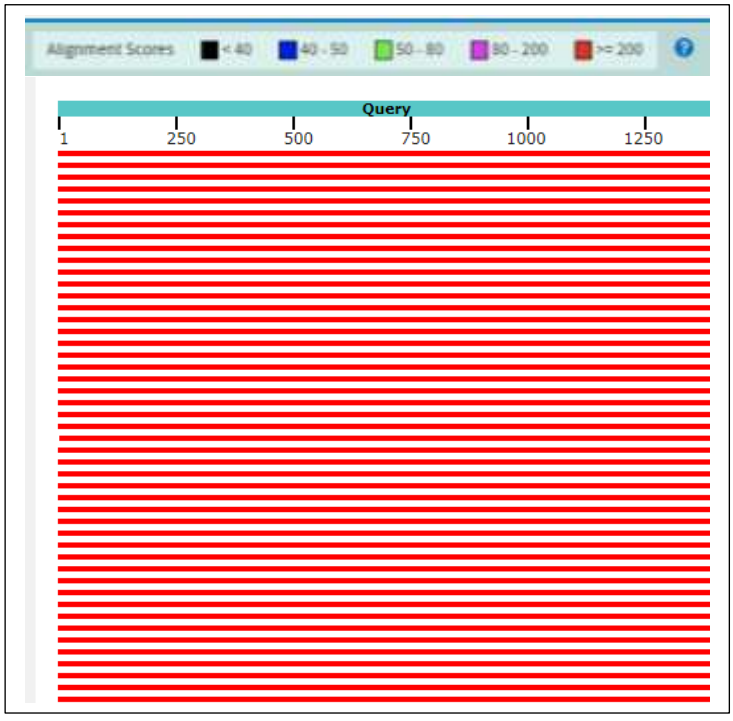
Plate 19: 16S rRNA sequence analysis of the $KgNF_1$

agtttgatca tggctcagga cgaacgctgg cggcgtgcct aatacatgca agtcgagcga atcgatggga gcttgctccc tgagattagc ggcggacggg tgagtaacac gtgggcaacc tgcctataag actgggataa cttcgggaaa ccggagctaa taccggatac gttcttttct cgcatgagag aagatggaaa gacggtttac gctgtcactt atagatgggc ccgcggcgcattagctagtt ggtgaggtaa tggctcacca aggcgacgat gcgtagccga cctgagaggg tgatcggcca cactgacact gagacacggc ccagactcct acgggaggca gcagtaggga atcttccgca atggacgaaa gtctgacgga gcaacgccgc gtgaacgaag aaggccttcgggtcgtaaag ttctgttgtt agggaagaac aagtaccaga gtaactgctg gtaccttgac ggtacctaac cagaaagcca cggctaacta cgtgccagca gccgcggtaa tacgtaggtggcaagcgttg tccggaatta ttgggcgtaa agcgcgcgca ggtggttcct taagtctgat gtgaaagccc acggctcaac cgtggactat cattggaaac tggggaactt gagtgcagaa gaggaaagtg gaattccaag tgtagcggtg aaatgcgtag agatttggag gaacaccagtggcgaaggcg actttctggt ctgtaactga cactgaggcg cgaaagcgtg gggagcaaacaggattagat accctggtag tccacgccgt aaacgatgag tgctaagtgt tagagggtt ccgcccttta gtgctgcagc taacgcatta agcactccgc ctggggagta cggccgcaag gctgaaactc aaaggaattg accattgccc gcacaagcgg tggagcatgt ggtttaattc gaagcaacgc gaagaacctt accaggtctt gacatcctct gacaacccta gagatagggc tacgtccagt gggggacaga gtgacaggtg gtgcatggtt gtcgtcagct cgtgtcgtgagatgttgggt taagtcccgc aacgagcgca acccttgatc ttagttgcca gcattcagtt gggcactcta aggtgactgc cggtgacaaa ccggaggaag gtggggatga cgtcaaatca tcatgcccct tatgacctgg gctacacacg tgctacaatg gatggtacaa agggctgcaa acctgcgaag gtaagcgaat cccataaagc cattctcagt tcggattgca ggctgcaact cgcctgcatg aagccggaat cgctagtaat cgcggatcag catgccgcgg tgaatacgtt cccgggcctt

Merged sequence

Description	Max. score	Query coverage (%)	Accession	Identity (%)	E value
Bacillus sp S12206	2479	100	KF 956662.1	98.85	0.0
Bacillus sp S12208	2479	100	KF 966646.1	98.85	0.0
Bacillus sp S10718	2479	100	KF 956566.1	98.85	0.0
Bacillus sp S10504	2479	100	JX173285.1	98.85	0.0
Bacillus simplex strain N25	2479	100	GU086427.1	98.85	0.0
Bacillus simplex strain JHCFS1	2479	100	Fj455076.1	98.85	0.0
Uncultured bacterium clone	2479	100	DQ129440.1	98.85	0.0

Sequences showing homology



BLASTN output

Plate 20: 16S rRNA sequence analysis of the AkNF$_3$

aacctagatacctctaagagatgatcccgccgcactgaatcccccgtgaatacccaagctcccgaaggttagactagctacttctggtgcaacccactcccat
ggtgtgacgggcggtgtgtacaaggcccgggaacgtattcaccgcgacattctgattcgcgattactagcgattccgacttcacgcagtcgagttgcagactg
cgatccggactacgatcggttttgtgagattaactccacctcgcggcttggcgaccctctgtaccgaccattgtagcacgtgtgtagcccaggccgtaagggc
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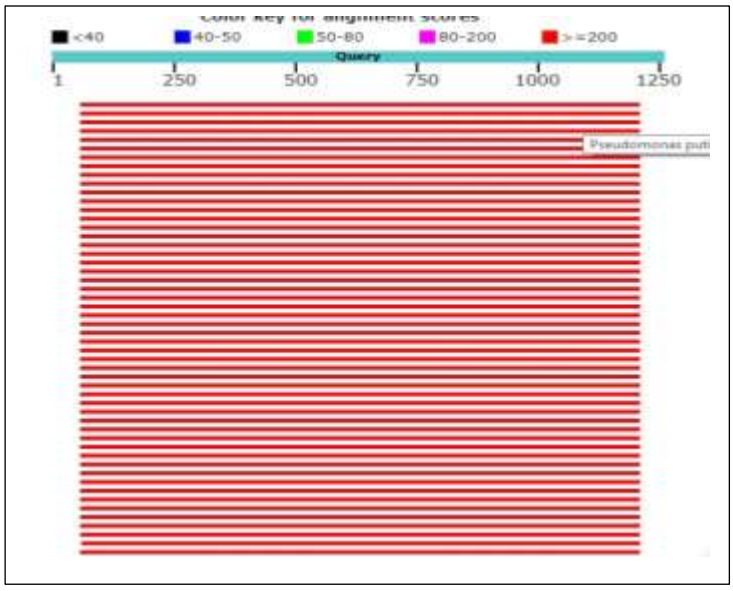
cgatgtcatattccgacttgctgcctcccgtaagacttggacgggttctcattcaggtgactgacttcttcgaaccgtacggaatctgaacttggtaccatactg
ctcccacag

Merged sequence

Description	Max. score	Query coverage (%)	Accession	Identity (%)	E value
Pseudomonas sp. VTAE169	1923	91	JN_886725.1	96.89	0.0
Pseudomonas putida strain JOF30	1917	91	MK737106.1	96.81	0.0
Pseudomonas sp. IAE244	1917	91	MK414950.1	96.81	0.0
Pseudomonas putida. strain MY1901	1917	91	MK578188.1	96.81	0.0
Pseudomonas sp. strain CA15-39	1917	91	MH769545.1	96.81	0.0
Pseudomonas sp. strain Pm1	1917	91	MK095774.1	96.81	0.0
Pseudomonas japonica strain CH-26	1917	91	MH712955.1	96.81	0.0

Sequences showing homology



BLASTN output

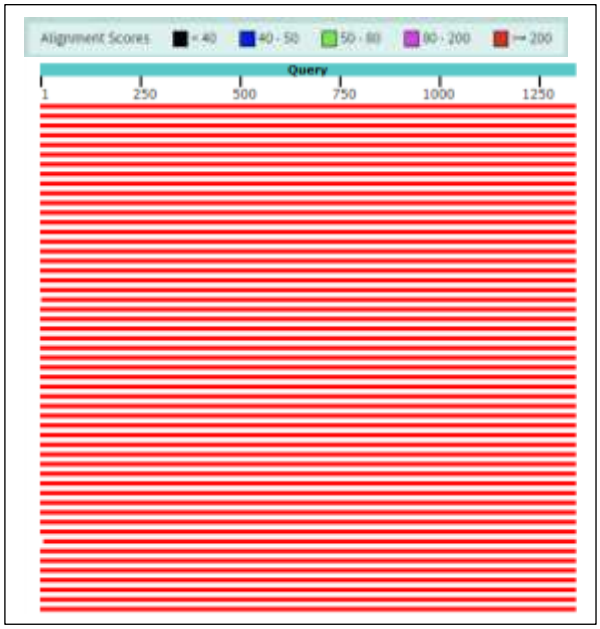
Plate 21: 16S rRNA sequence analysis of the $PkNF_4$

gtttgatcct ggctcagatt gaacgctagc gggatgcctt acacatgcaa gtcgaacggcagcacggact tcggtctgtt ggcgagtggc gaacgggtga gtaatgtatc ggaacgtgcc cagtagcgtt ggataactac gcgaaagccc agctaatacc gcatacgccc tacggatgaaagcaggccat cgcaagacct tgcactattg gagcggccga tatcggatta gctagttggtggtataacgg ctcaccaagg cgacgatccg tagctggtcc gagaggacga ccagccacac tgcgactgag atacggccca gactcctacg ggaggcagca gtggtcaatt ttggacaatg ggtgaaaccc tgatccagcc atcccgcgtg tgcgatgaag gccttcgggt tgtaaagcac ttcgggcagg aaagaaacgt catgggctaa tagcatgtga aactgacggt acctgcagaataagcaccgg ctaactacgt gccagcagcc gcggtaatac gtagagtgca agcgttaatc ggaattactg ggcgtaaagc gtgcgcaggc ggttcggaaa gaaagatgtg aaatcccagagcttaactcg ggaactgcat ttagtaacta cgggctagag tgtgtcagag ggaggtggaa ttagtcgtgt agcagtgaaa tgcgtagata tgcggaccaa caccgatggc gaaggcagcc tcctgggata acactgacgc tcatgcacga aagcgtgcgg agcaaacagg attagataccctgttagtcc acgccctaaa cgatgtcaac tagctgttgg ggccttcggg ccttggtagcgcagctaacg cgtgaagttg accgcctggg gagtacggtc gcaagattaa aactcaaag aattgacggg gacccgcaca agcggtggat gatgtggatt aattcgatgc aacgcgaaaaaccctaccta cagttgacat gtctggaatg ccgaagagat ttggcagtgc tcgcaagaga accgcaacac aggtgctgca tggctgtcgt cagctcgtgt cgtgagatgt tgggttaagt cccgcaacga gcgcaaccct tgtcattagt tgctacgaaa gggcactcta atgagactgccgatgactaa ccgcagcaag gtggtaatga cgtcaagtcc tcatggccct tatgggtagggcttcacacg tcatacaatg gtcgggacag agggtcgcca acccgcgtcc gggagccaat cccagaaacc cgatcgtagt gcctatcgca gtctgcaact cgactgcgtg aagtcggaat cgctagtaat cgcggatcag

Merged sequence

Description	Max. score	Query coverage (%)	Accession	Identity (%)	E value
Achromobacter sp. strain EIKU18	2193	100	MW063602.1	96.20	0.0
Achromobacter ruhlandii strain SCCH3ACH133-1365	2187	100	CP017433.1	96.12	0.0
Achromobacter xylosoxidans strain MN001	2187	100	CP012046.1	96.12	0.0
Achromobacter sp. strain GKA-1	2187	100	EU520399.1	96.12	0.0
Beta proteobacterium PII_GH12A7	2187	100	AY162060.1	96.12	0.0
Uncultured bacterium clone	2182	100	KJ457318.1	96.05	0.0
Achromobacter sp. ATY31	2182	100	HQ219950.1	96.05	0.0

Sequences showing homology



BLASTN output

Plate 22: 16S rRNA sequence analysis of the $AvPS_1$

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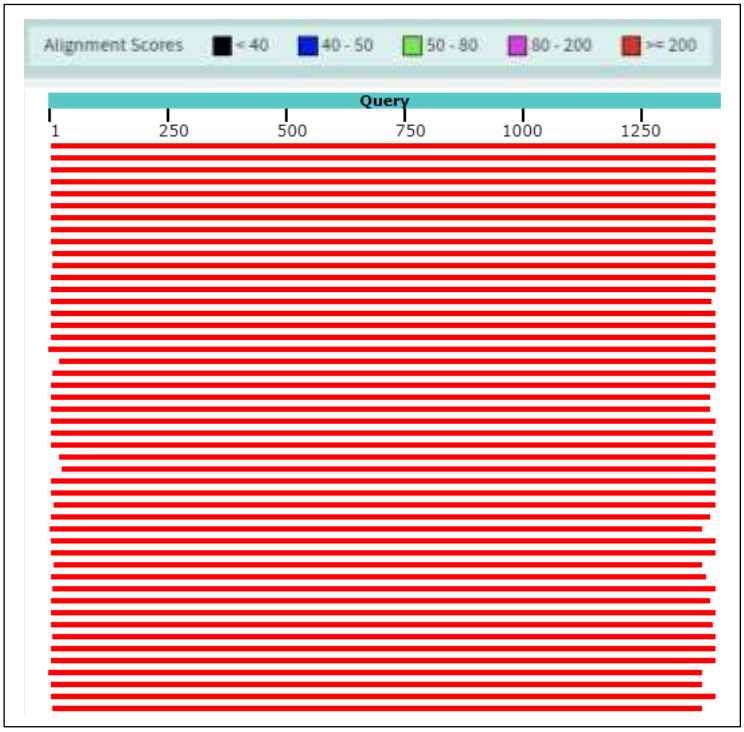
gatcagaatgccgcggtgaatacgttcccgggccttgtacacaccgcccgtcacaccatgggagtttgttgcaccagaagtaggtagtctaaccgcaaggaggac
gcttaccacggtgtggccgatgactgggggaagtcgaacaagaggctagccca

Merged sequence

Description	Max. score	Query coverage (%)	Accession	Identity (%)	E value
Acinetobacter schindleri strain ACE	2206	98	CP015615.1	94.54	0.0
Acinetobacter sp. BD189	2202	98	HM120259	94.52	0.0
Acinetobacter schindleri strain HZE30-1	2200	98	CP044483.1	94.47	0.0
Acinetobacter schindleri strain HZE23-1	2194	98	CP044463.1	94.40	0.0
Acinetobacter sp. strain KR26	2194	98	MK490975.1	94.40	0.0
Acinetobacter schindleri SGAir0122	2194	98	CP025618.2	94.40	0.0

Sequences showing homology



BLASTN output

Plate 23: 16S rRNA sequence analysis of the AkPS$_4$

aagggcttgcggcagctataatgcagtcgagcgaactgattagaagcttgcttctatgacgttagcggcggacgggtgagtaacacgtgggcaacctgcctgtaa
gactgggataacttcgggaaaccgaagctaataccggataggatcttctccttcatgggagatgattgaaagatggtttcggctatcacttacagatgggcccgcg
gtgcattagctagttggtgaggtaacggctcaccaaggcaacgatgcatagccgacctcagagggtaatcgccacactggaactgagaaccgcccagatcctac
gagcagcagtagaattccgcaatggacgaaagtcgaccgagcaacgccgcgtgagtgatgaaagctttccggtcgtaaaactctgttgttaaggaagaacaagt
acaagagtaactgctgtaccttgacggtactaaccagaaagccacggctaactacgtgccagcagccgcggtaatacgtaggtggcaagcgtatccggaattatt
gggcgtaaagcgcgcgcaggcggtttcttaagtctgatgtgaaagccacggctcaaccgtggagggtcattggaaactggggaacctgagtgcagaagagaaaa
gcggaattccacgtgtagcggtgaaatgcgtagagatgtggaggaacaccagtggcgaaggcggctttttggtctgtaactgacgctgaggcgcgaaagcgtgg
ggagcaaacaggattagataccctggtagtccacgccgtaaacgatgagtgctaagtgttagagggtttccgcccttagtgctgcagctaacgcattaagcactc
cgcctggggagtacggtcgcaagactgaaactcaaaggaattgacgggggcccgcacaagcggtggagcatgtgtttaattcgaagcaacgcgaagaaccttac
caggtcttgaaatcctctgacactctagagatagagcgttcccctcgggggacagagtgacagtggtgcatggttgtcgtcagctcgtgtcgtgagatgttgggtt
agtcccgcaacgagcgcaaccttgatcttaggtgccagcattagttgggcactctagtgactgcggtgacaaaccggaggaagtggggatgacgtcaatcatcat
gccccttatgacctgggccacacacgtgctacaatggatggtacaaagggctgcaagaccgcgaggtcaagccaatcccataaaaccattctcagttcggattgt
aggctgcaactcgcctacatgaagctggaatcgctagtaatcgcggatcagcatgccgcggtgaatacgttcccgggccttgtacacaccgcccgtcacaccacg
agagtttgtaacacccgaagtcggtggagtaaccgtaaggagctagccgcctaaggtgggacagatgattggggtgaagtcgaacaacgagccggacggg

Merged sequence

Description	Max. score	Query coverage (%)	Accession	Identity (%)	E value
Bacillus megaterium strain CP33	2667	100	MH667457.1	99.52	0.0
Bacillus megaterium strain ML257	2477	98	KC692200.1	97.29	0.0
Bacillus megaterium strain ML235	2475	98	KC692200.1	97.23	0.0
Bacillus megaterium strain ML479	2470	99	KC692206.1	97.16	0.0
Bacillus aryabhattai strain L42	2464	98	KU179345.1	97.09	0.0
Bacillus sp. B2(20106)	2464	99	HM104462.1	96.97	0.0
Bacillus sp. (in:Bacterium) XIXJ060	2460	98	MH801089.1	97.21	0.0

Sequences showing homology

BLASTn output

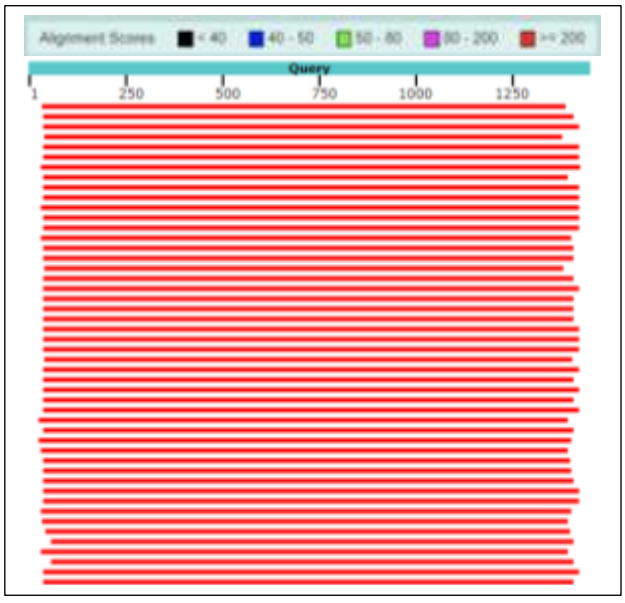
Plate 24: 16S rRNA sequence analysis of the $PkPS_1$

tacacttcaatctggagagcagcagatgcgctgctaccatgcagtcgaacggtgaaggagagcttgctctctggatcagtggcgaacgggtgagtaacacgtgag
caatctgcccctgactctgggataagcgctggaaacggcgtctaataccggatacgagctgcgaaggcatcttcagcagttggaaagaatttcggtcagggatga
gctcgcggcctatcagcttgttggtgaggtaacggctcaccaaggcgtcgacgggtagccggcctgagagggtgaccggccacactgggactgagacacggccc
agactcctacgggaggcagcagtggggaatattgcacaatgggcgcaagcctgatgcagcaacgccgcgtgagggacgacggccttcgggcggaaaacacacg
ccagcagggaacaagcgaaagagacgaaaacggaagaaaaagaccgctatacctgcagcagcgcgtatacgtaggtgcagcgtatccggactatggcgtaaa
gagctctagcgctttgtcgcgtctgctgtgaaaaccgagactcacatcggcctgcagtgggtacggcagactagagtgcggtagaggagataggaattcctggtgt
agcggtggaatgcgcagatatcaggaggaacaccgatggcgaaggcagatctctgggccgtaactgacgctgaggagcgaaagggtggggagcaaacaggct
tagataccctgatagtccaccccgtaaacgttgggaactagttgtggggtccattccacggattccgtgacgcagctaacgcattaagttccccgcctggagagtac
ggccgcaaggctaaaactcaaaggaattgacggggacccgcacagcggcggagcatgcggattaattcgatgcacgcgaagaccttaccaggcttgacatatag
aggaaacgtctgaaacagtcgccccgcagtctctatacaggtgctgcatgtggtcgtcagctcgttcatgaatgtggttaagtccgcaacgaggcaacctcgtccat
gtgcagacgtatggtgaactatgatctgcgggtcaactcagcaagtggatgacgaaaaatcatcatgccccctatgtcttgggcttcacgcatgctacaatggccg
gtacaaagggctgcaataccgtgaggtggagcgaatcccaaaaagccggtcccagttcggattgaggtctgcaactcgacctcatgaagtcggagtcgctagtaa
tcgcagatcagcaacgctgcggtgaatacgttcccgggtcttgtacacaccgcccgtcaagtcatgaaagtcggtaacacctgaagccggtggcccaacccttgtg
gagggagccgtcgaaggtgggatcggtaattagaaaagtcgaataagtgcgagcaacgaaataatcccaacgtccca

Merged sequence

Description	Max. score	Query coverage (%)	Accession	Identity (%)	E value
Microbacterium sp. strain 3B2	2050	93	MG763154.1	93.84	0.0
Uncultured bacterium partial 16S rRNA gene	2043	94	LR639600.1	93.42	0.0
Microbacterium sp. strain TM-B15	2028	95	MH698706.1	92.94	0.0
Microbacterium sp. strain RT111	2026	92	MK014278.1	93.78	0.0
Uncultured bacterium clone 1-11D	2026	95	EU289474.1	92.94	0.0
Microbacterium arborescense strain 13635B	2015	95	EU741114.1	92.79	0.0
Cellulosimicrobium cellulans strain IARI-BHI-13	2012	96	KF054857.1	92.57	0.0

Sequences showing homology



BLASTn output

Plate 25: 16S rRNA sequence analysis of the $MvKS_1$

gattgcggcggcgctcgctgctaccatgcagtcgagcggaagaggtagcttgctactgatcttagcggcggacgggtgagtaatgcttaggaatctgcctattagt
gggggacaacatctcgaaagggatgctaataccgcatacgtcctacgggagaaagcaggggatcttcggaccttgcgctaatagatgagcctaagtcggattag
ctagttggtggcgtaaaggcctaccaaggcgacgatctgtagcgggtctgagaggatgattcgcccactgactgagacggccaatctacgagcgctggaaatcgg
cattgcgaatctgattcagcatgccggtgtggaggagcctaagtgtaaagcctagcgaggaggaggctactttagttaatacctagagatagttgacgtactgcag
aatatgcaccggctaactctgtgccagcagcgcgtatacagagggtgcaagcgtaatcggatttactgggcgtaaagcgcgcgtaggcggctaaataagtcaaat
gtgatatccccgagcttaacttgggaattgcattcgatactggttagctagagtgtggagaggatggtagaattccaggtgtagcggtgaaatgcgtagagatctg
gaggaataccgatggcgaaggcagccatctggcctaacactgacgctgaggtgcgaaagcatggggagcaaacaggatagataccctggtagtccatgccgta
aacgatgtctactagccgttggggcctatgaggctttagtggcgcagctaacgcgataagtagaccgcctggggagtacggtcgcagactaaaactcaaatgaat
tgacggtggcccgcacaagcggtggagctgtggttaattcgatgcaacgcgaagaaccttacctgccttgaatagtaagaactttccagagtggattggtgccttc
gggaacttacatacaggtgctgcatggctgtcgtcagctcgtgtcgtgagatgttgggttaagtcccgcaacgagcgcaacccttatccttatttgccagcgagtaa
tgtcgggaactttaaggatactgccagtgacaaactggaggaaggcggccacgacgtcaagtcatcatggcccttacggccagggctacacacgtgctacaatgg
tcggtacaaagggttgctacacagcgtgtgatgctaatctcaaaccgccgatcgtagtccggattggagtctgcaactcgactccatgaagtcggaatcgctagta
atcgcggatcagaatgccgcggtgaatacgttcccgggccttgtacacaccgcccgtcaccccatgggagtatgttgcaccagaagtagctagcctaactgcaaa
gagggcggttaccacggtgtggccgatgactggtgc

Merged sequence

Description	Max. score	Query coverage (%)	Accession	Identity (%)	E value
Acinetobacter calcoaceticus strain Eu04	2392	99	MH368540.1	96.73	0.0
Acinetobacter seifenti strain WS1	1746	91	MT632639.1	97.43	0.0
Acinetobacter calcoaceticus strain MWU-2536	1746	90	MT101738.1	97.43	0.0
Acinetobacter sp. strain AA85	1746	91	MN540114.1	97.43	0.0
Acinetobacter nosocomialis strain ST02	1746	90	MH368653.1	97.43	0.0
Acinetobacter calcoaceticus strain 11TG-ORWB3	1746	91	MH0363446.1	97.43	0.0

Sequences showing homology

BLASTn output

Plate 26: 16S rRNA sequence analysis of the MvKS$_3$

gcggaatttgggttgcttaacatgcagtcgaacggcagcacggacttcgcttctggtggtgagtggcgaacgggtgaataatgtatcgaaacgtgccaatctacg
ggggatagctacacgaaagcgtaactcttaccgcatacccccctacgggggatagcaggggatcgcgggaccttgcactattgcgcggccgatatcggattagct
agttggtggggtaacggctcaccaaggcgacgatccgtagctggtttgagaggacgaccaggatgactgggactgagaacggcccaaactcctacgggaggca
gcagtggggaattttggactatgggggaaaccctgatcccccatcccgcgtgtgcgatgaaggccttcgggttgtaaagcacttttggccttttgtaaacgtcatg
ggctaataccccgtgaaactgacggtacctgcataataagcaccggctaactacctgccttcatgcccggtaatacgtaaggtgcaagcgttaatccgaattact
gggcttaaggcgtgcgcaggcggttcggagagagagatgtgaaatcccgaagcttaactttggaactgcatttttaactaccgggctggagtgtgtcagtgggag
gtggaattccgcgtgtcccagtgaatgcgtagatatgcggaggaacaccgatggctccgggcctcctggctaacactgacgctcatgcactgagcgtggggagc
agcaggattactaccctggtagtccggccctaacgatgtcaactagctgttggggccttcgggccttattgcgcgctaacgcgtgatttgaccgcctgtcgagtacg
gtcgcaagattaaaactcaatgaattgacggggacccgacagaagcgcggatgatgtggataaatctatgcaactcgaaaaccttacctacccttgacctgtctg
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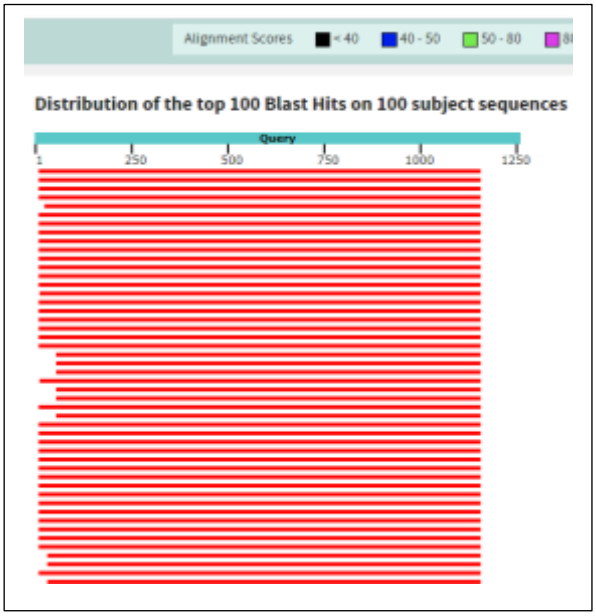
caaccattgtccatcattgctcctaatgccactctaatgagactgccagtctacagaccgccagcaggttgagaatgacgtcaggtccgtcaatgacccttatggc
ttacggctctacgtcactcacgagtatccgaactaggtgccgcactagcgctgtagctgaccactccgagatcccgaatcgtctcggaatcgccatacatc

Merged sequence

Description	Max. score	Query coverage (%)	Accession	Identity (%)	E value
Acromobacter marplatensis strain R-46660	1522	89	NR_117614.1	91.93	0.0
Acromobacter kerstersil strain LMG 3441	1507	89	NR_152015.1	91.65	0.0
Acromobacter delevi strain LMG 3458	1507	89	NR_152014.1	91.65	0.0
Acromobacter insuavis strain LMG 26845	1506	89	NR-117706.1	91.65	0.0
Acromobacter aegrifaciens strain LMG 26892	1498	89	NR_117707.1	91.47	0.0
Acromobacter insolitus strain LMG 6003	1493	89	NR_025685.1	91.38	0.0

Sequences showing homology



BLASTn output

Plate 27: 16S rRNA sequence analysis of the $ThZnS_2$

cggctggctccaaaaggttaccccaccgacttcgggtgttacaaactctcgtggtgtgacgggcggtgtgtacaaggcccgggaacgtattcaccgcggcatg
ctgatccgcgattactagcgattccgacttcatgtaggcgagttgcagcctacaatccgaactgagaatggttttatgggattggctaaacctcgcggtcttgc
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ttagagtgcccaactaaatgctggcaactaagatcaagggttgcgctcgttgcgggacttaacccaacatctcacgacacgagctgacgacaaccatgcacc
acctgtcactctgtccccgaaggggaaagtcctatctctaggattgtcagaggatgtcaagacctggtaaggttcttcgcgttgcttcgaattaaaccacatg
ctccaccgcttgtgcgggcccccgtcaattcctttgagtttcaaccttgcggtcgtactccccaggcggagtgcttaatgcgtttgctgcagcactaaagggcgg
aaaccctctaacacttagcactcatcgtttacggcgtggactaccagggtatctaatcctgtttgctccccacgctttcgcgcatcagcgtcagttacagaccag
aaagtcgccttcgccactggtgttcctccaaatctctacgcatttcaccgctacacttggaattccactttcctcttctgcactcaagttccccagtttccaatgac
cctccacggttgagccgtgggctttcacatcagacttaaggaaccgcctgcgcgcgctttacgcccaataattccggacaacgcttgccacctacgtattaccg
cggctgctggcacgtagttagccgtggctttctggttaggtaccgtcaaggtaccggcagttactccgatacttgttcttccctaacaacagagttttacgatcc
gaaaaccttcatcactcacgcggcgttgctccgtcagactttcgtccattgcggaagattccctactgctgcctcccgtaggagtctgggccgtgtctcagtccc
agtgtggccgatcaccctctcaggtcggctacgcatcgtcgccttggtgagccgttacctcaccaactagctaatgcgccgcgggcccatctgtaagtgatagc
agaaccatctttcaatctaattatatgtataaatagatattatccggtattagccccggtttcccggagttatcccagtcttacaggcaggttgcccacgtgttac
tcacccgtccgccgctaatctttgggagcaagctccctcagattcgctcgacttg

Merged sequence

Description	Max. score	Query coverage (%)	Accession	Identity (%)	E value
Cytobacillus kochii strain BDGP4	2601	100	CP0222983.1	99.86	0.0
Bacillus sp. mixed culture J2-13	2601	100	KR029186.1	99.86	0.0
Bacillus pocheonensis strain C6DMVR	2601	100	KR140187.1	99.86	0.0
Bacillus sp. R-27341	2601	100	AM910198.1	99.86	0.0
Bacillus sp. Fen_H	2599	99	JN247735.1	99.86	0.0
Bacillus solani strain BGS-CAP-2	2597	99	MF076230.1	99.86	0.0
Bacillus kochii strain 58312	2597	99	KX418574.1	98.89	0.0
Bacillus fleus KSC	2597	99	DQ870687.1	98.89	0.0

Sequences showing homology

BLASTn output

Plate 28: 16S rRNA sequence analysis of the PKZnS$_3$

tcatgaatcccaccgtggtaagcgccctccttacggttaggctacctacttctggtgaaacccactcccatggtgtgacgggcggtgtgtacaagacccgggaacg
tattcaccgcgacattctgatccgcgattactagcgattccgacttcacgcagtcgagttgcagactgcgatccggactacgatcgggtttctgagattggctcccc
ctcgcgggttggcgaccctctgtcccgaccattgtatgacgtgtgaagccctacccataagggccatgaggacttgacgtcatccccaccttcctccggtttgtcac
cggcagtctcattagagtgctcttgcgtagcaactaatgacaagggttgcgctcgttgcgggacttaacccaacatctcacgacacgagctgacgacagc
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acggccccaacagctagttgacatcgtttagggcgtggactaccagggtatctaatcctgtttgctccccacgctttcgtgtctgagcgtcagtattatcccagggg
gctgccttcgccatcggtattcctccacatatctacgcatttcactgctacacgtggaattctacccccctctgacatactctagctcggcagttaaaaatgcagttc
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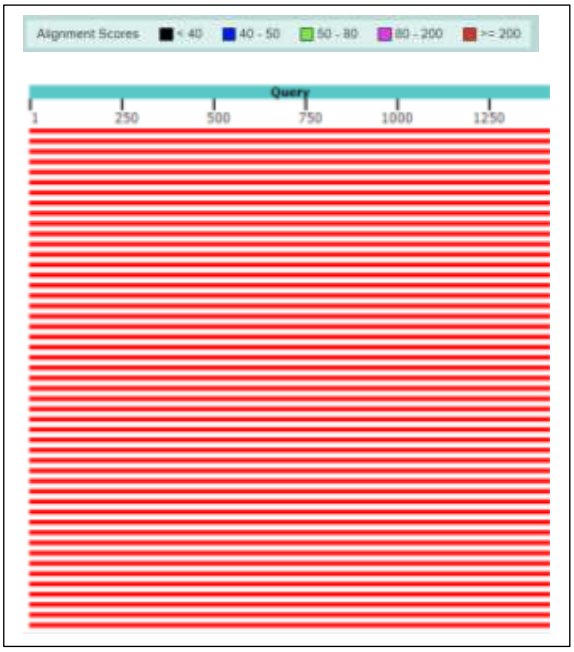
tttcccccgtagggcgtatgcggtattagccactctttc

Merged sequence

Description	Max. score	Query coverage (%)	Accession	Identity (%)	E value
Alcaligenes faecalis strain FC2960	2601	100	MK517568.1	99.93	0.0
Alcaligenes faecalis strain FDAARGOS	2601	100	CP033861.1	99.93	0.0
Alcaligenes faecalis strain FC2960	2601	100	MK312671.1	99.93	0.0
Alcaligenes faecalis strain DSM 30030	2601	100	CP023667.1	99.93	0.0
Alcaligenes faecalis strain P156	2601	100	KT748643.1	99.93	0.0
Alcaligenes faecalis strain C_9	2601	100	KT748643.1	99.93	0.0
Alcaligenes sp. BN3	2601	100	KR051026.1	99.93	0.0

Sequences showing homology



BLASTn output

Plate 29: 16S rRNA sequence analysis of the $KpNF_5$

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tccgcgattactagcgattccagcttcatgcactcgagttgcagagtgcaatccggactacgatcggttttctgggattagctccccctcgcgggttggcaaccctctgtt
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agcaactaaggacaagggttgcgctcgttgcgggacttaacccaacatctcacgacacgagctgacgacagccatgcagcacctgtgcgccggttctctttcgagcac
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agttttaatcttgcgaccgtactccccaggcggtcaacttcacgcgttagctacgttactaaggaaatgaatccccaacaactagttgacatcgtttagggcgtggacta
ccagggtatctaatcctgtttgctccccacgctttcgtgcatgagcgtcagtattggcccagggggctgccttcgccatcggtattcctccacatctctacgcatttcactg
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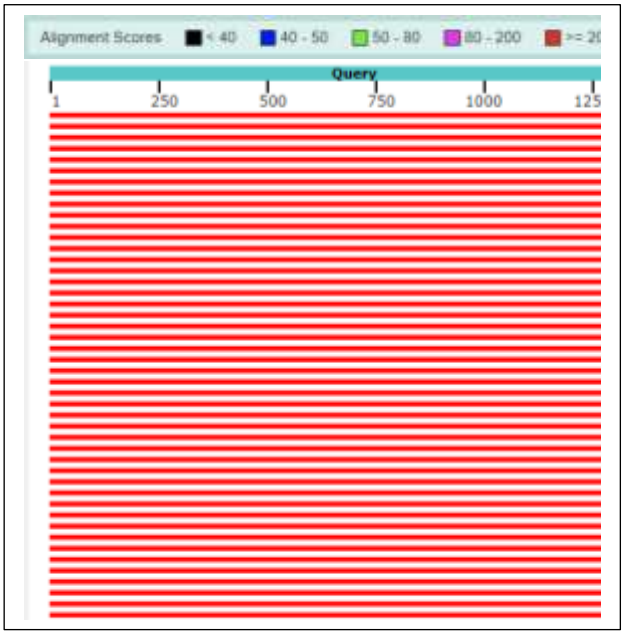
atcagccatcggccaaccctatagcgcgaggcccgaaggtcccccgctttcatccatagatcgtatgcggtattaatccggctttcgccgggctatccccccactacagg
acatgttccgatgtattactcacccgttcgccactcgccaccaggtgcaagcacccgtgctgcc

Merged sequence

Description	Max. score	Query coverage (%)	Accession	Identity (%)	E value
Burkholderia sp. strain DDMZ1-1	2567	100	MK583567.1	100	0.0
Burkholderia vietnamiensis BE10	2567	100	MKD41542.1	100	0.0
Burkholderia sp. strain TC129	2567	100	MK459497.1	100	0.0
Burkholderia vietnamiensis lala l4	2567	100	MK393863.1	100	0.0
Burkholderia vietnamiensis strain TVV75	2567	100	MH547402.1	100	0.0
Burkholderia sp. strain EIKU9	2567	100	MK393863.1	100	0.0
Burkholderia vietnamiensis strain FDAARGOS	2567	100	CP020394.1	100	0.0

Sequences showing homology



BLASTn output

Plate 30: 16S rRNA sequence analysis of the $MtNF_4$

ccccagtcgctgaccctaccgtggtcggctgcctccattgctggttagcgcaccgccttcgggtagaaccaactcccatggtgtgacgggcggtgtgtacaaggcccgggaa
cgtattcaccgcggcatgctgatccgcgattactagcgattccaacttcatgccctcgagttgcagaggacaatccgaactgagacgacttttaaggattaaccctctgtagtc
gccattgtagcacgtgtgtagcccaccctgtaagggccatgaggacttgacgtcatccccaccttcctccggcttagcaccggcagtcccattagagttcccaactaaatgat
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cgtctccgtggcggtccaggcatgtcaaaaggtggtaaggttctgcgcgttgcttcgaattaaaccacatgctccaccgcttgtgcgggcccccgtcaattcctttgagtttta
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taatcctgtttgctccccacgctttcgcgcctcagcgtcagtaatgagccagtatgtcgccttcgccactggtgttcttccgaatatctacgaatttcacctctacactcggagtt
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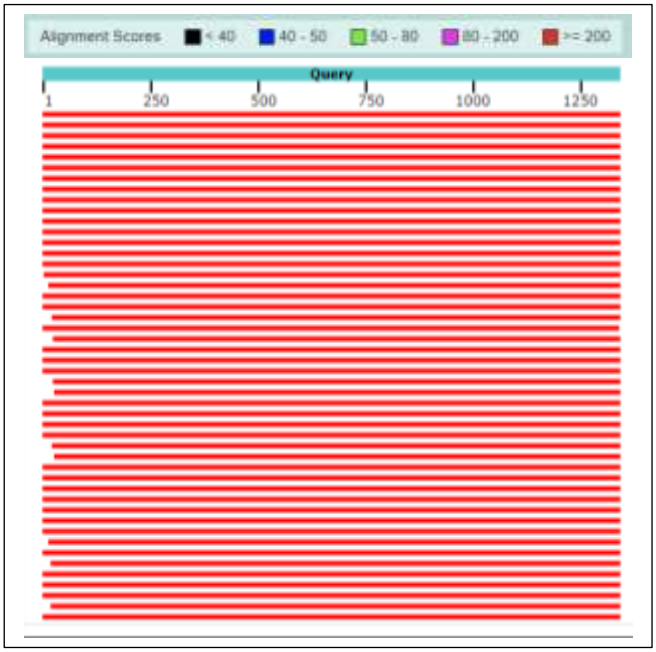
attcggtattagcacaagtttccctgagttattccgaaccaaaaggcacgttcccacgtgttactcacccgtccgccactaactccgaagagttc

Merged sequence

Description	Max. score	Query coverage (%)	Accession	Identity (%)	E value
Bruvendimonas naejangsanensis strain FS1091	2475	100	CP038027.1	100	0.0
Bruvendimonas sp. strain alpha-24	2475	100	MH686076.1	100	0.0
Bruvendimonas naejangsanensis strain B1					
Parastrongyloides trichosuri	2475	100	LM523554.1	100	0.0
Bruvendimonas diminuta JANV-6	2475	100	KF453789.1	100	0.0
Bruvendimonas naejangsanensis strain JANV- 1	2475	100	KF453785.1	100	0.0

Sequences showing homology



BLASTn output

Plate 31: 16S rRNA sequence analysis of the AkNF$_2$

ccccttttttaaactagatcaacgtggtgacgtcctccttgcggttagactagccacttctggtaaaacccactcccatggtgtgacgggcggtgtgtacaag
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acgagctgacgacagccatgcagcacctgtgcgccggttctctttcgagcactcccgaatctcttcaggattccgaccatgtcaagggtaggtaaggttttc
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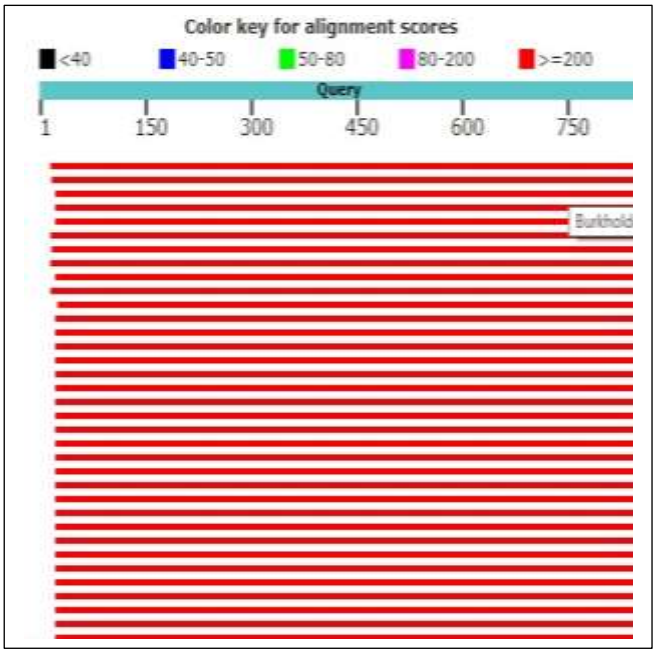
gcgttagctacgttactaaggaaatgaatccccaacaactagttgacatcgtttagggcgtggactaccagggtatctaaatcctgtttgctccccacgcttt
cgtgcatgagcgtcagtattggcccaggggctgccttcgccatcggtattccctccaatctctacgcattttcactgctacacatggaaattctacccccccct
ctgccatactctaccctgcc

Merged sequence

Description	Max. score	Query coverage (%)	Accession	Identity (%)	E value
Burkholderia vietnamiensis strain RTA	1482	98	MK014280.1	98.92	0.0
Burkholderia cepecia strain HYRE2	1480	97	KU942389.1	98.92	0.0
Burkholderia multivorans strain OGO71	1478	97	MK353957.1	99.03	0.0
Burkholderia cepacia strain KLA-2	1476	97	MG846077.1	99.03	0.0
Burkholderia sp. strain AAAr-129	1476	97	KY810655.1	99.03	0.0
Uncultured prokaryote clone	1474	97	KP409338.1	99.03	0.0

Sequences showing homology



BLASTn output

Plate 32: 16S rRNA sequence analysis of the $PkPS_2$

gtcatgaatcacaaagtggtaagcgccctcccgaaggttaagctacctacttcttttgcaacccactcccatggtgtgacgggcggtgtgtacaaggcccgggaac
gtattcaccgtagcattctgatctacgattactagcgattccgacttcatggagtcgagttgcagactccaatccggactacgacgtactttatgagttccgcttgctc
tcgcgaggtcgcttctctttgtatacgccattgtagcacgtgtgtagccctactcgtaagggccatgatgacttgacgtcatccccaccttcctccggtttatcaccgg
cagtctcctttgagttcccgaccgaatcgctggcaacaaaggataagggttgcgctcgttgcgggacttaacccaacatttcacaacacgagctgacgacagccat
gcagcacctgtctcagagttcccgaaggcaccaaagcatctctgctaagttctctggatgtcaagagtaggtaaggttcttcgcgttgcatcgaattaaaccacatg
ctccaccgcttgtgcgggcccccgtcaattcatttgagttttaaccttgcggccgtactccccaggcggtcgatttaacgcgttagctccggaagccacgcctcaagg
gcacaacctccaaatcgacatcgtttacagcgtggactaccagggtatctaatcctgtttgctccccacgctttcgcacctgagcgtcagtctttgtccaggggggccg
ccttcgccaccggtattcctccacatctctacgcatttcaccgctacacatggaattctacccccctctacaagactctagctgaccagtcttagatgccattcccagg
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acgcggcatggctgcatcaggcttgcgcccattgtgcaatattccccactgctgcctcccgtaggagtctgggccgtgtctcagtcccagtgtggctgatcatcctct
cagaccagctagggatcgtcgcctaggtgagccattaccccacctactagctaatcccatatgggttcatccgatagcgcaaggaccgaagttcccctgctttgctc
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cttcccctgttaccgctcgactgcat

Merged sequence

Description	Max. score	Query coverage (%)	Accession	Identity (%)	E value
Providencia vermicola strain Gol 12	2630	100	MT263027.1	99.33	0.0
Providencia vermicola strain KUBT-1	2630	100	KX098543.1	99.33	0.0
Providencia sp. strain SMKRBT-TS9	2652	100	MN204490.1	99.86	0.0
Providencia rettgeri strain BFM1	2625	100	KU870748.1	99.86	0.0
Uncultured *Providencia* sp. clone M	2619	100	MF327082.1	99.79	0.0
Providencia vermicola strain 6G	2614	100	KP204501.1	99.72	0.0

Sequences showing homology

BLASTn output

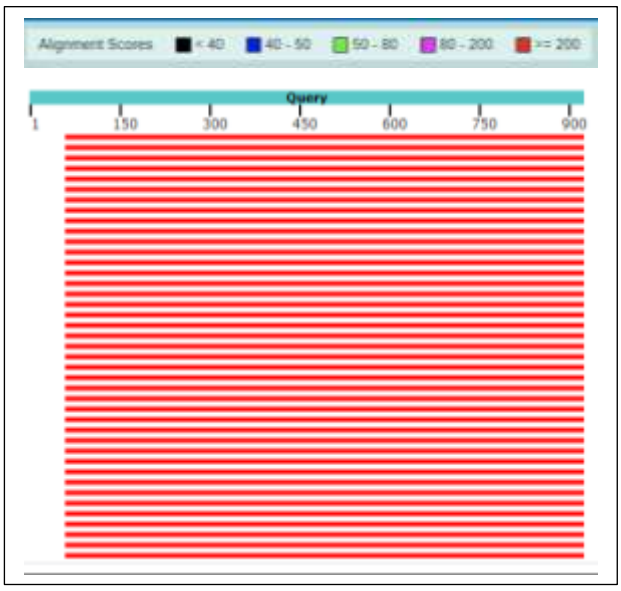
Plate 33: 16S rRNA sequence analysis of the $PkPS_3$

aaggtagttctctctcgagatgacccgccgcactgagacccaatgtgaatctcaaacagtccttgcggttagactagccacttctggtaaaacccactcccatg
gtgtgacgggcggtgtgtacaagacccgggacgtattcaccgcggcatgctgatccgcgattactagcgattccagcttcatgcactcgagttgcagagtgca
atccggactacgatcggttttctgggattagctccccctcgcgggttggcaaccctctgttccgaccattgtatgacgtgtgaagccctacccataagggccatg
aggacttgacgtcatccccaccttcctccggtttgtcaccggcagtctccttagagtgctcttgcgtagcaactaaggacaagggttgcgctcgttgcgggactt
aacccaacatctcacgacacgagctgacgacagccatgcagcacctgtgcgccggttctctttcgagcactcccgcctctcagcaggattccgaccatgtcaa
gggtaggtaaggtttttcgcgttgcatcgaattaatccacatcatccaccgcttgtgcgggtccccgtcaattcctttgagttttaatcttgcgaccgtactcccc
aggcggtcaacttcacgcgttagctacgttactaaggaaatgaatccccaacaactagttgacatcgtttagggcgtggactaccagggtatctaatcctgttt
gctctccacgctttcgtgcatgagcgtcagtattggcccaggggctgccttcgccatcggtattcctccacatctctacgcatttcactgctacacgtggaattc
taccccctctgccatactctagcctgccagtcaccattgcagttcccaggttgagcccggggatttcaatcggtctta

Merged sequence

Description	Max. score	Query coverage (%)	Accession	Identity (%)	E value
Burkholderia cepacia strain FDAARGOS 345	1568	93	CP0220832	99.65	0.0
Burkholderia cepacia strain FDAARGOS 388	1568	93	CP023518.1	99.65	0.0
Burkholderia cepacia strain ATCC 25416	1568	93	CP012981.1	99.65	0.0
Burkholderia cepacia strain ATCC 25416	1568	93	CP0077748.1	99.65	0.0
Burkholderia cepacia strain MK 834705.1	1567	93	MK834705.1	99.65	0.0
Burkholderia sp. strain Y13	1567	93	MH266122.1	99.65	0.0
Bacterium strain BS2104	1567	93	MK825292.1	99.65	0.0

Sequences showing homology



BLASTn output

Plate 34: 16S rRNA sequence analysis of the $MvPS_3$

atgggggtctcgctagagattgtcctgggtctcgtgcgcgggtgcccccagccggattaataccgcatacgatctacggatgaaagcgggggaccttcgggcctcgc
gctatagggttggccgatggcagattagctagttgaggtaaggcctacaggacgatcagtagctggtcgaagaacgaccagccacactggactagacacggccca
actcctacggagcagcagtgggaatttggacaatggcgaagctgatccagcaatgccgcgtgtgtgagaagcttcgggtgtaaagcactttgtccggaaagaaatc
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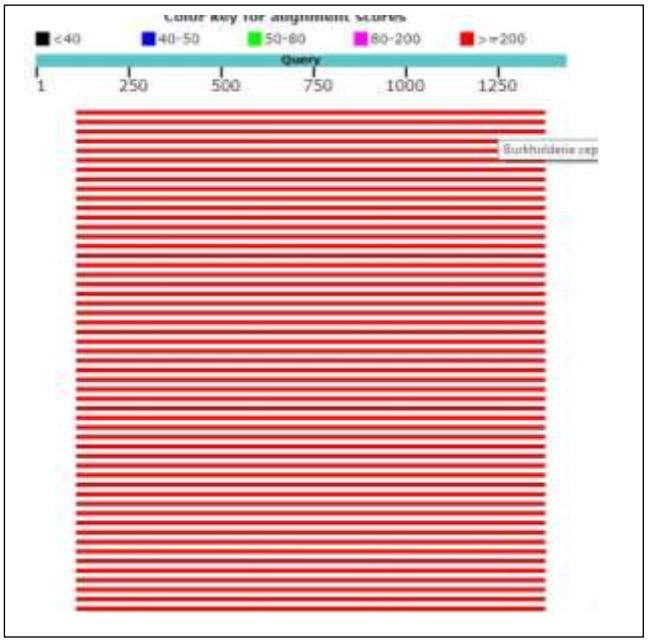
ctgcaactcgagtgcatgaagctggaatcgctagtaatcgcggatcagcatgccgcggtgaatacgttcccgggtcttgtacacaccgcccgtcacaccatgggagt
gggttttaccagaagtggctagtctaaccgcaaggactcttgagattcacactgggactcagtgcggcgggtcatctcgtagagtacgacgt

Merged sequence

Description	Max. score	Query coverage (%)	Accession	Identity (%)	E value
Burkholderia cepacia strain HSC	2145	92	MT565309.1	97.05	0.0
Burkholderia sp strain Isyb 42	2145	92	KY678897.1	97.05	0.0
Burkholderia cepacia strain QEH47	2145	92	MN508423.1	97.05	0.0
Burkholderia cepacia strain QEH9	2145	92	MN508422.1	97.05	0.0
Burkholderia cepacia strain QEH3	2145	92	MN508421.1	97.05	0.0
Burkholderia territori strain SCAT001	2145	92	MN54845.1	97.05	0.0
Burkholderia territori strain yy02	2145	92	MN177180.1	97.05	0.0
Burkholderia cepacia ATCC25416	2145	92	CP034553.1	97.05	0.0

Sequences showing homology



BLASTn output

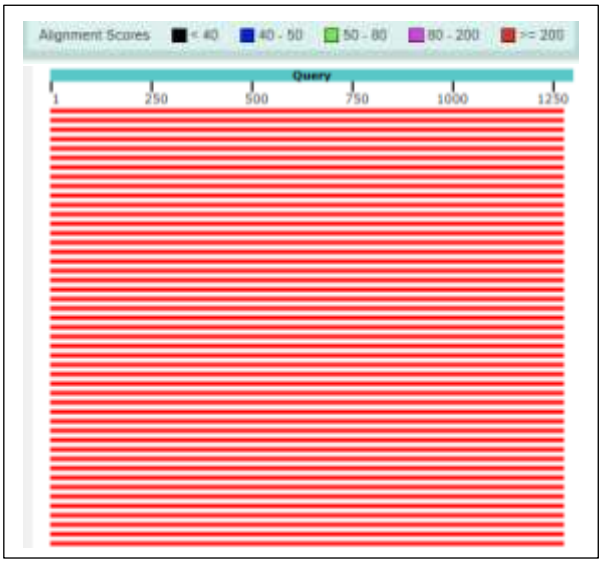
Plate 35: 16S rRNA sequence analysis of the AkKS$_1$

cagatgaacg ctggcggcag gcttaacaca tgcaagtcga gcgggggaag gtagcttgctaccggaccta gcggcggacg ggtgagtaat gcttaggaat ctgcctatta gtgggggaca acatctcgaa agggatgcta ataccgcata cgtcctacgg gagaaagcag gggatcttcg gaccttgcgc taatagatga gcctaagtcg gattagctag ttggtggggt aaaggcctac caaggcgacg atctgtagcg ggtctgagag gatgatccgc cacactggga ctgagacacg gcccagactc ctacgggagg cagcagtggg gaatattgga caatgggggg aaccctgatccagccatgcc gcgtgtgtga agaaggcctt atggttgtaa agcactttaa gcgaggagga ggctacttta gttaatacct agagatagtg gacgttactc gcagaataag caccggctaa ctctgtgcca gcagccgcgg taatacagag ggtgcgagcg ttaatcggat ttactgggcg taaagcgtgc gtaggcggct tattaagtcg gatgtgaaat ccccgagctt aatgcattcga tactggtgag ctagagtatg ggagaggatg gtagaattcc aggtgtagcg gtgaaatgcg tagagatctg gaggaatacc gatggcgaag gcagccatct ggcctaatac tgacgctgag gtacgaaagc atggggagca aacaggatta gataccctgg tagtccatgc cgtaaacgat gtctactagc cgttggggcc tttgaggctt tagtggcgca gctaacgcga taagtagacc gcctggggag tacggtcgca agactaaaac tcaaatgaat tgacgggggc ccgcacaagc ggtggagcat gtggtttaat tcgatgcaac gcgaagaacc ttacctggcc ttgacatact agaaactttc cagagatgga ttggtgcctt cgggaatcta gatacaggtg ctgcatggct gtcgtcagct cgtgtcgtga gatgttgggt taagtcccgc aacgagcgca acccttttcc ttacttgcca gcatttcgga tgggaacttt aaggatactg ccagtgacaa actggaggaa ggcggggacg acgtcaagtc atcatggccc ttacggccag ggctacacac gtgctacaat ggtcggtaca aagggttgct acacagcgat gtgatgctaa tctcaaaaag ccgatcgtag tccggataca cacgtgctac aatggtcggt

Merged sequence

Description	Max. score	Query coverage (%)	Accession	Identity (%)	E value
Acinetobacter sp. strain ZX01	2359	98	KY704098.1	100.00	0.0
Acinetobacter bummanii strain M14413	2353	98	CP054302.1	99.52	0.0
Acinetobacter bummanii strain ABF9692	2353	98	CP048827.1	99.52	0.0
Acinetobacter bummanii strain VB82	2353	98	CP050385.1	99.52	0.0
Acinetobacter bummanii strain VB2486	2353	98	CP050403.1	99.52	0.0
Acinetobacter bummanii strain PM193665	2353	98	CP050415.1	99.52	0.0
Acinetobacter bummanii strain PM194229	2353	98	CP054332.1	99.52	0.0

Sequences showing homology



BLASTn output

Plate 36: 16S rRNA sequence analysis of the $MvZnS_1$

4.7. Genetic variability of selected isolates

Phylogenetic analysis was carried out for studying the relationship of the isolates collected during the present study with already existing strains of relevant species. The 16S rDNA nucleotide sequence of eighteen isolates were aligned with the closely related sequences of 16S rDNA gene retrieved from NCBI database. The sequences were aligned using MAFFT software and a phylogenetic tree was constructed employing the MEGA 7 software (Fig. 1).

Sequence similarity search in NCBI for the nitrogen fixing isolates, $AkNF_3$, $KgNF_1$, $PkNF_4$, $KpNF_5$, $AkNF_2$ and $MtNF_4$ showed 100% similarity with available sequences of *Bacillus* sp, *Pseudomonas putida*, *Pseudomonas* sp., *Alcaligens faecalis*, *Brevundimonas naejangsanensis* and *Burkholderia* sp. respectively.

Phosphate solubilizing PGPR isolates *viz*, $PkPS_1$, $AkPS_4$, $AvPS_1$, $PkPS_2$, $PkPS_3$ and $MvPS_3$ showed homology with *Bacillus megaterium*, *Acinetobacter shindleri*, *Achromobacter* sp, *Burkholderia vietnamiensis*, *Providencia vermicola* and *Burkholderia cepacia* with available sequences of NCBI database.

Identification of potassium solubilizing isolates namely $MvKS_1$, $MvKS_3$ and $AkKS_1$ through 16S rDNA sequences analysis indicated that the new strains were highly homology with *Microbacterium* sp. *Acinetobacter calcoaceticus* and *Burkholderia cepacia*. Zinc solubilizing isolates $ThZnS_1$, $PkZnS_3$ and $MvZnS_1$ showed homology with *Achromobacter marplatensis*, *Cytobacillus kochii* and *Acinetobacter* sp. respectively.

The phylogenetic analysis revealed that 10 isolates out of 18 identified isolates *viz*, *Bacillus* sp $AkNF_3$, *Pseudomonas putida* $KgNF_1$, *Bacillus megaterium* $PkPS_1$, *Acinetobacter shindleri* $AkPS_4$, *Achromobacter* sp $AvPS_1$, *Microbacterium* sp. $MvKS_1$, *Acinetobacter calcoaceticus* $MvKS_3$, *Burkholderia cepacia* $AkKS_1$, *Achromobacter marplatensis* $ThZnS_1$ and *Acinetobacter* sp. $MvZnS_1$ clustered with respective species obtained from NCBI databank and confirmed they were belonging to the identified species. However other eight isolates were clustering separate clades together and these isolates showed the phylogenetic distance with sequences obtained from NCBI database.

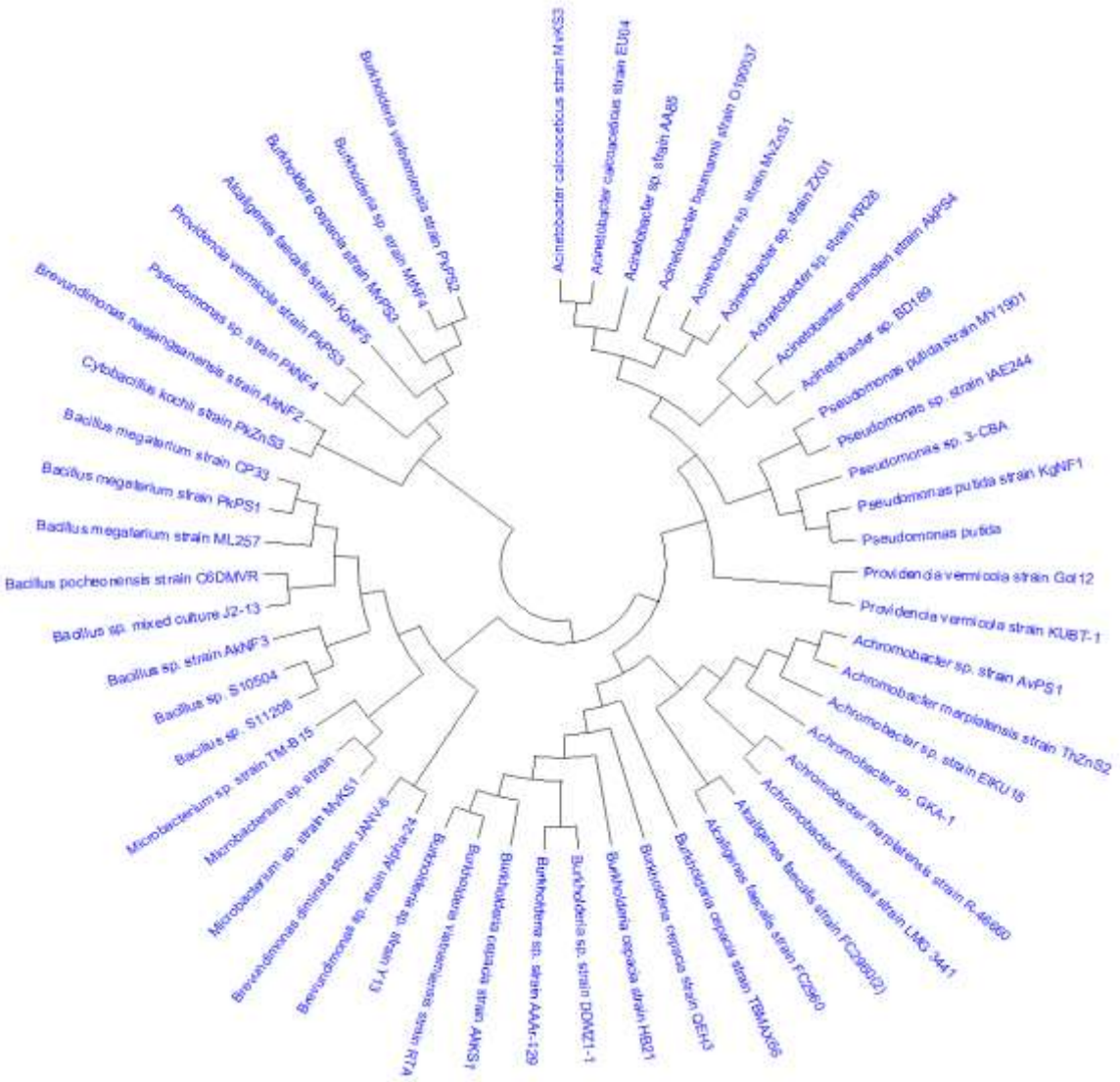


Fig 1. Maximum likelihood phylogenetic tree produced using multiple alignment of 16S rRNA gene sequence of all bacterial isolates with related species found in GeneBank database

4.8. Selection of potential isolates of PGPR for further experiments

Potential isolates under different functional categories were selected based on their efficiency of N fixation and solubilization of P, K and Zn and considering all other PGP activities tested. Weightage was given for each function of isolates according to our requirement for biofertilizer production and ranking was obtained.

4.8.1. Selection of potential isolates of N-fixers

Efficiency of the isolates to fix N and other tested PGP activities were considered for selection of the isolates. Isolates were ranked based on the sum of weightage and arranged in descending order (Table 35). Based on the ranking, first three isolates ($KgNF_1$, $AkNF_3$ and $PkNF_4$) were selected for consortial formulation.

Table 35. Ranking of N-fixers

Isolate	N fixed (mg g^{-1} of sucrose)	Production of IAA	Production of NH_3	Production of HCN	Production of siderophore	Antagonistic activity		Rank
						R. solani	*X. oryzae*	
$KgNF_1$	8.86	34.83	++	-	+	-	+	1
$AkNF_3$	9.33	23.16	++	-	++	-	-	2
$PkNF_4$	9.33	20.66	++	-	+	-	-	3
$AvNF_4$	1.86	25.00	+	-	+	-	-	4
$KpNF_2$	7.93	14.83	+	-	-	-	-	5
$KpNF_6$	4.26	19.33	+	-	-	-	-	6
$AvNF_2$	2.33	18.83	+	-	++	-	-	7
$PkNF_3$	6.06	12.33	+	-	+	-	-	8
$KpNF_7$	3.73	15.83	+	-	-	-	-	9
$PkNF_2$	4.66	11.16	++	-	+	-	-	10
$KpNF_5$	8.40	-	++	-	++	+	+	11
$AvNF_3$	5.60	6.16	+	-	+	-	-	12
$KgNF_9$	6.06	3.50	+++	-	-	-	-	13
$AkNF_2$	7.93	-	++	-	++	-	-	14
$KpNF_4$	4.66	5.00	+	-	-	-	-	15
$MtNF_4$	7.00	-	++	-	-	+	-	16

$AvNF_1$	6.53	-	+	-	++	-	-	17
$PkNF_1$	6.06	-	+	-	+	-	-	18
$MtNF_3$	4.20	2.50	++	-	-	-	-	16
$AkNF_5$	4.16	-	++	-	+	-	-	20

NF: nitrogen fixers
Kg: Kolagappara, Kp: Kuppamudi, Av: Ambalavayal, Mt: Marathat, Ak: Ambukuthi
Pk: Pottankoli

4.8.2. Selection of potential isolates of phosphate solubilizers

Ranking was obtained based on the efficiency in terms of quantity of phosphate solubilized in µg ml^{-1} and other PGP characters (Table 36). Isolates with first three ranks ($PkPS_1$, $AkPS_4$ and $AvPS_1$) were selected for checking the compatibility followed by consortial formulation.

Table 36. Ranking of phosphate solubilizers

Isolate	Quantity of P solubilized µg ml^{-1}	Production of IAA µg ml^{-1}	Production of NH3	Production of HCN	Production of siderophore	Antagonistic activity		Rank
						R. solani	*X. oryzae*	
$PkPS_1$	134.88	4.66	+	-	+	-	-	1
$AkPS_4$	121.33	4.66	+	-	+	-	-	2
$AvPS_1$	113.33	8.16	++	-	+	-	-	3
$PkPS_2$	108.33	4.83	+	-	+	-	-	4
$PkPS_3$	103.83	2.66	+	-	-	-	-	5
$MtPS_5$	82.00	4.83	-	-	-	-	-	6
$MvPS_4$	81.66	3.66	+	-	+	-	-	7
$MvPS_3$	77.66	7.00	+	-	++	-	+	8
$EdPS_4$	82.33	-	+	-	-	-	-	9
$AkPS_5$	81.33	-	+	-	-	-	-	10
$MtPS_2$	66.83	19.80	+	-	+	-	-	11
$ThPS_4$	74.00	-	+	-	-	-	-	12
$AkPS_7$	72.50	-	-	-	+	-	-	13
$EdPS_5$	55.66	12.00	-	-	-	-	-	14

$MtPS_6$	49.83	5.33	-	-	-	-	-	15
$PkPS_5$	50.00	3.33	+	-	-	-	-	16

PS: phosphate solubilizers
Th: Thanivayal, Ed: Edakkal, Av: Ambalavayal, Mt: Marathat, Ak: Ambukuthi
Mv: Malavayal, Pk: Pottankoli

4.8.3. Selection of potential isolates of potassium solubilizers

Based on the efficiency in terms of quantity of K solubilized in µg $ml^{-1}$and other PGP activities, two potential isolates ($MvKS_1$ and $MvKS_3$) were selected for compatibility test and formulation of PGPR consortium (Table 37).

Table 37. Ranking of potassium solubilizers

Isolate	Quantity of K solubilized µg ml^{-1}	Production of IAA	Production of NH3	Production of HCN	Production of siderophore	Antagonistic activity		Rank
						R. solani	*X. oryzae*	
$MvKS_1$	4.19	-	+	-	-	-	-	1
$MvKS_3$	3.01	-	+	-	-	-	-	2
$AkKS_1$	1.81	-	+	-	-	-	-	3
$MvKS_2$	1.62	-	-	-	-	-	-	4

KS: potassium solubilizers
Ak: Ambukuthi, Mv: Malavayal

4.8.4. Selection of potential isolates of zinc solubilizers

Based on ranking, two most potential isolates ($PkZnS_3$ and $ThZnS_2$) were selected for testing the compatibility and formulation of consortium (Table 38).

Table 38. Ranking of zinc solubilizers

Isolate	Quantity of Zn solubilized µg ml^{-1} (ZnO)	Quantity of Zn solubilized µg ml^{-1} (ZnCO3)	Production of IAA	Production of NH3	Production of HCN	Production of siderophore	Rank
$PkZnS_3$	18.57	13.26	-	+	-	-	1
$ThZnS_1$	17.43	10.33	-	+	-	-	2
$MvZnS_1$	17.65	-	-	+	-	++	3
$MvZnS_3$	16.08	-	-	+	-	++	4

$AvZnS_1$	14.67	-	-	-	-	-	5
$AkZnS_4$	14.05	-	-	-	-	-	6

ZnS: zinc solubilizers
Th: Thanivayal, Av: Ambalavayal, Ak: Ambukuthi, Mv: Malavayal

Based on the ranking, ten most potential isolates were selected for development of PGPR consortia. These included three nitrogen fixers ($KgNF_1$, $AkNF_3$ and $PkNF_4$), three P solubilizers ($AvPS_1$, $PkPS_1$ and $AkPS_4$), two K solubilizers ($MvKS_1$ and $MvKS_3$) and two Zn solubilizers ($ThZnS_2$ and $PkZnS_3$). Selected PGPRs for consortial development are detailed in Table 39.

Table 39: Selected isolates for consortial development

Isolate	Identity based on 16S rRNA gene sequence
$KgNF_1$	*Pseudomonas putida* strain KgNF1
$AkNF_3$	*Bacillus* sp. strain AkNF3
$PkNF_4$	*Pseudomonas* sp. strain PkNF4
$PkPS_1$	*Bacillus megaterium* strain PkPS1
$AkPS_4$	*Acinetobacter schindleri* strain AkPS4
$AvPS_1$	*Achromobacter* sp. strain AvPS1
$MvKS_1$	*Microbacterium* sp. strain MvKS1
$MvKS_3$	*Acinetobacter calcoaceticus strain* MvKS3
$PkZnS_3$	*Cytobacillus kochii* strain PkZnS3
$ThZnS_2$	*Achromobacter marplatensis* strain ThZnS2

4. 9. Compatibility of selected potential isolates under *in vitro* conditions for developing consortial formulations

In order to develop the consortial formulation of PGPR, compatibility among the ten selected isolates was tested by using cross streak method and further confirmed with dual culture method (Table 40 and Plates 37, 38). All possible combinations of ten promising isolates were tested and the results revealed that there was no inhibition of growth among the isolates. Hence, all the isolates were considered compatible.

Fig. a. Compatibility test of $PkNF_4$ and $PkPS_1$

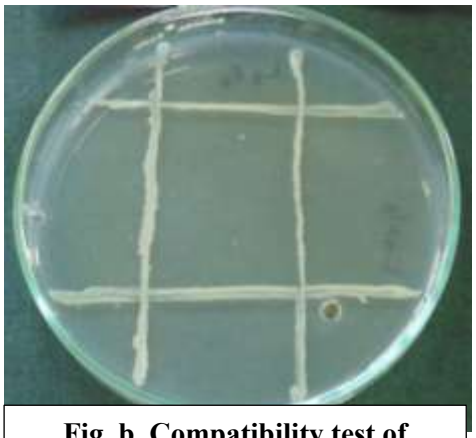


Fig. b. Compatibility test of $PkNF_4$ and $AkPS_4$

Fig. c. Compatibility test of $AvPS_1$ and $PkZnS_3$

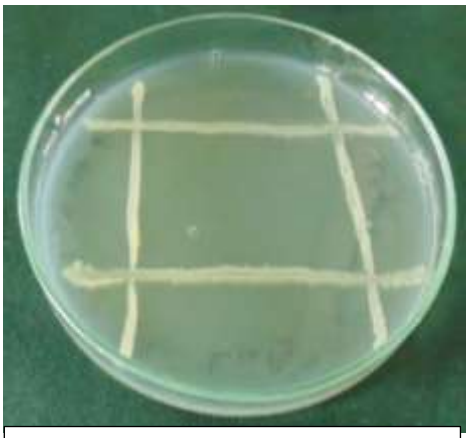


Fig. d. Compatibility test of $PkPS_1$ and $PkNF_4$

Fig. e. Compatibility test of $MvKS_1$ and $PkPS_1$

Fig. f. Compatibility test of $ThZnS_2$ and $PkPS_1$

Plate 37: Compatibility test of promising isolates using cross streak method

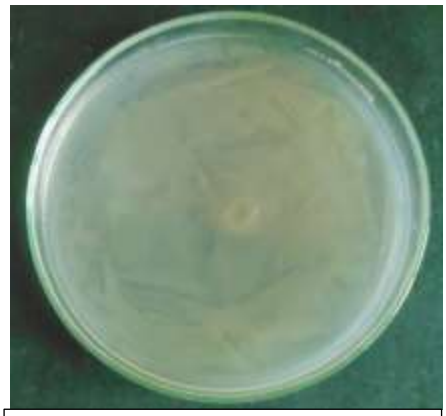


Fig. a. Compatibility test of $PkNF_4$ and $PkPS_1$

Fig. b. Compatibility test of $PkNF_4$ and $AkPS_4$

Fig. c. Compatibility test of $AkNF_3$ and $ThZnS_2$

Fig. d. Compatibility test of $KgNF_1$ and $PkPS_1$

Fig. e. Compatibility test of $PkNF_4$ and $PkZnS_3$

Fig. e. Compatibility test of $MvKS_1$ and $PkPS_1$

Plate 38: Compatibility test of promising isolates using dual culture method

Table 40: Compatibility of potential PGPRs selected for consortial development

Isolates	$KgNF_1$	$AkNF_3$	$PkNF_4$	$PkPS_1$	$AkPS_4$	$AvPS_1$	$MvKS_1$	$MvKS_3$	$ThZnS_2$	$PkZnS_3$
$KgNF_1$		+	+	+	+	+	+	+	+	+
$AkNF_3$	+		+	+	+	+	+	+	+	+
$PkNF_4$	+	+		+	+	+	+	+	+	+
$PkPS_1$	+	+	+		+	+	+	+	+	+
$AkPS_4$	+	+	+	+		+	+	+	+	+
$AvPS_1$	+	+	+	+	+		+	+	+	+
$MvKS_1$	+	+	+	+	+	+		+	+	+
$MvKS_3$	+	+	+	+	+	+	+		+	+
$ThZnS_2$	+	+	+	+	+	+	+	+		+
$PkZnS_3$	+	+	+	+	+	+	+	+	+	

Compatibility: (+)
NF: nitrogen fixers, PS: phosphate solubilizers, KS: potassium solubilizers
ZnS: zinc solubilizers
Th: Thanivayal, Kg: Kolagappara, Av: Ambalavayal, Ak: Ambukuthi, Mv: Malavayal Pk: Pottankoli

Three PGPR based consortial formulations were prepared by using the ten potential isolates from different functional groups. Every consortium included five isolates, consisting of two nitrogen fixers, one P solubilizer, one K solubilizer and one zinc solubilizer. Details of the consortial formulations are presented in Table 41.

Table 41: Details of consortial formulations of PGPR isolates

Components	**Consortial formulation**		
	1	**2**	**3**
N fixers	*Bacillus* sp. AkNF3 *Pseudomonas* sp. PkNF4	*Bacillus* sp. AkNF3 *Pseudomonas putida* KgNF1	*Pseudomonas* sp. PkNF4 *Pseudomonas putida* KgNF1
Phosphate solubilizer	*Achromobacter* sp. AvPS1	*Bacillus megaterium* PkPS1	*Acinetobacter schindleri* AkPS4
K solubilizer	*Microbacterium* sp. MvKS1	*Acinetobacter calcoaceticus* MvKS3	*Microbacterium* sp. MvKS1
Zn solubilizer	*Achromobacter marplatensis* ThZnS2	*Cytobacillus kochii* PkZnS3	*Cytobacillus kochii* PkZnS3

4.10. Evaluation of PGPR based consortia for growth promotion in rice

4.10.1. Pot culture experiment

Three PGPR based consortial formulations were evaluated for plant growth promotion and yield increase in rice under pot culture conditions, as detailed under chapter Materials and Methods. Soil collected from the rice fields at RARS, Ambalavayal was used for the pot culture experiments. The initial microbial population and physico-chemical properties of soil were determined at the start of the experiment (Table 42). According to the results, soil sample was acidic and low in organic carbon content and available potassium. The content of available phosphorus of soil was medium and available zinc was sufficient.

Table 42: Initial microbial population and physico-chemical properties of soil

Initial microbial population		**Physico-chemical properties**		
Micro-organism	**Population cfu g^{-1} of soil**	**Parameter**	**Value recorded**	**Critical limits as per Tandon (2004)**
Bacteria	3.66×10^7	pH	5.1	
Nitrogen fixers	1.66×10^6	EC (dSm^{-1})	0.07	< 1.0
Phosphate solubilizers	3.66×10^5	Organic carbon %	0.47	0.5-0.75
Potassium solubilizers	3.33×10^4	Available phosphorus (kg ha^{-1})	11.04	10-25
Zinc solubilizers	2.66×10^4	Available potassium (kg ha^{-1})	79.03	120-280
Fluorescent pseudomonads	ND	Zinc content (mg kg^{-1})	1.37	0.6

ND: not detected

4.10.1.1. Effect of PGPR isolates on the bacterial population in soil

The population of total bacteria, nitrogen fixers, phosphate solubilizers, potassium solubilizers and zinc solubilizers was enumerated at monthly intervals after transplanting of rice up to fourth month by serial dilution and plating techniques (Tables 43, 44,45,46 and 47).

Population of total bacteria on nutrient agar medium after one month of transplanting up to fourth month are presented in Table 43. In all the treatments which received bio-inoculants, an increased population was observed during the 2nd month of planting, except in T_1, T_7 and T_8. In T_1 and T_8, the highest population was observed at 3 MAP and in T_7 there was no change up to 3 MAP (Table 43). In general, no significant difference in population could be observed at 50 per cent RDF and 75 per cent RDF. Significantly higher population was observed in inoculated treatments, than the uninoculated treatments, except in T_9 at one MAP. In this treatment, the population was statistically on par with other inoculated treatments. The lowest population of bacteria was obtained in T_{12} (control) at all the intervals. The higher population of total bacteria was obseverd in all treatments at all intervals comaperd to the initial population of total bacteria (3.66×10^7 cfu g^{-1} of soil) except T_{10}, T_{11} and T_{12}.

In the case of N-fixing bacteria, the population was significantly higher in treatments which received 50 per cent RDF, than 75 per cent RDF, with the exception of T_5 at one MAP. Higher population of N-fixers was noticed at two MAP in all the treatments, and thereafter gradually decreased. However, in T_4 the highest population was recorded at three MAP (Table 44). At all intervals, the population of N-fixers was significantly lower in uninoculated treatments, as compared to the inoculated ones. Initial nitrogen fixers population was 1.66×10^6 cfu g^{-1} of soil and all treatments which recievd biofertilizer showed higher population of nitrogen fixers at all intervals than initial population.

The population of phosphate solubilizing bacteria are presented in Table 45. Initial population of phosphate solubilizers was 3.66×10^5 cfu g^{-1} of soil. Results showed no significant differences among the treatments at first month after transplanting. However, population of phosphate solubilizers was high at 1 MAP in all the treatments, except T_9 and T_{12} compared to the initial soil population of phosphate solubilizers. After two MAP, on par population of phosphate solubilizers were recorded in treatments T_1 to T_7, T_{10} and T_{11}. At three MAP, population of phosphate solubilizers was significantly higher in treatments which had combined application of inoculum with inorganic fertilizers than uninoculated treatments such as T_9, T_{10}, T_{11} and T_{12}. At fourth month, high population of 33.33×10^4 cfu g^{-1} was recorded in T_6 and on par with

all other treatments which had combined application of bioinoculants and inorganic fertilizers than uninoculated treatments.

Population of potassium solubilizers are detailed in Table 46 and results showed significant differences at every month after transplanting. First month after transplanting, significantly higher population of K solubilizers was recorded in all treatments except T_3 and T_{12}. After two months of transplanting, population of potassium solubilizers was higher in treatments with combined application of inoculum with inorganic fertilizer than uninoculated treatments such as T_9, T_{10}, T_{11} and T_{12}. Higher population of K solubilizers was recorded in treatments T_1, T_2, T_3, T_5, T_6 and T_7 at two MAP than initial population of potassium solubilizers (3.33×10^4 cfu g^{-1} of soil). At third month of transplanting, population of K solubilizing bacteria was significantly higher in T_1, T_2, T_5, T_6, T_7 and T_8. Lowest population of K-solubilizers was recorded in T_{11} and T_{12} (1.00×10^4 cfu g^{-1}). At fourth month, population of K solubilizers was higher in the treatments T_1, T_2, T_4, T_5, T_6, T_7 and T_8, as compared to the other treatments.

Population of zinc solubilizing bacteria was significantly different at all the intervals (Table 47). Higher population of zinc solubilizing bacteria was recorded in T_2, T_3, T_5, T_6, T_7 and T_8 at one month after planting. Lower population was observed in T_4, T_{10}, T_{11} and T_{12}. At two, three and four months after planting significantly higher population of zinc solubilizers was observed in all the treatments which received integrated application of biofertilizer with inorganic fertilizer (both 50 per cent and 75 per cent) than uninoculated treatments. However, all the treatments showed lower population of zinc solubilizers, except T_6 than the initial population of zinc solubilizers (2.66×10^4 cfu g^{-1} of soil).

Table 43: Population of total bacteria in rhizosphere soil during growth period of rice under pot experiment

Treatments	Population of total bacteria (cfu g^{-1} of soil)			
	1 MAP	2 MAP	3 MAP	4 MAP
T_1: Consortium 1 + 50% RDF	4.33×10^7 (7.515) ^{cde}	12.33×10^7 (8.085) ^{abc}	14.66×10^7 (8.161) ^{a}	10.00×10^7 (7.999) ^{ab}
T_2: Consortium 2 + 50% RDF	10.66×10^7 (7.962) ^{abc}	22.00×10^7 (8.327) ^{a}	12.33×10^7 (8.085) ^{ab}	12.66×10^7 (8.097) ^{a}
T_3: Consortium 3 + 50% RDF	9.33×10^7 (7.920) ^{abc}	30.00×10^7 (8.460) ^{a}	10.66×10^7 (8.026) ^{ab}	11.66×10^7 (8.059) ^{a}
T_4: PGPR Mix 1 + 50% RDF	8.00×10^7 (7.793) ^{abcd}	14.00×10^7 (8.134) ^{ab}	12.66×10^7 (8.079) ^{ab}	9.66×10^7 (7.981) ^{ab}
T_5: Consortium 1 + 75% RDF	11.66×10^7 (8.064) ^{a}	26.00×10^7 (8.408) ^{a}	13.00×10^7 (8.103) ^{ab}	9.33×10^7 (7.952) ^{ab}
T_6: Consortium 2 + 75% RDF	6.66×10^7 (7.774) ^{abcd}	22.66×10^7 (8.348) ^{a}	16.00×10^7 (8.191) ^{a}	9.33×10^7 (7.969) ^{ab}
T_7: Consortium 3 + 75% RDF	12.33×10^7 (8.087) ^{a}	12.33×10^7 (8.089) ^{abc}	12.33×10^7 (8.085) ^{ab}	9.33×10^7 (7.968) ^{ab}
T_8: PGPR Mix 1 + 75% RDF	13.00×10^7 (8.101) ^{a}	14.00×10^7 (8.141) ^{ab}	15.33×10^7 (8.168) ^{a}	8.00×10^7 (7.901) ^{ab}
T_9: Organic POP	10.00×10^7 (7.995) ^{ab}	6.66×10^7 (7.816) ^{bcd}	7.33×10^7 (7.859) ^{b}	6.66×10^7 (7.816) ^{b}
T_{10}: 100% RDF	4.00×10^7 (7.560) ^{bcde}	6.00×10^7 (7.700) ^{cd}	7.00×10^7 (7.816) ^{bc}	2.66×10^7 (7.418) ^{c}
T_{11}: POP, KAU	2.66×10^7 (7.333) ^{de}	6.66×10^7 (7.648) ^{d}	7.33×10^7 (7.835) ^{b}	3.66×10^7 (7.534) ^{c}
T_{12}: Control	2.33×10^7 (7.301) ^{e}	4.33×10^7 (7.560) ^{d}	4.66×10^7 (7.534) ^{c}	2.66×10^7 (7.360) ^{c}

MAP: months after planting, Log transformation values are given in parenthesis

Table 44: Population of nitrogen fixing bacteria in rhizosphere soil during growth period of rice under pot experiment

Treatments	Population of nitrogen fixing bacteria (cfu g^{-1} of soil)			
	1 MAP	2 MAP	3 MAP	4 MAP
T_1: Consortium 1 + 50% RDF	5.00×10^6 (6.693) ^{abcd}	21.00×10^6 (7.320) ^{ab}	7.33×10^6 (6.835) ^{bcd}	6.33×10^6 (6.749) ^{bc}
T_2: Consortium 2 + 50% RDF	13.00×10^6 (6.979) ^{ab}	21.66×10^6 (7.323) ^{ab}	12.33×10^6 (7.085) ^{ab}	12.00×10^6 (7.065) ^{a}
T_3: Consortium 3 + 50% RDF	12.33×10^6 (6.916) ^{abc}	16.66×10^6 (7.220) ^{ab}	11.33×10^6 (6.984) ^{abc}	6.66×10^6 (6.803) ^{bc}
T_4: PGPR Mix 1 + 50% RDF	13.33×10^6 (7.100) ^{a}	7.66×10^6 (6.800) ^{c}	13.66×10^6 (7.023) ^{ab}	5.33×10^6 (6.725) ^{bc}
T_5: Consortium 1 + 75% RDF	5.00×10^6 (6.693) ^{abcd}	19.66×10^6 (7.256) ^{ab}	6.66×10^6 (6.774) ^{bcde}	6.33×10^6 (6.800) ^{bc}
T_6: Consortium 2 + 75% RDF	2.33×10^6 (6.360) ^{de}	27.00×10^6 (7.431) ^{a}	22.33×10^6 (7.327) ^{a}	13.66×10^6 (7.134) ^{a}
T_7: Consortium 3 + 75% RDF	4.33×10^6 (6.583) ^{bcde}	17.66×10^6 (7.212) ^{ab}	12.00×10^6 (7.027) ^{ab}	8.00×10^6 (6.901) ^{ab}
T_8: PGPR Mix 1 + 75% RDF	3.00×10^6 (6.460) ^{cde}	14.00×10^6 (7.136) ^{b}	4.33×10^6 (6.625) ^{cdef}	4.33×10^6 (6.634) ^{cd}
T_9: Organic POP	5.00×10^6 (6.548) ^{bcde}	12.33×10^6 (7.077) ^{bc}	3.33×10^6 (6.519) ^{def}	2.66×10^6 (6.360) ^{de}
T_{10}: 100% RDF	2.00×10^6 (6.259) ^{de}	13.66×10^6 (7.126) ^{b}	2.66×10^6 (6.401) ^{f}	2.66×10^6 (6.418) ^{de}
T_{11}: POP, KAU	1.66×10^6 (6.201) ^{e}	11.33×10^6 (7.049) ^{bc}	3.00×10^6 (6.460) ^{ef}	3.00×10^6 (6.460) ^{ef}
T_{12}: Control	2.00×10^6 (6.201) ^{e}	7.00×10^6 (6.793) ^{c}	2.66×10^6 (6.418) ^{ef}	1.33×10^6 (6.100) ^{f}

MAP: months after planting, Log transformation values are given in parenthesis

Table 45: Population of phosphate solubilizing bacteria in rhizosphere soil during growth period of rice under pot experiment

Treatments	Population of phosphate solubilizing bacteria (cfu g^{-1} of soil)			
	1 MAP	2 MAP	3 MAP	4 MAP
T_1: Consortium 1 + 50% RDF	50.00×10^4 (5.634)	43.33×10^4 (5.600) ^{abc}	56.66×10^4 (5.719) ^{a}	13.33×10^4 (5.100) ^{ab}
T_2: Consortium 2 + 50% RDF	60.00×10^4 (5.752)	50.00×10^4 (5.693) ^{ab}	33.33×10^4 (5.502) ^{a}	16.66×10^4 (5.201) ^{ab}
T_3: Consortium 3 + 50% RDF	46.66×10^4 (5.619)	56.66×10^4 (5.752) ^{a}	30.00×10^4 (5.401) ^{a}	13.33×10^4 (5.100) ^{ab}
T_4: PGPR Mix 1 + 50% RDF	40.00×10^4 (5.541)	43.33×10^4 (5.583) ^{abc}	33.33×10^4 (5.434) ^{a}	10.00×10^4 (5.000) ^{ab}
T_5: Consortium 1 + 75% RDF	56.66×10^4 (5.710)	56.66×10^4 (5.752) ^{a}	30.00×10^4 (5.401) ^{a}	23.33×10^4 (5.301) ^{ab}
T_6: Consortium 2 + 75% RDF	60.00×10^4 (5.761)	33.33×10^4 (5.519) ^{abc}	30.00×10^4 (5.434) ^{a}	33.33×10^4 (5.434) ^{a}
T_7: Consortium 3 + 75% RDF	60.00×10^4 (5.774)	40.00×10^4 (5.560) ^{abc}	26.66×10^4 (5.333) ^{a}	26.66×10^4 (5.360) ^{ab}
T_8: PGPR Mix 1 + 75% RDF	46.66×10^4 (5.641)	23.33×10^4 (5.301) ^{cd}	40.00×10^4 (5.482) ^{a}	26.66×10^4 (5.418) ^{ab}
T_9: Organic POP	36.66×10^4 (5.551)	13.33×10^4 (5.100) ^{d}	3.33×10^4 (4.434) ^{b}	2.33×10^4 (4.301) ^{c}
T_{10}: 100% RDF	43.33×10^4 (5.625)	30.00×10^4 (5.460) ^{abc}	2.00×10^4 (4.259) ^{b}	3.00×10^4 (4.460) ^{c}
T_{11}: POP, KAU	43.33×10^4 (5.634)	40.00×10^4 (5.593) ^{abc}	3.00×10^4 (4.434) ^{b}	2.66×10^4 (4.333) ^{c}
T_{12}: Control	23.33×10^4 (5.301)	30.00×10^4 (5.360) ^{bcd}	1.33×10^4 (4.100) ^{b}	1.66×10^4 (4.159) ^{c}

MAP: months after planting, Log transformation values are given in parenthesis

Table 46: Population of potassium solubilizing bacteria in rhizosphere soil during growth period of rice under pot experiment

Treatments	Population of potassium solubilizing bacteria (cfu g^{-1} of soil)			
	1 MAP	2 MAP	3 MAP	4 MAP
T_1: Consortium 1 + 50% RDF	3.33×10^4 (4.519) ^{abc}	3.66×10^4 (4.560) ^{abc}	3.33×10^4 (4.519) ^{abcd}	2.33×10^4 (4.360) ^{a}
T_2: Consortium 2 + 50% RDF	4.00×10^4 (4.593) ^{ab}	4.66×10^4 (4.667) ^{abc}	3.66×10^4 (4.534) ^{abcd}	1.66×10^4 (4.201) ^{abc}
T_3: Consortium 3 + 50% RDF	2.00×10^4 (4.259) ^{cd}	4.33×10^4 (4.619) ^{abc}	3.33×10^4 (4.502) ^{bcd}	1.33×10^4 (4.100) ^{bc}
T_4: PGPR Mix 1 + 50% RDF	2.66×10^4 (4.418) ^{abc}	3.33×10^4 (4.519) ^{bc}	3.00×10^4 (4.401) ^{d}	2.00×10^4 (4.259) ^{ab}
T_5: Consortium 1 + 75% RDF	2.33×10^4 (4.360) ^{abcd}	4.00×10^4 (4.593) ^{abc}	5.00×10^4 (4.693) ^{abc}	2.33×10^4 (4.360) ^{a}
T_6: Consortium 2 + 75% RDF	4.66×10^4 (4.641) ^{a}	5.66×10^4 (4.752) ^{a}	6.33×10^4 (4.800) ^{a}	2.66×10^4 (4.401) ^{a}
T_7: Consortium 3 + 75% RDF	4.00×10^4 (4.593) ^{ab}	4.00×10^4 (4.560) ^{ab}	6.00×10^4 (4.767) ^{ab}	2.00×10^4 (4.301) ^{ab}
T_8: PGPR Mix 1 + 75% RDF	3.66×10^4 (4.534) ^{abc}	3.00×10^4 (4.693) ^{abc}	6.00×10^4 (4.761) ^{ab}	2.66×10^4 (4.418) ^{a}
T_9: Organic POP	2.66×10^4 (4.333) ^{bcd}	3.00×10^4 (4.460) ^{c}	3.00×10^4 (4.460) ^{cd}	1.33×10^4 (4.100) ^{bc}
T_{10}: 100% RDF	3.00×10^4 (4.460) ^{abc}	1.66×10^4 (4.201) ^{d}	3.00×10^4 (4.460) ^{cd}	1.00×10^4 (4.000) ^{c}
T_{11}: POP, KAU	3.33 (4.502) ^{abc}	1.66×10^4 (4.201) ^{d}	1.33×10^4 (4.100) ^{e}	1.00×10^4 (4.000) ^{c}
T_{12}: Control	1.33×10^4 (4.100) ^{d}	1.00×10^4 (4.000) ^{d}	1.33×10^4 (4.100) ^{e}	1.00×10^4 (4.000) ^{c}

MAP: months after planting, Log transformation values are given in parenthesis

Table 47: Population of zinc solubilizing bacteria in rhizosphere soil during growth period of rice under pot experiment

Treatments	Population of zinc solubilizing bacteria (cfu g^{-1} of soil)			
	1 MAP	2 MAP	3 MAP	4 MAP
T_1: Consortium 1 + 50% RDF	13.33×10^3 (4.100) ^{bcd}	16.66×10^3 (4.159) ^{ab}	13.33×10^3 (4.100) ^{ab}	$13.3 \times 10^3 3$ (4.100) ^{a}
T_2: Consortium 2 + 50% RDF	26.66×10^3 (4.418) ^{a}	23.33×10^3 (4.360) ^{a}	20.00×10^3 (4.259) ^{a}	13.33×10^3 (4.100) ^{a}
T_3: Consortium 3 + 50% RDF	16.66×10^3 (4.201) ^{abc}	20.00×10^3 (4.259) ^{a}	13.33×10^3 (4.100) ^{ab}	10.00×10^3 (4.000) ^{ab}
T_4: PGPR Mix 1 + 50% RDF	10.00×10^3 (4.000) ^{cde}	23.33×10^3 (4.360) ^{a}	16.66×10^3 (4.201) ^{ab}	16.66×10^3 (4.201) ^{a}
T_5: Consortium 1 + 75% RDF	16.66×10^3 (4.201) ^{abc}	23.33×10^3 (4.360) ^{a}	20.00×10^3 (4.259) ^{a}	16.66×10^3 (4.201) ^{a}
T_6: Consortium 2 + 75% RDF	26.66×10^3 (4.418) ^{a}	26.66×10^3 (4.401) ^{a}	23.33×10^3 (4.360) ^{a}	16.66×10^3 (4.201) ^{a}
T_7: Consortium 3 + 75% RDF	23.33×10^3 (4.301) ^{ab}	23.33×10^3 (4.360) ^{a}	13.33×10^3 (4.100) ^{ab}	13.33×10^3 (4.100) ^{a}
T_8: PGPR Mix 1 + 75% RDF	20.00×10^3 (4.259) ^{abc}	23.33×10^3 (4.301) ^{a}	16.66×10^3 (4.201) ^{ab}	13.33×10^3 (4.100) ^{a}
T_9: Organic POP	13.33×10^3 (4.100) ^{bcd}	8.33×10^3 (3.900) ^{bc}	9.33×10^3 (3.968) ^{bc}	6.66×10^3 (3.799) ^{bc}
T_{10}: 100% RDF	5.66×10^3 (3.735) ^{e}	5.00×10^3 (3.699) ^{c}	6.66×10^3 (3.799) ^{c}	5.00×10^3 (3.699) ^{c}
T_{11}: POP, KAU	7.66×10^3 (3.884) ^{de}	6.66×10^3 (3.799) ^{c}	6.00×10^3 (3.671) ^{c}	5.00×10^3 (3.699) ^{c}
T_{12}: Control	6.33×10^3 (3.784) ^{e}	5.00×10^3 (3.699) ^{c}	5.00×10^3 (3.699) ^{c}	5.00×10^3 (3.699) ^{c}

MAP: months after planting, Log transformation values are given in parenthesis

4.10.1.2 Effect of PGPR isolates on growth and yield of rice under pot culture conditions

All attributes of growth and yield of rice were recorded. These included growth parameters like plant height, number of tillers per pot and number of leaves per tiller and yield parameters such as number of panicles /pots, number of grains/ panicles, 1000 grains weight and grain yield/ pot. Results are presented in Table 48.

4.10.1.2.1. Plant height

Plant height at maximum tillering stage was not significantly different among the treatments and the plant height ranged from 64.33 to 89.00 cm.

4.10.1.2.2. Number of tillers per pot and leaves per tiller

Number of tillers per pot at maximum tillering stage was significantly different in different treatments and ranged from 6.00 to 13.00 (Table 48). Significantly higher number of tillers per pot was recorded in treatments T_{11}, T_6, T_1, T_2, T_3, T_5, T_7 and T_{10}, all these treatments being on par. Number of tillers per pot was significantly lower in treatments T_{12} (control), T_8 and T_9. There was no significant difference in the number of leaves per tiller among the treatments.

4.10.1.2.3. Number of panicles per pot

Number of panicles per pot was recorded at harvesting stage and the data are presented in Table 48. Number of panicles per pot was significantly different among the treatments. Number of panicles was significantly higher in plants treated with T_{11} (8.3), T_2 (6.60), T_5 (6.60), T_6 (8.00) and T_7 (7.00). Treatment T_{12} (control) and T_4 (PGPR Mix-1 + 75% RDF) recorded significantly lower number of panicles (3.30 and 3.60 per pot) respectively.

4.10.1.2.4. Number of grains per panicle and per cent filled grains

Number of grains per panicle was significantly different in different treatments and it ranged from 80.00 (in treatment T_{12}) to 99.33 (in T_{11}). It was generally higher in

treatments which received bio-inoculants, at 75 per cent RDF, as compared to 50 per cent RDF of the respective treatments.

Per cent filled grains was significantly different in different treatments and it ranged from 85.12 (recorded in T_{12}) to 92.30 (in T_{11}). Plants treated with T_{11} (PoP, KAU) was recorded 92.30 per cent filled grains and which was on par with treatments which received integrated application of biofertilizer with inorganic fertilizer except T_1. Significantly lower per cent filled grains were observed in treatments T_{12}, T_1, T_3 and T_9.

4.10.1.2.5. Grain and straw yield

Grain yield per pot ranged from 12.71 g (observed in treatment T_{12}) to 19.05 g (in T_{11}). Significantly higher grain yield was recorded in treatments T_{11} (19.05 g per pot), T_{10} (18.97 g) and T_6 (16.47 g), as compared to other treatments.

There was no significant difference in straw yield per pot, among the treatments.

Table 48: Effect of PGPR on the growth and yield of rice under pot culture

Treatments	Plant height (cm)	No. of tillers per pot	No. of leaves per tiller	No. of panicles per pot	No. of grains per panicle	Filled grain (%)	Grain yield per pot (g)	Straw yield per pot (g)
T_1: Consortium 1 + 50% RDF	78.66	11.00 ^{abc}	5.33	6.00 ^{c}	83.00 ^{cd}	87.93 ^{bc}	13.88 ^{bc}	17.58
T_2: Consortium 2 + 50% RDF	78.33	11.66 ^{ab}	5.00	6.60^{abc}	86.00 ^{bcd}	89.53 ^{ab}	14.61 ^{bc}	18.04
T_3: Consortium 3 + 50% RDF	76.00	10.33 ^{abc}	5.33	6.30 ^{bc}	83.33 ^{cd}	89.01 ^{abc}	14.18 ^{bc}	15.15
T_4: PGPR Mix 1 + 50% RDF	82.66	8.33 ^{cd}	5.16	3.60^{d}	84.33 ^{bcd}	89.13 ^{ab}	14.40 ^{bc}	13.28
T_5: Consortium 1 + 75% RDF	86.33	12.33 ^{ab}	5.83	6.60^{abc}	96.00 ^{ab}	90.24 ^{ab}	14.44 ^{bc}	18.85
T_6: Consortium 2 + 75% RDF	87.66	13.00 ^{a}	6.33	8.00 ^{ab}	99.00 ^{a}	92.27 ^{ab}	16.47 ^{ab}	19.13
T_7: Consortium 3 + 75% RDF	81.66	12.66 ^{ab}	5.83	7.00 ^{abc}	96.66 ^{ab}	91.58 ^{ab}	15.09 ^{bc}	16.62
T_8: PGPR Mix 1 + 75% RDF	83.00	9.66 ^{bcd}	5.66	5.60 ^{c}	93.00 ^{abc}	91.61 ^{ab}	14.74 ^{bc}	15.47
T_9: Organic POP	71.66	9.66 ^{bcd}	3.16	6.30 ^{bc}	89.33 ^{abcd}	88.04 ^{bc}	16.18 ^{b}	16.02
T_{10}: 100% RDF	80.66	12.00 ^{ab}	5.66	6.30 ^{bc}	95.00 ^{abc}	91.53 ^{ab}	18.97 ^{a}	14.45
T_{11}: POP, KAU	89.00	13.00 ^{a}	6.50	8.3 ^{a}	99.33 ^{a}	92.30 ^{a}	19.05 ^{a}	23.70
T_{12}: Control	64.33	6.00 ^{d}	4.80	3.3 ^{d}	80.00 ^{d}	85.12 ^{c}	12.710 ^{c}	15.08

Consortium 1: *Bacillus* sp. AkNF3, *Pseudomonas* sp. PkNF4, *Achromobacter* sp. AvPS1, *Microbacterium* sp. MvKS1, *Achromobacter marplatensis* ThZnS2

Consortium 2: *Bacillus sp.* AkNF3, *Pseudomonas putida* KgNF1, *Bacillus megaterium* PkPS1, *Acinetobacter calcoaceticus* MvKS3, *Cytobacillus kochii* kZnS3

Consortium 3: *Pseudomonas* sp. PkNF4, *Pseudomonas putida* KgNF1, *Acinetobacter schindleri* AkPS4, *Microbacterium* sp. MvKS1, *Cytobacillus kochii* PkZnS3

4.10.1.3. Effect of PGPR application on plant nutrient content

The content of nutrients including nitrogen, phosphorus, potassium and zinc in plants, at panicle initiation stage of the crop is presented in Table 49.

4.10.1.3.1. Nitrogen content of plant

Nitrogen content in plant samples varied significantly among the treatments and it ranged from 0.525 to 1.342 per cent. Nitrogen content of T_{11} was recorded as 1.342 per cent which was statistically on par with T_6 and T_7. N content was low in T_{12} (0.525 per cent), and this was on par with T_3, T_4, T_8 and T_9.

4.10.1.3.2. Phosphorus content of plant

The content of phosphorus in plants also differed significantly among the treatments and it ranged between 0.269 per cent to 0.462 per cent. The highest P content of 0.462 per cent was observed in T_{11} and this was significantly superior to all other treatments. This was followed by T_6, which recorded a P content of 0.398 per cent, and this was statistically on par with treatments T_3, T_5, T_7, T_8 and T_{10}.

4.10.1.3.3. Potassium content of plant

Potassium content of the plant samples differed significantly with different treatments. The potassium content was higher and statistically on par with treatments in T_2, T_3, T_5, T_6, T_7, T_8, T_{10} and T_{11}. Significantly lower content was noticed in the treatments T_1, T_4, T_9 and T_{12}, which recorded 1.133, 1.173, 1.177 and 0.857 per cent K in the plant tissue respectively.

4.6.1.3.4. Zinc content of plant

Zinc content of plant samples was significantly different among the treatments. The content of Zn varied from 0.105 ppm (T_{12}) to 0.195 ppm (recorded in T_3 and T_6). Significantly higher Zn content was observed in treatments T_1, T_2, T_3, T_5, T_6, T_7, T_8 and T_9 as compared to the other treatments (Table 49).

Table 49: Effect of PGPR application on nutrient content in plant tissue

Treatments	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Zinc (ppm)
T_1: Consortium 1 + 50% RDF	1.050 ^{bc}	0.333 ^{de}	1.133 ^{bc}	0.156 ^{ab}
T_2: Consortium 2 + 50% RDF	0.992 ^{bcd}	0.308 ^{def}	1.480 ^{ab}	0.162 ^{ab}
T_3: Consortium 3 + 50% RDF	0.642 ^{ef}	0.359 ^{bcd}	1.260 ^{abc}	0.195 ^{a}
T_4: PGPR Mix 1 + 50% RDF	0.700 ^{ef}	0.340 ^{cde}	1.173 ^{bc}	0.139 ^{bc}
T_5: Consortium 1 + 75% RDF	0.992 ^{bcd}	0.352 ^{bcde}	1.350 ^{ab}	0.155 ^{ab}
T_6: Consortium 2 + 75% RDF	1.108 ^{ab}	0.398 ^{b}	1.677 ^{a}	0.195 ^{a}
T_7: Consortium 3 + 75% RDF	1.167 ^{ab}	0.391 ^{bc}	1.690 ^{a}	0.160 ^{ab}
T_8: PGPR Mix 1 + 75% RDF	0.758 ^{def}	0.352 ^{bcde}	1.330 ^{ab}	0.169 ^{ab}
T_9: Organic POP	0.700 ^{ef}	0.301 ^{ef}	1.177 ^{bc}	0.156 ^{ab}
T_{10}: 100% RDF	0.817 ^{cde}	0.346 ^{bcde}	1.507 ^{ab}	0.132 ^{bc}
T_{11}: POP, KAU	1.342 ^{a}	0.462 ^{a}	1.667 ^{a}	0.143 ^{bc}
T_{12}: Control	0.525 ^{f}	0.269 ^{f}	0.857 ^{c}	0.105 ^{c}

Consortium 1: *Bacillus* sp. AkNF3, *Pseudomonas* sp. PkNF4, *Achromobacter* sp. AvPS1, *Microbacterium* sp. MvKS1, *Achromobacter marplatensis* ThZnS2
Consortium 2: *Bacillus sp.* AkNF3, *Pseudomonas putida* KgNF1, *Bacillus megaterium* PkPS1, *Acinetobacter calcoaceticus* MvKS3, *Cytobacillus kochii* PkZnS3
Consortium 3: *Pseudomonas* sp. PkNF4, *Pseudomonas putida* KgNF1, *Acinetobacter schindleri* AkPS4, *Microbacterium* sp. MvKS1, *Cytobacillus kochii* PkZnS3

4.10.1.4. Effect of PGPR on physico-chemical parameters of soil at harvesting of the rice crop

Physical parameters like pH and EC and chemical parameters like nutrient status of the soil at harvest of the crop were assessed and the data are presented in Table 50.

4.10.1.4.1. Soil pH

Soil pH ranged between 6.73 to 7.68 and was not significantly different among the treatments.

4.10.1.4.2. Soil EC

Electrical conductivity ranged between 0.23 dS m^{-1} to 0.57 dS m^{-1} and different treatments recorded significantly different values. The highest EC was recorded in the treatment T_{10}, and this was significantly higher than all other treatments. However, the EC values recorded in all the samples were less than 1.00.

4.10.1.4.3. Soil organic carbon content

Soil organic carbon content ranged between 0.362 per cent to 0.812 per cent. Significantly higher soil organic carbon was recorded in the treatments T_2 (0.812 per cent), T_6 (0.756 per cent), T_7 (0.759 per cent) and T_9 (0.800), when compared to other treatments.

4.10.1.4.4. Soil microbial biomass carbon

Soil microbial biomass carbon was significantly different among the treatments and varied from 68.00 to 144.00 µg g^{-1} (Table 50). Significantly higher values of microbial biomass carbon were recorded in treatments T_6 (144.0 µg g^{-1}) and T_7 (120 µg g^{-1}).

4.10.1.4.5. Soil nitrogen content

Soil nitrogen content at harvesting was significantly different among the treatments and ranged from 83.62 kg per ha (in T_{12}) to 171.43 kg per ha (observed in T_6). The lowest soil nitrogen content was recorded in the treatment T_{12}, which was significantly lower to all other treatments.

4.10.1.4.6. Available soil phosphorus content after harvesting

Available phosphorus in soil was significantly different among different treatments and the values ranged from 4.04 kg per ha (observed in T_{12}) to 14.20 kg per ha (recorded in T_{11}). Significantly higher available soil P was observed in T_{11} (14.20 kg per ha), T_4 (12.22 kg per ha), T_6 (10.90 kg per ha), T_8 (13.21 kg per ha) and T_{10} (10.93 kg per ha).

4.10.1.4.7. Soil potassium content

Soil potassium content was significantly different among different treatments and the values ranged from 29.88 kg per ha (in treatment T_{12}) to 78.40 kg per ha (in T_{11}). The content of available potassium was significantly higher in treatments T_6 (66.82 kg per ha), T_7 (67.20 kg per ha), T_{10} (56.37 kg per ha) and T_{11} (78.40 kg per ha).

4.10.1.4.8. Soil zinc content

Available zinc in soil at harvest of the crop was found to vary significantly among treatments. The treatments T_6 and T_7 recorded significantly higher content of zinc (3.26 and 3.17 mg per kg respectively). The lowest zinc content was observed in T_{12}, which recorded 1.81 mg per kg and this was significantly lower than all other treatments.

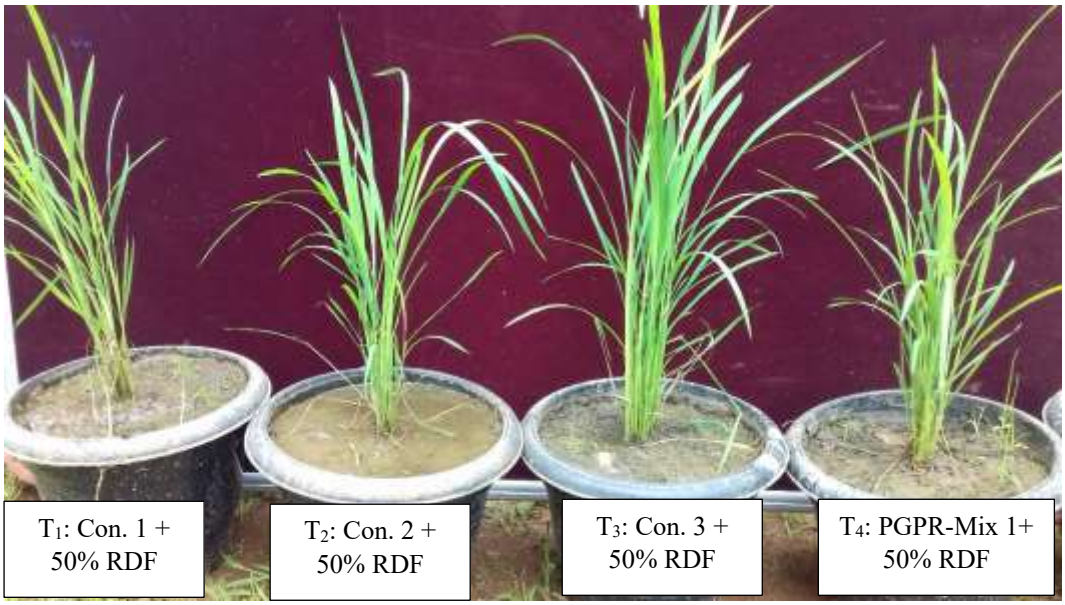


Fig. a. Biofertilizer with 50% recommended dosage of fertilizer applied treatments

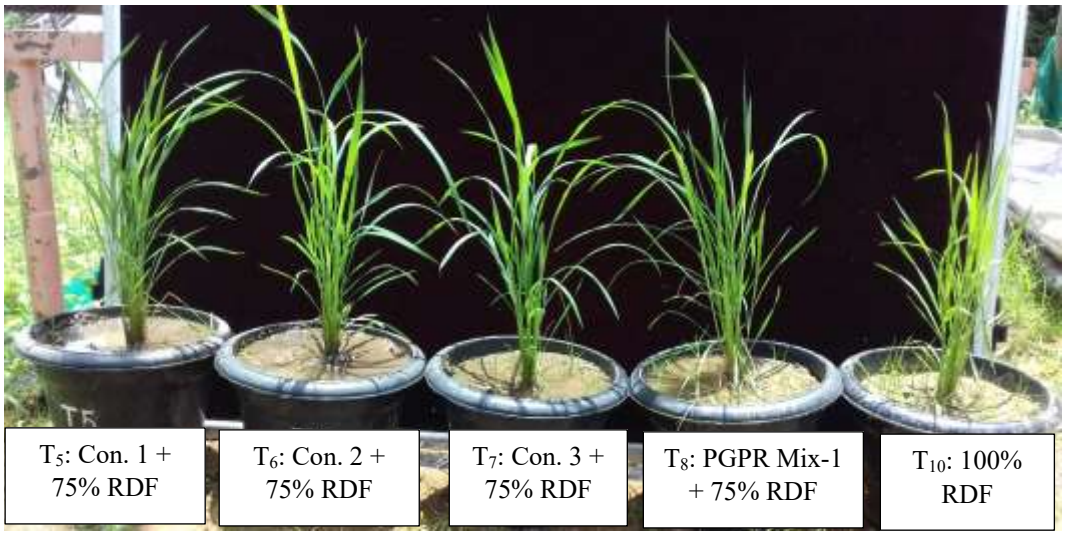


Fig. b. Biofertilizer with 75% recommended dosage of fertilizer and 100% RDF fertilizer applied treatments

Plate 39: Rice plants in pot culture, one month after planting

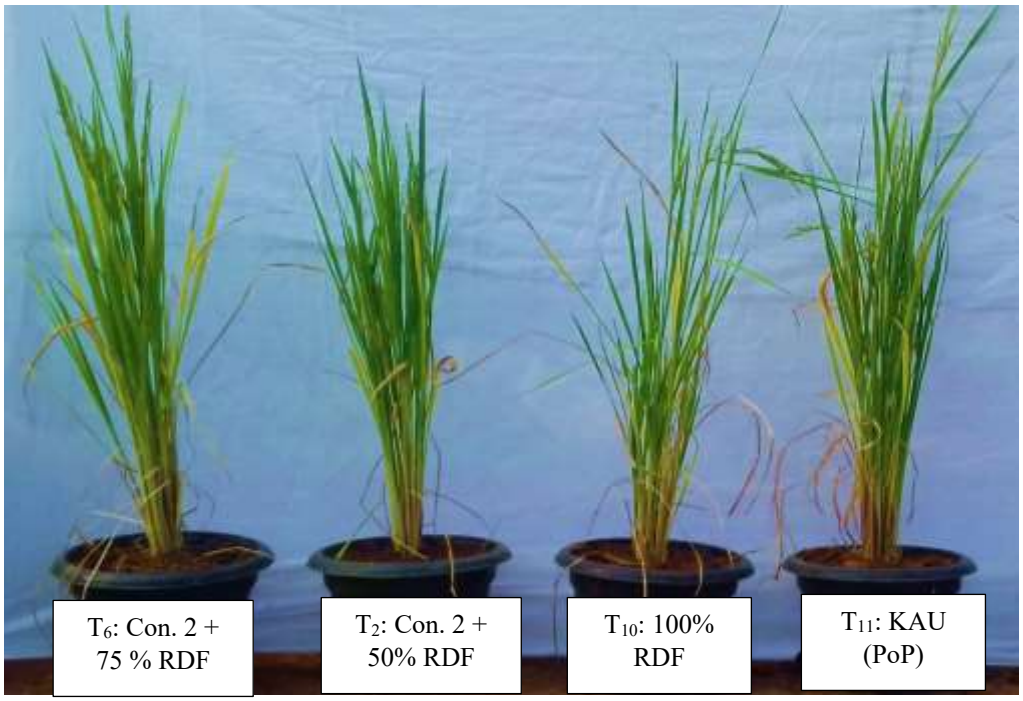


Fig. a. Comparison of consortium 2 applied treatments with 100% RDF applied treatments

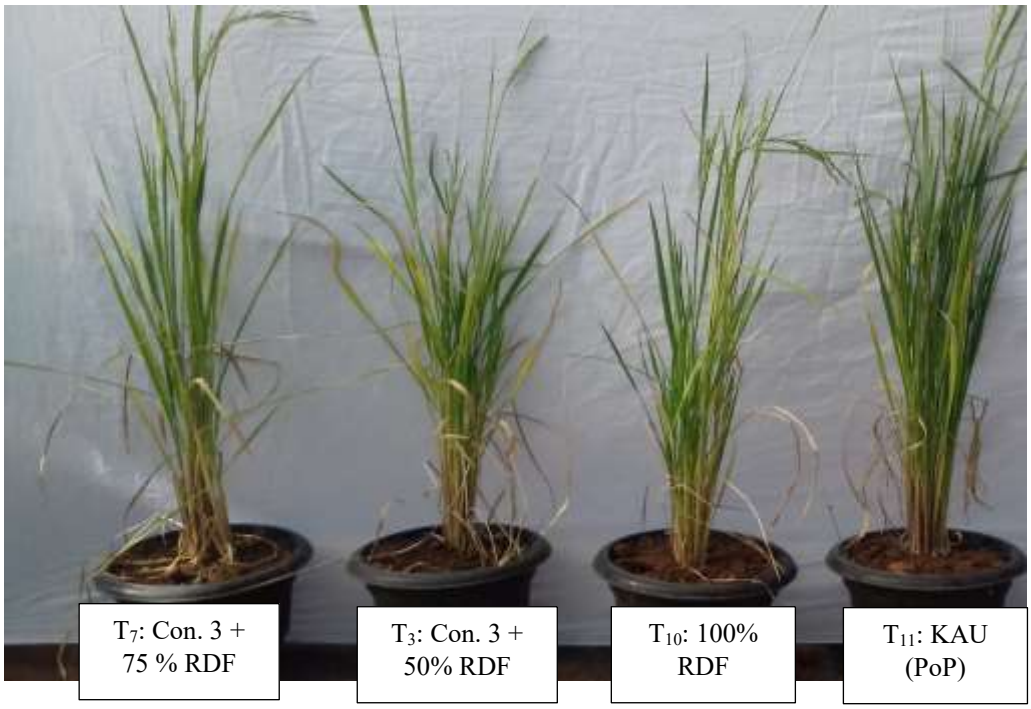


Fig. b. Comparison of consortium 3 applied treatments with 100% RDF applied treatments

Plate 40: Selected consortia for field evaluation

Table 50: Effect of different bioinoculants and chemical fertilizer on nutrient contents of soil after harvesting

Treatments	Soil pH	Soil EC (dS m^{-1})	Soil organic C %	Microbial biomass C (µg g^{-1})	Nitrogen (kg ha^{-1})	Phosphorus (kg ha^{-1})	Potassium (kg ha^{-1})	Zinc (mg kg^{-1})
T_1: Consortium 1 + 50% RDF	7.18	0.36 ^{bcd}	0.671^{bc}	88.00 ^{bcd}	133.80 ^{cd}	4.46 ^{de}	38.82 ^{c}	2.54 ^{cd}
T_2: Consortium 2 + 50% RDF	7.68	0.23 ^{e}	0.812^{a}	108.00 ^{bc}	146.34 ^{abcd}	7.01 ^{bcde}	39.20 ^{c}	2.37 ^{d}
T_3: Consortium 3 + 50% RDF	7.24	0.25 ^{e}	0.656^{c}	96.00 ^{bcd}	121.25 ^{de}	4.94 ^{de}	40.32 ^{bc}	2.47 ^{cd}
T_4: PGPR Mix 1 + 50% RDF	7.05	0.39 ^{bc}	0.630^{c}	96.00 ^{bcd}	96.16 ^{ef}	12.22 ^{abc}	32.48 ^{c}	2.36 ^{d}
T_5: Consortium 1 + 75% RDF	6.79	0.42 ^{b}	0.656^{c}	100.00 ^{bcd}	137.98 ^{bcd}	4.73 ^{de}	49.28 ^{bc}	2.33 ^{d}
T_6: Consortium 2 + 75% RDF	7.08	0.38 ^{bc}	0.756^{ab}	144.00 ^{a}	171.43 ^{a}	10.90 ^{abcd}	66.82 ^{ab}	3.26 ^{a}
T_7: Consortium 3 + 75% RDF	6.78	0.26 ^{de}	0.759^{ab}	120.00 ^{ab}	167.24 ^{ab}	6.11 ^{cde}	67.20 ^{ab}	3.17 ^{ab}
T_8: PGPR Mix 1 + 75% RDF	7.22	0.34^{bcde}	0.652^{c}	96.00 ^{bcd}	121.23 ^{de}	13.21 ^{ab}	37.70 ^{c}	2.83 ^{bc}
T_9: Organic POP	6.92	0.40 ^{bc}	0.800^{a}	100.00 ^{bcd}	117.07 ^{de}	4.68 ^{de}	39.64 ^{c}	2.36 ^{d}
T_{10}: 100% RDF	6.90	0.57 ^{a}	0.411^{d}	84.00 ^{cd}	125.43 ^{de}	10.93 ^{abcd}	56.37 ^{abc}	2.31 ^{d}
T_{11}: POP, KAU	7.10	0.31^{bcde}	0.357^{d}	76.00 ^{cd}	163.06 ^{abc}	14.20 ^{a}	78.40 ^{a}	2.41 ^{cd}
T_{12}: Control	6.73	0.29 ^{cde}	0.362^{d}	68.00 ^{d}	83.62 ^{f}	4.04 ^{e}	29.88 ^{c}	1.81 ^{e}

Consortium 1: *Bacillus* sp. AkNF3, *Pseudomonas* sp. PkNF4, *Achromobacter* sp. AvPS1, *Microbacterium* sp. MvKS1, *Achromobacter marplatensis* ThZnS2

Consortium 2: *Bacillus sp.* AkNF3, *Pseudomonas putida* KgNF1, *Bacillus megaterium* PkPS1, *Acinetobacter calcoaceticus* MvKS3, *Cytobacillus kochii* kZnS3

Consortium 3: *Pseudomonas* sp. PkNF4, *Pseudomonas putida* KgNF1, *Acinetobacter schindleri* AkPS4, *Microbacterium* sp. MvKS1, *Cytobacillus kochii* PkZnS3

4.10.2. Field evaluation of most promising consortia on rice growth and yield

Two best PGPR consortial (Consortium 2 and Consortium 3) were selected for further evaluation on rice growth and yield under field conditions at RARS, Ambalavayal, as detailed in Materials and Methods. Consortium 2, Consortium 3 and PGPR Mix-1 with 75% recommended dosage of fertilizer (RDF) were evaluated along with 100% RDF and farmer's practice. Farmer's practice consisted of organic manure (farm yard manure) at the rate of 5 t ha^{-1} (organic POP, KAU).

The initial microbial population and physico-chemical properties are depicted in Table 51. The soil was slightly acidic with a pH of 5.5. Soil organic matter content (0.34%), available phosphorus (4.61 kg ha^{-1}) and potassium content (96.21 kg ha^{-1}) were low. Available zinc content (1.09 mg kg^{-1}) was high in soil sample.

Weather data such as maximum and minimum temperature, rainfall and relative humidity (morning and evening) were recorded during rice crop in the field from July 2019 to December 2019. Weather data are presented as weekly average throughout the above-mentioned time period in Fig. 2, 3 and 4.

Highest maximum temperature (27.8 ^{0}C) was recorded in 5th week during the mentioned period and lowest maximum (23.6 ^{0}C) temperature was recorded during 6th week. Minimum temperature was recorded in third week of December. Highest weekly average rainfall received during 6th week of above-mentioned period and it was (102.22 mm). Lowest minimum temperature was recorded in December last week and it was 16.12 ^{0}C. From mid-November to December, recorded evening relative humidity was lower than other months.

There was no remarkable fluctuation of factors such as temperature (maximum and minimum) and relative humidity (morning and evening). However, it was noticed that highest rainfall (102.22 mm weekly average) received during the sixth week of crop growth period and all other weeks received less than 25 mm weekly average rainfall.

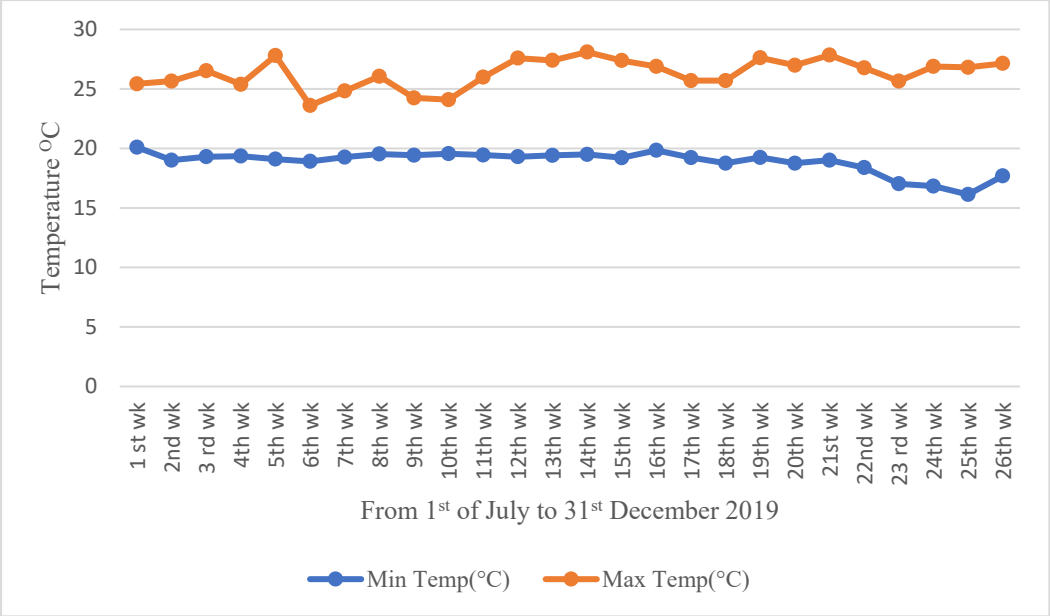


Fig. 2: Average maximum and minimum temperature (weekly) from 1st of July 2019 to 31st of December 2019

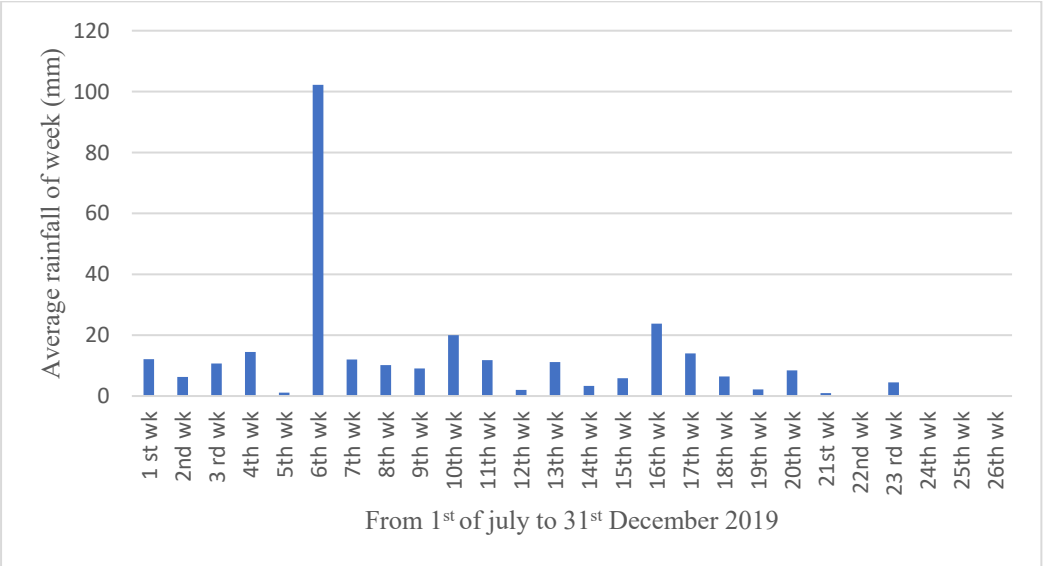


Fig. 3: Average rainfall (weekly) from 1st of July 2019 to 31st of December 2019

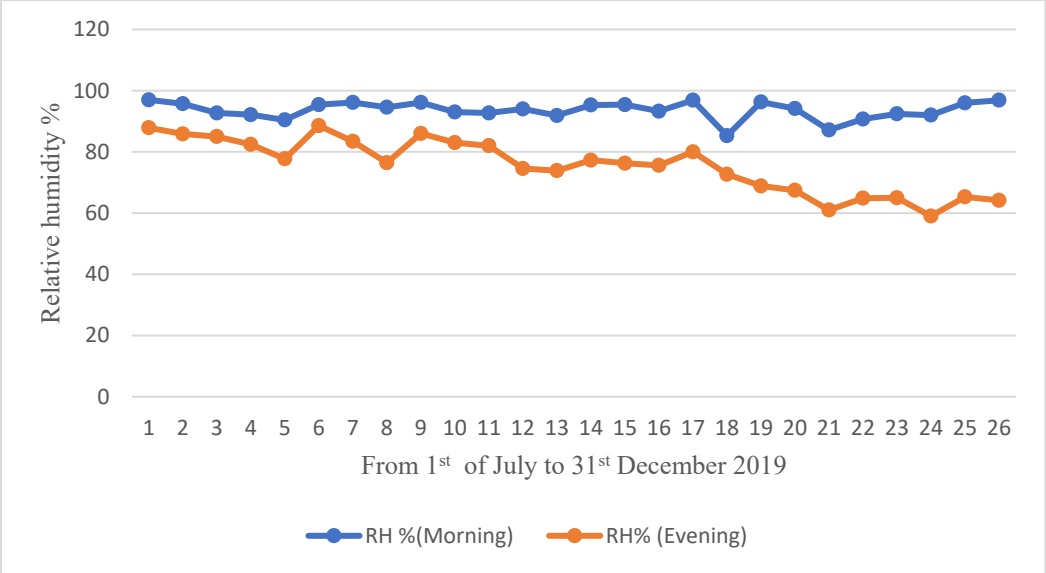


Fig. 4: Average relative humidity of week from 1st of July 2019 to 31st of December 2019

Table 51: Initial microbial population, physico-chemical properties of soil

Initial microbial population		**Physico-chemical properties**		
Micro-organism	**Population cfu g $^{-1}$ soil**	**Parameter**	**Value recorded**	**Critical limits as per Tandon (2004)**
Bacteria	4.50 x 10^7	pH	5.5	
N-fixers	8.00 x10^6	EC (dSm^{-1})	0.03	< 1.0
Phosphate solubilizers	1.50 x 10^5	Organic carbon (%)	0.34	0.5-0.75
Potassium solubilizers	4.50 x 10^4	Available phosphorus (kg ha^{-1})	4.61	10-25
Zinc solubilizers	1.50 x 10^4	Available potassium (kg ha^{-1})	96.21	120-280
Fluorescent pseudomonads	ND	Zinc content (mg kg^{-1})	1.09	0.6

ND: not detected

Population of total bacteria, nitrogen fixers, P solubilizers, K solubilizers and Zn solubilizers in the soil were enumerated at monthly interval up to four months. Rice plants growth and yield, nutrient content of plants at flowering stage were recorded. Nutrient content of soil was determined at harvesting stage.

4.10.2.1. Effect of PGPR isolates on microbial population in soil

The population of total bacteria, nitrogen fixers, phosphate solubilizers, potassium solubilizers and zinc solubilizers were assessed at monthly intervals after transplanting of rice up to fourth month by serial dilution and plating on respective media (Table 52, 53, 54,55 and 56).

. Population of total bacteria at monthly interval up to fourth month are presented in Table 52. Results showed that population of bacteria was significantly different at every month after transplanting. One month of transplanting, higher bacterial population was noticed in T_1 (17.00 x 10^7 cfu g^{-1}), T_5 (16.25 x 10^7 cfu g^{-1}), T_3 (16.00 x 10^7 cfu g^{-1}) and T2 (14.25 x 10^7cfu g^{-1}). T_4 which had 100% inorganic fertilizers recorded significantly lower population. Higher and on par total bacterial population was observed in T_1, $T_3$and T_5 at two MAP. At three and four MAP, treatments which received bioinoculants (T_1, T_2 and T_3) had significantly higher bacterial population than uninoculated treatments (T_4 and T_5). Initial populaton of total bacteria was 4.50 x 10^7cfu g^{-1} and all the treatments showed higher bacterial population than initial population at every month after transplanting except T_4.

Population of nitrogen fixing bacteria are shown in Table 53. Population of nitrogen fixers at one and three MAP, showed no significant differences among the treatments. During the second month of transplanting, significantly higher population of nitrogen fixers was recorded in T_1 (12.50 x 10^6 cfu g^{-1}), T_2 (11.25 x 10^6 cfu g^{-1} of soil) and T_3 (10.75 x 10^6 cfu g^{-1} of soil). Lowest population was noticed in T_4 (3.25 x 10^6 cfu g^{-1}). At four months after transplanting, higher population of N fixers was recorded in T_3 (7.00 x 10^6 cfu g^{-1}), T_2 (6.50x 10^6 cfu g^{-1}) and T_1 (6.00x 10^6 cfu g^{-1}). Significantly lower population of N fixers (2.75 x 10^6 cfu g^{-1} of soil) was noticed in T_4 which was 100% RDF and T_5 (3.00 x 10^6 cfu), Farmer's practice, both without any bio-inoculant. Treatments which received bioinoculants (T_1, T_2 and T_3) had higher

population of nitrogen fixers at noe, two and three months after planting than initial population of nitrogen fixers (8.00x 10^6 cfu g^{-1}).

Population of phosphate solubilizers was significantly different at all intervals, up to four MAP (Table 54). One month after transplanting, significantly higher population of phosphate solubilizers was observed in T_1 (11.00 x 10^5 cfu g^{-1}), T_3 (8.50 x 10^5 cfu g^{-1}) and T_2 (7.75 x 10^5 cfu g^{-1}) than uninoculated treatments (T_4 and T_5). Similar observations were recorded at two, three and four months after transplanting. Lowest population of phosphate solubilizers was recorded T_4 (100% RDF) during one, three and four MAP. All the treatments showed higher population of phosphate solubilizers than initial population (1.50 x 10^5 cfu g^{-1}) at one, two and three months after planting.

The population of potassium solubilizers in soil after transplanting are depicted in Table 55. After one month of planting, population of potassium solubilizers was higher in T_1 (6.75 x 10^4 cfu g^{-1}), T_2 (5.50 cfu g^{-1}) and T_3 (6.25 x 10^4 cfu g^{-1}). At second and fourth months, significantly higher population of K solubilizers was recorded in treatments which had combined application of biofertilizers with 75% RDF (T_1, T_2 and T_3). At three MAP, lowest population of K solubilizers was recorded in T_4. At one, two and four months after transplanting, T_4 was on par with T_5. Treatments which received bioinoculants (T_1, T_2 and T_3) had higher population of potassium solubilizers at all interval than initial population of potassium (4.50 x 10^4 cfu g^{-1}). Uninoculated treatments showed lower population of potassium solubizers than initial population.

The population of zinc solubilizing bacteria at different intervals are presented in Table 56. Population of zinc solubilizers was significantly higher and on par in T_1 and T_2, at all intervals. Significantly lower population was recorded in T_3, T_4 and T_5 at all intervals up to four MAP. Treatments which received biofertilizer showed higher population of zinc solubilizers at one, two and three months after transplanting than initial population (1.50 x 10^4 cfu g^{-1}).

Table 52: Effect of PGPR on the population of total bacteria in rice soil under field culture

Treatments	Population of total bacteria (cfu g^{-1})			
	1 MAP	2 MAP	3 MAP	4 MAP
T_1: Consortium 2 + 75% RDF	17.00×10^7 (8.228) [a]	17.75×10^7 (8.248) [a]	10.50×10^7 (8.019) [a]	9.50×10^7 (7.967) [a]
T_2: Consortium 3 + 75% RDF	14.25×10^7 (8.098) [ab]	12.00×10^7 (8.071) [bc]	10.75×10^7 (8.030) [a]	9.25×10^7 (7.964) [a]
T_3: PGPR Mix -1 + 75% RDF	16.00×10^7 (8.201) [a]	17.50×10^7 (8.226) [a]	10.50×10^7 (8.015) [a]	8.50×10^7 (7.926) [ab]
T_4: 100% RDF	8.25×10^7 (7.909) [b]	8.75×10^7 (7.936) [c]	4.50×10^7 (7.639) [b]	3.75×10^7 (7.551) [c]
T_5: Farmer's practice	16.25×10^7 (8.208) [a]	14.00×10^7 (8.139) [ab]	6.25×10^7 (7.764) [b]	5.75×10^7 (7.734) [bc]

MAP: month after planting, Log transformation values are given in parenthesis

Table 53: Effect of PGPR on the population of nitrogen fixing bacteria in rice soil under field culture

Treatments	Population of nitrogen fixing bacteria (cfu g^{-1})			
	1 MAP	2 MAP	3 MAP	4 MAP
T_1: Consortium 2 + 75% RDF	10.50×10^6 (7.011)	12.50×10^6 (7.095) [a]	12.0×10^6 (7.074)	6.00×10^6 (6.747) [a]
T_2: Consortium 3 + 75% RDF	9.00×10^6 (6.942)	11.25×10^6 (7.041) [a]	10.5×10^6 (6.834)	6.50×10^6 (6.801) [a]
T_3: PGPR Mix -1 + 75% RDF	8.25×10^6 (6.882)	10.75×10^6 (7.030) [a]	11.75×10^6 (7.063)	7.50×10^6 (6.850) [a]
T_4: 100% RDF	5.00×10^6 (6.690)	3.25×10^6 (6.496) [c]	3.50×10^6 (6.520)	2.75×10^6 (6.420) [b]
T_5: Farmer's practice	6.25×10^6 (6.782)	6.00×10^6 (6.758) [b]	5.50×10^6 (6.726)	3.00×10^6 (6.464) [b]

MAP: month after planting, Log transformation values are given in parenthesis

Table 54: Effect of PGPR on the population of phosphate solubilizing bacteria in rice soil under field culture

Treatments	Population of phosphate solubilizing bacteria (cfu g^{-1})			
	1 MAP	2 MAP	3 MAP	4 MAP
T_1: Consortium 2 + 75% RDF	11.00×10^5 (6.032) [a]	12.00×10^5 (6.074) [a]	12.00×10^5 (6.074) [a]	1.60×10^5 (5.167) [a]
T_2: Consortium 3 + 75% RDF	7.75×10^5 (5.878) [a]	8.50×10^5 (5.926) [b]	12.75×10^5 (6.084) [a]	1.40×10^5 (5.141) [a]
T_3: PGPR Mix -1 + 75% RDF	8.50×10^5 (5.926) [a]	9.50×10^5 (5.975) [ab]	13.00×10^5 (6.112) [a]	1.52×10^5 (5.178) [a]
T_4: 100% RDF	4.25×10^5 (5.615) [b]	4.25×10^5 (5.615) [c]	3.50×10^5 (5.520) [c]	0.70×10^5 (4.826) [b]
T_5: Farmer's practice	5.00×10^5 (5.695) [b]	2.75×10^5 (5.420) [d]	5.50×10^5 (5.726) [b]	0.72×10^5 (4.850) [b]

MAP: month after planting, Log transformation values are given in parenthesis

Table 55: Effect of PGPR on the population of potassium solubilizing bacteria in rice soil under field culture

Treatments	Population of potassium solubilizing bacteria (cfu g^{-1})			
	1 MAP	2 MAP	3 MAP	4 MAP
T_1: Consortium 2 + 75% RDF	6.75×10^4 (4.828) [a]	6.25×10^4 (4.790) [a]	9.25×10^4 (4.957) [ab]	6.50×10^4 (4.806) [a]
T_2: Consortium 3 + 75% RDF	5.50×10^4 (4.731) [ab]	6.50×10^4 (4.790) [a]	6.75×10^4 (4.823) [b]	5.75×10^4 (4.746) [a]
T_3: PGPR Mix -1 + 75% RDF	6.25×10^4 (4.792) [a]	9.50×10^4 (4.975) [a]	11.00×10^4 (5.040) [a]	6.75×10^4 (4.826) [a]
T_4: 100% RDF	3.25×10^4 (4.496) [c]	1.50×10^4 (4.151) [b]	1.25×10^4 (4.075) [d]	1.25×10^4 (4.075) [b]
T_5: Farmer's practice	4.00×10^4 (4.595) [bc]	2.50×10^4 (4.345) [b]	3.25×10^4 (4.496) [c]	2.25×10^4 (4.301) [b]

MAP: month after planting, Log transformation values are given in parenthesis

Table 56: Effect of PGPR on the population of zinc solubilizing bacteria in rice soil under field culture

Treatments	Population of zinc solubilizing bacteria (cfu g^{-1})			
	1 MAP	2 MAP	3 MAP	4 MAP
T_1: Consortium 2 + 75% RDF	62.50 x 10^3 (4.746) ^{a}	70.00 x 10^3 (4.814) ^{a}	42.50 x 10^3 (4.608) ^{a}	20.00 x 10^3 (4.270) ^{a}
T_2: Consortium 3 + 75% RDF	37.50 x 10^3 (4.525) ^{a}	55.00 x 10^3 (4.721) ^{ab}	30.00 x 10^3 (4.420) ^{ab}	17.50 x 10^3 (4.226) ^{ab}
T_3: PGPR Mix -1 + 75% RDF	17.50 x 10^3 (4.151) ^{b}	30.00 x 10^3 (4.420) ^{bc}	17.50 x 10^3 (4.226) ^{b}	10.00 x 10^3 (4.000) ^{bc}
T_4: 100% RDF	11.25 x 10^3 (4.048) ^{b}	6.00 x 10^3 (3.768) ^{d}	5.50 x 10^3 (3.726) ^{c}	3.00 x 10^3 (3.670) ^{d}
T_5: Farmer's practice	13.25 x 10^3 (4.109) ^{b}	17.50 x 10^3 (4.159) ^{c}	9.00 x 10^3 (3.956) ^{c}	6.25 x 10^3 (3.782) ^{cd}

MAP: month after planting, Log transformation values are given in parenthesis

4.10.2.2. Effect of PGPR isolates on growth and yield of rice under field conditions

Plant growth parameters such as plant height, number of tillers at maximum tillering stage and yield attributes such as number of panicles, number of grains per panicle, filled grain per cent and 1000 grain weight were recorded. Grain and straw yield per plot were recorded and extrapolated to the yield ton per hectare.

4.10.2.2.1. Plant height

Plant height is an important parameter related to growth and development. No significant difference was observed among the tested treatments. Plant height ranged between 72.51 cm to 85.22 cm as shown in Table 57.

4.10.2.2.2. Number of tillers

Higher number of tillers/hills was recorded in T_4 (9.65), T_1 (9.45) and T_2 (9.03). Significantly lowest no. of tillers/hill (7.30) was recorded in T_5 (Farmers' practice). Similar trend was observed in the number of tillers/m^2. T_4 recorded 309.5 tillers/m^2 and

was on par with T_1 (307.5) and T_2 (276.25). Significantly lowest number of tillers/m^2 was recorded in T_5 (223.75).

4.6.2.2.3. Yield attributes

Number of panicles at harvesting stage was significantly different among the treatments (Table 58). Significantly higher number of panicles/hills was recorded in treatments T_1 (9.17), T_2 (8.98), T_3 (8.20) and T_4 (8.95). Lowest no. of panicles/hill was observed in T_5 (7.15). Number of panicles/m^2 was recorded and results showed that statistically comparable no. of panicles/m^2 in T_1 (270.50), T_4 (261.50) and T_2 (254.25). Significantly lowest number of tillers/ m^2 was recorded in T_5 (207.00).

Number of grains/panicle and filled grain percentage were not significantly different among the treatments (Table 58). Per cent of filled grains/panicle ranged 89.89 per cent (recorded in T_5) to 94.49 per cent (recorded in T_4).

Results of 1000 grain weight showed that significantly higher 1000 grain weight in T_4 (32.50 g), T_1 (31.19 g), T_2 (31.04 g) and T_3 (30.95 g) than T_5.

4.10.2.2.4. Grain yield and straw yield

After harvesting grain yield and straw yield were measured separately (Table 59). Significantly higher values of grain yield were observed in T_1 (3.60 t ha^{-1}), T_2 (3.23 t ha^{-1}) and T_4 (3.58 t ha^{-1}) and lowest grain yield was observed in T_5 (2.49 t ha^{-1}). The results revealed that grain yield of treatments T_1 and T_2 which were inoculated with indigenous PGPR with 75% RDF was statistically comparable with grain yield of 100% RDF treated plots.

Straw yield was not significantly different among the treatments. Straw yield varied from 3.14 t ha^{-1} (observed in T_5) to 4.29 t ha^{-1} (observed in T_1).

Fig. a. View of rice field at tillering stage

Fig. b. View of rice field at harvesting stage

Plate 41: View of field experiment at RARS, Ambalavayal

Fig a: Plots of T_1(Consortium 2 + 75% RDF) and T_4 (Uninoculated + 100% RDF)

Fig b: Plots of T_1(Consortium 2 + 75% RDF) and T_3 (PGPR mix-1 + 75% RDF)

Fig c: Plots of T_5 (Farmer practice) and T_1 (Consortium 2 + 75% RDF)

Plate 42: View of different treatments in rice field at tillering stage

Fig a: Plots of T_4: Uninoculated + 100% RDF and **T_1: Consortium 2 + 75% RDF**

Fig. b: Plots of T_3: PGPR Mix -1 + 75% RDF and T_1: Consortium 2 + 75% RDF

Plate 43: View of different treatments in rice field at harvesting stage

Table 57: Effect of different treatments on plant height and number of tillers

Treatments	Plant height (cm)	No. tillers / hill	No. tillers /m^2
T_1: Consortium 2 with 75% RDF	82.40	9.45^{ab}	307.00 ^{a}
T_2: Consortium 3 with 75% RDF	80.67	9.03^{ab}	276.25 ^{ab}
T_3: PGPR Mix-1 with 75% RDF	77.32	8.48^{b}	262.50 ^{bc}
T_4: 100% RDF	85.22	9.65^{a}	309.50 ^{a}
T_5: Farmer's practice	72.51	7.30^{c}	223.75 ^{c}

Table 58: Effect of different treatments on yield attributes of rice

Treatments	No. panicles/ hill	No. panicles/m^2	No. grains / panicle	Filled grain %	1000 grain weight (g)
T_1: Consortium 2 with 75% RDF	9.17 ^{a}	270.50 ^{a}	113.52	91.09	31.19 ^{a}
T_2: Consortium 3 with 75% RDF	8.98 ^{a}	254.25 ^{ab}	108.15	90.30	31.04 ^{a}
T_3: PGPR Mix-1 with 75% RDF	8.20 ^{ab}	242.25 ^{b}	114.45	90.81	30.95 ^{a}
T_4: 100% RDF	8.95 ^{a}	261.50 ^{ab}	127.12	94.49	32.50 ^{a}
T_5: Farmer's practice	7.15 ^{b}	207.00 ^{c}	108.52	89.80	29.05 ^{b}

Table 59: Effect of different treatments on grain yield and straw yield

Treatments	Grain yield ($t\ ha^{-1}$)	Straw yield ($t\ ha^{-1}$)
T_1: Consortium 2 with 75% RDF	3.60 [a]	4.29
T_2: Consortium 3 with 75% RDF	3.23 [ab]	3.67
T_3: PGPR Mix-1 with 75% RDF	2.93 [b]	4.25
T_4:100% RDF	3.58 [a]	4.09
T_5: Farmers' practice	2.49 [c]	3.14

4.10.2.3. Effect of PGPR application on plant nutrient content

Plant samples were analyzed for the content of nutrients including nitrogen, phosphorus, potassium and zinc at panicle initiation stage and results are presented in Table 60.

4.10.2.3.1. Nitrogen content of plant

Nitrogen content of plants showed significant difference among the treatments. Higher and on par N content of plant tissue was recorded in T_4 (2.05%), T_1 (1.79%) and T_2 (1.75%). Lowest nitrogen content of plants (1.18%) was observed in T_5 (Farmers' practice).

4.10.2.3.2. Phosphorus content of plant

Significantly higher phosphorus content of 0.52% was recorded in T_4 (100 % RDF) which was statistically on par with T_1, which recorded a P content of 0.46%. P content was significantly low in T_2, T_3 and T_5.

4.10.2.3.3. Potassium content of plant

Potassium content of plant samples was significantly different and higher values of P content were recorded in in T_1, T_2, T_3 and T_4 which was statistically on par. Lowest potassium content was recorded in T_5 (1.080 %).

4.10.2.3.4. Zinc content of plant

Zinc content of plants was not significant different among the treatments and it ranged between 0.13 ppm to 0.15 ppm.

Table 60: Effect of PGPR on nutrient contents of plant tissue

Treatments	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Zinc (ppm)
T_1: Consortium 2 + 75% RDF	1.79 ^{ab}	0.46 ^{ab}	1.443 ^{a}	0.15
T_2: Consortium 3 + 75% RDF	1.75 ^{ab}	0.43 ^{b}	1.253 ^{ab}	0.14
T_3: PGPR Mix 1 + 75% RDF	1.58 ^{b}	0.41 ^{b}	1.310 ^{ab}	0.14
T_4: 100% RDF	2.05 ^{a}	0.52 ^{a}	1.463 ^{a}	0.14
T_5: Farmer's practice	1.18 ^{c}	0.40 ^{b}	1.080 ^{b}	0.13

4.10.2.4. Effect of PGPR on physico-chemical parameters of soil at harvesting of the rice crop

Soil samples collected at harvest of rice crop were analysed for soil nutrient status and results are presented in Table 61.

4.10.2.4.1. Soil pH

Soil pH was not significantly different among the treatments and it ranged from 5.79 to 6.18.

4.10.2.4.2. Soil EC

Soil EC was significantly different among the treatments and significantly highest EC of 0.09 dS m^{-1} was recorded in T_4 (100% RDF). However, all samples recorded EC of less than 1.0, which was the critical level.

4.10.2.4.3. Soil organic carbon content

Soil organic carbon content was not significantly different among the treatments at harvest.

4.10.2.4.4. Soil microbial biomass carbon

Soil microbial biomass carbon was significantly different and higher amount of microbial biomass carbon was observed in T_5 (102.60 µg g^{-1}) which was statistically on par with T_1 (98.70 µg g^{-1}), T2 (82.55 µg g^{-1}) and T3 (77.67). Lowest microbial biomass carbon recorded in T_4 (55.57 µg g^{-1}).

4.10.2.4.5. Soil nitrogen content

Available nitrogen content of soil was significantly different among the treatments. Higher and on par N content was recorded in treatments T_1 (163.07 kg ha^{-1}), T_2 (163.06 kg ha^{-1}), T_3 (147.39 kg ha^{-1}) and T_4 (159.43 kg ha^{-1}). Lowest N content of 128.57 kg $ha^{-1}$was recorded in T_5 (Farmer's practice).

4.10.2.4.6. Soil phosphorus content

Significantly higher phosphorus content of soil was observed in T_1 (17.35 kg ha^{-1}) and T_3 (17.03 kg ha^{-1}). Significantly lower phosphorus content of soil was recorded in T_2 (10.25 kg ha^{-1}), T_4 (9.91 kg ha^{-1}) and T_5 (5.88 kg ha^{-1}).

4.10.2.4.7. Soil potassium content

Soil potassium content was significantly higher in T_4 (83.33 kg ha^{-1}) and T_3: (72.95 kg ha^{-1}). All other treatments showed significantly lower potassium content.

4.10.2.4.8. Soil zinc content

Soil zinc content was not significant different among the treatments and ranged between 2.10 to 2.48 mg kg^{-1}.

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Table 61: Effect of different treatments on soil nutrient contents at harvesting

Treatments	Soil pH	Soil EC (dS m^{-1})	Soil organic C %	Microbial biomass C (µg g^{-1})	Nitrogen (kg ha^{-1})	Phosphorus (kg ha^{-1})	Potassium (kg ha^{-1})	Zinc (mg kg^{-1})
T_1: Consortium 2 + 75% RDF	5.79	0.08 ^{b}	1.07	98.70 ^{a}	163.07 ^{a}	17.35 ^{a}	50.40 ^{b}	2.48
T_2: Consortium 3 + 75% RDF	6.13	0.05 ^{b}	1.13	82.55 ^{ab}	163.06 ^{a}	10.25 ^{b}	48.72 ^{b}	2.33
T_3: PGPR Mix 1 + 75% RDF	6.07	0.07 ^{b}	1.04	77.67 ^{ab}	147.39 ^{ab}	17.03 ^{a}	72.95 ^{a}	2.26
T_4: 100% RDF	5.88	0.09 ^{a}	1.01	55.57 ^{b}	159.93 ^{a}	9.91 ^{b}	83.33 ^{a}	2.13
T_5: Farmer's practice	6.18	0.06 ^{b}	1.15	102.60 ^{a}	128.57 ^{b}	5.88 ^{c}	43.68 ^{b}	2.10
Critical limit as per Tandon (2004)		< 0.1	0.75		280 to 560	10 to 25	120 to 280	0.6

4.10.2.5. Benefit cost ratio of rice cultivation with different treatments

The cost of production of rice cultivation with 100% recommended dosage of inorganic fertilizer, organic fertilizer and integrated application of biofertilizer with 75% RDF was calculated. For calculation of cost of cultivation all the inputs for each treatment and operational costs were taken. For estimating the returns, the average sale price for bulk paddy seed was considered as Rs. 28 per one kg. Calculated benefit cost ratio (B:C ratio) is presented in Table 62. Highest benefit cost ratio (1.51) was recorded in T_1 which had consortium 2 + 75% RDF and lowest B:C ratio (0.92) was recorded in T_5 which was farmer's practice.

Table 62: Benefit cost ratio of rice with different treatments

Treatments	Total cost Rs ha^{-1}	Gross income Rs ha^{-1}	B:C ratio
T_1: Consortium 2 + 75% RDF	66791.85	100800.00	1.51
T_2: Consortium 3 + 75% RDF	66791.85	89600.00	1.34
T_3: PGPR Mix 1 + 75% RDF	66791.85	82040.00	1.22
T_4: 100% RDF	66855.80	100240.00	1.49
T_5: Farmer's practice	75200.00	69720.00	0.92

RDF: Recommended dosage of fertilizer

Discussion

5. DISCUSSION

Rice (*Oryza sativa* L.) is one of the principal crops in the world and about 90% of rice is being cultivated and produced in Asian countries. Among several factors, soil fertility and well-balanced mineral nutrition have immense effect on rice productivity. The high yielding rice varieties introduced in India during the Green Revolution resulted in increased rice production but demanded large amounts of chemical fertilizers. Non-judicious application of chemical fertilizers raised concerns over environment and health issues. Biofertilizers offer an alternative to decrease the use of chemical fertilizers, thereby solving environmental pollution and damage to human and animal health. Plant Growth Promoting Rhizobacteria (PGPR) are microorganisms that reside in the rhizosphere and improve the growth and yield of plants, and there have been several studies which indicated the beneficial effects of application of PGPR under different ecological situations (Zahid *et al*., 2015).

The commercial inoculants of PGPR are based on stimulation of plant growth by either the production of phytohormones or improving plant nutrient availability or enhancing the resistance of the plant against pathogens (Figueiredo *et al*., 2010). However, bacterial strains having multiple positive effects are few and selection of multiphasic characters of PGPR might make their application more efficient (Miao *et al*., 2015).

Several studies have shown that growth promoting activities of PGPR strains are highly specific to certain plant species, cultivar, genotype and soil. The performance of PGPR inoculants may vary widely when using them for different crops and environment (Lucy *et al*., 2004). Many authors reviewed efficiency of native isolates on crop growth and yield improvements over commercial inoculants. Sahoo *et al*. (2013) reported native *Azotobacter vinelandii* are highly efficient to improved growth and yield of rice crop over commercial bioinoculants. Khanghahi, *et al.* (2019) stated that commercial K biofertilizer had no significant effect on grain yield of rice, but native potassium solubilizing bacteria increased yield component of rice. Therefore, non-native strains, as a commercial biofertilizer, cannot compete with the native microbial communities. The native PGPRs isolated from rhizospheric soil may be more useful

than others because they are already competent, adapted and dominant in a particular geographical area (Bergottini *et al*., 2015).

Wayanad is generally considered as rich in biodiversity because of the unique sub-tropical climatic conditions. The name Wayanad is believed to have been derived from Vayal nadu meaning the land of paddy fields. Wayanad is a plateau with an altitude varying from 700 to 2100 m from sea level ensconced among Western Ghats. The soil of Wayanad district is mainly loamy type and generally shows wide variation in texture with a very high content of organic matter and slightly acidic pH (Silja *et al*., 2008). Therefore, it is important to explore region specific microbial strains which can be used for developing effective microbial consortia. In this context, present study was carried out with the following objectives (i) to isolate native PGPR strains from the rice rhizosphere in Wayanad district of Kerala, India (ii) to assess PGP and antagonistic activities of selected isolates *in vitro*, (iii) biochemical and molecular characterization of efficient PGPR isolates (iv) to formulate PGPR based consortia by using promising PGPR strains and (v) to evaluate performances of PGPR based consortia on rice plant growth and yield.

Ten rice growing fields (Thanivayal, Edakkal, Kolagappara, Nellara, Kuppamudi, Ambalavayal, Marathat, Ambukuthi, Malavayal and Pottankoli) in Wayanad district of Kerala were selected for collection of soil samples. Rhizosphere soil samples of ten different locations were analyzed for physio-chemical parameters and showed that all the soils were acidic and EC were highly variable among the locations. Prasanna and Nayak (2007) stated that paddy soil samples collected from Moncompu, Kerala were in the acidic range. According to the critical limits of soil nutrition (Tandon, 2004), soil organic matter content was higher in six locations (Thanivayal, Edakkal, Nellara, Kuppamudi, Marathat and Ambukuthi). All the tested soils were low in available nitrogen, potassium and zinc content as per the critical limits of Tandon (2004). Available P content was high in four locations (Thanivayal, Marathat, Ambukuthi and Pottankoli).

Isolation of different plant growth promoting rhizobacteria with potential PGP activities were attempted from rhizosphere soils of two months old rice plants collected

from ten different location in Wayanad. Various groups of PGPR including N-fixers, solubilizers of phosphate, potassium and zinc and fluorescent pseudomonads were isolated and enumerated on appropriate media. The total bacteria on nutrient agar ranged between 5.00 x 10^6 cfu to 23.00 x10^6 cfu g^{-1} of soil. The lowest population of 5.00 cfu was observed in Edakkal, which could be due to the low pH of soil (4.16). Significantly lower population of bacteria was also observed in Thanivayal and Nellara. These locations also had a low soil pH of 4.96 and 4.34. The location Ambukuthi also recorded a low soil pH of 4.16. However**,** bacterial population was significantly higher than Edakkal, which recorded the same soil pH. This could be due to the higher content of organic carbon in Ambukuthi soil (1.17 per cent). Laldinthar and Dkhar (2015), stated that bacterial population was positively correlated with organic carbon ($r = 0.90$; $p \leq 0.001$) and total nitrogen ($r = 0.76$; $p \leq 0.05$) at broad leaved forest of Meghalaya and concluded that these two constitute the major driving factors of bacterial population in soil. The structures of bacterial communities, in the rice rhizosphere are diverse and dynamic and primarily shaped by soil and plant related conditions such as geographic location, soil type and rice genotypes (Edwards *et al*., 2015).

The population of nitrogen fixing bacteria on Jensen's agar was significantly higher in Kolagappara (46.66 x 10^5 cfu g-^{1}), Ambukuthi (37.66 x10^5 cfu) and Pottankoli (36.33 x10^5 cfu) than other locations. Kolagappara and Pottankoli soils had a pH above 5.0. Ambukuthi soil recorded the lowest pH of 4.16 and the highest organic carbon content of 1.17 per cent. This high content of organic carbon could be responsible for the higher population of N-fixers in Ambukuthi soil, even though the pH was low.

Ashby's agar was another medium utilized for the isolation of N-fixers in the present study. Population of N-fixers on this medium was higher and on par in in Pottankoli, Malavayal, Ambukuthi, Kuppamudi and Marathat soils. In all the soil samples tested, population of N-fixing bacteria was higher in Jensen's agar, as compared to Ashby's agar. Both the media lack N in the medium and hence only microorganisms which can fix atmospheric N will be capable of growing in the media. Jensen's agar contains sucrose as the carbon and energy source and sodium molybdate helps in the functioning of nitrogenase enzyme. Ashby's agar is generally recommended for the isolation and culturing of *Azotobacter* and the population of this

genus may be lowers in rice soils, as they prefer aerobic conditions (Jnawali *et al*., 2015). Phiromtan *et al*. (2010) who stated that soil pH had significant effect on the population and density of *Azotobacter* and maximum population of *Azotobacter* was found at pH 5.1 to 5.5. In this study results showed pH had over 5.0 in locations Pottankoli, Malavayal and Marathat had higher *Azotobacter* population. Other two locations which showed higher *Azotobacter* population Ambukuthi and Kuppamudi had pH lower than 5.0 however, these two locations had higher organic matter content 1.17 % and 1.02 % respectively and this may be reason for the higher population level. Phiromtan *et al.* (2010) reported maximum amount of *Azotobacter* (4.06 x 10^6 cfu g^{-1}) was found in soil with 2.6 to 3.5 % soil organic matter (SOM) and suggested SOM highly impacts on *Azotobacter* population.

The population of N-fixers was significantly lower in soil sample collected from Edakkal on both Jensen's agar (8.33 x10^5 cfu g-^{1} of soil) and Ashby's agar (4.66x x10^5 cfu g-^{1} of soil). Lowest pH of 4.16 was recorded in Edakkal soil and the low pH could be responsible for the low population of N-fixers. The pH value played a more important role than other soil properties for population of diazotroph (Han *et al*., 2019). Optimal pH value for the growth of diazotroph is between 7.5 and 8.0 (Roper and Gupta, 2016).

Available N content was highest in Ambukuthi soil, which justifies the finding that the population of N-fixers was significantly higher in this soil. Higher population of N-fixers could be responsible for higher N-fixation in this soil. Lowest available N content of 137.98 g ha-^{1} was recorded in Edakkal soil, which also recorded lowest population of N-fixers in both Jensen's agar and Ashby's agar.

Cyanobacterial population was significantly highest (18.76 x 10^4 cfu g^{-1}) in soil sample collected from Kuppamudi. Second highest population of cyanobacteria (9.26 x 10^4 cfu g^{-1}) was recorded in soil sample of Ambukuthi. Among the environmental variables (soil pH, organic carbon (%) and conductivity) highly positive correlation was observed between cyanobacterial population and soil pH (Dey *et al*., 2010). Soil pH is important in determining growth, establishment and diversity of cyanobacterial flora, which is generally been reported to prefer neutral to slightly alkaline (Kaushik, 1994).

Population of phosphate solubilizers was enumerated on Pikovskaya's agar which contains insoluble tri-calcium phosphate. Microorganisms capable of phosphate solubilisation grow and produce clear zones around the colonies, as a result of solubilisation of tri-calcium phosphate. The population of phosphate solubilizers ranged from 2.66 $x10^5$ to 13.33 $x10^5$ cfu g^{-1} of soil. The higher and on par population of phosphate solubilizers was observed in Pottankoli, Ambukuthi, Malavayal, Edakkal, Kuppamudi and Thanivayal. Pottankoli soil recorded a pH of 5.07, which was higher as compared to many other locations. Lowest population of phosphate solubilizers was observed in Nellara (2.66 x 10^5), which was statistically on par with Kolagappara and Ambalavayal (4.66 x 10^5 cfu g^{-1} soil in both the locations). Nellara recorded a low pH of 4.34 and this could be a reason for the low population of phosphate solubilizers. Zheng *et al*. (2018) studied inorganic phosphate solubilizing bacteria (iPSB) community structures of the pH-adjusted soils based on database alignment and marker gene quantification methods, and stated that diversity of the iPSB communities increased significantly with pH ($P<0.05$). Besides the iPSB community, abundance also significantly increased with soil pH. Krishnakumar *et al*., (2014) reported that the population of phosphate solubilizing bacteria varied among different crops and highest population was reported in groundnut rhizosphere soils (4.7 x 10^5 cfu g^{-1} of soil) and lowest in rice rhizosphere soils (1.5 $x10^5$ cfu g^{-1}).

In the present investigation, Nellara soil recorded a low content of available P (7.44 kg ha^{-1}) and lowest population of phosphate solubilizers. Vikram *et al*. (2007) studied factors related to the occurrence of phosphate solubilizing bacteria (PSB) and found organic carbon and available nitrogen of soil showed significant positive corelation with population of PSB. While soil pH, available P and total P of the soil showed a positive but insignificant correlation with the PSB population. Our results are agreed with these results and phosphate solubilizers was recorded as low in soil from Nellara which had low available P content. In contrasting to findings of Vikram *et al*. (2007) population of phosphate solubilizers was recorded low in Nellara even though soil organic carbon and available N content was high.

Higher population of potassium solubilizers (4.66 $x10^5$ cfu $g^{-1}$of soil) was recorded in soil from Malavayal and Kolagappara while minimum population was

observed in Marathat soil (0.40 $x10^5$ cfu g^{-1}). Marathat soil also recorded a comparatively higher content of available K (80.54 kg ha^{-1}), though all the soils were deficient in available K.

Zinc solubilizing bacterial population was higher in Malavayal soil (6.00 $x10^5$ cfu g^{-1}) followed by Pottankoli (5.33 $x10^5$ cfu $g^{-1}$of soil) and these were also on par with Ambukuthi, Thanivayal and Kuppamudi. Lowest population of Zn solubilizers was observed in Nellara (0.433 cfu g^{-1}). All the locations were deficient in Zn, as the values were below the critical level of 0.6 mg ha^{-1}. Comparatively lower population of Zn solubilizers was found in all the locations, as compared to N-fixers and phosphate solubilizers. Abaid-Ullah *et al*. (2015) also reported low population of rhizobacteria as Zn solubilizers in wheat rhizosphere. Shakeel *et al*. (2015) recovered a large number of endophytic isolates from the rice plants, but a very few strains depicted Zn solubilization potential.

Fluorescent pseudomonads were obtained on King's B agar only from two locations (Kuppamudi and Pottankoli) at 10^{-4} dilution.

Habibi *et al*. (2014) studied nitrogen fixers of rhizosphere of different crops and found 83.4 % of bacteria isolated from the rhizosphere soil of rice showed nitrogen-fixing abilities; this was the highest proportion of nitrogen-fixing bacteria among the tested rhizosphere soils. Our results also corroborated with earlier results and higher population of nitrogen fixing bacteria was observed than P, K and Zn solubilizers in all the locations.

A total of 149 isolates (including 60 N-fixers, 59 phosphate solubilizers, seven K-solubilizers, 21 Zn-solubilizers and 2 fluorescent pseudomonads) obtained on different media from various locations in Wayanad were subjected to preliminary screening for growth on N-free media in case of N-fixers, solubilisation zone for phosphate, K and Zn-solubilizers. This preliminary screening yielded 32 N-fixers, 16 phosphate solubilizers, four isolates each of K and Zn-solubilizers and two fluorescent pseudomonads. These isolates were further screened for PGP traits and efficient isolates selected for *in vivo* evaluation.

Biological nitrogen fixation by bacteria that present in the rhizosphere is important property contributing for growth of plants (Kumar and Gera, 2014). Biological nitrogen fixation has been reported to be exclusively carried out by few members of the prokaryotic organisms and can be used as an alternate to chemical fertilizers. Biological nitrogen fixation of different strains is highly location specific and therefore, resident strains would be better suited (Kannan and Ponmurugan, 2010). In the present investigation, sixty N-fixers were isolated on N-free media. Thirty-two N-fixers were screened for PGP traits including production of the plant growth hormone IAA, production of ammonia, HCN and siderophores. Fourteen N-fixers were positive for production of IAA, 30 for ammonia production and 13 for siderophore production (Table 63 and Fig. 5). Two isolates exhibited antagonism against each of the rice pathogens tested *viz. Rhizoctonia solani* and *Xanthomonas oryzae*. Thirty isolates (except $KgNF_5$ and $NrNF_6$) possessed at least one PGP activity. Nine isolates ($KgNF_1$, $KpNF_5$, $AvNF_2$, $AvNF_3$, $AvNF_4$, $AkNF_3$, $PkNF_2$, $PkNF_3$ and $PkNF_4$) exhibited three PGP traits each (Table 64).

Table 63: Plant growth promotion and antagonistic activities exhibited by nitrogen fixers

PGP trait	IAA production	NH3 production	HCN production	Siderophore production	Antagonism	
					R. solani	***X. oryzae***
No. of isolates positive	14	30	0	13	2	2
No. of isolates negative	18	2	32	19	30	30

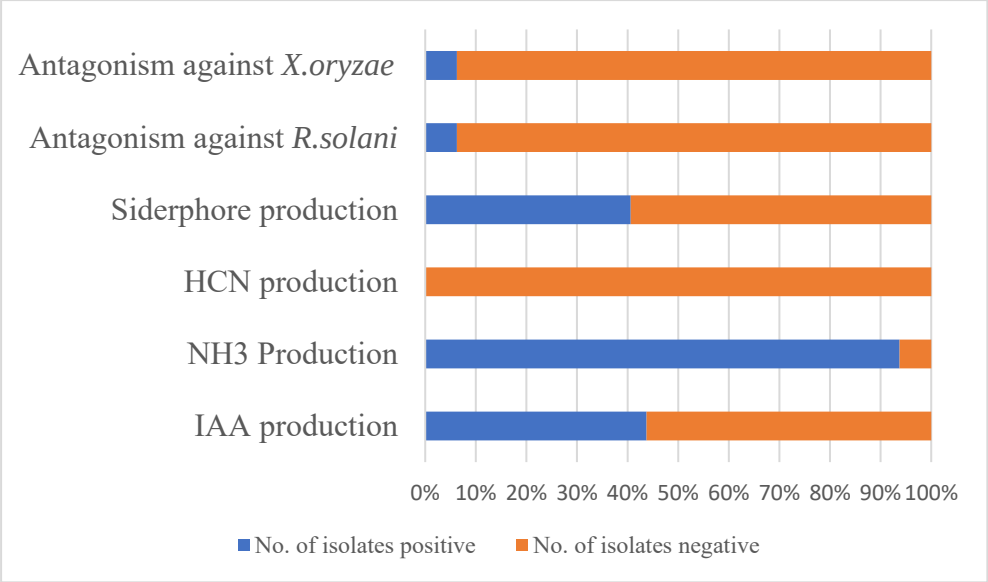

Fig.5: PGP and antagonism activities showed by selected nitrogen fixers

Table 64: Details of N-fixers exhibiting multiple PGP traits

Number of PGP traits exhibited	No. of isolates	Details of isolates
Three	9	$KgNF_1$, $KpNF_5$, $AvNF_2$, $AvNF_3$, $AvNF_4$, $AkNF_3$, $PkNF_2$, $PkNF_3$, $PkNF_4$
Two	11	$KgNF_9$, $KpNF_2$, $KpNF_4$, $KpNF_6$, $KpNF_7$, $AvNF_1$, $MtNF_3$, $MtNF_4$, $AkNF_2$, $AkNF_5$, $PkNF_1$
One	10	$ThNF_1$, EdNF1, $EdNF_2$, $KgNF_4$, $NrNF_2$, $MtNF_1$, $MtNF_2$, $AkNF_4$, $MvNF_1$, $MvNF_2$
Nil	2	$KgNF_5$ and $NrNF_6$

Phytohormones are organic substances that influence physiological process of plants at extremely low concentrations and 80% of rhizobacteria are capable for IAA production (Patten and Glick, 1996). In the present investigation, all the fourteen isolates which tested positive for IAA production were further subjected to the quantification of IAA production. Quantity of IAA production was measured using

Salkowski method (Brick *et al*., 1991). Luria-Bertani broth supplemented with tryptophan was inoculated with bacterial isolates and incubated for 7 days. After incubation period, supernatants were mixed with Salkowski's reagent. Appearance of pink color indicates IAA production and further measured at 530 nm by using spectrophotometer.

Tryptophan is natural exudate from roots of plants and has an impact on IAA concentration produced by bacteria and help maintain plant-microbe interaction (Bhutani *et al*., 2018). When tryptophan, a precursor of IAA, is present, bacteria produce increased amount of IAA. There have been reports on significant increase in IAA production by various bacteria on application of exogenous tryptophan (Patten and Glick, 2002; Bhutani *et al*., 2018).

The amount of IAA produced under *in vitro* conditions ranged from 2.50 to 34.83 µg ml^{-1}. Highest production of IAA was recorded by the isolate $KgNF_1$ (34.83 µg ml^{-1}), which was significantly superior to all other isolates. Islam *et al*. (2013) screened nitrogen fixers for their PGP activities and found all the strains had ability to produce IAA. Rana *et al*. (2011a) tested 10 isolates for IAA production and all the isolates produced significant amount of IAA and it ranged from 12.68 to 46.88 µgml^{-1}. Habibi *et al*. (2014) studied nitrogen fixing PGPR from rice rhizosphere and results revealed that out of 166 isolates 115 isolates positive for IAA production and it ranged between 0.04 µg ml^{-1} to 231 µg ml^{-1}.

Twenty N-fixers with multiple PGP traits were screened for the efficiency of nitrogen fixation under *in vitro* conditions, by micro-Kjeldahl method. Amount of N fixed during a period of 14 days by the selected isolates varied from 1.86 to 9.33 mg of N g^{-1} of sucrose utilized. Two isolates, $AkNF_3$ and $PkNF_4$ recorded the highest amount of N-fixed (9.33 mg of N) and this was statistically on par with isolates $KgNF_1$ and $KpNF_5$.

The isolates $AkNF_3$ and $PkNF_4$ were identified as *Bacillus* sp. and *Pseudomonas* sp. based on 16S rRNA gene sequencing. $KgNF_1$ was identified as *Pseudomonas putida* and $KpNF_5$ as *Alcaligenes faecalis*. Habibi *et al.* (2014) isolated PGPR from the

rhizosphere of crops and stated that strains of *Bacillus altitudinis*, *Pseudomonas monteilii*, and *Pseudomonas mandelii* formed associations with rice plants and fixed nitrogen. Akintokun *et al*. (2019) studied the nitrogen-fixing ability of the PGPR isolates from maize rhizosphere by Micro-Kjeldahl method and estimated the N fixation by *Pseudomonas syringae pv. syringae* strain HS191, *Bacillus cereus* strain 20UPMNR, and *Pseudomonas aeruginosa* strain ZSL-2 to be 6.3, 8.2, and 9.4 mg N $g^{-1}$of sucrose respectively.

Bacillus sp. $AkNF_3$ had multiple PGP traits, produced IAA, ammonia and siderophore. Nitrogen fixation ability of *Bacillus* sp. has been reviewed by many authors. Diverse species of *Bacillus viz. B*. *cereus*, *B. circulans*, *B. firmus*, *B. pumilus*, *B. licheniformis*, *B. subterraneous*, *B. megaterium*, *B. aquimaris*, *B. vietnamensis* and *B. aerophilus* are known to fix atmospheric nitrogen (Xie *et al*., 1998; Ding *et al*. 2005; Yousuf *et al*., 2017; Saxena *et al*., 2020). Yousuf *et al*. (2017) tested 19 different *Bacillus* strains and reported that 16 isolates had *nifH* gene. Ambrosini *et al*. (2016) showed highest nitrogenase activity of *B. cereus* among 42 different strains of *Bacillus* sp. isolated from sunflower rhizosphere. Singh *et al*. (2020) isolated PGPR from sugarcane rhizosphere and selected 22 most prominent isolates based on plant growth promotion traits, biocontrol and nitrogenase activity. All 22 the isolates were proved to harbor *nif*H gene and possessed nitrogen fixation ability and the most prominent strains were *Bacillus megaterium* and *Bacillus mycoides*.

Shultana *et al*. (2019) isolated PGPRs from rice rhizosphere and the isolate UPMRB9 (identified as *Bacillus tequilensis* based on 16S rRNA sequence) exhibited multiple PGP traits, such as nitrogen fixation, phosphate and potassium solubilization, IAA production as well siderophore and hydrolyzing enzyme production. The results of the present study are also in line with this finding, as the isolate *Bacillus* sp. $AkNF_3$ exhibited multiple PGP traits.

The isolate *Pseudomonas* sp. $PkNF_4$ also recorded highest amount of fixed nitrogen and possessed the ability to produce IAA, ammonia and siderophore. Mirza *et al*. (2006) identified a nitrogen fixing phytohormone-producing bacterial isolate from Kallar grass (strain K1) as *Pseudomonas* sp by 16S rRNA gene sequencing. Further

studies revealed that *Pseudomonas* strain K1 enhanced the yield of rice, as compared to non-*Pseudomonas* nitrogen fixing PGPR. Akintokun *et al*. (2019) also stated that nitrogen fixing *Pseudomonas syringae pv. syringae* also produced IAA.

A nitrogen fixing bacterium *Pseudomonas stutzeri* A15, isolated from rice rhizosphere, was used as crop growth promoting inoculum in China. Root colonization, invasion of rice root epidermis and nitrogen fixation abilities have been well-characterized (Rediers *et al*., 2009; Pham *et al*., 2016). Effect of *P. stutzeri* A15 inoculation on the growth of rice seedlings induced significant growth promotion and clearly pointing the potential of this bacterium as biofertilizer (Pham *et al*, 2016). Li *et al*. (2017) studied nitrogen fixing plant growth promoting *Pseudomonas* spp. from sugarcane rhizosphere and reported that two strains *P. koreensis* (CY4) and *P. entomophila* were very efficient in terms of enhancing plant growth and disease control.

Another nitrogen fixing bacterium *Pseudomonas putida* $KgNF_1$ produced highest amount of IAA (34.83 µg ml^{-1}) among the selected isolates and also showed antagonistic activity against rice pathogen X*anthomonas oryzae*. Laskar *et al.* (2013) reported nitrogen fixing ability of *Pseudomonas putida*. They reported that all diazotrophs isolated from paddy soils proved to have nitrogen fixing potential and among them seven isolates were better nitrogen fixers. *Pseudomonas putida* showed maximum ARA activity of 280 ethylene/h/mg protein. Habibi *et al.* (2019) isolated 98 indigenous PGPR from different rice cultivars in Afghanistan soils. Results revealed that the isolate *Pseudomonas putida* AF137exhibited highest nitrogenase activity (647.4 nmol ethylene h^{-1}). *Pseudomonas putida* showed high level of IAA (110 µg ml^{-1}) production and high levels of IAA excreted by *P. putida* was most consistent in enhancing plant growth (Meliani *et al*., 2017).

Pseudomonas spp. are known to be good biocontrol agents against plant pathogens. Among the different species *P. fluorescens, P. putida* and *P. aeruginosa* are the most well-characterized (Kandaswamy *et al*., 2019). Sharma and Dubey (2017) reported the inhibitory activity of *P. putida* CRN-09 against *Macrophomina phaseolina* under *in vitro* studies. In a study conducted on endophytic bacteria from eight different rice cultivars, four isolates *viz.* *Bacillus* sp, *Bacillus subtilis*, *Pseudomonas putida* and

Enterobacter sp. exhibited antagonistic activity against *Xanthomonas oryzae* (Yousefi *et al*., 2018). In the present study, nitrogen fixing isolate *Pseudomonas putida* $KgNF_1$ exhibited the ability to produce IAA, NH_3 and siderophore and expressed antagonistic activity against *X. oryzae*, proving its multiphasic PGP activities and the potential for exploiting as a bioinoculant.

The isolate $KpNF_5$ fixed atmospheric nitrogen to the tune of 8.40 mg g^{-1} of sucrose, which was on par with the highest amount of N fixed. This isolate also exhibited ability produce ammonia and siderophore and antagonistic effect against rice pathogens *Rhizoctonia solani* and *Xanthomonas oryzae*. 16SrRNA gene sequencing revealed that this isolate $KpNF_5$ was close to *Alcaligenes faecalis*. Akintokun *et al*. (2019) reported significant level of nitrogen fixing ability with respect to *Alcaligenes faecalis* strain P156 (11.4 mg N g^{-1} of sucrose utilized). Kakar *et al*. (2017) examined the biocontrol activities of five rhizobacterial strains and proved that *Alcaligenes faecalis* strains (P1 and BK1) were able to suppress the mycelial growth of *Rhizoctonia solani* and *Magnoportha oryzae*. Four PGPR isolates (KJKB5.4, LMTSA5.4, *Bacillus cereus* and *Alcaligenes faecalis* AJ14) exhibited plant growth promotion on rice seedlings and inhibited the growth of Xanthomonas *oryzae* pv. o*ryzae*, with diameter of inhibition zone greater than 11.50 mm (Rahma *et al*., 2019).

The isolate $AkNF_2$ obtained from the location Ambukuthi and identified as *Brevundimonas naejangsanensis* also possessed multiple PGP traits. This isolate fixed 7.93 mg N under *in vitro* conditions and was able to produce ammonia and siderophore. Kumar and Gera (2014) also reported that bacterial strain isolated from sugarcane rhizosphere showed multiple PGP activities including nitrogen-fixation, production of IAA and ammonia and. This isolate was identified as *Brevundimonas* sp. on the basis of phenotypic, biochemical, phylogenetic and 16S rDNA gene sequencing data. Naqqash *et al.* (2020) isolated four strains of *Bruvendimonas* from potato rhizosphere and evaluated their nitrogen fixing potential through acetylene reduction assay (ARA), which was further confirmed by presence of the *nif*H gene in their genome except one strain.

Nitrogen fixing isolate $MtNF_4$ fixed nitrogen to the extent of 7.00 mg g^{-1} of sucrose and had ability to produce ammonia. Further this isolate showed antagonistic effect against rice pathogen *Rhizoctonia solani*. This isolate was identified as *Burkholderia* sp. based on molecular characterization These results are in agreement with Miao *et al*. (2015) who reported that *Burkholderia* sp 7016 isolated from soybean rhizosphere exhibited nitrogenase activity, in addition to 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity and phosphate solubilizing ability. It also inhibited the growth of *Sclerotinia sclerotiorum*, *Gibberella zeae* and *Verticillium dahliae* and produced small quantities of IAA. In another study, antagonistic activity of *Burkholderia* isolates was tested by dual culture method and two isolates (*Burkholderia thailandensis* and *Burkholderia vietnamiensis*) inhibited two plant pathogens *Macrophomina phaseolina* and *Rhizoctonia solani.* (Khambalkar and Sridar, 2015). Araujo *et al*. (2013) reported that diazotrophic *B. vietmaniensis* strain AR1122 was a good biofertilizer candidate for inoculation of traditional rice varieties. All these findings indicate ability of *Burkholderia* as nitrogen fixer with multiple PGP activities its potential to be explored as a good bio-inoculant.

Phosphorus is one of the major essential macronutrients for plant growth and development. Phosphorus is abundant in many agricultural soils but large fraction of P is insoluble and relatively unavailable to plants in both acidic and alkaline conditions. Phosphate solubilizing bacteria (PSB) have the ability to solubilize insoluble forms of soil phosphorus (Bhattacharyya and Jha, 2012). Phosphate solubilization mechanism of PSB strains are associated with the release of inorganic acids or organic acids, mainly gluconic or keto-gluconic acids (Zhao *et al*., 2014). PSB facilitates P solubilization by several ways such as organic acid production, extracellular enzyme production, chelation and exchange reactions (Khan *et al*. 2009; Stephan *et al*., 2015). Bacteria hold foremost position among phosphate solubilizing microorganisms in soils than fungi and actinomycetes with a population of 1–50 % among total soil microbial populations (Alam *et al*., 2002). Phosphorus nutrition management in rice plants through microbial inoculants has gained attention, as it provides an eco-friendly and lowcost alternative for P nutrition.

In the present study, sixteen P solubilizing bacterial isolates were obtained from rice rhizosphere soils in Wayanad. These isolates were screened for phosphate solubilization on Pikovskaya's agar medium. This medium act as specific medium for selecting P solubilizers due to the presence of tricalcium phosphate (TCP) which is known for halo zone formation (Sharma, 2005).

Further these selected P solubilizers were screened *in vitro* for PGP activities such as production of IAA, ammonia, HCN and siderophore. All the isolates had at least one PGP activity (Table 65 and Fig. 6). Twelve isolates showed positive reaction for production of IAA and ammonia. Based on the intensity of pink colour developed, two isolates ($EdPS_5$ and $MtPS_2$) were identified as high IAA producers and seven as medium producers. None of the isolates produced HCN, but eight isolates were positive for siderophore. Batool and Iqbal (2019) studied phosphate solubilizing rhizobacteria from different crops and different regions and screened their PGP activities apart from P solubilization ability. They reported that 83.33% strains were IAA and ammonia producers while 66.66% strains were HCN and siderophore producers. All these characters of P solubilizers help in plant growth promotion and make them able to survive in harsh environmental conditions. In the current investigation, none of the isolates produced HCN.

None of the isolates exhibited antagonistic activity against *R. solani* and one isolate $MvPS_3$ was antagonistic to *X. oryzae*.

Table 65: Details on the PGP activities of phosphate solubilizers isolated from Wayanad

PGP trait	IAA production	NH3 production	HCN production	Siderophore production	Antagonism	
					R. solani	*X. oryzae*
No. of isolates positive	12	12	0	8	0	1
No. of isolates negative	4	4	16	8	16	15

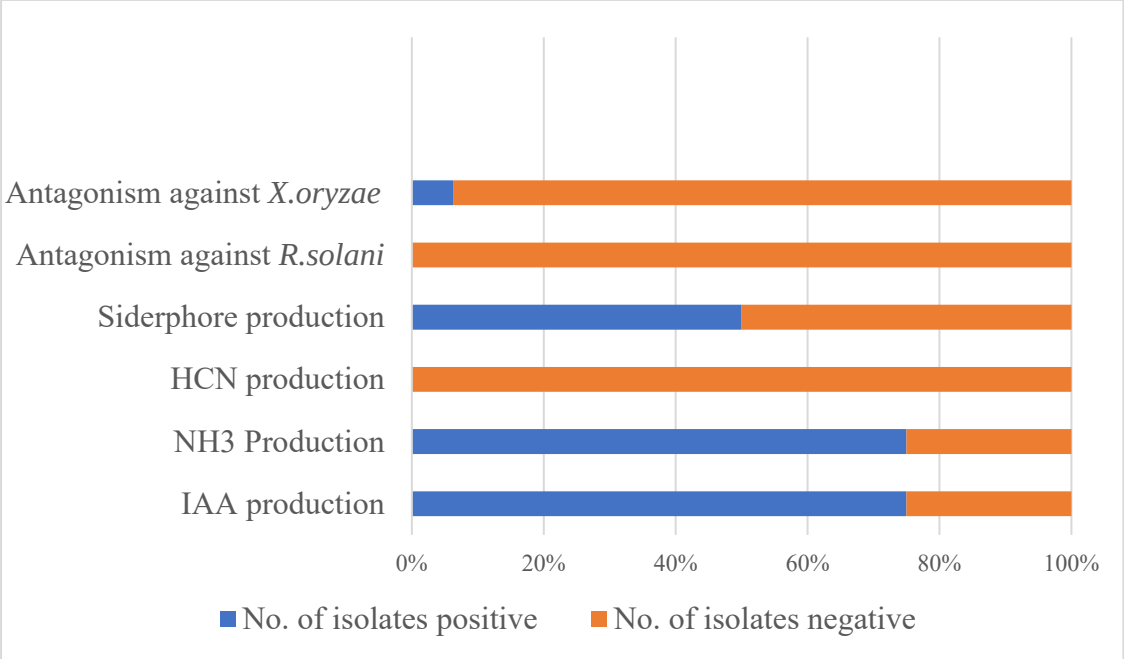

Fig.6: PGP and antagonism activities showed by selected phosphate solubilizers

All the isolates exhibited at least one PGP activity. Seven isolates ($AvPS_1$, $MtPS_2$, $AkPS_4$, $MvPS_4$, $MvPS_3$, $PkPS_1$, $PkPS_2$) exhibited three PGP traits and two isolates ($PkPS_3$, $PkPS_5$), two PGP traits (Table 66). Seven isolates exhibited only one PGP activity and there were no isolate which was lacking in any PGP trait.

Duarah *et al*. (2011) screened seven phosphate solubilizing bacteria for production of siderophore, plant growth regulators indole-acetic acid (IAA) and gibberellic acid and reported all isolates were positive for all three PGP activities. Panhwar *et al*. (2012) isolated sixteen phosphate-solubilizing bacteria (PSB) from aerobic rice grown in Penang Malaysia and reported all isolates were positive for production of IAA and among them four isolates were positive for production of siderophore. Apart from this, all isolates had antagonistic effect against *R. solani*. Batool and Iqbal (2019) isolated and selected ten phosphate solubilizing rhizobacteria from wheat rhizosphere and screened for PGP activities reported nine isolates were positive for IAA and ammonia production, eight were siderophore production and six were HCN production and exhibited multi-PGP traits.

Table 66: Details of phosphate solubilizers exhibiting multiple PGP traits

Number of PGP traits exhibited	No. of isolates	Details of isolates
Three	7	$MvPS_3$, $AvPS_1$, $MtPS_2$, $AkPS_4$, $MvPS_4$, $PkPS_1$, $PkPS_2$
Two	2	$PkPS_3$, $PkPS_5$
One	7	$ThPS_4$, $EdPS_4$, $EdPS_5$, $MtPS_5$, $PtPS_6$, $AkPS_5$, $AkPS_7$

IAA was determined spectrophotometrically at 530 nm using Salkowski's reagent and it ranged 2.66 to 19.80 µg ml^{-1}. Isolate $MtPS_2$ (19.80 µg ml^{-1}) had significantly highest production of IAA followed by isolate $EdPS_5$ (12.00 µg ml^{-1}). Batool and Iqbal (2019) reported production of IAA of P solubilizers and ranged between 25 to 120 µg ml^{-1} and suggested IAA production may highly varied with strains. Saranya (2016) isolated native phosphate solubilizing microorganisms from acidic soil of Kerala and screened for IAA production and reported four isolates had ability to produce IAA. Modi and Jacob (2017) screened five best phosphate solubilizing *Bacillus* isolates for production of IAA and reported all isolates were positive and ranged 34 to 54 µg ml^{-1}.

All the 16 isolates were subjected to screening for the efficiency of phosphate solubilization efficiency (PSE). PSE was calculated based on the diameter of the clearing zone around the colony on Pikovskaya's agar after seven days of incubation. Diameter of clear zone is indicative of the amount of phosphate solubilized. PSE ranged between 30.7 per cent to 127.7 per cent among tested isolates. Isolate $PkPS_1$ obtained from Pottankoli recorded maximum PSE (127.7 per cent) followed by $PkPS_3$ (123.8 per cent) and $AvPS_1$ (122.5 per cent). Saranya (2016) screened native phosphate solubilizers for solubilize insoluble P and reported PSE ranged between 106.6 per cent to 555.5 per cent among tested bacterial isolates. Mumtaz *et al*. (2017) studied efficacy of rhizobacterial isolates to solubilize insoluble P and results showed percent PSE ranged between 133 to 303 per cent. In another study, phosphate solubilizers isolated from the rhizosphere, rhizoplane and non-rhizosphere soil in ten different leguminous plants recorded PSE ranging from 13.04 to 85.71 per cent (Selvi *et al.*, 2017).

Assessment of PSE in solid medium is qualitative in nature. Estimation of the amount of phosphate solubilized is quantitatively estimated in liquid medium, which is more sensitive for detecting P solubilization by microorganisms. Therefore, the selected isolates were further subjected to quantitative estimation of the amount of P solubilized in liquid medium containing insoluble P by Mo blue method (Olsen *et al*., 1954). Results of the experiment indicated that the amount of P solubilized by the selected isolates in Pikovskaya's broth after fourteen days of incubation period ranged from 49.83 to 134.88 µg ml^{-1}. Among the tested P solubilizers $PkPS_1$, identified as *Bacillus megaterium* solubilized maximum amount of P (134.88 µg ml^{-1}). The same isolate was found to record highest PSE in the qualitative assay also. This was followed by $AkPS_4$ (121.33 µg ml^{-1}), identified as *Acinetobacter schindleri,* $AvPS_1$ (113.33 µg ml^{-1}), identified as *Achromobacter* sp., $PkPS_2$ (108.33 µg ml^{-1}) identified as *Burkholderia vietnamiensis* and $PkPS_3$ (103.83 µg ml^{-1}) identified to be *Providencia vermicola*.

Bacillus megaterium has been reported to be a good phosphatic biofertilizer by several workers. Kang *et al*. (2014) reported that *B. megaterium* solubilized phosphate and enhanced the growth and yield of mustard. Zheng *et al*. (2018) isolated inorganic phosphate-solubilizing bacteria from agricultural field and reported four *Bacillus megaterium* isolates Y95, Y99, Y924 and Y1412 released 134.49 µg ml^{-1}, 159.48 µg

ml^{-1},136.83 µg ml^{-1} and 138.68 µg ml^{-1} soluble P respectively when cultured in Pikovskaya's broth. They also suggested that *B. megaterium* solubilized more P than other genera, including *Streptomyces*, *Arthrobacter* and *Pseudomonas*. The results of the present investigation are in agreement with these reports, *Bacillus megaterium* PkPS1 solubilizing higher amount of phosphate *in vitro,* than other genera *Acinetobacter*, *Achromobacter*, *Burkholderia* and *Providencia*.

Another phosphate solubilizer obtained in the present study was $AkPS_4$, which fixed 121.33 µg ml^{-1} phosphorus in liquid medium and identified as *Acinetobacter schindleri*. Ogut *et al*. (2010) reported that phosphate solubilization potential of *Acinetobacter* sp. WR922 was relatively high *in vitro* and it significantly increased plant phosphorus content and dry matter accumulation in wheat. Rasul *et al*. (2019) isolated PGPR from rice rhizosphere and screened for phosphate solubilization and other PGP traits. The solubilized P ranged from 20 to 315 µg ml^{-1} with pH decline up to 4.2 in Pikovskaya's broth. Maximum P release and acidification was observed for *Acinetobacter* sp. Further rice seedling inoculation studies suggested that gluconic acid producing *Acinetobacter soli* was a potent P solubilizer for rice plants (Rasul *et al*., 2019). Wan *et al*. (2020) reported *Acinetobacter pittii* strain gp-1 had good performance for solubilizing $Ca_3(PO_4)_2$, $FePO_4$, and $AlPO_4$, and for digesting phytate, with corresponding P solubilizing levels 250.77, 46.10, 81.99, and 7.91 mg l^{-1} respectively. They further stated that soil-derived *Acinetobacter pittii* gp-1 possessing the ability to utilize multiple P sources could be a good candidate in improving soil fertility and quality (Wan *et al*., 2020).

Phosphate solubilizing isolate $AvPS_1$ exhibited multiple PGP traits (production of IAA, ammonia and siderophore) and solubilized 113.33 µg ml^{-1} P in Pikovskya's broth. 16S rRNA gene sequence analysis revealed that it was *Achromobacter* sp. Ahmad *et al*. (2006) studied PGP characters of free living diazotrophs and reported that isolate *Achromobacter* PNF_{11} had the ability to solubilize phosphate and produce IAA and siderophore. Vyas *et al*. (2018) isolated large number of bacterial isolates from the rhizosphere of oil seed palm and identified multifarious PGP traits (phosphate solubilization, production of IAA and ammonia) associated with isolate *Achromobacter xylosoxidense* AKDJ2.

Molecular characterization results revealed isolate $PkPS_2$ identified as *Burkholderia vietnamiensis.* Many studies revealed the efficiency of *Burkholderia* sp. as P solubilizer (Pande *et al*., 2017, Zhao *et al*., 2014, Singh *et al*., 2011). Park *et al*. (2010) observed *B. vietnamiensis* strain M6 able to solubilize different phosphate sources and has ability to produce 2-ketogluconic acids and amount of acid production increased with the age of bacterial culture. Miao *et al*., (2015) identified multitrait isolate 7016 as *Burkholderia* sp. based on 16S rDNA sequence analysis. This bacterium presented phosphate solubilizing ability, nitrogenase activity, and 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity and produced small quantities of indole acetic acid (IAA).

The phosphate solubilizer $PkPS_3$ was able to solubilize 103.83 µg ml^{-1} in liquid medium, and identified to be *Providencia vermicola*. It also exhibited PGP traits like. production of IAA and ammonia. Hussain *et al*. (2015) isolated two strains of *Providencia vermicola* from the rhizosphere of rapeseed and found promising in phosphate solubilization, auxin biosynthesis and N-fixation. Rana *et al*. (2011b) also stated that multiphasic characters of *Providencia* sp. such as production of ammonia, IAA, HCN, and siderophore and antifungal activity in addition to its phosphate and zinc solubilizing potential. Three species of *Providencia* namely *P. rettgeri*, *P. stuarti*, *P. vermicola,* isolated from vegetable rhizosphere from North Eastern Region of India were found to produce IAA, ACC deaminase and protease enzyme. In addition, the strains *P. rettgeri* CIAH-3MK and *P. vermicola* TRB-2 solubilized phosphate and produced siderophore (Gowtham *et al*., 2015).

Isolate $MvPS_3$, identified as *Burkholderia cepacia,* solubilized 77.66 µg ml^{-1} P in broth, and was a good producer of IAA and siderophore. This isolate exhibited antagonism against *Xanthomonas oryzae* in dual culture. Singh *et al*. (2011) isolated six diazotrophic bacteria from rice roots and one isolate *Burkholderia cepacia* RREM25 recorded appreciable level of nitrogenase activity, production of IAA and solubilization of phosphate. *Burkholderia cepacia* strain SCAUK0330, isolated from maize rhizosphere, exhibited phosphate solubilization of 452.19 µg ml^{-1} from insoluble $Ca_3(PO_4)_2$. This isolate was also antagonistic to nine pathogenic fungi (Zhao *et al*., 2014).

Phosphate solubilizing microorganisms generally convert insoluble phosphate into soluble forms through the process of acidification, chelation and exchange reaction. In the present study, initial pH of Pikovskaya's broth 7.20 was brought down by all the isolates. The minimum pH at the end of incubation period was recorded by the isolate $AvPS_1$ (4.10) and highest pH in broth recorded with $EdPS_5$ (6.66). The major mechanism of phosphate solubilization is through the production of acids by microorganisms and this could have led to pH drop. Many reports indicate that the major mechanism of phosphate solubilisation by microorganisms is through organic or inorganic acids (Goldstein, 1995 and Rashid *et al*., 2004). A study conducted with phosphate solubilizers revealed that there was a significant negative correlation between the amount of solubilized P in the medium and pH drop (Liu *et al.,* 2016).

Potassium is an essential macro nutrient and a major constituent of all living cells. Naturally soils contain larger amount of potassium however most of the K are unavailable for plants uptake. Potassium solubilizing bacteria (KSB) can solubilize potassium bearing minerals and convert insoluble K to soluble form of K available for plant uptake. Potassium solubilizing bacteria can release K from feldspar and alumina-silicate minerals by acidolysis, chelation, exchange reactions as well as the decomposition of organic matter and crop residues (Etesami *et al*., 2017). KSB provide an alternative technology to make potassium available for uptake by plants. Therefore, identification of efficient bacterial strains capable of solubilizing K minerals can conserve our existing resources.

In this present study, potassium solubilizing bacteria were isolated using Aleksandrov agar medium containing insoluble potassium bearing minerals (mica) as sole source of potassium. Potassium solubilizing bacteria are generally screened by a plate assay using Aleksandrov agar medium based on exopolysaccharide production (Rajawat *et al*., 2016).

A total of seven K-solubilizers were obtained from rice rhizosphere soils of ten selected locations. In primary screening, colonies producing clear zones around the colonies were considered as potassium solubilizers and were purified on Aleksandrov agar. After purification, four distinct isolates were selected for screening for PGP activities. These four isolates were evaluated for K solubilization efficiency by both

qualitative and quantitative methods. However very poor performances of PGP activities showed by K solubilizers and three isolates out of four showed production of ammonia and all other tested PGP activities were negative such as production of IAA, siderophore and HCN (Table 67).

Table 67: Details on the PGP activities of potassium solubilizers isolated from Wayanad

PGP trait	IAA production	NH3 production	HCN production	Siderophore production	Antagonism	
					R. solani	*X. oryzae*
No. of isolates positive	0	3	0	0	0	0
No. of isolates negative	4	1	4	4	4	4

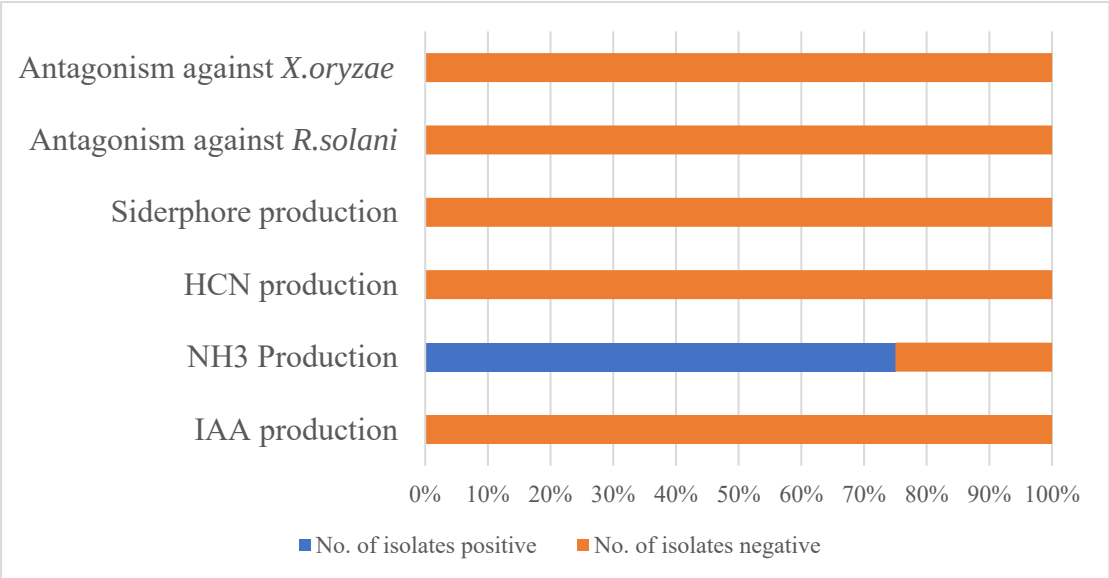

Fig.7: PGP and antagonism activities showed by selected potassium solubilizers

Table 68: Details of potassium solubilizers exhibiting multiple PGP traits

Number of PGP traits exhibited	No. of isolates	Details of isolates
One	3	$AkKS_1$, $MvKS_1$, $MvKS_3$
Nil	1	$MvKS_2$

Four isolates ($MvKS_1$, $MvKS_3$, $AkKS_1$ and $AkKS_2$) produced distinct clear zones on Aleksandrov agar. Potassium solubilization efficiency (KSE) of these isolates was calculated by measuring diameter of colony and clear zones. The KSE ranged between 51.13 to 142.69 per cent among the isolates. Prajapathi and Modi (2012) isolated fourteen strains of KSB from soil using mineral potassium as sole source and calculated potassium solubilization efficiency based on diameter of zone of clearance (D) and diameter of growth (d). The ratio D/d is ranged between 1.04 to 1.66.

Highest solubilisation efficiency of 142.69 per cent was observed in the isolate $MvKS_1$, which was later identified as *Microbacterium* sp. *Microbacterium* is a rod-shaped, non-spore forming bacteria that belongs to Actinobacteria, which are Gram positive bacteria with high G+C content. Diep and Hieu (2013) isolated 25 strains from Ha Tien mountain, Vietnam and results showed that eleven strain had ability to solubilized both P and K. Among them strain DNV16 was recorded 67.27 μg ml^{-1} released K in Aleksandrov broth and identified as *Microbacterium hominis*. *Microbacterium foliorum* isolated from tobacco rhizosphere in China also solubilized potassium (Zhang and Kong, 2014).

The isolate $MvKS_3$ recorded solubilisation efficiency of 111.18 per cent and this isolate was identified as *Acinetobacter calcoaceticus,* based on 16S rRNA gene sequencing. Kang *et al*. (2009) reported new strain *Acinetobacter calcoaceticus* SE370, ability to produced extracellular GA and also had phosphate solubilizing potential. The isolate lowered the pH of the medium during the process of K-solubilization. Bhattacharya *et al*. (2016) isolated several bacteria from salt pan and one of the

promising bacterial strains was identified as *Acinetobacter soli*. It showed potassium solubilizing potential under *in vitro* conditions.

The isolate $AkKS_1$ recorded a KSE of 79.34 per cent and this bacterium was identified as *Burkholderia cepacia. B. cepacia*, previously known as *Pseudomonas cepacia,* is a Gram negative, rod-shaped bacterium found in soil and water. It is multi-drug resistant and often associated with hospital specimens and considered as an opportunistic human pathogen. It has been reported to have phosphate and potassium solubilisation ability. Zhang and Kong (2014) also reported *Burkholderia cepacia* isolated from tobacco rhizosphere was capable of K-solubilization. Sun *et al*. (2020) isolated KSB from *M. micarantha* rhizosphere soil and evaluated solubilizing ability using Aleksandrov broth and revealed *Burkholderia* genus had the highest solubilization ability with 1.75 mg l^{-1}. Our results also lined up with these results and isolate $AkKS_1$ showed solubilization ability 1.81 µg ml^{-1} and was identified as *Burkholderia cepacia*.

Four isolates of K-solubilizers were screened for their ability to release of K in Aleksandrov broth containing mica as the sole source of K. Quantitative estimation of K solubilization was performed by flame photometry (Sugumaran and Janarthanam, 2007). Respective isolates were inoculated to the Aleksandrov broth and incubated for 14 days. After incubation period released K in the broth was measured by using flame photometry. Potassium released in the broth varied significantly among the different isolates and ranged from 1.89 to 4.19 µg ml^{-1}. Sun *et al*. (2020) screened KSB strains in Aleksandrov broth supplemented with K-feldspar powder as a sole source of K and found K solubilization ranged from 0.07 to 1.75 mg l^{-1}. In the present investigation, significantly higher K-solubilization (4.19 µg ml^{-1}) was observed in the isolate *Microbacterium* sp. $MvKS_1$ than the other three isolates. Parmar and Sindhu (2013), tested twenty K solubilizing strains for solubilizing K from mica on Aleksandrov broth supplemented with mica and it was ranged from 15 mg l^{-1} to 48 mg l^{-1}. However, in the present investigation, the isolates solubilized lower amounts of K in broth culture. In general, microbial solubilization of K was strongly influenced by pH, oxygen, the bacterial strain used, chemical composition of the potassium bearing minerals (Sheng and Huang, 2001). Sun *et al*. (2020) isolated KSB from *M. micarantha* rhizosphere soil

and evaluated solubilizing ability using Aleksandrov broth and revealed *Burkholderia* genus had the highest solubilization ability with 1.75 mg l^{-1}.

Microbial transformation of unavailable forms of soil zinc in plant available zinc is an important approach contributing to plant zinc nutrition. Plant can uptake zinc as divalent cation but only a very minor portion of soluble form of zinc present in soil solution (Kamran *et al*., 2017). Bacterial strains capable for utilizing different insoluble forms of zinc may be useful to make zinc available in the soil system.

Therefore, in this study, attempts were made to isolate and characterize zinc solubilizing bacteria. Total twenty-one colonies were picked up as zinc solubilizers from rice rhizosphere. In the primary screening process, colonies which were showing clear zones around the colonies on mineral salt medium with 1 per cent ZnO were considered as zinc solubilizing strains. Six isolates retained the ability to solubilize Zn even after three sub-cultures and these were selected for further studies of qualitative and quantitative assays of Zn solubilization.

All the selected isolates were screened for their growth promotion characters such as production of IAA, ammonia, HCN and siderophore (Table 69). Among selected isolates only two isolates ($MvZnS_1$ and $MvZnS_2$) exhibited two PGP characters *viz.* production of ammonia and siderophore. Very good yellow halo zones produced by isolates $MvZnS_1$ and $MvZnS_3$. Shaikh and Saraf (2017) who studied zinc solubilizing plant growth fungi and bacteria and found all tested isolates capable for producing ammonia and five isolates out of seven were positive for production siderophore. None of isolates were positive for HCN and IAA production in this study. All six isolates were screened antagonistic activity against *Rhizoctonia solani* and *Xanthomonas oryzae* and found no antagonism of isolates against tested pathogens.

Table 69: Details on the PGP activities of zinc solubilizers isolated from Wayanad

PGP trait	IAA production	NH3 production	HCN production	Siderophore production	Antagonism	
					R. solani	*X. oryzae*
No. of isolates positive	0	4	0	2	0	0
No. of isolates negative	6	2	6	4	6	6

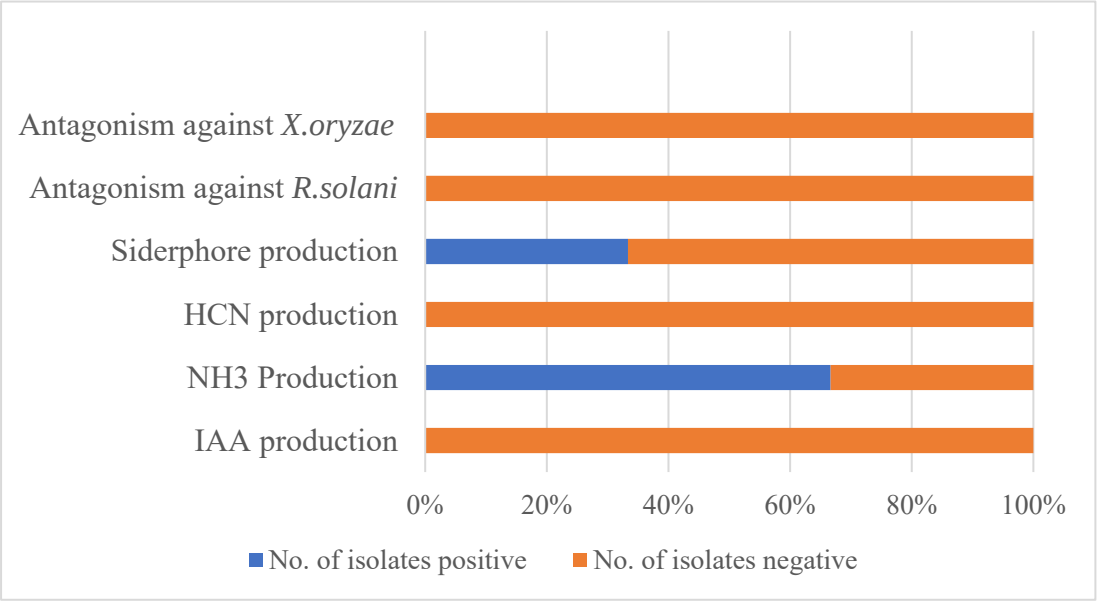

Fig.8: PGP and antagonism activities showed by selected zinc solubilizers

Table 70: Details of zinc solubilizers exhibiting multiple PGP traits

Number of PGP traits exhibited	No. of isolates	Details of isolates
Two	2	$MvZnS_1$, $MvZnS_3$
One	2	$ThZnS_2$, $PkZnS_3$
Nil	2	$AvZnS_1$, $AkZnS_4$

Zinc solubilizing efficiency was calculated by measuring colony growth and halo zone on mineral salt medium amended with 0.1% ZnO after seven days of incubation period. All the tested isolates solubilized zinc oxide with solubilization efficiency ranging from 27.30 to 143.98 per cent. The isolate $PkZnS_3$ (*Cytobacillus kochii*) recorded highest dissolution with 143.98 per cent, followed by $ThZnS_2$ (*Achromobacter marplatensis*) with 121.78 per cent solubilization. Zn solubilizing isolate $MvZnS_1$ which had the ability to produce siderophore, was identified as *Acinetobacter sp.* by 16S rRNA gene sequencing. *Acinetobacter* was reported as a good Zn solubilizer by earlier workers Vaid *et al*. (2013) and Gandhi and Muralidharan (2016).

Mumtaz *et a*l. (2017) revealed that out of seventy rhizobacterial isolates from maize rhizosphere, thirteen isolates had the potential to solubilize Zn and zinc solubilization efficiency in plate assay ranged from 181.6 to 296.0 per cent.

Plate assay has some limitations, it is not taken as authentic procedure to assess the zinc solubilization and mineralization (Abaid-Ullah *et al*., 2015). Therefore, rhizobacterial isolates showing good potential of zinc solubilization on agar plates were further tested in a mineral salts broth supplemented with insoluble ZnO at 0.1 per cent. The results revealed that *Cytobacillus kochii* PkZnS3 recorded highest amount of soluble zinc in the broth (18.53µg ml^{-1}), after 14^{th} days of incubation. This was statistically on par with *Achromobacter marplatensis* $ThZnS_2$ (solubilization 17.44 µg ml^{-1}) and *Acinetobacter* sp. $MvZnS_1$ (17.66 µg ml^{-1}). Saravanan *et al*. (2004) reported that solubilizing ability was more with in the zinc oxide than $ZnCO_3$ and isolate ZSB-S-2 (*Pseudomonas* sp.) solubilized 13.40 mg kg^{-1} of zinc in broth assay. Sharma *et al*. (2012) reported that inoculation of *Bacillus* isolates solubilized 3.65µg ml^{-1} zinc in liquid broth supplemented with ZnO after 14^{th} days of incubation. However great differences of zinc quantification among the strain reported by Abaid-Ullah *et al*. (2015) and *in vitro* quantification of zinc solubilization varied 8 µg ml^{-1} (*Serratia liquefaciens*) and 93 µg ml^{-1} (*Serratia marcescens*) ZnO as insoluble source. All the tested isolates lowered pH in broth after 14 days of incubation and the final pH varied from 6.12-6.80. pH drop was highest in *Cytobacillus kochii* PkZnS3 (6.12).

The genus *Cytobacillus* is closely related to *Bacillus.* Many authors reviewed zinc solubilization ability of *Bacillus* sp. and *B. aryabhattai*, *B. subtilis*, *B. thuringiensis* and *B. tequilensis* are well known (Sharma *et al*., 2012; Shakeel *et al*., 2015; Singh *et al.* 2017). Mumtaz *et al*. (2017) isolated rhizobacterial colonies from maize rhizosphere. These isolates were screened for their ability to solubilize zinc oxide. The results revealed that *Bacillus* sp. (ZM20), *Bacillus subtilis* (ZM63) and *Bacillus aryabhattai* (ZM31 and S10) had the ability to solubilize sources of zinc with multitrait PGP activities.

Further these six isolates were screened for zinc solubilization potential using $ZnCO_3$ as insoluble source. Halo zone indicated solubilization of Zn and two isolates out of six were solubilized insoluble $ZnCO_3$ with solubilization efficiency 77.77 per cent (*Cytobacillus kochii* PkZnS3) and 42.85 per cent (*Achromobacter marplatensis* $ThZnS_2$). Sharma *et al.* (2012) also reported only few isolates had the capacity of solubilization potential on zinc carbonate. Ability to solubilize different zinc insoluble sources is an added advantage of isolates for being exploited as biofertilizers. Further dissolution of Zn in mineral salt broth amended with $ZnCO_3$ as insoluble source recorded highest quantity of Zn (13.26 µg ml^{-1}) by *Cytobacillus kochii* $PkZnS_3$ and 10.33 µg ml^{-1} by *Achromobacter marplatensis* $ThZnS_2$. Goteti *et al*. (2013) screened ten zinc solubilizing bacteria for zinc solubility with insoluble ZnO and $ZnCO_3$ in plate and broth assays. Isolates P29 and B40 showed significant release of Zn in broth amended with $ZnCO_3$ (17 and 16.8 ppm) and ZnO (18 and 17 ppm) respectively. From the present study, it can be concluded that two isolates ($PkZnS_3$ and $ThZnS_2$) have the ability to solubilize both insoluble ZnO and $ZnCO_3$ and hence possess great potential for being used as a zinc biofertilizer.

A PGPR strain isolated from water canal, Egypt was identified as *Achromobacter marplatensis* B2. *In vitro* analysis revealed that the isolate was non-pathogenic and capable of producing IAA and gibberellin (GA3), and solubilizing rock phosphate (Abdel-Rahman *et al*., 2017). Oves *et al*. (2019) reported *Achromobacter xylosoxidense* strain which can survive well at high doses of heavy metal such as Cr, Ni, Cu and Zn and could solubilize tri calcium phosphate up to 363 µg ml^{-1}. In the present investigation, *Achromobacter marplatensis* $ThZnS_2$ showed potential to

solubilize both ZnO and $ZnCO_3$ and therefore could be used as potential strains for zinc solubilizing for biofertilizers.

Vaid *et al*. (2013) isolated three bacterial strains from a Zn-deficient rice-wheat field namely; BC, AX and AB belonging to the genera *Burkholderia* and *Acinetobacter*. Gandhi and Muralidharan (2016) isolated 143 zinc solubilizing bacteria from rice rhizosphere and screened for zinc solubilization ability *in vitro*, results revealed that two strains AGM3 and AGM9 were efficient zinc solubilizers and both strains belong to *Acinetobacter*. In this study, zinc solubilizing isolate $MvZnS_1$ identified as *Acinetobacter* sp. based on molecular characterization.

Two isolates of fluorescent pseudomonads, *viz.* $KpFP_1$ and $PkFP_2$, when screened for PGP traits, exhibited production of ammonia. The isolate $KpFP_1$ recorded multiple PGP traits including the production of ammonia, HCN and siderophore. $PkFP_2$ exhibited no other PGP trait than ammonia production. Sharma *et al*. (2014) isolated 30 indigenous fluorescent *Pseudomonas* from temperate zone of Himachal Pradesh and screened for plant growth promoting activities. Results revealed that two isolates (An-1-kul and An-13- kul) possessed multiple PGP traits. Subramanian and Satyan (2014) isolated 144 fluorescent pseudomonad isolates from rhizosphere soil samples and they were screened for PGP activities *viz.* production of IAA, ammonia, HCN and siderophore. All isolates were positive for ammonia production, 46 per cent were positive for HCN production and 40 per cent isolates were positive for siderophore production. Karimzadeh, *et al*. (2020) isolated fifteen fluorescent pseudomonads and characterized for PGP traits and reported that all isolates had ability to grow at different drought stress level and salinity level in addition, all the isolates were able to produce multiple PGP traits.

Once the PGP traits of all the isolates was assessed, the next phase of the experiment was to select potential isolates of PGPR for further characterization by cultural, morphological, biochemical and molecular tests and further tests for compatibility among the isolates to develop a consortium. Based on the efficiency in N-fixation, solubilization of phosphate, potassium and zinc, a total of twenty isolates from different groups of PGPR were selected as potential isolates. These included seven N-fixers ($PkNF_4$, $AkNF_3$, $KgNF_1$, $KpNF_5$, $KpNF_2$, $AkNF_2$ and $MtNF_4$), six phosphate

solubilizers ($PkPS_1$, $AkPS_4$, $AvPS_1$, $PkPS_2$, $PkPS_3$ and $MvPS_3$), three K-solubilizers ($MvKS_3$, $MvKS_1$ and $AkKS_1$), three Zn-solubilizers ($PkZnS_3$, $ThZnS_2$ and $MvZnS_1$) and one fluorescent pseudomonad.

Colony morphology of twenty potential isolates including shape, margin, elevation and colour of the colonies on nutrient agar were studied. Colonies of most of the isolates were circular, cream or creamy white in colour, raised with entire margins. Most of isolates were Gram negative and short rods and isolates $AkNF_3$, $PkPS_1$, $PkZnS_3$ and $MvKS_1$ were Gram positive rods. Gram staining technique is used as a tool for the differentiation of Gram-positive and Gram-negative bacteria, as a first step to determine the identity of particular bacteria.

Biochemical tests were carried out as per Cappuccino and Sherman (1992) for the identification of the bacterial isolates. All the bacterial isolates used in present study produced oxidase and catalase. Catalase enzyme helps in degrading hydrogen peroxide and releasing oxygen, which can be detected as effervescence. Bacteria having catalase activity are highly resistant to environmental, mechanical and chemical stress. Positive reaction in oxidase test indicates the presence of cytochrome oxidase, which is the enzyme that catalyses oxidation of reduced cytochrome by oxygen.

All bacteria must be utilizing the energy sources for their survival and every bacterium has its own collection of enzymes that enable it to utilize diverse carbohydrates. Competitiveness of microorganisms depends on number of energy sources utilized by them. Strains which can utilize diverse substrate can survive more competitively under different environmental conditions. In this study all the isolates were evaluated for the utilization of sugars (glucose, lactose, maltose, fructose, mannitol, dulcitol and sorbitol). Two isolates ($AkNF_3$ and $PkZnS_3$) were able to utilize all tested sugar sources, which indicated their wide adaptability of utilizing different sugars.

Twenty selected isolates were further subjected to molecular characterization by 16S rRNA gene sequencing by the Sanger's method. Woese and others, stated that phylogenetic relationships of bacteria, and, indeed, all life-forms, could be determined by comparing a stable part of the genetic code (Woese, 1985). The part of the DNA

now most commonly used for taxonomic purposes for bacteria is the 16S rRNA gene and exists universally among bacteria, includes regions with species-specific variability, which makes it possible to identify bacteria to the genus or species level by comparison with databases in the public domain (Clarridge, 2004). The conserved regions are used for designing primers, which will amplify the gene. In this study universal primers 8F and 1522R (Zhou *et al*., 1995) were used to amplify the 1500bp 16S rRNA. Molecular characterization provides added advantage that it is not affected by environmental factors or nutrient composition of the medium, unlike phenotypic characters. Therefore, 16S rRNA gene sequences allow bacterial identification that is more strong, reproducible, and precise than that obtained by phenotypic testing.

PCR amplification could not be obtained in two strains $KpNF_2$ and $KpPF_1$ and hence sequence could not be obtained. The sequences obtained from 18 isolates were analysed using BLASTn, to find out the similarity with sequences available in NCBI databank. GenBank (www.ncbi.nlm.nih.gov/genbank) is a comprehensive databse that contains publicly available nucleotide sequences for 370 000 formally described species and daily data exchange with the European Nucleotide Archive (ENA) and the DNA data Bank of Japan (DDBJ) ensure worldwide coverage (Benson *et al*., 2017).

The accession sharing maximum homology with the query sequence was considered for identification of the isolate. 16S rRNA gene sequence of all the 18 isolates were submitted to the GeneBank of NCBI, using BankIt tool, to get accession numbers. The tentative names assigned to each isolate based on the location and PGP trait was retained as strain number.

Nitrogen fixing isolates included $KgNF_1$, $AkNF_3$, $PkNF_4$, $KpNF_5$, $MtNF_4$ and $AkNF_2$ were identified and submitted to the GeneBank under accession numbers MW288152 (*Pseudomonas putida* KgNF1), MW288141 (*Bacillus* sp. AkNF3), MW269608 (*Pseudomonas* sp. PkNF4), MW307349 (*Alcaligenes faecalis* KpNF5), MW307253 (*Burkholderia* sp. MtNF4) and MW307254 (*Brevundimonas naejangsanensis* AkNF2).

Phosphate solubilizing isolates were deposited in the GeneBank under accession numbers MW290515 (*Bacillus megaterium* PkPS1), MW291534

(*Acinetobacter schindleri* AkPS4), MW290516 (*Achromobacter* sp. AvPS1), MW307345 (*Burkholderia vietnamiensis* PkPS2), MW356852 (*Providencia vermicola* PkPS3) and MW307346 (*Burkholderia cepacia* MvPS3).

Three potassium solubilizing isolates $MvKS_1$, $MvKS_3$ and, $AkKS_1$ were identified as *Microbacterium* sp. $MvKS_1$, *Acinetobacter calcoaceticus* $MvKS_3$ and *Burkholderia cepacia* $AkKS_1$ respectively. Sequences of isolates $MvKS_1$, $MvKS_3$ and, $AkKS_1$were deposited in the GeneBank under accession numbers MW295415, MW295416 and MW307347 respectively.

Zinc solubilizers $ThZnS_2$, $PkZnS_3$ and $MvZnS_1$ were identified as *Achromobacter marplatensis* $ThZnS_2$*, Cytobacillus kochii* $PkZnS_3$ and *Acinetobacter* sp. $MvZnS_1$and deposited under accession numbers MW284891, MW295426 and MW307349 respectively.

Phylogenetic analysis was carried out for studying the relationship of the isolates collected during the present study with already reported strains of relevant species. Phylogenetic trees were constructed using MEGA 7 software.

The phylogenetic tree showed two major clusters. Cluster 1 had two sub-clusters: sub-cluster 1 contained Gram negative bacteria *viz*. *Pseudomonas, Providencia, Alcaligenes* and *Burkholderia*. Sub-cluster 2 consisted of *Brevundimonas* and *Cytobacillus kochii*.

The second major cluster was again divided into two sub-clusters: sub-cluster 1 which consisted of all Gram positive isolates like *Bacillus* and *Microbacterium*. However, two Gram negative bacteria *(Brevundimonas)* also were grouped under this sub-cluster, as a small sub-cluster. Sub-cluster 2 was again divided into two clusters: one containing *Burkholderia, Alcaligenes* and *Achromobacter* and the second one consisting of *Acinetobacter, Pseudomonas* and *Providencia*.

The accession sharing maximum homology with the query sequence in the NCBI database was considered for identification of the isolates. However, in the phylogenetic tree, a few isolates did not cluster with the similar sequences obtained from GeneBank viz, *Pseudomonas* sp. $PkNF_4$ clustered with *Providencia vermicola*

PkPS$_3$, *Cytobacillus kochii* PkZnS$_3$ clustered with *Brevundimonas naejangsanensis* AkNF$_2$, *Alcaligens faecalis* KpNF$_5$ with *Burkholderia cepacia* MVPS$_3$. More investigation will be required to find out the possibility of occurrence of new strain/species of this genus.

In the present study, based on the efficiency of nitrogen fixation, P, K and Zn solubilization and considering their PGP activities, 10 efficient PGPR isolates were selected from each functional group. Three nitrogen fixers with multitrait PGP activities AkNF$_3$ (*Bacillus* sp.), PkNF*4* (*Pseudomonas* sp.) and KgNF$_1$ (*Pseudomonas putida*) and three most efficient phosphate solubilizers with multitrait PGP activities PkPS$_1$ (*Bacillus megaterium*), AkPS$_4$ (*Acinetobacter schindleri*) and AvPS$_1$ (*Achromobacter* sp.) were selected for consortium preparation. Two potassium solubilizers MvKS$_1$ (*Microbacterium* sp) and MvKS$_3$ (*Acinetobacter calcoaceticus*) were selected. Isolate ThZnS$_2$ (*Acromobacter marplatensis*) and PkZnS$_3$ (*Cytobacillus kochii*) were selected as efficient zinc solubilizers.

Promising isolates were subjected to compatibility test by using cross streaking method followed by dual culture method and confirmed their compatibility of each other.

Three PGPR based consortial were formulated and each consortium included five bacterial isolates: two nitrogen fixers, one P solubilizers, one K solubilizers and one Zn solubilizers. Consortium 1 included *Bacillus* sp. AkNF3, *Pseudomonas* sp. PkNF4, *Achromobacter* sp. AvPS1, *Microbacterium* sp. MvKS1, *Achromobacter marplatensis* ThZnS2. Consortium 2 included *Bacillus* sp. AkNF3, *Pseudomonas putida* KgNF1, *Bacillus megaterium* PkPS1, *Acinetobacter calcoaceticus* MvKS3 and *Cytobacillus kochii* PkZnS3. Consortium 3 was made from *Pseudomonas* sp. PkNF4, *Pseudomonas putida* KgNF1, *Acinetobacter schindleri* AkPS4, *Microbacterium* sp. MvKS1 and *Cytobacillus kochii* PkZnS3.

Raja *et al*. (2006) studied the effect of individual and microbial consortium *viz., Azospirillum lipoferum*-Az 204, *Bacillus megaterium* var. phosphaticum and *Pseudomonas fluorescens* Pf-1 on rice root exudates and plant growth under hydroponic culture conditions. Results revealed that bioinoculants consortium

improves the colonization potential, sustainability within the inoculants and enhances crop growth positively by a multitude of synergistic mechanisms when compared to single inoculants application. Kumar *et al*. (2016) investigated the effects of four rhizobacterial strains *viz*., UKA-24 (*Rhizobium radiobacter*), UKA-27 (*Bacillus pumillus*), UKA-72 (*Stenotrophomonas maltophilia*) and AKA-1(*Pseudomonas putida*) on the growth promotion of basmati rice cultivar Pusa Sugandha 4. Results reported that rhizobacterial consortia (UKA-24, UKA-27, UKA-72 and AKA -1) were more effective bio-inoculants than single inoculant.

In order to make rice cultivation sustainable and less dependent on chemical fertilizers, it is important to use PGPRs that can biologically fix nitrogen, solubilize phosphorus and produce growth promoting substances like indole acetic acid (IAA) that can contribute to enhancement of growth and yield. The consortial formulations prepared in the study were evaluated in pot culture experiment, followed by field experiment, for enhancing the growth and yield of rice crop.

Pot culture experiment was carried out with rice (local variety Valichoori) as the test crop. KAU commercial formulation of plant growth promoting rhizobacteria, sold under the name "PGPR Mix-1" was used as reference biofertilizer. KAU commercial formula of biofertilizer PGPR Mix-1 contains two nitrogen fixers (*Azotobacter chroococcum* and *Azospirillum lipoferum*), phosphate solubilizing bacterium (*Bacillus megaterium*) and potassium solubilizing bacterium (*Pseudomonas* sp.). Recommended dose of fertilizer was applied according to the soil test data and N, P, K were supplied by urea, rock phosphate and muriate of potash (MOP) respectively. The effect of integrated application of biofertilizer along with inorganic fertilizer was assessed and the impact on soil microbial population, soil physio-chemical parameters and rice plant growth and yield attributes were assessed.

Soil used for the pot culture was analyzed for physico-chemical properties before rice planting and showed that soil was slightly acidic in nature with low organic and potassium content. Available soil phosphorus content was medium and zinc content was sufficient in soil as per critical limits of Tandon (2004). Normally laterite soils of Kerala are acidic in nature (Chandran et al., 2005). The soil of Wayanad district is mainly loamy type and generally shows very high content of organic matter and slightly

acidic pH (Silja, *et al*., 2008). In contrast to that our soil sample showed low level of organic matter content.

With respect to the microbial population, total bacteria, nitrogen fixing bacteria, P, K and Zn solubilizers were evaluated in soil samples at monthly interval up to the fourth month after transplanting.

Data on the population of total bacteria on nutrient agar at different intervals is presented in Figure 9. During the second month an increased population was observed all the treatments except T_9. This may be due to the enhanced availability of roots exudates and growth promoting substances during active growth stage of the rice crop. The treatments with combined application of biofertilizers and inorganic fertilizers showed higher bacterial population compared to the uninoculated treatments, throughout the growth period and it clearly indicated the beneficial effect of bioinoculants on the microbial population in the rhizosphere.

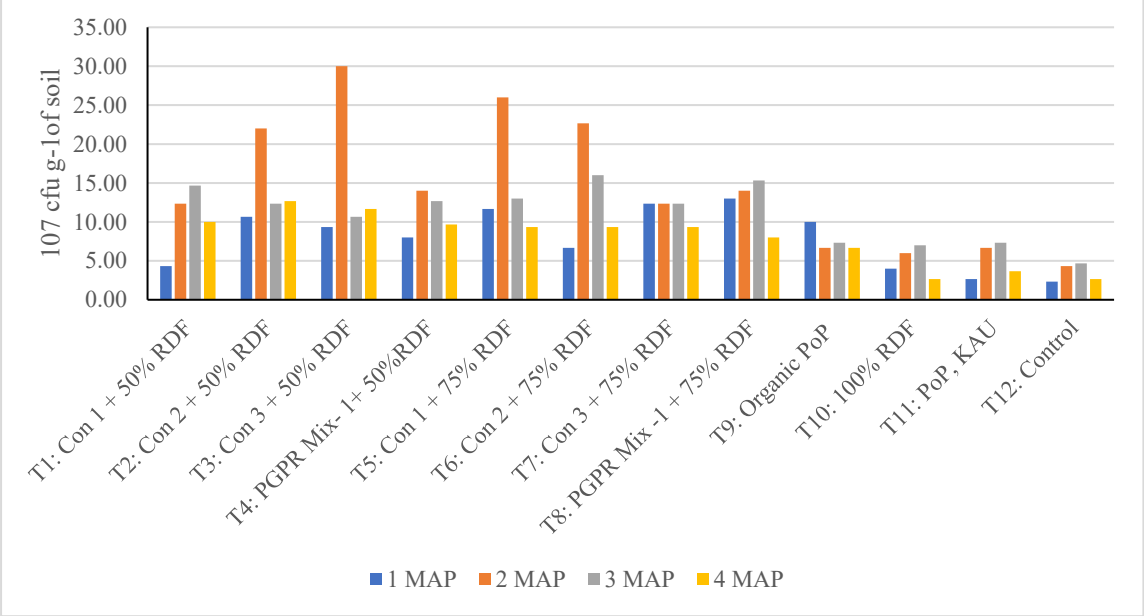

Figure 9. Comparison of total bacteria population in different treatments at monthly interval after transplanting

Bacteria is an important component of soil microflora that participate in soil nutrient cycling and play a significant role in maintaining the stability of the entire soil ecosystem. Liang *et al*. (2020) studied the effect of different fertilizers on rhizosphere

bacterial community diversity and structure. Results revealed that microbial diversity was higher in soil which received bio/ organic fertilizer than soils without biofertilizer application or with N-fertilizer alone. Shanthpure *et al*. (2019) analysed the long-term effect of fertilizer regimes *viz.,* organic inputs (ONM), integrated inputs (INM) and inorganic (INF) inputs on bacterial community in paddy soils using NGS sequencing of 16S rRNA genes. The results revealed that long term application of organic and integrated fertilizers clearly increased the beneficial microbes such as *Bacillus, Arthrobacter, Bradyrhizobium, Frankia* etc. in soil and continuous chemical fertilizer suppressed the growth of *Rhizobium, Azospirillum, Pseudomonas* and *Burkholderia* in rhizosphere soil.

Statistical analysis revealed that nitrogen fixers were significantly different at different intervals and in general, higher population of nitrogen fixers was observed after two months of transplanting irrespective of treatments, except T_4 as shown in Figure 10. Thereafter, population of nitrogen fixers declined. Highest population of N fixers ($27x10^6$ cfu) was observed in T_6 at two MAP and this was statistically on par with T_7, T_1, T_2 and T_3. The population of nitrogen fixers was comparatively low in T_8, when compared to the other bio-inoculants at 75% RDF including T_5, T_6 and T_7. Treatments T_5, T_6 and T_7 contained indigenous nitrogen fixers isolated from rice rhizosphere, whereas T_8 consisted commercial formula of biofertilizer (PGPR mix-1) with 100% RDF.

Population of N-fixers was higher in treatments with bio-inoculants at 50 per cent RDF, than 75 per cent RDF. This is in agreement with Wu *et al*. (2005) who noticed in preliminary studies, the propagation of *A. chroococcum* was seriously inhibited when the ammonium N concentration exceeded 200 mg kg^{-1}. In this study, results showed successful adaptation and proliferation of the indigenous nitrogen fixing isolates in natural rhizosphere habitats.

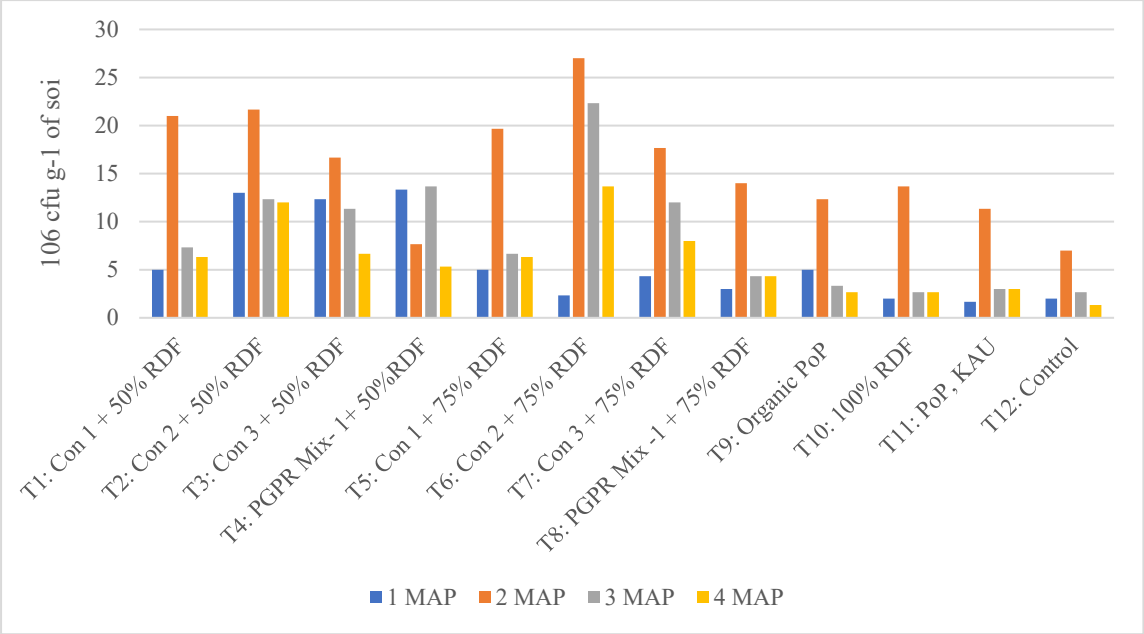

Figure 10. Comparison of the population of nitrogen fixers in different treatments at monthly interval after transplanting

The population of phosphate solubilizers was higher in all treatments at one and two MAP than other months irrespective of treatments except treatments T_1 and T_8. Population of P solubilizers drastically declined three and four MAP in treatments such as T_9, T_{10}, T_{11} and T_{12}. It implies high indigenous population of P solubilizes in rhizosphere soils at early stages of rice but latter stages of rice plants had lower population of P solubilizers. Results showed that all the treatments which had application of bioinoculants, maintained comparatively high population of P solubilizers throughout the growth period.

Two indigenous rhizospheric phosphate solubilizing isolates PSB 12 (*Gluconacetobacter* sp.) and PSB 73 (*Burkholderia* sp.) were examined for their growth enhancement potential of rice (Jyothi PTB 39) under pot culture assay and results showed that receiving both the inocula and rock phosphate (RP) significantly increased microbial count in soil when compared to uninoculated pot soils with RP (Stephen *et al*., 2015). The present study indicates that native P solubilizers are more advantageous, because of their high adaptability.

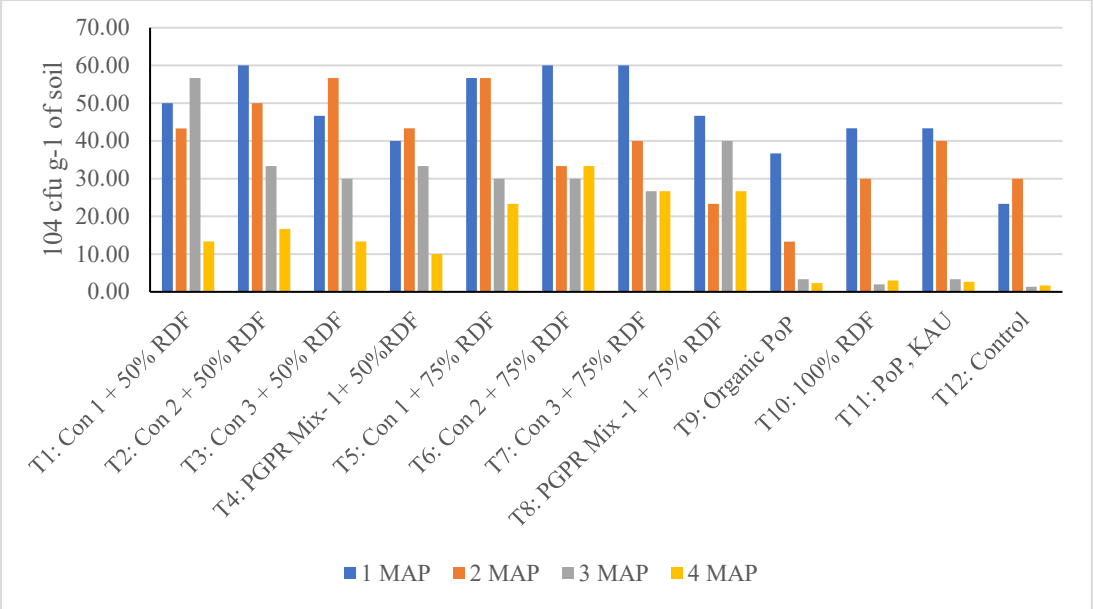

Figure 11. Comparison of phosphate solubilizing bacterial population in different treatments at monthly interval after transplanting

In general, the population of potassium solubilizers was significantly higher in treatments which consisted of consortial formulation along with inorganic fertilizers at two, three and four MAP compared to uninoculated treatments including T_9, T_{10}, T_{11} and T_{12} as shown in Figure 12. Highest K population was recorded in T_6 at two and third MAP.

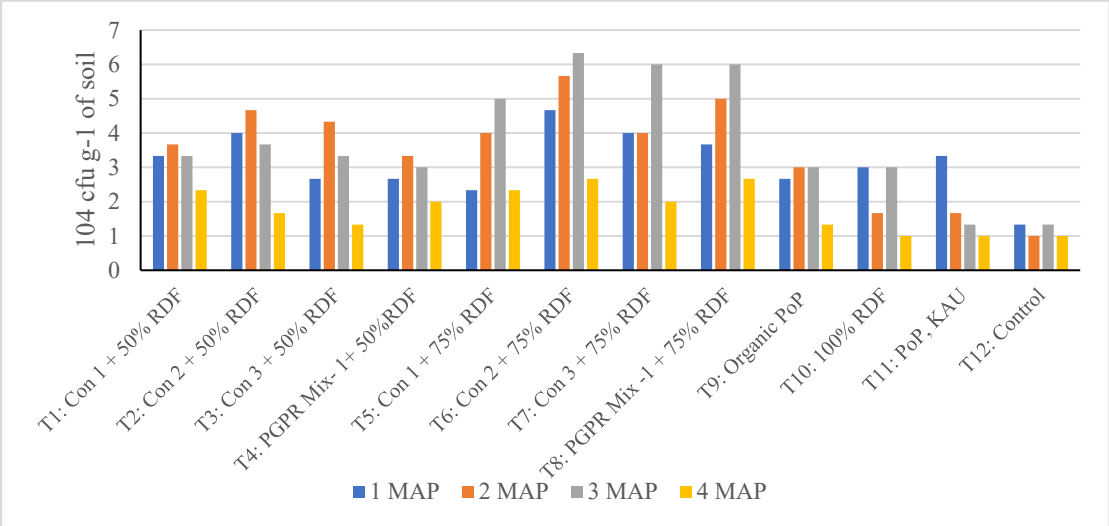

Figure 12. Comparison of potassium solubilizing bacterial population in different treatments at monthly interval after transplanting

Population of zinc solubilizing bacteria up to fourth MAP are shown in Figure 13. Highest population of zinc solubilizers ($26.66x10^3$) was recorded in treatment T_2 at one MAP and in T_6 at one and two MAP. However, this was on par with other treatments with bioinoculants. The population was higher in treatments that received bio-inoculants, compared to the treatments with no bio-inoculants, even at 4 MAP. Hence the study revealed that the inoculation of zinc solubilizers helps to maintain the population zinc solubilizers at higher levels, throughout the growth stage of rice plants than uninoculated treatments. At two MAP, highest population was recorded in treatment T_6 followed by T_2.

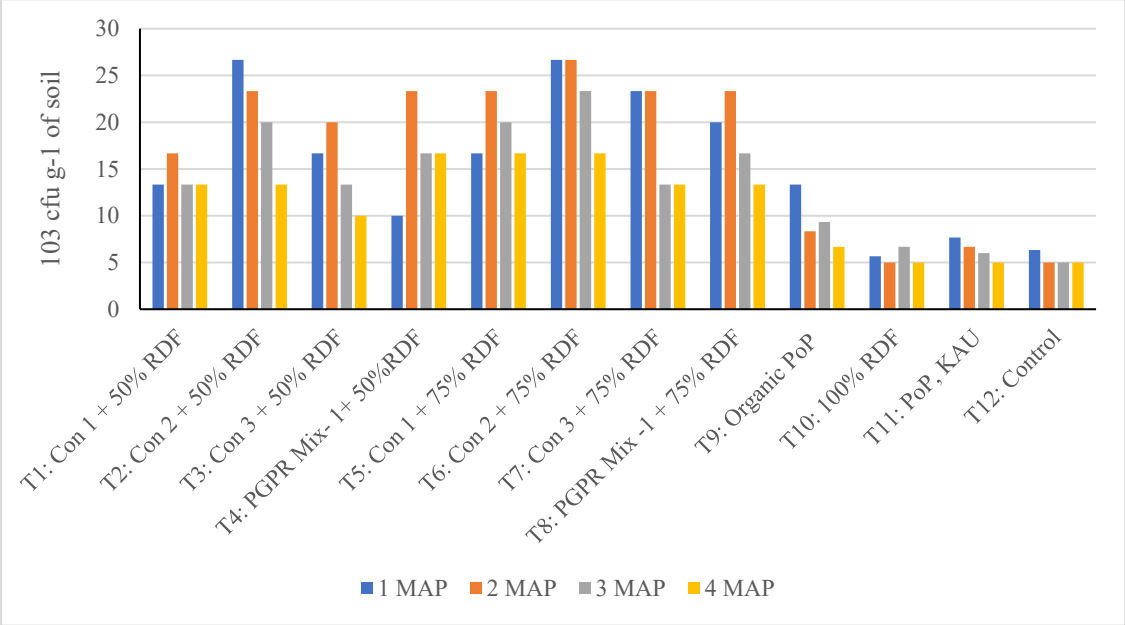

Figure 13. Comparison of zinc solubilizing bacterial population in different treatments at monthly interval after transplanting

The results of the investigation revealed that combined application of PGPR based biofertilizer with 50 per cent or 75 per cent of the recommended dose of fertilizers maintained high level of PGPR population than uninoculated treatments. It implies that application of bioinoculants helps to maintain rhizosphere bacterial population at significantly higher levels, which will contribute to soil and plant health. Kuan *et al*. (2016) reported PGPR inoculants increased rhizobacterial colonization around maize roots and their surrounding soils compared to uninoculated control at harvest and

inoculation with *Klebsiella* sp. Br1 and *Acinetobacter* sp. S3r2 gave significantly higher bacterial population in rhizosphere around 250-350% over the uninoculated control.

It was hypothesized that multi-strain biofertilizer consortium would promote plant growth and yield through BNF, solubilization of insoluble minerals and other beneficial effects of PGPR. To determine the effect of locally isolated PGPR based consortia on growth and yield of rice, growth parameters were recorded at maximum tillering stage. Grain and straw yields were recorded after harvesting.

Highest number of tillers (13 tillers/pot) were recorded in treatments T_{11}: POP (KAU) which had 100% RDF with organic manure and T_6: Consortium 2 + 75% RDF. These two treatments were statistically on par with treatments T_7: Consortium 3 + 75% RDF, T_5: Consortium 1 + 75% RDF, T_{10}: 100% RDF and T_2: Consortium 2 + 50% RDF. PGPR Mix-I recorded significantly lower number of tillers than consortia of native isolates.

Number of panicles/pots was highest (8.3 panicles/pot) in T_{11}: POP (KAU) and this was statically on par with T_2, T_5, T_6 and T_7. This result revealed that Consortium 2 with 50% RDF was performing as good as other native consortia with 75 % RDF, indicating the superiority of Consortium 2. Consortia of native PGPR at 75 % RDF performed as good as PoP recommendation. Results indicate possibility of reducing 25% inorganic fertilizer without affecting the growth of plants.

Number of grains/panicles, filled grain percentage and yield/pot were significantly different among the treatments. The highest no. of grains per panicle, per cent filled grains, grain yield and straw yield were recorded in T_{11} (PoP). However, these were statistically on par with a few other treatments also. In the case of grain yield, T_6 was statistically on par with T_{10} and T_{11}. This indicated that Consortium 2, in the presence of 75% RDF was able to perform as good as 100% inorganic fertilizer applied treatments (T_{10} and T_{11}). This also brings out the possibility of replacing 25% of the recommended dosage of chemical fertilizer, with integration application of PGPR based consortium 2 without affecting growth and yield of plants.

Tan *et al*. (2015) assessed PGPR and rhizobia as multi-strain biofertilizers on growth and yield of rice plants and reported that mixed inoculum significantly promoted plant and root growth, tiller numbers, plant dry weight and nutrient accumulation with minimal usage of nitrogen fertilizer. The results of the present investigation are in agreement with the report by Elekhtyar (2016) that PGPR biofertilizer (*Pseudomonas fluorescens* + *Bacillus subtilis* + *Azospirillum brasilense*) with 75% nitrogen fertilizer was able to improve growth and yield of rice. PGPR contributed in reducing 25% of chemical nitrogen fertilizer as well as minimizing cost of inputs and environmental pollution.

In the present study, plant content of nutrients including nitrogen, phosphorus, potassium and zinc were analysed to assess the effect of PGPR based biofertilizer on plant nutrient content. Nitrogen content of plant samples was highest (1.342%) in T_{11} (PoP), which was statistically on par with treatments T_6 and T_7 (Consortia 1 and 2 at 75 % RDF). Phosphorus content was also highest in T_{11}. Potassium content was highest in T_7 (Consortium 3+ 75 % RDF) and this was statistically on par with T_6 (Consortium 2 + 75 % RDF) and T_{11} (PoP). Zinc content of plant sample, revealed that all plants treated with integrated application of biofertilizer with inorganic fertilizer had high Zn content than plants treated with inorganic fertilizer alone without inoculum.

The above results are in agreement with Rana *et al*. (2012) who studied enhancement of nutrient uptake of wheat through bacterial consortia in pot culture experiment. The treatment involving bacterial consortia (*Bacillus* sp. + *Providencia* sp.) with two third of N, full dosage of P and K recorded highest value of P and N contents in plants, compared to plants which received full dosage of N, P and K fertilizers.

Shakeel *et al*. (2015) inoculated two zinc solubilizing strains (*Bacillus* sp. and *Bacillus cereus*) on Basmathi rice varieties and found that consortium of two indigenous inoculants had Zn translocation to the rice grains in similar way as that of chemical Zn fertilizer.

Kumar *et al*. (2016) suggested that rhizobacterial consortia (*Rhizobium radiobacter* + *Bacillus pumillus* + *Stenotrophomonas maltophila* + *Pseudomonas*

*putid*a) were more effective bio-inoculants than single inoculant and could be used effectively for enhancement of growth promotion and nutrient uptake in rice plants. Uptake of nitrogen, phosphorus and potassium were positively correlated with plant height, shoot and root dry weights (Kumar *et al*., 2016).

Results of Khangahahi *et al*. (2019), revealed that native potassium solubilizers (*Pantoea agglomerans, Rahnella aquatilis and Pseudomonas orientalis*) inoculations increased K uptake in the grain, especially when they were applied in combination with 1/2 K chemical fertilizer. Bakhshandeh *et al*. (2017) reported that K uptake by plant increased as influenced by bio-inoculation.

In the present investigation, soil-physico chemical properties were analysed at harvesting stage to find out integrated application of PGPR consortial effect of soil quality improvement. Soil pH was not significantly different among the treatments. However, EC was significantly higher in soil treated with 100% RDF. Soil organic carbon was significantly high in treatments T_2, T_9 and T_6 than other treatments and microbial biomass carbon was highest soil treated with T_6 which had integrated application of PGPR based consortium 2 + 75% RDF. This was statistically on par with T_7 (Consortium 3 + 75% RDF). Total bacteria count in T_6 also significantly highest at two, three and four MAP. Therefore, it suggested increased proliferation of soil microbes led to increase of microbial biomass carbon in T_6.

Available soil nitrogen was significantly higher in T_6 (Consortium 2 + 75% RDF) and this was on par with T_2 (Consortium 2 + 50% RDF), T_7 (Consortium 3 + 75 % RDF) and T_{11} (PoP). This indicated that Consortium 2 at 50 % and 75% RDF was comparable to PoP, with respect to N nutrition. Available P was highest in T_{11} (PoP) and this was on par with T_4 (PGPR Mix 1 + 50% RDF), T_6 (Consortium 2 + 75% RDF), T_8 (PGPR Mix I + 75% RDF) and T_{10}. Available zinc content was high in integrated application of PGPR based Consortium 2 + 75% RDF than 100% RDF applied uninoculated soils.

The results of the present investigation revealed that soil organic carbon, available microbial biomass carbon, N, K and Zn at harvest stage significantly

increased due to the integrated inoculation of PGPR based consortia with application of 75% RDF which was statistically on par with 100% RDF treated soils and implies reduction of 25% of N, P, and K fertilizer compensate by applying effective PGPR inoculants. Meena *et al*. (2014a) also stated soil organic carbon, available N, P, K, Zn, Fe and Cu in soil at crop harvest stage significantly increased due to the integrated inoculation of PGPR.

Shen *et al*. (2016) evaluated complex inoculum (*Bacillus amyloliquefaciens, B. pumilus, B. circulans*) on growth of kiwi fruits and results indicated that the complex inoculant with half rate of chemical fertilizer had the capacity to increased soil available N, P, and K.

Collectively, these results indicate that all the three PGPR based consortia which had indigenous isolates showed the capacity to increase soil available N, P, K and Zn. Combined application of 75% of chemical fertilizers with PGPR based consortia (five indigenous bacterial isolates) may have the potential to replace the amount of chemical fertilizer needed to obtain optimum levels of essential nutrients. Importantly, soil N, P and K concentrations with 100% RDF statistically on par with integrated application of consortial with 75% RDF.

Based on the efficiency to improve the growth and yield of rice crop in pot culture experiment, two best consortia were selected for further evaluation of growth and yield of rice crops in field conditions. Consortium 2 was selected as the best consortium among three tested consortia and which contains, *Bacillus* sp. AkNF3, *Pseudomonas putida* KgNF1, *Bacillus megaterium* PkPS1, *Acinetobacter calcoaceticus* MvKS3 and *Cytobacillus kochii* PkZnS3. Consortia 3 was selected as second-best consortia and it contained *Pseudomonas* sp. PkNF4, *Pseudomonas putida* KgNF1, *Acinetobacter schindleri* AkPS4, *Microbacterium* sp. MvKS1 and *Cytobacillus kochii* PkZnS3.

Synergistic effects of combined inoculations of PGPRs have also been reported in various crops, for example rice (Lavakush *et al*., 2014; Vaid *et al*., 2014; Tan *et al*., 2015; Kumar *et al*., 2016) wheat (Rana *et al*., 2012), pepper and cucumber (Han *et al*.,

2016) kiwifruit (Shen *et al*., 2016) and further stated that combined inoculant consistently performed better than single inoculum.

Field experiment was carried out for further evaluation of two promising native PGPR based consortia on rice growth and yield under field conditions, at RARS, Ambalavayal, using traditional rice variety Valichoori as the test crop. Five treatments were used for the experiment, *viz* T_1: Consortium 2 + 75% RDF, T_2: Consortium 3 + 75% RDF, T_3: PGPR- Mix 1 + 75% RDF, T_4: 100% RDF and T_5: Farmers' practice (farm yard manure at 5.00 t ha^{-1}).

Farmers in the Wayanad region generally use farmyard manure, compost, mango leaves, and jack fruit leaves as organic fertilizers for rice cultivation. In this study, farmyard manure was applied at the rate of 5 t ha^{-1} as farmer's practice.

Rice field soil sample was analyzed for initial microbial count and physico-chemical properties before planting. Results showed that the soil was acidic in nature with low content of nitrogen, phosphorus and potassium and sufficient zinc content.

During the crop growth period in the field from July to December 2019, temperature, rainfall and humidity were recorded. However, there was not much variation in the temperature and relative humidity during the crop growth period in the field. One week after transplanting, heavy rainfall was recorded and this was followed by dry spell.

With respect to the microbial population, total bacteria, N, P, K and Zn solubilizers were enumerated on selective media, at one month interval, up to four months, to assess the impact of PGPR application on soil microflora. Total bacterial counts were significantly different among treatments during the growth period of rice. Results showed that T_4 had lowest population at every month tested. All the other treatments recorded significantly higher population than T_4 (100% RDF) (Figure 14). This could be due to the application of PGPR and/ or organic manure in T_1, T_2, T_3 and T_5 and indicated that application of PGPR and organic manure helps in maintaining higher bacterial population in soil. Ramalakshmi *et al*. (2008) studied the influence of biofertilizer on soil physico-chemical and biological properties and reported that the total bacteria increased significantly in all treatments including the uninoculated, but in

the biofertilizer inoculated plots, the increase was relatively higher than uninoculated soil. Nakho and Dkhar (2010) reported that organically treated plot had a higher bacterial population than inorganically treated plots and the results of the present investigation are in agreement with their observations. In the present study it was noticed that in all the treatments, total population of bacteria decreased during third and fourth months. This could be attributed to the better availability of nutrients in the rhizosphere through root exudates up to the tillering stage. Sahu *et al*. (2016) reported that the population of rice rhizosphere microflora was a function of the physiological requirements of the plant at various developmental stages of the crop. They reported an increase in the population of phosphate solubilizers and ammonia producers from seedling to booting stage and a decrease in the population during maturity stage.

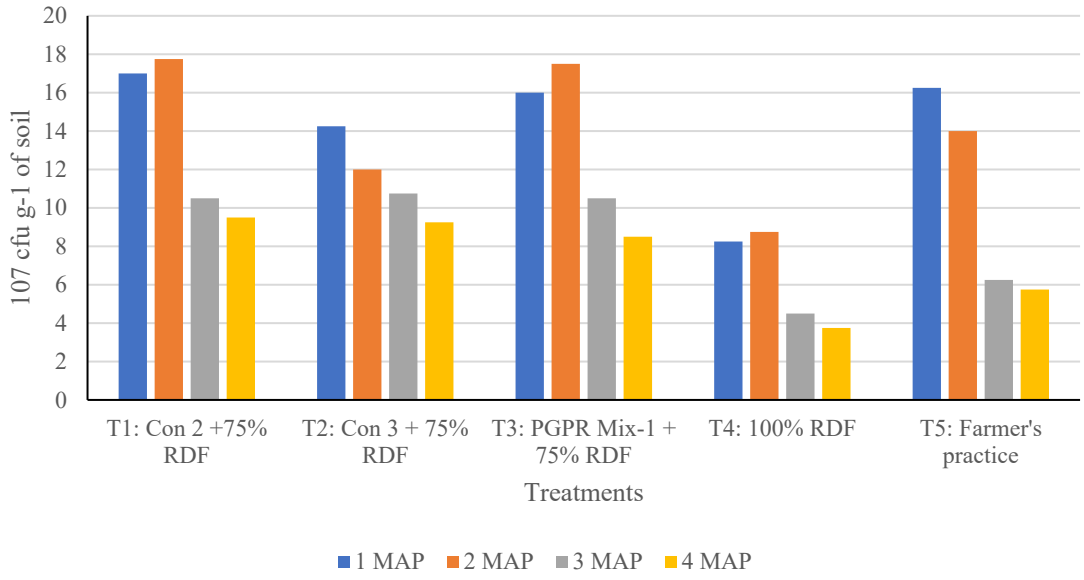

Figure 14. Effect of PGPR application on the population of total bacteria at monthly interval after transplanting

Results of the enumeration of nitrogen fixers in rhizosphere soil revealed that nitrogen fixing bacterial population was higher in all the treatments having integrated application of PGPR based consortia at second and fourth months of transplanting (Figure 15). Lower population was found in T_4 and T_5 which were uninoculated treatments. Results indicated that strains included in consortia have high adaptability to the natural rhizosphere. High level of colonization of nitrogen fixers helps to biological

nitrogen fixation at higher level and thereby enhances plant nitrogen uptake. In the present investigation, the higher population of N-fixers in the PGPR applied treatments reflected on the growth and yield of rice.

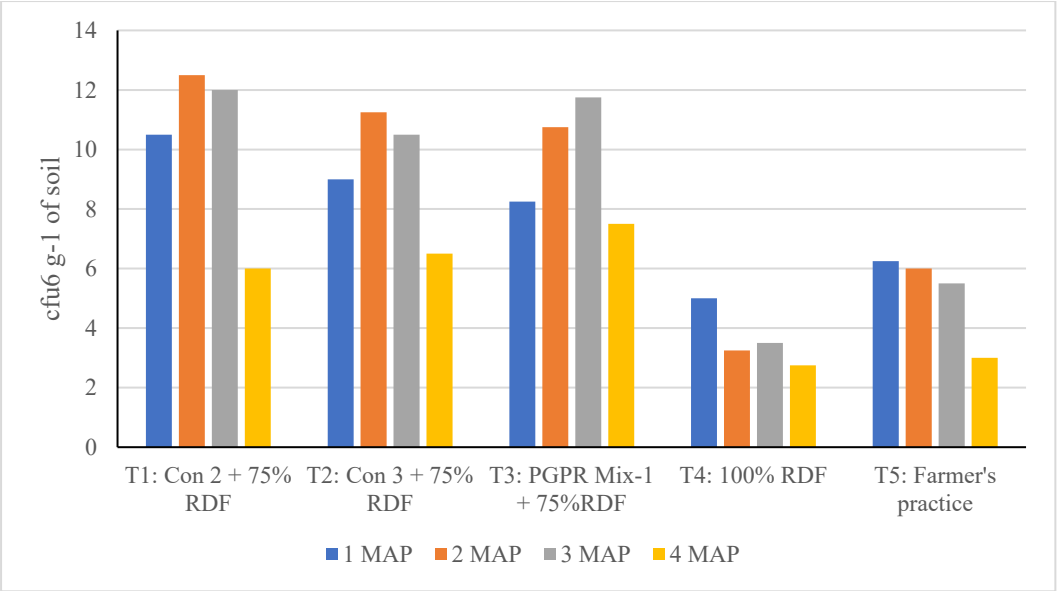

Figure 15. Effect of PGPR application on the population of nitrogen fixers at monthly interval after transplanting

Phosphate solubilizers were significantly higher in treatments with consortia, throughout the rice growth period than 100% RDF applied soils and organic manure applied soils, both devoid of any bio-inoculant. Results indicated that the colonization of phosphate solubilizers in rice rhizosphere was enhanced by artificial inoculation of selected PGPR in the form of consortia. Population of phosphate solubilizers was high at first three months after transplanting and lower population was observed at fourth month of transplanting irrespective of treatments (Figure 16). Since the populations of phosphate solubilizing bacteria was significantly higher in the biofertilizer + 75% RDF, these results suggested that the selected phosphate solubilizers had ecological adaptation of the paddy soil.

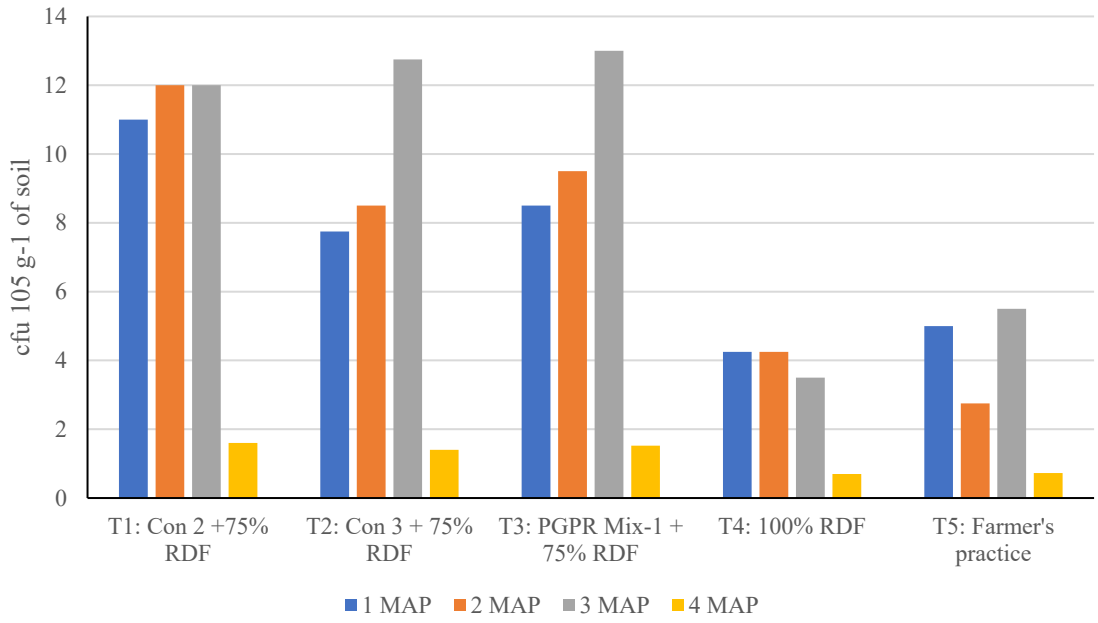

Figure 16. Effect of PGPR application on the population of phosphate solubilizers at monthly interval after transplanting

Statistical analysis revealed that the population of potassium solubilizers was higher in integrated application of consortia with 75% RDF than uninoculated 100% inorganic and organic fertilizer applied treatments at every month up to 4 MAP. Results revealed that integrated application of PGPR based consortia + 75% RDF had ability to maintain population of K solubilizers in higher level comparable to the uninoculated treatments (T_4 and T_5) throughout the growth period. Results clearly indicated that potassium solubilizers included in the consortial formulation were highly adaptable and proliferated in the natural rice rhizosphere.

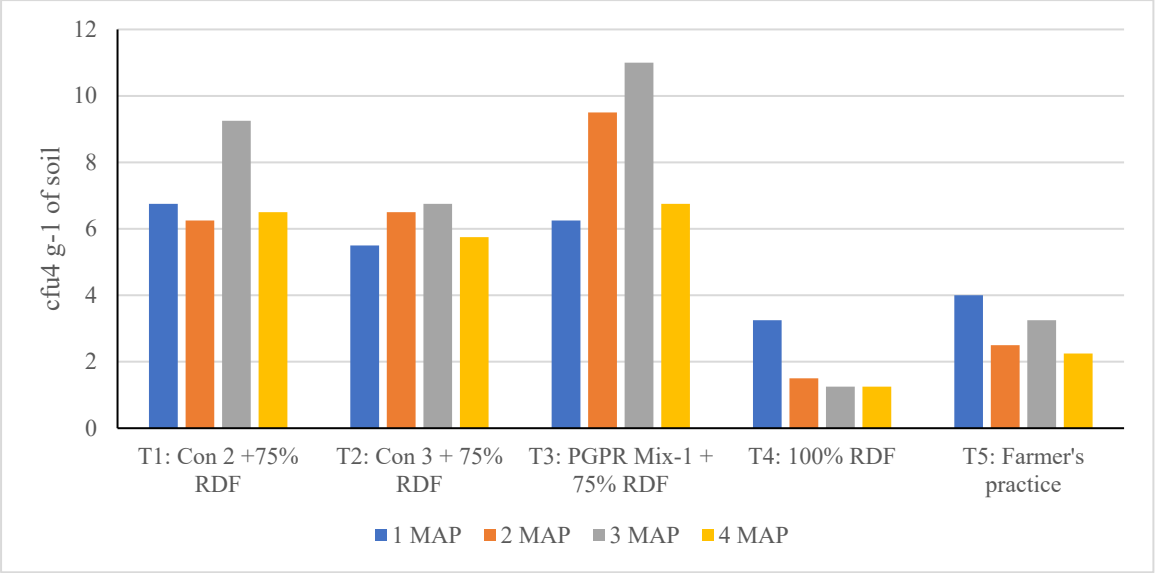

Figure 17. Effect of PGPR application on the population of potassium solubilizers at monthly intervals after transplanting

In general, the population of Zn solubilizers was significantly higher in T_1 and T_2 than treatments T_3, T_4 and T_5. Results clearly indicates, zinc solubilizers in native PGPR based consortia highly adaptable and proliferated in rice rhizosphere. Mumtaz *et al*. (2017) stated that zinc solubilizing *Bacillus* strains *i.e., Bacillus* sp. (ZM20), *Bacillus subtilis* (ZM63) and *Bacillus aryabhattai* (ZM31 and S10) have multiple plant growth promoting traits which can well colonize plant roots and improving the productivity and nutrient quality of maize grains.

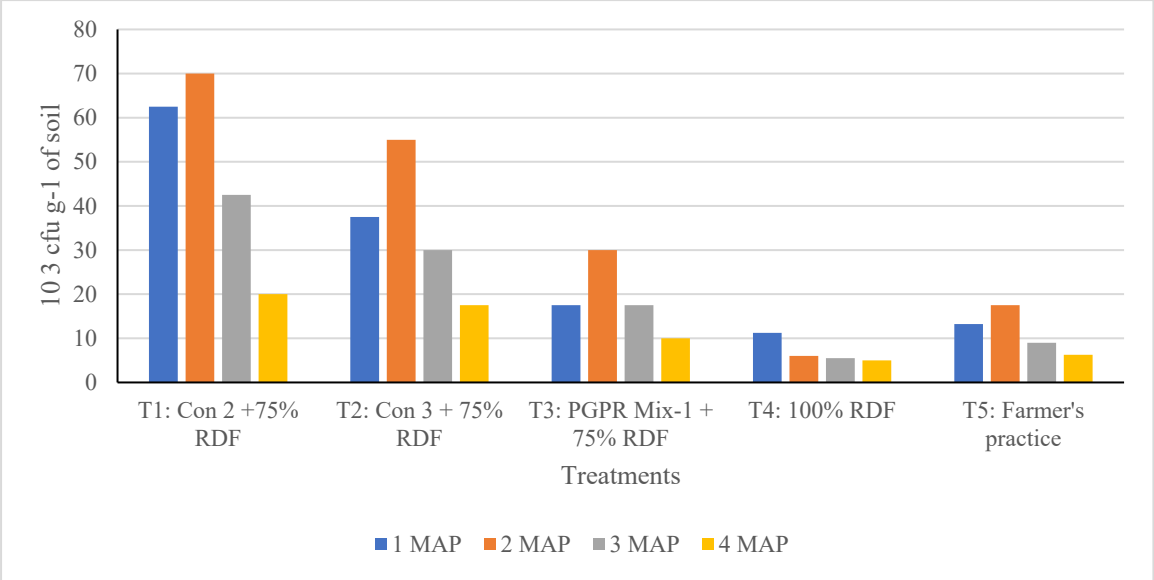

Figure 18. Effect of PGPR on the population of zinc solubilizers at monthly intervals after transplanting

The results of the present investigation are in agreement with Shen *et al*. (2016) studied colonization of complex inoculants application on rhizosphere of kiwifruit and results revealed that total colony forming units of bacteria was highest in half rate chemical fertilizer + complex inoculants (64.52 x 10^6 cfu g^{-1} of soil) than complex inoculants alone (40.42 x 10^6 cfu g^{-1} of soil), chemical fertilizer alone (11.63 x 10^6 cfu g^{-1} of soil) and control (3.98 x 10^6 cfu g^{-1} of soil) and results indicated that population of total bacteria, N_2 fixing, P and K solubilizing bacteria were strongly enhanced with complex inoculum application compare to the control and chemical fertilizer alone applied treatments.

Since the populations of total bacteria, N_2-fixing bacteria, as well as P, K and Zn solubilizing bacteria were significantly higher in the PGPR based consortia + 75% RDF irrespective of combination of inocula, these results proved that the isolates which were used for the preparation of consortial formulation, had ecological adaptation to the tested paddy soil at Ambalavayal. Many biotic and abiotic factors influence the colonization of plants and host specificity may be one factor influencing the colonization of PGPR to diverse crops.

Evaluation of PGPR based consortia on growth and yield of rice, revealed that most of important parameters like number of tillers/m^2, number of panicles/m^2 and grain yield (t ha^{-1}) were statistically on par in 100% RDF and PGPR based consortia + 75% RDF application treatments. Thousand grain weight also was statistically on par in 100% RDF and PGPR based consortia +75% RDF. All the checked parameters were significantly lower in T_5 which had organic fertilizer (farm yard manure 5 t ha^{-1}) than other treatments. The release of nutrients from organic amendments depends on the nature of nutrient and also microorganism's community in the rhizosphere. Therefore, release of nutrient from organic amendment may be slow compared to the inorganic fertilizer. Lowered growth and yield of T_5 may be slow release of nutrient from farm yard manure compared to the other treatments.

The present data pertains to growth and yield attributes, implies plant growth with 100% recommended dosage of N, P and K can be achieved without any significant difference by reduction of 25% recommended dosage of N, P and K chemical fertilizer and that reduction amount can be substituted with applying of PGPR based consortial. These enhancements of growth and yield of rice by the PGPR might be linked with their PGP traits recorded under the in vitro experiments as well as synergistic effects due to co- inoculation.

Further for confirmation of effectiveness of PGPR based consortial on rice growth, plant nutrient content of rice plants was evaluated at panicle initiation stage and found to be significantly different. Higher values of N, P and K were given by treatment T_4, T_1 and T_2 (N and K). Significantly lowest N, P and K was given by farmer practice (farm yard manure 5t ha^{-1}). Commercial formulation of PGPR based biofertilizer developed by KAU (PGPR Mix-1) with 75% RDF treatments showed significantly low values of N and P content of plants than 100% RDF treated plants. Results indicated native nitrogen fixers in PGPR consortia were highly adaptable in rhizosphere than nitrogen fixers in commercial PGPR Mix-1. Zinc content of plants were not significantly different among the treatments. However, K content of plants treated with T_3 (PGPR Mix -1 + 75% RDF) was statistically comparable with 100% RDF treated plants. These results showed locally isolated PGPR consortia were efficiently enhanced nutrient contents of plants than commercial PGPR formula and

also integrated application of PGPR based consortia were able to compensate 25% reduction of N, P and K chemical fertilizers without any significant reduction of plant nutrients.

These results are in agreement with Batool and Altaf (2017) who studied microorganisms that recover soil fertility and improve plant nourishment, revealed the quantity of nitrogen per gram of chili root and shoot tissues were statistically alike for 100% fertility without PGPR and 75% fertilizer plus inoculant. The plants that received 75% fertility plus PGPR produced stable results which were equivalent to 100% fertilizer without inoculant. Bechtaoui *et al*. (2020) also reported regardless of the applied phosphorus source, co-inoculation significantly improved the P concentrations of bean plants as well as in bean pods.

Effect of bioinoculants on soil physico-chemical parameters at harvesting stage was evaluated to confirm whether soil nutrient availability had enhanced due to applying PGPR based biofertilizer. Soil organic carbon was not significantly different among the treatments. However, microbial biomass carbon was found to be higher in integrated application of biofertilizer with inorganic fertilizer (T_1, T_2 and T_3) and farm yard manure applied treatments than uninoculated inorganic fertilizer applied treatment. Results revealed that available nitrogen content of soil was highest in T_1 statistically on par with T_4, T_2 and T_3. Significantly lowest nitrogen content was found to be in T_5 which was farm yard manure applied treatment. Considering the results, a phosphorus content of soil showed significantly high in T_1 and T_3 treated soil than T_4 which had 100% RDF. Soil nitrogen and phosphorus content was enhanced by PGPR based consortia and had improved the soil quality. However, soil K was significantly higher in T_4 and T_3. Significantly lower K content was observed in treatments T_1, T_2 and T_5. Available zinc content of soil at harvesting stage was not significantly different among the treatments.

Ramalakshmi *et al.* (2008) reported significant increase in the soil available nitrogen, phosphorus and potassium in the biofertilizer inoculated plots over the uninoculated control. The available nitrogen was higher in the co-inoculation of Azophos and mycorrhiza with 182 kg ha^{-1} at harvest and in this study results also similar

to that biofertilizer treated soil which gave higher N content in soil at harvest T_1 (Consortium 2 +75% RDF), T_2 (Consortium 3 +75% RDF) and which was statistically comparable with T_4 (100% RDF). Higher soil phosphorous content was observed in the treatment with Azophos and mycorrhiza, phosphobacteria and mycorrhiza at 90 days after germination of cotton seeds (Ramalakshmi *et al*., 2008). According to our results, higher P content of soil observed in treatments T_1 (17.35 kg/ha) and T_3 (17.03 kg/ha) at harvest and P content of soil with 100% RDF applied soils found to be significantly lower than T_1 and T_3. Collectively, these results indicated that the complex inoculants had the capacity to increase soil available N and P. Integrated application of 75% chemical fertilizer with the PGPR consortial containing five indigenous PGPR isolates may have the potential to replace the amount of chemical fertilizer needed to obtain optimum levels of essential nutrients.

Finally, benefit cost ratio with different treatments revealed that B:C ratio of consortium 2 + 75% RDF had highest value 1.51 due to high yield and low cost of cultivation comparable to other treatments. Cost of cultivation for integrated application of biofertilizer did not exceed than 100% chemical fertilizer applied treatment. Lowest B:C ratio 0.92 was observed in farm yard manure applied treatments due to its high cost for farm yard manure and also return very less due to low yield. Based on the benefit cost ratio, it could be inferred that rice cultivation with biofertilizer + 75% inorganic fertilizer was the more profitable than organic farming and inorganic farming.

Based on the growth and yield of rice plant, nutrient content of plant tissues and soil nutrient content at harvest, consortium 2 with 75% RDF performed best in both pot and field conditions than other biofertilizers. Therefore, it can be concluded that native plant growth rhizobacteria included in consortium 2 were successful in colonizing well in rice rhizosphere and enhancing plant growth and yield. Consortium 2 consisted of five native PGPRs *viz, Bacillus* sp. AkNF3, *Pseudomonas putida* KgNF1, *Bacillus megaterium* PkPS1, *Acinetobacter calcoaceticus* MvKS3 and *Cytobacillus kochii* PkZnS3.

Majority of *Bacillus* are non-pathogenic, and many species have been used for biotechnological and industrial applications (Price, 2007). Only a few species of *Bacillus* are known to cause diseases in animals and humans and medically significant species of *Bacillus* are *B. anthracis* and *B. cereus* (Spencer, 2003). A few reports on *Bacillus megaterium* have indicated low level of human pathogenicity. *Cytobacillus kochii* belongs to the genus *Bacillus* and so far no pathogenicity has been reported.

The genus *Pseudomonas* has many years been used in commercial inoculants (Martínez-Hidalgo *et al.*, 2019). *Pseudomonas aeruginosa* is an opportunistic human pathogen, but other species of *Pseudomonas* are non-pathogenic when used in agriculture (Novik *et al*., 2015). Interestingly, *P. putida* has been reported to have relatively low harmful effects on human and the environment (Fernández *et al*., 2015).

Genus *Acinetobacter* have been implicated in a wide spectrum of infectious disease and within the genus *Acinetobacter baumannii* appears to be the species of greatest clinical importance (Joly-Guillou, 2005). However, other species of the '*A. baumannii* complex' (comprising *A. baumannii, A. calcoaceticus*, and the unnamed sp. 3 and sp. 13 of Tjernberg and Ursing) are also reported as clinical importance (Joly-Guillou, 2005). Chu *et al*. (2020) stated that prior to recommendation of microorganisms for agronomic purposes as a bioinoculants safety testing and risk assessment evaluation is required.

The increased yield due to biofertilizers inoculants not be solely due to N fixation, P, K and Zn solubilization but because of several other PGP activities such as release of growth promoting substances, control of pathogens or proliferation of beneficial organisms in rhizosphere. The enhanced microbial dynamics in turn increased plant growth parameters, rice yield as well as soil health due to more availability of nutrients and plant growth promoting substances, thus satisfying all the criteria for sustainability. The finding of this research could be used for development of an eco-friendly and cost effective biofertilizer for rice cultivation.

The success of biofertilizers depends on innovative strategies related to the functions of PGPRs and their proper application to the field of agriculture.

Identification of various strains of PGPRs and their properties and synergistic effects for improved efficiency are key factors in successful production of PGPR biofertilizer. As future line of work from the outcome of this study, it can be proposed that further confirmation of N-fixation by selected PGPR using Acetylene Reduction Assay (ARA) or detection of *nif* gene, cross checking of the isolates for PGP traits other than the identified traits (eg N-fixers for phosphate, K and Zn solubilization, N-fixation by phosphate solubilizers, Zn solubilization by phosphate and K solubilizers) and evaluation of abiotic and biotic stress tolerance may be carried out. Novel formulations of biofertilizers to increase the efficiency, shelf-life studies of biofertilizers during storage period and survival studies of inoculated strains in the field, using markers may also be attempted. Therefore, deep rooted research in this area is highly needed and PGPRs are the potential tools for sustainable agriculture and trend for the future.

Summary

6. SUMMARY

The study entitled "Characterization and evaluation of plant growth promoting rhizobacteria from rice soils of Wayanad" was conducted during 2018 to 2020 at Department of Agricultural Microbiology, College of Horticulture, Kerala Agricultural University, Thrissur. The salient features of this study are summarized in this chapter.

It is important to identify the effective strains of beneficial microorganisms to maximize the beneficial plant growth responses. This study comprised isolation of efficient plant growth promoting rhizobacteria (PGPR) from rice rhizosphere from Wayanad, screening *in vitro* for plant growth promoting activities, formulation of consortial biofertilizer with efficient N fixing P, K and Zn solubilizing isolates and evaluation of the consortia on rice growth and yield in pot culture and field experiments.

Isolation, evaluation and characterization of PGPR from rice rhizosphere

- Rice rhizosphere soil samples from ten locations in Wayanad were collected for the isolation of PGPR.
- A total of 60 N-fixers, 59 phosphate solubilizers, seven K-solubilizers, 21 Zn-solubilizers and two fluorescent pseudomonads were isolated on selective media.
- Thirty-two N fixers, 16 phosphate solubilizers, four K-solubilizers, six Zn-solubilizers and two fluorescent pseudomonads were selected for preliminary screening of PGP traits including production of IAA, NH_3, HCN and siderophore and antagonistic activity against two economic important rice pathogens *Rhizoctonia solani* and *Xanthomonas oryzae*.
- Estimation of IAA production in the N-fixers revealed that $KgNF_1$ recorded the highest value of 34.83 µg ml^{-1} and was identified as *Pseudomonas putida*. Amount of N fixed was significantly higher in six isolates.
- Among the phosphate solubilizing bacteria, the isolate $MtPS_2$ recorded significantly higher IAA production of 19.8 µg ml^{-1}. Highest phosphate solubilization of 134.88 µg ml^{-1} was recorded in the isolate $PkPS_1$, which was

identified as *Bacillus megaterium. B. megaterium* has earlier been reported from different parts of the world, as a good phosphate solubilizing bacterium.

- None of the K-solubilizers were found to produce IAA. The isolate $MvKS_1$ solubilized significantly higher amount of K (4.19 µg ml^{-1}) in Aleksandrov broth, and was identified as *Microbacterium* sp. based on 16S rRNA gene sequencing.
- Among the Zn-solubilizers, isolates $PkZnS_3$, $MvZnS_3$ and $ThZnS_2$ solubilized significantly higher amount of ZnO (18.53 µg ml^{-1}, 17.66 µg ml^{-1} and 17.44 µg ml^{-1} respectively) in mineral salts medium. These isolates were identified as *Cytobacillus kochii*, *Acinetobacter* sp. *Achromobacter marplantensis* based on 16S rDNA gene sequencing. Further it is was confirmed that isolates $PkZnS_3$ (*Cytobacillus kochii*) and $ThZnS_2$ (*Achromobacter marplantensis*) could also solubilize $ZnCO_3$.
- Twenty isolates including seven N-fixers, six phosphate solubilizers, three K-solubilizers, three Zn-solubilizers and one fluorescent pseudomonad were selected based on the functional efficiency of the isolates to fix N and solubilize phosphate, potassium and zinc. Cultural, morphological and biochemical characterization of these isolates were carried out. Four isolates *viz,* $AkNF_3$, $PkPS_1$, $PkZnS_3$ and $MvKS_1$ were Gram positive rods and all others were Gram negative short rods.
- Eighteen isolates were identified by 16S rRNA gene sequencing by the Sanger's method. 16S rRNA gene sequences of all the 18 isolates were submitted to the GeneBank and accession numbers obtained.
- Phylogenetic tree constructed using MEGA 7 software showed two major clusters and several sub-clusters. A few of the native isolates did not cluster with the similar sequences obtained from GeneBank, indicating that they are genetically diverse.
- Based on the efficiency of nitrogen fixation, P, K and Zn solubilization and considering their PGP activities, ranking was done for all the 20 isolates and 10 potential PGPR isolates selected for compatibility checking and consortial formulation. These included *Bacillus* sp. AkNF3, *Pseudomonas* sp. PkNF4, *Pseudomonas putida* KgNF1, *Bacillus megaterium* PkPS1, *Acinetobacter*

schindleri AkPS4, *Achromobacter* sp. AvPS1, *Microbacterium* sp. MvKS1, *Acinetobacter calcoaceticus* MvKS3, *Cytobacillus kochii* PkZnS3 and *Achromobacter marplatensis* ThZnS2.

- Compatibility of selected isolates was tested by cross streaking and dual culture methods. Results revealed that all the isolates were compatible.
- Talc-based formulation of three consortia were developed, each consisting of five efficient PGPR isolates (Two N-fixers, one phosphate solubilizer, one K-solubilizer and one Zn-solubilizer).

Pot culture evaluation of PGPR based consortia on rice growth and yield

- Above mentioned three consortia at 50% and 75% recommended dosage of fertilizer (RDF) were evaluated under pot culture conditions, with traditional variety 'Valichoori', at Regional Agricultural Research Station, Ambalavayal, Wayanad. KAU commercial formulation (PGPR mix-1), 100 % RDF, PoP (KAU) and organic PoP (KAU) were also included in the treatments for comparison.
- Results revealed that the population of total bacteria, N fixers, P, K, and Zn solubilizers were higher in treatments having combined application of PGPR biofertilizer and inorganic fertilizer than inorganic fertilizer alone.
- Result of pot culture evaluation suggested that PGPR based biofertilizer with 75% chemical fertilizer (N, P and K) was able to improve growth and yield of rice and contributed in reducing 25% of chemical fertilizer and minimizing environmental pollution.
- The nutrient content of plants such as nitrogen, potassium and zinc at panicle initiation stage revealed that combined application of biofertilizer with 75% inorganic fertilizer was statistically on par with sole 100% inorganic fertilizer treatments.
- Soil physico-chemical parameters at the time of harvesting showed that soil microbial biomass carbon, available N and Zn were significantly higher in T_6: (Consortium 2+75% RDF) than 100% RDF.
- Among the three PGPR based consortia, Consortium 2 and Consortium 3 were selected for field evaluation of rice growth and yield.

Field evaluation of PGPR based consortia on rice growth and yield

- Field experiment was conducted to study to evaluate Consortium 2 and 3 in combination with 75% RDF (N, P and K) on the soil microbial dynamics, plant nutrient content, soil physico-chemical properties, plant growth and yield.
- The results showed that combined application of consortial biofertilizer with 75% inorganic fertilizer significantly improved the microbial population compared to inorganic and organic fertilizers alone.
- Plant nutrient content at panicle initiation stage showed combined application of biofertilizer with 75% inorganic fertilizer was statistically on par with 100% inorganic fertilizer treatment and suggested a 25% reduction in N, P, K in the form of chemical fertilizer may replaced by application of biofertilizer.
- Enhanced soil microbial biomass C was observed in all treatments which received combined application of biofertilizer with 75% RDF over the 100% inorganic fertilizer alone.
- The yield attributes were significantly higher in treatments having a combination of PGPR and inorganic fertilizer over control treatment (farmer practice). G rain yield of treatments T_1 and T_2 which were inoculated with indigenous PGPR with 75% RDF was statistically comparable with grain yield of 100% RDF treated plots.
- The results indicated that integrated application of a consortium of biofertilizer containing native PGPR, with 75% RDF contributed to the reduction of 25% inorganic fertilizer application (N, P, K) without affecting the yield of rice.
- Based on the field studies, consortium 2 can be recommended as best PGPR consortium and it consisted of native PGPRs *viz. Bacillus* sp. AkNF3, *Pseudomonas putida* KgNF1, *Bacillus megaterium* PkPS1, *Acinetobacter calcoaceticus* MvKS3 and *Cytobacillus kochii* PkZnS3.
- Present investigation concluded that integrated application of native PGPR consortia with inorganic fertilizer enhanced plant nutrients, soil nutrient content at harvest, plant growth and yield attributes than commercial PGPR

formulation. The enhanced microbial dynamics in turn increased plant growth parameters and yield as well as soil health due to more availability of nutrients, thus satisfying all the criteria for sustainability.

References

REFERENCES

Aarab, S., Ollero, F. J., Megias, M., Laglaoui, A., Bakkali, M., and Arakrak, A. 2015. Isolation and screening of bacteria from rhizospheric soils of rice fields in Northwestern Morocco for different plant growth promotion (PGP) activities: An *in vitro* study. *Int. J. Curr. Microbiol. App. Sci*. 4(1): 260-269

Abaid- Ullah, M., Hassan, M. N., Jamil, M., Brader, G., Shah, M. K. N., Sessitsch, A., Haffez, F. Y. 2015. Plant growth promoting rhizobacteria an alternative way to improve yield and quality of wheat (*Triticum aestivum*). *Int. J. Agric. Biol.* 17: 51-60

Abdel-Rahman, H. M., Saleem, A. A., Moustafa, M. M. A., El-Garhy, H. A. S. 2017. A novice *Acromobacter* sp. EMCC1936 strain acts as a plant growth promoting agents. *Acta. Physiol. Plant*. 39:61. doi:10.1007/311738-017-2360-6.

Adesemoye, A. O., and Kloepper, J.W. 2009. Plant-microbes interactions in enhanced fertilizer-use efficiency. *Appl. Microbiol. Biotechnol*. 85: 1-12.

Agbodjato N. A., Noumavo, P. A., Baba-Moussa, F., Salami, H. A., Sina, H., Sèzan, A., Bankolé, H., Adjanohoun, A., and Baba-Moussa L. 2015. Characterization of Potential Plant Growth Promoting Rhizobacteria Isolated from Maize (*Zea mays* L.) in Central and Northern Benin (West Africa). *Appl. Environ. Soil Sci*.2015. https://doi.org/10.1155/2015/901656

Ahemad, M., and Kibret, M. 2014. Mechanisms and plant growth promoting rhizobacteria: current perspective. *J. King Saud Univ. Sci*. 26: 1-20.

Ahmad, F., Ahmad, I., Aqil, F., Wani, A. A., and Sousche, Y. S. 2006. Plant growth promoting potential of free-living diazotrophs and other rhizobacteria isolated from Northern Indian soil. *Biotechnol. J*. 1(10): 1112-1123.

Ahmad, F., Ahmad, I., and Khan, M. S. 2005. Indole acetic acid production by the indigenous isolates of *Azotobacter* and fluorescent Pseudomonas in the presence and absence of tryptophan. *Turk. J. Biol*. 29: 29-34.

Ahmad, F., Ahamad, I., and Khan, M. S. 2008. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiol. Res*. 163: 173-181.

Ahmad, M., Nadeem, S. M., Naveed, M., and Zahir, Z. A. 2016. Potasium solubilizing bacteria and their application in agriculture. In. Meena, V. S., Maurya, B. R., Verma, J. P., Meena, R. S (eds). *Potassium Solubilizing Microorganisms for Sustainable Agriculture*, Springer India, New Delhi. pp 293-313.

Akintokun, A. K., Ezaka, E., Akintokun, P. O., Shittu, O. B., and Taiwo, L. B. 2019. Isolation, screening and response of maize to plant growth promoting rhizobacteria inoculants. *Sci. Agric. Bohem.* 50 (3): 181-190

Alam, S., Khalil, S., Ayub, N., and Rashid, M. 2002. *In vitro* solubilization of inorganic phosphate by phosphate solubilizing microorganism (PSM) from maize rhizosphere. *Intl. J. Agric. Biol*. 4:454–458

Ambrosini, A., Stefanski, T., Lisboa, B. B., Beneduzi, A., Vargas, L. K. and Passaglia, L. M. P. 2016. Diazotrophic bacilli isolated from the sunflower rhizosphere and the potential of *Bacillus mycoides* B38V as biofertilizer. *Ann. Appl. Biol*. 168: 93–110

Araujo, A. E. S., Baldani, V. L. D., Galisa, P. S., Pereira, J. A., and Baldani, J. I. 2013. Response of traditional rice varieties to inoculation with selected diazotrophic bacteria isolated from rice cropped at the Northeast region of Brazil. *Apl. Soil Ecol.* 64: 49-55

Araujo, F. F., Henning, A. A. and Hungria, M. 2009. Phytohormones and antibiotics produced by *Bacillus subtilis* and their effects on seed pathogenic fungi and on soya bean root development. *World J. Microbiol. Biotechnol*. 21: 1639-1645

Armada, E., Portela, G., Roldam, A., Azcon, R. 2014. Combined use of beneficial soil microorganism and agro waste residue to cope with plant water limitation under semi-arid conditions. *Geoderma*. 232: 640-648.

Babalola, O. O. 2010. Beneficial bacteria of agricultural importance. *Biotech. Lett*. 32 (11):1559-1570

Babu, B. K., Meena, V., Agarwal, V., and P. K. Agrawal. 2014. Population structure and genetic diversity analysis of Indian and exotic rice (*Oryza sativa* L) accessing using SSR markers. *Mol. Biol. Reprod.* 41 (7): 4328 -4339.

Backer, R., Rokem, J. S., Illangumaran, G., Lamont, J., Praslickova, D., Ricci, E., Subramaniam, S., and Smith, D. L. 2018. Plant-growth promoting rhizobacteria: context, mechanisms of action, and roadmap to

commercialization of biostimulants for sustainable agriculture. *Front. Plant Sci*. 9:1473. Doi:10.3389/fpls.2018.01473

Badri, D. V., Weir, T. L., van der Lelie, D., and Vivanco, J. M. 2009. Rhizosphere chemical dialogues: plant-microbe inter- actions. *Curr. Opin. Biotech*. 20 (6): 642–650.

Bakhshandeh, E., Pirdashti, H., and Lendeh, K.S. 2017. Phosphate and potassium-solubilizing bacteria effect on the growth of rice. *Ecol. Eng*. 103: 164 – 169

Bakhshandeh, E., Rahimian, H., Pirdashti, H., and Nematzadeh, G. A. 2015. Evaluation of phosphate-solubilizing bacteria on the growth and grain yield of rice (*Oryza sativa* L.) cropped in northern Iran. *J. Appl. Microbiol*. 119 (5): 1371-1382

Bakker, A.W., and Schippers, B. 1987. Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* spp. mediated plant growth stimulation. Soil *Biol. Biochem.* 19(4): 451-457

Baldani, V. L. D., Baldani, J. I., and Dobereiner, J. 2000. Inoculation of rice plants with the endophytic diazotrops *Herbaspirillum serepedicae* and *Burkholderia* spp. *Biol. Fert. Soils*. 30: 485 -491

Bapiri, A., Asgharzadeh, A., Mujallali, H., Khavazi, K., and Pazira, E. 2012. Evaluation of zinc solubilization potential by different strains of Fluorescent Pseudomonads. *J. Appl. Sci. Environ. Manage*. 16(3): 295-298.

Barman, M., Paul, S., Choudhury, A. G., Roy, P., and Sen, J. 2017. Biofertilizer as prospective input for sustainable agriculture in India. *Int. J. Curr. Microbiol. App. Sci*. 6 (11): 1177-1186

Bashan, Y., and de-Bashan, L. E. 2010. How the plant growth-promoting bacterium *Azospirillum* promotes plant growth. Critical assessment. *Adv. Agron*. 108: 77-136.

Batool. S., and Altaf, M. A. 2017. Plant growth promoting rhizobacteria (PGPR) reduces application of fertilizer in chili (*Capsicum frutescens* L.) cultivation. *J. Hortic.* 215. Doi: doi: 10.4172/2376-0354.1000215

Batool, S., and Iqbal, A. 2019. Phosphate solubilizing rhizobacteria as alternative of chemical fertilizers for growth and yield of *Triticum aestivum* (var. Galaxy 2013). *Saudi. J. Biol. Sci.* 26:1400-1410

Bechtaoui, N., Raklami, A., Benidire, L., Tahiri, A., Göttfert, M., Oufdou, K. 2020. Effects of PGPR co-inoculation on growth, phosphorus nutrition and phosphatase/phytase activities of faba bean under different phosphorus availability conditions. *Pol. J. Environ. Stud*. 29 9(2): 1557-1565

Benson, D. A., Cavanaugh, M., Clark, K., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J., and Sayers, E.W. 2017. GenBank. *Nucleic Acids Res*. 45. doi: 10.1093/nar/gkw1070

Bergottini, V. M., Otegui, M. B., Sosa, A., Zapata, P. D., Mulot, M., Rebord, M., Zopfi, J., Wiss, F., Benrey, B., and Junier. P. 2015. Bio-inoculation of yerba mate seedlings (*Ilex paraguariensis* St. Hill.) with native plant growth - promoting rhizobacteria: A sustainable alternative to improve crop yield. *Biol. Fertl. Soils*. 51 (6): 749–755.

Bhattacharjee, S. N., Singh, S., and Mukhopadhyay, S. N. 2008. Use of nitrogen-fixing bacteria as biofertlizer for non-legumes: prospects and challenges. *Appl. Microbiol. Biotechnol*. 80:199-209

Bhattacharya, S., Bachani, P., Jain, D., Patidar, S. K. and Mishra, S. 2016. Extraction of potassium from K-feldspar through potassium solubilization in the halophilic *Acinetobacter soli* (MTCC 5918) isolated from the experimental salt farm. *Int. J. Miner. Process*. 152: 53-75.

Bhattacharyya, P. N., and Jha, D. K. 2012. Plant groth promoting rhizobacteria (PGPR): emergence in agriculture. *World J. Microbial. Biotechnol*. 28 (4): 1327-1350

Bhutani, N., Maheshwari, R., Negi, M., and Suneja, P. 2018. Optimization of IAA production by endophytic Bacillus spp. from Vigna radiata for their potential use as plant growth promoters. *Israel J. Plant Sci*. 65: 83-96

Biswakarma, B., Verma, H., and Sarka, N. C., 2018. Effect of phosphate solubilizing bacteria on yield of transplanted rice under lateritic belt of West Bengal, India. *Int. J. Curr. Microbiol. App. Sci.* 7(2):3192-3204.

Boyer, M., Bally, R., Perrotto, S., Chaintreuil, C., and Wisniewski-Dye, F. 2008. A quorum-quenching approach to identify quorum-sensing-regulated functions in *Azospirillum lipoferum*. *Res. Microbiol.* 159 (9–10):699-708

Bremner, J. M. 1960. Determination of nitrogen in soil by the Kjeldahl method. *J. Agric. Sci*. 55: 11-33.

Brick, J. M., Bostock, R. M., and Silverstone, S. E. 1991. Rapid *in-situ* assay for indole acetic acid production by bacteria immobilized nitrocellulose membrane. *Appl. Environ. Microbiol.* 57(2):535-538

Calvo, P., Nelson, L. M., Kloepper, J. W. 2014. Agricultural uses of plant biostimulants. *Plant soil*. 383: 3-41.

Cappuccino, J. C. and Sherman, N. 1992. *Microbiology: A Laboratory Manuel*. Wesley Publication, New York, 179p.

Chandran, P., Ray, S. K., Bhattacharyya, K., Krishnan, P., and Pal, D. K. 2005. Clay minerals in two ferruginous soils of southern India. *Clay Res*. 19:77–85

Chaparro, J. M., Badri, D. V., and Vivanco, J. M. 2014. Rhizosphere microbiome assemblage is affected by plant development. *ISME J*. 8 (4): 790-803

Chaudhary, S.K., Thakur, S.K., and Pandey, A.K. 2007. Response of wetland rice to nitrogen and zinc. *Oryza*. 44(1): 44-47

Chen, Y. P., Rekha, P. D., Arun, A. B., Shen, F.T. Lai, W. A., and Young, C. C. 2006. Phosphate solubilizing bacteria from biofertilizer for non- legumes: subtropical soil and their tricalcium phosphate solubilizing abilities. *Appl. Soil Ecol*. 34: 33-41

Choudhury, A. T. M. A., and Kennedy, I. R. 2004. Prospects and potentials for systems of biological nitrogen fixation in sustainable rice production. *Biol. Fertil. Soils* 39:219–227

Chu, T. N., Bui, L. V., and Hoang, M. T. T. 2020. *Pseudomonas* PS01 isolated from maize rhizosphere alters root system architecture and promotes plant growth. *Microorganisms*. 8(4): doi:10.3390/microorganisms8040471

Clarridge, J. E. 2004. Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. *Clin. Microbiol. Rev.* 17(4): 840–862

Compant, S., Duffy, B., Nowak, J., Clement, C., and Barka, E. A. 2005. Use of plant growth-promoting bacteria for biocontrol of plant disease: principles, mechanisms of action, and future prospects. *Appl. Environ. Microbiol.* 71(9): 4961-4959

Desbrosses, G. J., and Stougaard, J. 2011. Root nodulation: a paradigm for how plant microbe symbiosis influences host developmental pathways. *Cell Host Microbe*.10: 348-358

De Souza, R., Ambrosini, A., and Passaglia, L. M. P. 2015. Plant growth-promoting bacteria as inoculants in agricultural soil. *Genet. Mol. Biol.* 38(4): 401-419

Dey, H.S., Tayung, K., and Bastia, A.K. 2010. Occurrence of nitrogen-fixing cyanobacteria in local rice fields of Orissa, India. *Ecoprint Int. J. Ecol*. 17: 77-85

Diep, C. N., and Hieu, T. N. 2013. Phosphate and potassium solubilizing bacteria from weathered materials of denatured rock mountain. *Am. J. Life Sci*. 1(3): 88-92.

Ding, Y., Wang, J., Liu, Y. and Chen, S. 2005. Isolation and identification of nitrogen-fixing bacilli from plant rhizospheres in Beijing region. *J. Appl. Microbiol.* 99: 1271– 1281

Duarah, I., Deka, M., Saikia, N., and Boruah, H. P. D. 2011. Phosphate solubilizers enhance NPK fertilizer use efficiency in rice and legume cultivation. *3 Biotech*. 1: 227–238. doi. 10.1007/s13205-011-0028-2

Edwards, J., Johnson, C., Santos-Medellin, C., Lurie, E., Podishetty, N. K., Bhatnagar, S., Eisen, J. A., and Sunderesan, V. 2015. Structure, variation, and assembly of the root-associated microbiomes of rice. *Proc. Natl. Acad. Sci. USA.* 201.www.pnas.org cgi/ doi/ 10.1073/ pnas.1414592112

Egamberdieva, D., Jabborova, D., and Hashem, A. 2015. *Pseudomonas* induces salinity tolerance in cotton (*Gossypium hirsutum*) and resistance to *Fusarium* root rot through the modulation of indole-3-acetic acid. *Saudi J. Biol. Sci.* 22: 773–779

Elekhtyar, N. M. 2016. Influence of different plant growth promoting bacteria (PGPR) strains on rice promising lines. *Proc. The sixth field crop conference*, FCRI, ARR, Giza, Egypt.

Estrada, G. A., Baldani, V. L. D., de Oliveira, D. M., Urquiaga and Baldani, J. I. 2013. Selection of phosphate-solubilizing diazotrophic *Herbaspirillum* and *Burkholderia* strains and their effect on rice crop yield and nutrient uptake. *Plant Soil*. 369:115–129. Doi. 10.1007/s11104-012-1550-7

Etesami, H., Emami, S., Alikhani, H. A. 2017. Potassium solubilizing bacteria (KSB): mechanisms, promotion of plant growth, and future prospects- a review. *J. Soil Sci. Plant Nutrition.* 17(4). 897-911

FAI [Fertilizer Association of India]. 2018. Specialty Fertilizer Statistics. New Delhi, India.

FAOSTAT [Food and Agriculture Organization of the United Nations Statistics Division}. 2018. Economic and Social Development Department. FAO, Rome, Italy.

Faramarzi, M. A., and Brandl, H. 2006. Formation of water-soluble metal cyanide complexes from solid minerals by *Pseudomonas plecoglossicida*. *FEMS Microbiol. Lett*. 259, 47–52

Fayez, M. 1990. Untraditional N_2 fixing bacteria as biofertilizers for wheat and barley. *Folia. microbiol.* 35: 218-226

Fernández, M., Porcel, M., de la Torre, J., Molina-Henares, M. A., Daddaoua, A., Llamas, M.A., Roca, A., Carriel, V., Garzón, I., Ramos, J. L. Alaminos, M., and Duque, E. 2015. Analysis of the pathogenic potential of nosocomial *Pseudomonas putida* strains. *Front. Microbiol*. 6. doi.org/10.3389/ fmicb.2015.00871

Ferreria, C. M. H., Vilas-Boas, A., Sousa, C. A., Soares, H. M. V. M., and Soares, E. V. 2019. Comparison of five bacterial strains producing siderophores with ability to chelate iron under alkaline conditions. *AMB Expr*. 9(78). doi.org./10.1186/s13568-019-0796-3

Figueiredo, M. V. B., Bonifacio, A., Rodrigues, A.C., and Arujo, F. F. 2016. Plant growth -promoting rhizobacteria: Key mechanisms of action. In. Chaudhary, D. K., and Verma, A. (Ed.) *Microbial-mediated Induced Systemic Resistance in Plants*. Springer Science and Business Media, Singapore. 23-37.

Figueiredo, M. V. B., Seldin, L., Araujo, F. F., and Mariano, R. L.R. 2010. Plant growth promoting rhizobacteria: Fundamental and applications. In: Maheshwari, D. K. (Ed.). *Plant Growth and Health Promoting Bacteria*. Springer- Verlag, Berlin, Heidelberg. 21-42.

Fischer, S. E., Fischer, S. I., Margis, S., and Mori, G. B. 2007. Isolation and characterization of bacteria from rhizosphere of wheat. *World J. Microbiol. Biotechnol.* 23: 895-903

Gandhi, A., and Muralidharan, G. 2016.Assessment of zinc solubilizing potentiality of *Acinetobacter* sp. isolated from rice rhizosphere. *Eur. J. soil. Biol*. 76: 1-8.

Ghosh, R., Barman, S., Mukherjee, R., and Mandala, N. C. 2016. Role of phosphate solubilizing *Burkholderia* spp. for successful colonization and growth promotion of *Lycopodium cernuum* L. (Lycopodiaceae) in lateritic belt of Birbhum district of West Bengal, India. *Microbiol. Res*. 183: 80-91

Glick, B. R. 2012. Plant growth promoting bacteria: Mechanism and applications. Hindawi publishing corporation. *Scientifica*. 15p

Glick, B. R., Patten, C. L., Holguin, G., Penrose, G.M., 1999. Biochemical and Genetic mechanisms used by Plant Growth promoting Bacteria, Imperial College Press. London.

Goldstein, A. H. 1995. Recent progress in understanding the molecular genetics and biochemistry of calcium phosphate solubilization by Gram negative bacteria. *Biol. Agr. Hortic*, 12 (2):185-193.

Goteti, P.K., Emmanual, L.D.A., Desai, S., and Shaik, M. M. A. 2013.Prospective zinc solubilizing bacteria for enhanced nutrient uptake and growth promotion in Maize. *Int. J. Microbiol*. http://dx.doi.org/10.1155/2013/869697

Govindarajan, M., Balandreau, J., Kwan, S., Weon, H. Lakshminarasimhan, C. 2008. Effect of the inoculation of *Burkholderia vietnamesis* and related endophytic diazotrophic bacteria on grain yield of rice. *Microb. Ecol*. 55: 21-37. Doi. 10-1007/s00248-007-9247-9

Gowtham, H. G., Singh, S. B., and Niranjana, S. R. 2015. Evaluation of *Providencia spp*. collected from North Eastern region of India in crucifers. *Int. J. Agrc. Sci. Res.* 5(3). http://www.researcggate.net/publication/282909593

Grobelak, A., and Hiller, J. 2017. Bacterial siderophores promote plant growth: Screening of catechol and hydroxamate siderophores. *Int. J. Phytoremdiat.* 19(9): 825-833

Grobelak, A., Napora, A., and Kacprzak, M. 2015. Using plant growth-promoting rhizobacteria (PGPR) to improve plant growth. *Ecol. Eng.* 84: 22–28.

Habibi, S., Djedidi, S., Ohkama-Ohtsu, N., sarhedi, W.A., Kojima, K., Rallos, R. V., Ramirez, M. D. A., Yamaya, H., Sekimoto, H., and Yokoyama, T. 2019. Isolation and screening of indigenous plant growth promoting rhizobacteria from different rice cultivars in Afghanistan. *Microbe Environ*. 34(4): 347-355

Habibi, S., Djedidi, S., Prongjunthuek, K., Mortuza, M. F., Ohkama – Ohtsu, N., Sekimoto, H., and Yokoyoma, T. 2014. Physiological and genetic characterization of rice nitrogen fixer PGPR isolated from rhizosphere soils of different crops. *Plant soil*. 379: 51-66

Hameeda, B., Harini, G., Rupela, O.P., Wani, S. P., and Reddy, G. 2008. Growth promotion of make by phosphate solubilizing bacteria isolated from compost and macrofauna. *Microbiol. Res.* 163: 234-242

Han, H. S., Supanjani, and Lee, K.D. 2016. Effect of co-inoculation with phosphate and potassium solubilizing bacteria on mineral uptake and growth of pepper and cucumber. *Plant Soil Environ*. 52 (3): 130-136

Han, L., Wang, Q., Shen, J., Di, H. J., Wang, J., Wei, W., Fang, Y., Zhang, L., and He, J. 2019. Multiple factors drive the abundance and diversity of the diazotrophic community in typical farmland soils of China. FEMS Microbiol. Ecol. 95 (8). doi:10.1093/femsec/fiz113

Hayward, A. C. 1960. A method for characterizing *Pseudomonas solanacearum*. *Nature*. 186: 405-406

Huang, X. F., Chaparro, J.M., Reardon, K. F., Zhang, R., Shen, Q., and Vivanco, J. M. 2014. Rhizosphere interactions: root exudates, microbes, and microbial communities. *Botany* 92 (4):267–275.

Hussain, K., Hameed, S. Shahid, M., Ali, A. Iqbal, J., and Hahn, D. 2015. First report of *Providencia vermicola* strain characterized for enhanced rapeseed growth attributing parameters. *Int. J. Agric. Biol*. 17 (6): 1110-1116

Islam, M. R., Sultana, T., Joe, M. M., Yim, W., Cho, J. C., and Sa, T. 2013. Nitrogen-fixing bacteria with multiple plant growth-promoting activities enhance growth of tomato and red pepper. *J. Basic Microbiol*. 53, 1004–1015. doi: 10.1002/jobm.201200141

Islam, M.T., Deora, A., Hashidokoa, Y., Rahmana, A., Itoa, T., and Tahara, S. 2007. Isolation and identification of potential phosphate solubilizing bacteria from

the rhizoplane of *Oryza sativa* L. cv. BR29 of Bangladesh. *Z. Naturforsch C J. Biosciences*. 62:103-110. doi. 10.1515/znc-2007-1-218.

Jackson, M. L. 1958. *Soil Chemical Analysis*. Prentice-Hall, Englewood Cliffs. 498p.

Jackson, M. L. 1973. *Soil Chemical Analysis* (2nd Ed). Prentice Hall of India, New Delhi. 498 p.

Jiao, W. T., Chen, W. P., Chang, A. C., and Page, A. L. 2012. Environmental risk of trace elements associated with long term phosphate fertilizer application: a review. *Environ. Pollut.* 168: 44-53.

Jnawali, A. D., Ojha, R. B., and Marathatta, S. 2015. Role of Azotobacter in soil fertility and sustainability–a review. *Adv. Plants Agric. Res*. 2 (6):250-253.

Joly-Guillou, M. 2005. Clinical impact and pathogenicity of Acinetobacter. *Clin. Microbiol. Infect*. 11(11): 868-873

Joseph, B., Patra, R. R., and Lawrence, R. 2007. Characterization of plant growth promoting rhizobacteria associated with chickpea (*Cicer arietinum* L.). *Int. J. Plant Prod*. 2: 141 152

Kakar, K. U., Nawaz, Z., Cui, Z., Almoneafy, A. A., Ullah, R., and Shu, Q. Y. 2017. Rhizosphere-associated *Alcaligenes* and *Bacillus* strains that induce resistance against blast and sheath blight diseases, enhance plant growth and improve mineral content in rice. *J. Appl. Microbiol*. Doi: org/10.1111/jam.13678

Kamran, S., Shahid, I., Baig, D. N., Rizwan, M., Malik, K. A., and Mehnaz, S. 2017. Contribution of zinc solubilizing bacteria in growth promotion and zinc content of wheat. *Front. Microbiol*. 8:2593. doi.10.3389/fmicb.2017.02593

Kandaswamy, R., Ramasamy, M. K., Palanival, R., and Balasundaram, U. 2019. Impact of *Pseudomonas putida* RRF3 on root transcriptome of rice plants. *J. Biosci*. 44 (98). DOI: 10.1007/s12038-019-9922-2

Kang, S., Joo, G., Hamayun, M., Na, C., Shin, D., Kim, Y., Homg, J., and Lee I. 2009. Gibberellin production and phosphate solubilization by newly isolated strain of *Acinetobacter calcoaceticus* and its effect on plant growth. *Biotechnol. Lett*. 31:277–281

Kang, S., Radhakrishnan, R., You, Y., Joo, G., Lee, I., Lee, K., and Kim, J. 2014. Phosphate solubilizing *Bacillus megaterium* mj1212 regulates endogenous

plant carbohydrates and amino acids contents to promote mustard plant growth. *Ind. J. Microbiol.* 54(4):427–433

Kannan, T., and Ponmurugan, P. 2010. Response of paddy (*Oryza sativa* L.) varieties to *Azospirillum brasilense* inoculation. *J. Phytol* 2: 8–13

Karimzadeh, J., Alikhani, H. A., Etesami, H., and Pourbabaei, A. A. 2020. Improved phosphorus uptake by wheat plant (*Triticum aestivum* L.) with rhizosphere fluorescent Pseudomonads strains under water-deficit stress. *J. Plant Growth Regul*. 10.1007/s00344-020-10087-3

Kaushik, B. D. 1994. Algalization of rice in salt-affected soils. *Ann. Agric. Res.* 14:105–106

Kaymak, D. C. Potential of PGPR in agricultural innovations. 2010. In Plant Growth and Health Promoting Bacteria: Maheshwari, D. K. (Ed) Springer-Verlag: Berlin/Heidelberg, Germany,

Khambalkar, P. and Sridar, R. 2015. A novel multifunctional *Burkholderia* ap. for plant growth promotion. *J. Pure Appl. Microbiol.* 9 (3): 2513-2521

Khan, A. A., Jilani, G., Akhtar, S.M., Naqvi, S. M. S., and Rasheed, M. 2009. Phosphorus solubilizing bacteria: occurrence, mechanisms and their role in crop production. *J. Agric. Biol. Sci*. 1: 48–58

Khan, A., Geetha, R., Akolkar, A., Pandya, A., Archana, G., and Desai, A. J. 2006. Differential cross- utilization of heterologous siderophores by nodule bacteria of *Cajanus cajan* and its possible role in growth under iron limited conditions. *Appl. Soil Ecol*. 34: 19–26

Khan, M. M. A., Haquei, E., Paul, N. C., Khaleque, M. A., Al-Garni, S. M. S., Rahmans, M., and Islami, M.T. 2017. Enhancement of growth and grain yield of rice in nutrient deficient soils by rice probiotic bacteria. *Rice Sci*. 24(5): 264-273

Khanghahi, M. Y., Pirdashti, H., Rahimian, H., Nematzadeh, G., and Sepanlou, M. G. 2017. Potassium solubilizing bacteria (KSB) isolated from rice paddy soil: from isolation, identification to K use efficiency. *Symbiosis*. 77(3): 1-11. doi:10.1007/s13199-017-0533-0.

Khanghahi, M. Y., Pirdashti, H., Rahimian, H., Nematzadeh, G., Sepanlou, M. G. 2019. The role of potassium solubilizing bacteria (KSB) inoculations on grain yield,

dry matter remobilization and translocation in rice (*Oryza sativa* L). *J. Plant Nutr*. 42(10): 1165-1179

Kim, J., and Rees, D.C. 1994. Nitrogenase and biological nitrogen fixation. *Biochemistry*. 33: 389-387.

Kloepper, J.W. 1994. Plant-growth promoting rhizobacteria (other systems). In. Okon, Y. (Ed.), *Azospirillum* / Plant Associations. CRC press, Bocca Raton, Fl., USA. 111-118

Kloepper, J. W., and Schorth, M. N. 1978. Plant growth promoting rhizobacteria on radishes. In. *Proceedings of the 4th International Conference on Plant Pathogenic Bacteria*, Angers, France. 879-882.

Kloepper, J. W., and Schroth. M. N. 1981. Relationship of *in vitro* antibiosis of plant growth promoting rhizobacteria to plant growth and the displacement of root microflora. *Phytopathology* 71:1020–1024

Krishna, K. R. 2014. Agroecosystems: Soils, climate, crops, nutrient dynamics and productivity. Waretown, Apple Academic Press, Inc. 552 p.

Krishnakumar, S., Bai, V. D. M., and Rajan, R. A. 2014. Evaluation of phosphate solubilizing microorganisms (PSMs) from rhizosphere soil of different crop plants and its antagonistic activity. *J. Microbiol. Biotech. Food Sci*. 3(5): 412-415

Kuan, K. B., Othman, R., Rahim, K. A., and Shamsuddin, Z, H. 2016. Plant growth promoting rhizobacteria inoculation to enhance vegetative growth, nitrogen fixation and nitrogen remobilization of maize under greenhouse conditions. *PLOS ONE*. Doi: 10.1371/ journal. pone.0152478

Kumar, A., Maurya, B.R., and Raghuwanshi, R. 2014. Isolation and Characterization of PGPR and their effect on growth, yield and nutrient content in wheat (*Triticum aestivum* L.). *Biocatal. Agril. Biotechnol*. 3:121-128

Kumar, M. S., Reddy, G. C., Phogat, M., and Koray, S. 2018. Role of biofertilizers towards sustainable agricultural development: a review. *J. Pharmacogn. Phytochemistry*. 7(6):1915-1921

Kumar, P., Dubey, R. C., and Maheshwari, D. K. 2012. *Bacillus* strains isolated from rhizosphere showed plant growth promoting and antagonistic activity against phytopathogens. *Microbiol. Res*. 167: 493-499.

Kumar, U., Panneerselvam, P., Jambhulkar, N. N., and Annapurna, K. 2016. Effect of inoculation of rhizobacterial consortia for enhancement of growth promotion and nutrient uptake in Basmathi rice. *Oryza*. 53(3): 282-287

Kumar, V., and Gera, R. 2014. Isolation of a multi-trait plant growth promoting *Brevundimonas* sp. and its effect on the growth of Bt-cotton. *3 Biotech*. 4: 97-101

Kwon, J.W., and Kim, S. D. 2014. Characterization of an antibiotic produced by *Bacillus subtilis* JW-1 that suppresses *Ralstonia solanacearum*. *J. Microbiol. Biotechnol*. 24:13-18

Ladha, J. K., de Bruijin, F. J., and Malik, K. A. 1997. Introduction: assessing opportunities for nitrogen fixation in rice – a frontier project. *Plant soil*. 124: 1-10

Laldinthar, R., and Dkhar, M. S. 2015. Relationship between soil bacterial population and various physicochemical properties at two broadleaved forest stands of Meghalaya differing in altitudes. *Transcriptomics*. 3(2). doi: 10.4172/2329-8936.1000125

Laskar, F., Sharma, F. D., and Deb, B. 2013. Characterization of plant growth promoting traits od diazotrophic bacteria and their inoculating effects on growth and yield of rice crops. *Glob. Res. Anal*. 2 (4): 3-5.

Lavakush., Yadev, J., Verma, J. P., Jaiswal, D. K., and Kumar, A. 2014. Evaluation of PGPR and different concentration of phosphorus level on plant growth, yield and nutrient content of rice (*Oryza sativa*). *Ecol. Eng.* 62: 123-128.

Li, H. B., Singh, R.K., Singh, P., Song, Q.Q., Xing, Y.X., Yang, L.T., and Li, Y.R. 2017. Genetic diversity of nitrogen-fixing and plant growth promoting Pseudomonas species isolated from sugarcane rhizosphere. *Front. Microbiol*. 8: doi.org/10.3389/fmicb.2017. 01268

Liang, R., Hou, R., Li, J., lyu, Y., Homg, S., Gang, H., and Ouyang, Z. 2020. Effect of different fertilizers on rhizosphere bacterial communities of winter wheat in the North China plain. *Agronomy*. 10:93. Doi. 10.3790/agronomy.10010093

Lindsay, W. L. and Norvell, W. A. 1978. Development of a DTPA soil test for zinc, iron, manganese and copper. *Soil Sci. Am. J*. 42(3): 421-428

Liu, M., Liu, X., Cheng, B., Ma, X., Lyu, X., Zhao, X., Ju, Y., Min, Z., and Fang, Y. 2016. Selection and evaluation of phosphate-solubilizing bacteria from grapevine rhizospheres for use as biofertilizers. *Span. J. Agric. Res*. 14 (4). doi.org/10.5424/sjar/2016144-9714

Liu, Q., Ma, H., Lin, X., Zhou, X., and Zhao, Q. 2019. Effects of different types of fertilizers application on rice grain quality. *Chil. J. Agric. Res*. 79 (2): 202-209.

Loaces, I., Ferrando, L., and Scavino, A. F. 2011. Dynamics, diversity and function of endophytic siderophore-producing bacteria in rice. *Microb. Ecol*. 61: 606–618.

Lucy, M., Reed, E., and Glick, B.R. 2004. Application of free-living plant growth-promoting rhizobacteria. *Antonie Van Leeuwenhoek*. 86: 1-25.

Lugtenberg, B., and Kamilova, F. 2009. Plant growth promoting rhizobacteria. *Annu. Rev. Microbiol*. 63:541-556.

Martínez-Hidalgo, P., Maymon, M., Pule-Meulenberg, F., and Hirsch, A. M. 2019. Engineering root microbiomes for healthier crops and soils using beneficial, environmentally safe bacteria. *Can. J. Microbiol*. 6(2): 91-104

Meena, G., and Maurya, B. R. 2017. Potentiality of potassium solubilizing bacteria on enhancing the growth, yield and nutrient acquisition on wheat (*Triticum aestivum* L.) *Intr. J. Curr. Microbiol. Appl. Sci.* 6(4): 2443 – 2450

Meena, R. K., Singh, Y. V., Prasanna, L. R., Kaur, C., Kumar, A., and Bana, R.S. 2014a. Influence of plant growth promoting rhizobacteria inoculation on nutrient availability, soil microbial population and defense enzymes in rice (Oryza sativa) crop. *Indian. J. Agrl. Sci*. 84(6): 761-764

Meena, V, S., Maurya, B. R., Verma, J. P., and Meena, R. S. 2016. Potassium solubilizing microorganisms for sustainable agriculture. Springer. 133p

Meena, V. S., Maurya, B. R., Verma, J. P. 2014b. Does a rhizospheric microorganisms enhance K+ availability in agricultural soils? *Microbial Res*. 169: 337-347

Meena, V. S., Maurya, B. R., Verma, J. P., Aeron, A., Kumar, A., Kim, K., Bajpai, V. K. 2015. Potassium solubilizing rhizobacteria (KSR): Isolation, identification and K-release dynamics from waste mica. *Ecol. Eng*. 81: 340-347

Mehta, P., Walia, A., Kulshrestha, S., Chauhan, A., and Shirkot, C. K. 2014. Efficiency of plant growth-promoting P-solubilizing *Bacillus circulans* CB7 for

enhancement of tomato growth under net house conditions. *J. Basic Microbiol*. 53. doi: 10.1002/ jobm.201 300562

Meliani, A., Bensoltane, A., Benidire, L., and Oufdou, K. 2017. Plant Growth-Promotion and IAA Secretion with *Pseudomonas fluorescens* and Pseudomonas putida. *Res. Rev. J. Bot. Sci*. 6(2): 16-24.

Miao, G., Jiang-jiao, Z., En-tao, W., Qian, C., Jing. X., and Jian-guang, S. 2015. Multiphasic characterization of a plant growth promoting bacterial strain, *Burkholderia* sp. 7016 and its effect on tomato growth in the field. *J. Integr. Agric*. 14(9): 1855-1863.

Mirza, M. S., Ahmad, W., Latif, F., Haurat, J., Bally, R., Normand, P., and Malik, K. A. 2001. Isolation, partial characterization, and the effect of plant growth-promoting bacteria (PGPB) on micro-propagated sugarcane *in vitro*. *Plant Soil*. 237: 47-54.

Mirza, M.S., Mehnaz, S., Normand, P., Prigent -Combaret, C., Moenn-Loccoz, Y., Bally, R., and Malik, K. A. 2006. Molecular characterization and PCR detection of a nitrogen fixing *Pseudomonas* strain promoting rice growth. *Biol. Fertil. Soils*. 43: 163-170.

Modi, K., and Jacob, F. 2017. Isolation and characterization of phosphate solubilizing *Bacillus* consortia for plant growth promotion. *Int. J. Pure App. Biosci*. 5 (3): 278-283

Montano, P. F., Alias-Villegas, C., Bellogin, R. A., del-Cerro, P., Espuny, M. R., Jimenez-Guerrero, I., Lopez-Baena, F. J., Ollero, F. J., and Cubo, T. 2014. Plant growth promotion in cereal and leguminous agricultural important plants: from microorganism capacities to crop production. *Microbiol. Res*. 169, 325–336.

Moraes, M.G. 1998. Mechanisms of acquired systemic resistance in plants. *Revis. Anu. Patol. Plantas.* 6:261–284

Mumtaz, M. Z. Ahamed, M., Jamil, M., and Hussain. T. 2017. Zinc solubilizing *Bacillus* spp. Potential candidates for biofortification in maize. *Microbiol. Res*. 202: 51-60

Nahar, U.A., Othman, R., Latif, M.A., Panhwar, Q. A., Ammadin, P.A. M., and Shamsuddin, Z. H. 2013. Biomolecular Characterization of Diazotrophs

Isolated from the Tropical Soil in Malaysia. *Int. J. Mol. Sci.*14: 17812-17829. doi:10.3390/ijms140917812

Nahas, E. 1996. Factors determining rock phosphate solubilization by microorganisms isolated from soil. *World J. Microbiol. Biotechnol*. 12: 567–572

Nakho, N., and Dkhar, M. 2010. Impact of organic & inorganic fertilizer on microbial population and biomass in paddy soil. *J. Agron*. 9(3): 102-110.

Naqqash, T., Imran, A., Hameed, S., Shahid, M., Majeed, A., Iqbal, J., Hanif, M. K., Ejaz, S., and Malik, K. A. 2020. First report of diazotrophic *Brevundimonas* spp. As growth enhancer and root colonizer of potato. *Sci. Rep*.10:1-14. doi.org/10.1038/s41598.02069782-6

Novik, G., Savich, V., and Kiselev, E. 2015. An insight into beneficial Pseudomonas bacteria. In microbiology in agriculture and human health. Shah, M. M. Ed: Intech Open. Rijeka, Croatia. 73-105 pp.

Ogut, M., Er, F., and Kandemir, N. 2010. Phosphate solubilization potentials of soil *Acinetobacter* strains. *Biol. Fertil. Soils.* 46: 707-715.

Olsen, S. R., Cole, C. V., Watanabe, F. S., and Dean, L. A. 1954.Estimation of available phosphorus in sols by extraction with sodium bicarbonate. USDA. 939 p.

Oves, M., Khan, M. S., and Qari, H. A. 2019. Chromium reducing and phosphate-solubilizing Acromobacter xylosoxidans bacteria from heavy metal-contaminated soil of Brass city, Moradabad, India. *Int. J. Envron. Te*. doi.10.1007/s13762-019-02300-y.

Panda, B., Rahman, H., and Panda, J. 2016. Phosphate solubilizing bacteria from the acidic soils of Eastern Himalayan region and their antagonistic effect on fungal pathogens. *Rhizosphere* 2: 62–71

Panda, D., Nayak A. K., and Mohanty, S. 2019. Nitrogen management in rice. *Oryza*. 56: 125-135

Panda, H. 2011. Manufacture of Biofertilizer and Organic Farming. Asia Pacific Business Press Inc. pp 103-121

Pande, A., Pandey, P., Mehra, S., Singh, M., and Kaushik, S. 2017. Phenotypic and genotypic characterization of phosphate solubilizing bacteria and their efficiency on the growth of maize. *J. Genet. Eng. Biotechnol*. 15(2): 379-391.

Panhwar, Q. A. R., Othman, Z. A., Rahman, S. M., and Ismail, M. R. 2012. Isolation and characterization of phosphate- solubilizing bacteria from aerobic rice. *Afr. J. Biotechnol*. 11 (11):2711-2719

Park, K. H., Lee, O. M., Jung, H. I., Jeong, J. H., Jeon, Y.D., Hwang, D.Y., Lee, C.Y., Son, H.J. 2010. Rapid solubilization of insoluble phosphate by a novel environmental stress-tolerant *Burkholderia vietnamiensis* M6 isolated from ginseng rhizospheric soil. *Appl. Microbiol. Biot*. 86:947–995

Parmar, K. B., Meheta, B. P., and Kunt, M. D. 2016. Isolation, characterization and identification of potassium solubilizing bacteria from rhizosphere soil of maize (*Zea mays*). *Int. J. Sci. Environ. Te.* 5(5): 3030-3037.

Parmar, P., and Sindhu, S. S. 2013. Potassium solubilization by rhizosphere bacteria: influence of nutritional and environmental conditions. *J. Microbiol. Res.* 3 (1): 25-31

Patten, C. L., and Glick, B. R. 1996. Bacterial biosynthesis of indole-3-acetic acid. *Can. J. Microbiol.* 42: 207-220

Patten, C.L., and Glick, B.R. 2002. Role of *Pseudomonas putida* indole acetic acid in development of the host plant root system. *Appl. Environ. Microbiol.* 68:3795–3801

Pawar, A., Ismail, S., Mundhe, S., and Patil, V. D. 2015. Solubilization of insoluble zinc compounds by different microbial isolates *in vitro* condition. *Int. J. Trop. Agric*. 33, 865–869.

Pham, V. T. K., Rediers, H., Ghequirel, M. G. K., Nguyen, H. H., De Mot, R. Vanderleyden, J., and Spaepen, S. 2016. The plant growth-promoting effect of the nitrogen-fixing endophyte *Pseudomonas stutzeri* A15. *Arch. Microbiol*. doi:10.1007/s00203-016-1332-3

Phiromtan, M., Mala, T., and Srinives, P. 2010. Effect of various habitats in Thailand on the abundance of *Azotobacter* in rhizospheric soils. ISSAAS 2009 January 11-15, 2010 Pattaya, Thailand. 235-241

Pieterse, C. M. J, van Pelt, J. A. Knoester, M, Laan, R., Gerrits, H., Weisbeek, P.J., and van Loon, L. C. 1998. Novel signaling pathway controlling induced systemic resistance in *Arabidopis*. *Plant Cell*. 10: 1571-1580

Prajapathi, K. B., and Modi, H.A. 2012. Isolation and characterization of potassium solubilizing bacteria from ceramic industry. *CIBtech J. Microbiol.* 1(2-3): 8-14

Prasad, M. N., Freitas, H., Fraenzle, S., Wuenschmann, S., Markert, B. 2010. Knowledge explosion in phytotechnologies for environmental solutions. *Environ. Pollut*. 158:18–23.

Prasanna, R., and Nayak, S. 2007. Influence of diverse rice soil ecologies on cyanobacterial diversity and abundance. *Wetlands Ecol. Manage*.15:127–134

Price, N. P., Rooney, A. P., Swezey, J. L., Perry, E., and Cohan, F. M. 2007. Mass spectrometric analysis of lipopeptides from *Bacillus* strains isolated from diverse geographical locations. *FEMS Microbiol. Lett.* 271, 83–89. doi: 10.1111/j.1574-6968.2007.00702.x

Rabindran, R. and Vidyasekaran, P. 1996. Development of a formulation of Pseudomonas fluorescens PFALR2 for management of rice sheath blight. *Crop Prot.* 15: 715-721

Rahma, H., Nurbilis, and Kristina, N. 2019. Characterization and potential of plant growth promoting rhizobacteria on rice seedling growth and the effect on *Xanthomonas oryzae* pv. *oryzae*. *Biodiversitas*. 20 (12): 3654-3661.

Raja, N. 2013. Biopesticides and biofertilizers: ecofriendly sources for sustainable agriculture. J. *Boifertil. Biopestici*. 4 (1). Doi. 10.4172/2155-6202.2000e112

Raja, P., Uma, S., Gopal, H., and Govindarajan, K. 2006. Impact of bio inoculants consortium on rice root exudates, biological nitrogen fixation and plant growth. *J. Biol. Sci*. 6 (5): 815-823.

Rajawat, M.V. S., Singh, S., Tyagi, S. P., and Saxena, A. K. 2016. A modified plate assay for rapid screening of potassium solubilizing bacteria. *Pedosphere*. 26 (5): 768–773.

Ramalakshmi, A., Iniyakumar, M. and Anthoni, R. 2008. Influence of biofertilizers on soil physico-chemical and biological properties during cropping period. *Asian J. Bio. Sci*. 3 (2): 348-351

Rana, A, Saharan B, Joshi M, Prasanna R, Kumar K, Nain L. 2011a. Identification of multi-trait PGPR isolates and evaluating their potential as inoculants for wheat. *Ann. Microbiol*. 61:893-900

Rana, A. Saharan, B., Kabi, S. R., Prasanna, R., and Nain, L. 2011b. *Providencia*, a PGPR with biocontrol potential elicits defense enzymes in wheat. *Ann. Plant Protec. Sci.* 19 (1): 138-141

Rana, A., Saharan, B., Nain, L., Prasanna, R., and Shivay, Y. S. 2012. Enhancing micronutrient uptake and yield of wheat through bacterial PGPR consortia. *Soil Sci. Plant Nutr*. 58: 573-582

Rashid, M., Khalil, S., Ayub, N., and Alam, S. 2004. Organic acids production and phosphate solubilization by phosphate solubilizing microorganisms (Psm) under in vitro conditions. *Pak. J. Biol. Sci.* 7 (2): 187-196.

Rasul, M., Yasmina, S., Sulemana, M., Zaheerc, A., Reitzed, T., Tarkka, M. T., Islama, E., and Mirza, M. S. 2019. Glucose dehydrogenase gene containing phosphobacteria for biofortification of phosphorus with growth promotion of rice. *Microbiol. Res*. 223-225: 1-12.

Rediers, H., Vanderleyden, J., and De Mot, R. 2009. Nitrate respiration in *Pseudomonas sturzeri* A15 and its involvement in rice and wheat root colonization. *Micrbiol. Res*. 164: 461-468

Richardson, A.E., Hocking, P.J., Simpson, R. J., and George, T. S. 2009. Plant mechanisms to optimize access to soil phosphorus. *Crop Pasture Sci*. 60: 124-143

Rijavec, T., and Lapanje, A. 2016. Hydrogen Cyanide in the Rhizosphere: Not Suppressing Plant Pathogens, but Rather Regulating Availability of Phosphate. *Front. Microbiol*. 7 (1785). doi: 10.3389/fmicb.2016.01785

Rodrigues, A. A., Forzani, M. V., Soares, R, D. S., Sibov, S. T., Vieira, D. G. 2016. Isolation and selection of plant growth-promoting bacteria associate with sugarcane. *Pesq. Agropec. Trop*. 46 (2): 149-158

Rodriguez, H., and Fraga, R. 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnol. Adv*. 17: 319-339

Roper, M. M., and Gupta, V. V. S. R. 2016. Enhancing non-symbiotic N_2 fixation in agriculture. *Open. Agr. J.* 10:7–27.

Saeid, A., Prochownik, E. and Dobrowolska-Iwanek, J. 2018. Phosphorus solubilization by *Bacillus* species. *Molecules* 23(11): 2897. doi:10.1016/j.biortech.2017.09.092

Sahoo, R. K., Ansari, M. W., Dangar, T. K., Mohanty, S., and Tuteja, N. 2013. Phenotypic and molecular characterization of efficient nitrogen- fixing *Azotobacter* strains from rice fields for crop improvement. *Protoplasma*. 251: 511-523.

Sahu, P. K., Sahu, U., Bhoyar, M. S., Singh, R. K., Kumar, M., and Shukla, R. 2016. Microbial dynamics in rhizosphere at different growth stages of rice (*Oryza sativa* L.) based on plant growth promoting functions. *Article*. 37: 434-444.

Sambrook, J., Fritschi, E. F., and Maniatis, T. 1989. *Molecular cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, New York, 752p.

Saranya, K. S. 2016. Exploration of native mineral phosphate solubilizing microorganisms on biofertilizer for the acidic soils of Kerala. M.Sc (Ag) thesis, Kerala Agricultural University. 269p.

Saravanan, V. S., Madhaiyan, M., and Thangaraju, M. 2007. Solubilization of zinc compounds by the diazotrophic, plant growth promoting bacterium *Gluconacetobacter diazotrophicus*. *Chemosphere*. 66, 1794–1798.

Saravanan. S., Subramaniam, S. R., and Raj, S. A. 2004. Assessing *in vitro* solubilizing potential of different zinc solubilizing bacterial (ZSB) isolates. *Bra. J. Microiol*. 35:121-125

Saxena, A. K., Kumar, M., Chakdar, H., Anuroopa, N., and Bagyaraj, D. J. 2020. *Bacillus* species in soil as a natural resource for plant health and nutrition. *J. Appl. Microbiol*. 128: 1583-1594.

Schippers, B., Bakker, A. W., and Bakker, H. M. 1987. Interactions of deleterious and beneficial rhizosphere microorganisms and the effect of cropping practice. *Annu. Rev. Phytopathol*. 25: 339-358

Schippers, B., Bakker, A. W., Bakker, P. A. H. M., Van Peer, R. 1990. Beneficial and deleterious effects of HCN producing pseudomonas on rhizosphere interactions. *Plant soil*.129: 75-83

Schwyn, B., and Neilands, J.B. 1987. Universal chemical assay for the detection and determination of siderophores. *Anal. Chem.*160: 47-56.

Selvi, K. B., Paul, J. J. A., Vijaya, V., and Saraswathi, K. 2017. Analyzing the Efficacy of Phosphate Solubilizing Microorganisms by Enrichment Culture Techniques. *Biochem. Mol. Biol. J.* 3.doi.10.21767/2471-8084.100027

Setiawati, T. C., and Mutmainnah, L. 2016. Solubilization of potassium containing mineral by microorganisms from sugarcane rhizosphere. *Agr. Agrl Sci. Procedia*. 9: 108-117.

Shaheena, K.M.F., Girija, D., Gopal, K.S., Sureshkumar, P., and Kumar, P. G. S. 2016. Novel free living diazotrophs associated with black pepper in Wayanad. *J. Trop. Agric.* 54(1): 71-78

Shaikh, S. S., and Saraf, M. 2017. Optimization of growth conditions for zinc solubilizing plant growth associated bacteria and fungi. *J. Adv. Res. Biotech*. 2(1): Doi: httpdx.doi.org/10.15 226/2475-4714/2/1/00115

Shakeel, M., Rais, M., Hassan, M. N., and Hafeez, F. Y. 2015. Root associated *Bacillus* sp. improves growth, yield and zinc translocation for basmathi rice (*Oryza sativa*) varieties. *Front. Microbiol*. 6:1286. doi:10.3389/fmicb.2015.01286

Shanthpure, A., Girija, D., Sidharthan, P. P., Peedikakkal, M, P., and Nair, S. S. 2019. Variation in soil bacterial communities by long-term application of organic and inorganic fertilizers in rice. *J. Cereal Res*. 11(3):286-292

Sharma, C., and Dubey, R. C. 2017. Plant growth promoting and antagonistic properties of *Pseudomonas putida* CRN-09 against *Macrophomina phaseolina* (TASSI) GOID. *Ind. J. Sci. Res*.13(2):01-05.

Sharma, K. 2005. Manual of Microbiology. In: Isolation, purification and identification of bacteria. Ane books Pub. New Delhi. P. 41.

Sharma, L., Shukla, S. K., Jaiswal, V. P., and Sharma, K. U. 2019. Characterization of cultural beneficial bacterial diversity for plant growth promoting attributes associated with rice rhizosphere. *J. Environ. Biol*. 40: 1180-1187.

Sharma, S., Kauri, M., and Prashad, D. 2014. Isolation of fluorescent *Pseudomonas* strain from temperate zone of himachal pradesh and their evaluation as plant growth promoting rhizobacteria (PGPR). *The Bioscan*. 9:323-328.

Sharma, Suhil, K., Sharma, M. P., Ramesh, A., and Joshi, O. P. 2012. Characterization of zinc solubilizing *Bacillus* isolates and their potential to influence zinc assimilation in soybean seeds. *J. Microbiol. Biotechnol.* 22 (3):352-359.

Sharon, J. A., Hathwaik, L.T., Glenn, G. M., Imam, S. H., and Lee, C.C. 2016. Isolation of efficient phosphate solubilizing bacteria capable of enhancing tomato plant growth. *J. Soil Sci. Plant Nutr*. 16(2): 525-536.

Shen, H., He, X., Liu, Y., Chen, Y., Tang, J., and Gua, T. 2016. A complex inoculant of N2-fixing P and K solubilizing bacteria from a purple soil improves the growth of kiwifruit (*Actinidia chinenesis*) plantlets. *Front. Microbiol*. 7: 841. doi: 10.3389/fmicb.2016.00841

Sheng, X., and Huang, W. 2001. Mechanism of potassium release from feldspar affected by the sprain Nbt of silicate bacterium. *Acta. Pedologica. Sinica*. 39: 863-871.

Shultana, R., Zuan, A. T. K., Othman, R., and Yusop, M. R. 2019. Growth and nutrients uptake of rice at early seedling stage as inoculated with *Bacillus* spp. *Plant Archives*. 19(2): 1995-2001.

Silja, V. P, Varma, K.S., and Mohanan, K. V. 2008. Ethnomedicinal plant knowledge of the *Mullu kuruma* tribe of Wayanad district, Kerala. *Indian J. Tradit. Know*. 7(4): 604-612.

Sims, J. T. and Johnson, G. V. 1991. *Micronutrients in Agriculture* (2nd ED). Soil Science Society of America. Madison, 476p.

Singh, D., Rajawat, M.V.S., Kaushik, R., Prasanna, R., and Saxena, A.K. 2017. Beneficial role of endophytes in biofortification of Zn in wheat genotypes varying in nutrient use efficiency grown in soils sufficient and deficient in Zn. *Plant Soil*. 416: 107–116.

Singh, M. J., Singh, D. P., Mesapogu, S. Babu, B. K., and Bontemps. C. 2011. Concomitant colonization of *nif*H positive endophytic *Burkholderia* sp. in rice (Oryza sativa L.) promotes plant growth. *World J. Microbiol. Biotechnol.* 27: 2023-2031

Singh, M.V. 2009. Evaluation of current micronutrient stocks in different Agro Ecological zones of India for sustainable crop production. *Fert. News*. 46: 25-42.

Singh, R. K., Singh, P., Li, H., Song, Q., Guo, D., Solanki, M. K., Verma, K. K., Malviya, M. K., Song, X., Lakshmanan, P. Yamg, L. and Lil, Y. 2020. Diversity of nitrogen-fixing rhizobacteria associated with sugarcane: a comprehensive study of plant-microbe interactions for growth enhancement in *Saccharum* spp. *BMC plant Biol.* 20(1): 1-21.

Sirohi, G., Upadhyay, A., Srivastava, P. S., and Srivastava, S. 2015. PGPR mediated Zinc biofertilization of soil and its impact on growth and productivity of wheat. *J. Soil. Sci. Plant. Nutr,* 15:202-216

Sivaji, M., Vinoth, R., Tamilkumar, P., Suresh K. S., Chandrasekara, A., and Syamala, M. 2016. Isolation and characterization of plant growth promoting *Berkholderia* spp. *Int. J. Plant Ptrot.* 9: 223-229

Skidmore, A. M. and Dickinson, C. H. 1976. Colony interactions and hyphal interference between *Septoria nodarum* and phylloplane fungi. Trans. *Br. Mycological. Soc*.66: 57-64

Somasegaran, P., and Hoben, H. J. 1994. Handbook for Rhizobia. Methods in Legume–Rhizobium Technology. Heidelberg, NY: Springer. doi: 10.1007/978-1-4613-8375-8

Spencer, R. C. 2003. *Bacillus anthracis. J. Clin. Pathol.* 56: 182–187. doi: 10.1136/jcp.56.3.182

Stephen, J., Shabanmol, S., Rishad, K. S., and Jisha, M.S. 2015. Growth enhancement of rice (*Oryza sativa*) by phosphate solubilizing *Gluconacetobacter* sp. (MTCC 8368) and *Burkholderia* sp. (MTCC 8369) under greenhouse conditions. *3 Biotech*. 5: 831-837

Subbaiah, B.V. and Asija, G. L. 1965. A rapid procedure for determination of available nitrogen in soil. *Curr. Sci*. 25: 259-260.

Subramanian, J. and Styan, K. 2014. Isolation and selection of fluorescent pseudomonads based on multiple plant growth promotion traits and siderotyping. *Chil. J. Agric. Res.*74(3): 319-325.

Sugumaran, P and Janarthanam, B. 2007. Solubilization of potassium containing minerals by bacteria and their effect on plant growth. *World J. Agrl. Sci.* 3: 350-355

Sun, F., Ou, Q., Wang, N., Guo, Z., Ou, Y., Li, N., Peng, C. 2020. Isolation and identification of potassium solubilizing bacteria from *Mikania micrantha* rhizospheric soil and their effect on *Mikania Micrantha*. *Glob. Ecol. Consrv*. 23. doi.org/10.1016/j.gecco.2020.e01141

Tandon, H. L. S. 2004. Fertilizers in Indian Agriculture from 20th to 21st Century. Fertilizer development and consultation organization, New Delhi, India. 240 p.

Tan, K. Z., Radziah, O., Halimi, M. S., Khairuddin, A. R. and Shamsuddin, Z. H. 2015. Assessment of plant growth promoting rhizobacteria (PGPR) and rhizobia as multi-strain biofertilizeron growth and N2 fixation of rice plants. *Aust. J. Crop. Sci*. 9 (12):1257-1264

Tan, K. Z., Radziah, O., Halimi, M. S., Rahim, K. B. A., Abdullah, M. Z., and Shamsuddin. 2017. Growth and yield responses of rice cv. MR219 to rhizobial and plant growth-promoting rhizobacterial inoculations under different fertilizer-n rates. *Bangladesh J. Bot*. 46: 481-488.

Tan, K.Z., Radziah, O., Halimi, M. S., Khairuddin, A.R., Habib, S. H., and Shamsuddin, Z. H. 2014. Isolation and characterization of rhizobia and plant growth-promoting rhizobacteria and their effects on growth of rice seedlings. *Am. J. Agric. Biol. Sci*. 9 (3): 342-360

Tejera. N., Luch, C., Martinez-Toledo, M. V., and Gonzalez-Lopez, J. 2005. Isolation and characterization of *Azotobacter* and *Azospirillum* strains from the sugarcane rhizosphere. *Plant Soil* 270: 223–232

Timmusk, S., behers, L., Muthoni, J., Muraya, A., and Aronsson, A. C. 2017. Perspectives and challenges of microbial application for crop improvement. *Front. Plant Sci*. 8:49. Doi:10.3389/fpls.2017.00049

Uroz, S., Calvaruso, C., Turpault, M.P., and Frey-Klett, P. 2009. Minerals weathering by bacteria: ecology, actors and mechanisms. *Trend Microbiol*. 17: 378-387

Vaid, S.K., Gangwar, B.K., Sharma, A., Srivastava, P.C., and Singh, M.V. 2013. Effect of zinc solubilizing bioinoculants on zinc nutrition of wheat (*Triticum aestivum* L.). *Int. J. Adv. Res*. 1(9): 805-820

Vaid, S. K., Kumar, B., Sharma, A., Shukla, A. K., and Srivastava, P. C. 2014. Effect of zinc solubilizing bacteria on growth promotion and zinc nutrition of rice. *J. Soil Sci. Plant Nutr.* 14 (4): 889-910

Vance, E D., Brookes, P. C., and Jenkinson, D. S. 1987. An extraction method for measuring soil microbial biomass C. *Soil. Biol. Biochem.* 19(6): 703-707.

Verma, J. P., Yadev, J., Tiwari, K. N., and Kumar, A. 2013. Effect of indigenous *Mesorhizobium spp*. and plant growth promoting rhizobacteria on yields and

nutrients uptake of chickpea (*Cicer aurentium* L) under sustainable agriculture. *Ecol. Eng*. 51:282-286.

Vessey, J. K. 2003. Plant growth promoting rhizobacteria as biofertilizers. Plant soil. 255 (2): 571-586.

Vikram, A., Alagawadi, A. R., Hamzehzarghani, H., and Krishnaraj, P. U. 2007. Factors related to the occurrence of phosphate solubilizing bacteria and their isolation in vertisols. *Int. J. Agric. Res*. 2 (7): 571-580

Vincent, J. M., and Humphrey, B. 1970. Taxonomically significant group antigens in Rhizobium. *J. Gen. Microbiol.* 63: 379–382.

Voisard, C., Keel, C., Haas, D., and Dèfago, G. 1989. Cyanide production by *Pseudomonas fluorescens* helps suppress black root rot of tobacco under gnotobiotic conditions. *EMBO J.* 8 (2): 351-358.

Vyas, P., Kumar, D., Dubey, A., and Kumar, A. 2018. Screening and characterization of Acromobacter xylosoxidans isolated from rhizosphere of Jatropha curcas L. (Energy crop) for plant growth-promoting traits. Adv. Res. Biotech. 3(2):1-8

Walkley, A. and Black, I. A. 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Sci*. 37(1):29-38.

Wan, W., Qin, Y., Wu, H., Zuo, W., He, H., Tan, J., Wang, Y., and He, D. 2020. Isolation and characterization of phosphorus solubilizing bacteria with multiple phosphorus sources utilizing capability and their potential for lead immobilization in soil. *Front. Microbiol*. 11: doi: 10.3389/fmicb.2020.00752

Watanabe, F. S. and Olsen, S. R. 1965. Test of an aerobic acid method for determine phosphorus in water and NaHCO3 extracts from soil. *Soil Sci. Soc. Am. J*. 29(6): 677-678.

Weller, D. M., and Thomashow, L. S. 1994. Current challenges in introducing beneficial microorganisms into the rhizosphere. In Molecular Ecology of Rhizosphere Microorganisms: Biotechnology and Release of BMOs: O'Gara, F., Dowling, D. N., Boesten, B., Eds: VCH: New York, NY, USA. 1-18.

Woese, C. R., Stackebrandt, E., Macke, T. J., and Fox, G. E. 1985. A phylogenetic definition of the major eubacterial taxa. *Syst. Appl. Microbiol*. 6:143–151.

Wongfun, N., Plötze, M., Furrer, G., and Brandl, H. 2014. Weathering of granite from the Damma glacier area: the contribution of cyanogenic bacteria. *Geomicrobiol. J*. 31: 93 – 100.

Woodman, M. E. 2008. Direct PCR of intact bacteria (colony PCR). *Curr. Protocol. Microbiol*. 9 (1): 1-3.

Wu, S. C., Cao, Z. H., Li, Z. G., Cheung, K. C., and Wong, M. H. 2005. Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. *Geoderma*. 125(2):155-166.

Xie, G. H., Su, B. L, and Cui, Z. J. 1998. Isolation and identification of N2-fixing strains of *Bacillus* in rice rhizosphere of the Yangtze River valley. *Acta. Microbiol. Sin*. 38: 480–483.

Xie, H., Pasternak, J. J., and Glick., B. R. 1996. Isolation and characterization of mutants of the plant growth-promoting rhizobacterium *Pseudomonas putida* GR12-2 that overproduce indoleacetic acid. *Curr. Microbiol*. 32: 67-71.

Yadav, J., Verma, J. P., and Tiwari, K. N. 2010. Effect on plant growth promoting rhizobacteria on seed germination and plant growth chickpea (Cicerarietinum L.) under in vitro conditions. *Biol. Forum*. 2 (2) 15-18.

Yasmin, S., Haffeez, F. Y., Mirza, M.S., Rasul, M., Arshad, H. M. T.., Zubair, M., and Iqbal, M. 2017. Biocontrol of bacterial leaf blight of rice and profiling of secondary metabolites produced by rhizospheric *Pseudomonas aeruginosa* BRp3. *Front. Microbiol*. 8: 1895. doi: 10.3389/fmicb.2017.01895

Yousefi, H., Hassanzadeh, N., Behboudi, K., and Firouzjahi, F. B. 2018. Identification and determination of characteristics of endophytes from rice plants and their role in biocontrol of bacterial blight caused by *Xanthomonas oryzae* pv. *Oryzae*. *Hellenic Plant Prot. J.* 11: 19-33

Yousuf, J., Thajudeen, J., Rahiman, M., Krishnankutty, S.P., Alikunj, A., and Abdulla, M.H. 2017. Nitrogen fixing potential of various heterotrophic *Bacillus* strains from a tropical estuary and adjacent coastal regions. *J. Basic. Microbiol.* 57: 922–932

Zahid, M., Abbasi, M. K., Hameed, S., and Rahim, N., 2015. Isolation and identification of indigenous plant growth promoting rhizobacteria from Himalayan region of

Kashmir and their effect on improving growth and nutrient contents of maize (Zea mays L.) *Front. Microbiol.* 6: 207.doi:10.3389/fmicb.2015.00020

Zahir, Z. A., Muhammad, A. F., and Frankenberger. W. T. 2004. Plant growth promoting rhizobacteria: applications and perspectives in agriculture. *Adv. Agron*. 81: 97-168.

Zhang, C., and F. Kong. 2014. Isolation and identification of potassium solubilizing bacteria from tobacco rhizospheric soil and their effect on tobacco plants. *Appl. Soil Ecol.* 82:18–25.

Zhao, K., Penttinen, P., Zhang, X., Ao, X., Liu, M., Yu, X., and Chen, Q. 2014. Maize rhizosphere in Scichuan, China, hosts plant growth promoting *Burkholderia cepacia* with phosphate solubilizing and antifungal abilities. *Microbiol. Res.* 169: 76-82

Zheng, B., Ibrahim, M., Zhang, D., Bi, Q., Li, H., Zhou, G., Ding, K., Penuelas, J., Zhu, Y., and Yang, X. 2018. Identification and characterization of inorganic-phosphate-solubilizing bacteria from agricultural fields with a rapid isolation method. *AMB Expr*. 8: doi.org/10.1186/s13568-018-0575-6

Zheng, B., Zhang, D., Wang, Y., Hao, X., Wadaan, M. A. M, Hozzein, W. N., Penuelas, J., Zhu, Y., and Yang, X. 2019. Responses to soil pH gradients of inorganic phosphate solubilizing bacteria community. *Sci. Rep*. 9 (25). doi:10.1038/s41598-018-37003

Zhou, J., Burns, M. A., and Tjedje, J. M. 1995. DNA recovery from soils of diverse composition. *Appl. Environ. Microbiol*. 62: 316-322

Appendices

Appendix 1

Media used and composition

Ashby's Mannitol agar

Mannitol	20.0 g
Dipotassium phosphate	0.20 g
Magnesium sulphate	0.20 g
Sodium chloride	0.20 g
Potassium sulphate	0.10 g
Calcium carbonate	5.00 g
Agar	20.00 g
Distilled water	1000 ml

Aleksandrov agar

Dextrose	5.00 g
Magnesium sulphate	0.50 g
Calcium carbonate	0.10 g
Potassium allumino silicate	2.00 g
Ferric chloride	0.005 g
Calcium phosphate	2.00 g
Agar	20.00 g
Distilled water	1000 ml

Jensen's agar

Sucrose	20.00 g
Dipotassium phosphate	1.00 g
Magnesium sulphate	0.50 g
Sodium chloride	0.50 g
Ferrous sulphate	0.10 g
Sodium molybdate	0.005 g
Calcium carbonate	2.00 g

Agar	20.00 g
Distilled water	1000 ml

King's medium B base

Proteose peptone	1.00 g
Dipotassium hydrogen phosphate	1.50 g
Magnesium sulphate heptahydrate	1.50 g
Agar	20.00 g
Glycerol	15 ml
Distilled water	1000 ml
pH	7.20 $\pm$ 0.2

Mineral salts medium amended with 0.1% ZnO

Dextrose	100.00 g
Ammonium sulphate	10.00 g
Potassium chloride	2.00 g
Dipotassium phosphate	1.00 g
Magnesium sulphate	2.00 g
Zinc oxide	1.00 g
Agar	20.00 g
Distilled water	1000 ml

Nutrient agar

Beef extract	3.00 g
Peptone	5.00 g
NaCl	5.00 g
Agar	20.00 g
Distilled water	1000 ml

Pikovskaya's agar

Dextrose	10.00 g
Calcium phosphate	5.00 g
Ammonium sulphate	0.50 g
NaCl	0.20 g
Magnesium sulphate	0.10 g
Potassium chloride	0.20 g
Yeast extract	0.50 g
Manganese sulphate	0.001 g
Ferrous sulphate	0.001 g
Distilled water	1000 ml
Agar	20.00 g
pH	7.2 ± 0.2

Potato dextrose agar

Potato infusion	200.00 g
Dextrose	20.00 g
Agar	20.00 g
Distilled water	1000 ml

Appendix 11

Equipment used in the present study

Sterilization of culture media - Equitron SLEFA and NatSteel horizontal autoclave

Centrifugation - Eppendorf 58004R and SPINWIN MC- 02

pH of culture media and buffers - Eutech pH Tutor

Amplification of 16S rRNA gene -. Eppendorf Master Cycler

Visualization of agarose gel - UV Benchtop Transilluminator

Photomicrography -Leica ICC50

Laminar air flow chamber - Rotek Laboratory Instruments

CHARACTERIZATION AND EVALUATION OF PLANT GROWTH PROMOTING RHIZOBACTERIA FROM RICE SOILS OF WAYANAD

by

W. R. K. D. W. K. V. WICKRAMASINGHE

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ABSTRACT OF THE THESIS

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(Agricultural Microbiology)

Faculty of Agriculture

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Abstract

Plant growth promoting rhizobacteria (PGPR) are a group of bacteria that colonize the plant rhizosphere and enhance the growth and yield of plants. The present investigation entitled "Characterization and evaluation of plant growth promoting rhizobacteria from rice soils of Wayanad" was undertaken at the Department of Agricultural Microbiology" during the year 2018-2020, with the objective of isolation, characterization and evaluation of plant growth promoting rhizobacteria from rice soils of Wayanad and formulation of a consortium to improve the growth and yield of rice.

Isolation of rhizobacteria with potential plant growth promoting (PGP) activities was attempted from rice rhizosphere soils collected from ten locations in Wayanad district of Kerala. Selective media were used for the isolation of PGPRs including nitrogen fixers, solubilizers of phosphate, K and Zn and fluorescent pseudomonads. A total of 149 isolates obtained on different media were subjected to preliminary screening for growth on selective media, which yielded 32 N-fixers, 16 phosphate solubilizers, four K solubilizers, six Zn solubilizers and two fluorescent pseudomonads. These isolates were evaluated *in vitro* for PGP activities (production of IAA, NH_3, HCN and siderophore) and antagonistic activities against *R. solani* and *X. oryzae*. Twenty promising isolates were selected based on their functional efficiency for further characterization using cultural, morphological, biochemical and molecular methods.

Four isolates were found to be Gram-positive rods and sixteen isolates were Gram-negative short rods. Eighteen isolates were identified based 16S rRNA gene sequencing and the sequences of all the eighteen isolates deposited in the GenBank of the NCBI. Phylogenetic analysis using MEGA 7 software showed two major clusters and several sub-clusters. A few of the native isolates stood out distinctly from the available accessions in the database, showing that they are genetically diverse.

Based on the efficiency of N fixation, P, K and Zn solubilization and other PGP activities, isolates were ranked. Based on ranking, three N-fixers (*Bacillus* sp. $AkNF_3$, *Pseudomonas* sp. $PkNF_4$ and *Pseudomonas putida* $KgNF_1$), three phosphate solubilizers (*Bacillus megaterium* $PkPS_1$, *Acinetobacter schindleri* $AkPS_4$ and

Achromobacter sp. AvPS$_1$), two K-solubilizers (*Microbacterium* sp. MvKS$_1$ and *Acinetobacter calcoaceticus* MvKS$_3$) and two zinc solubilizers (*Achromobacter marplatensis* ThZnS$_2$ and *Cytobacillus kochii* PkZnS$_3$) were selected for consortial formulation.

Compatibility of ten promising isolates was tested by cross streaking and dual culture methods. Three PGPR based consortia (Consortium 1, 2 and 3) were formulated, each consisting of 5 native isolates (two N-fixers, one each of phosphate, K and Zn solubilizers). These consortia were evaluated in pot culture experiment, along with KAU commercial formulation (PGPR mix-1), at RARS, Ambalavayal, with rice (variety Valichoori) as the test crop. PGPR application was combined with two levels (50% and 75%) of recommended dosage of inorganic fertilizers (RDF). Population of total bacteria, N fixers, P, K and Zn solubilizers was higher in combined application of biofertilizer with inorganic fertilizers than uninoculated treatments and this was indicative of better colonization of native PGPRs in the rice rhizosphere. Growth and yield parameters indicated that application of PGPR consortium with 75% RDF was statistically on par with PoP (KAU) and 100% RDF. Results suggested that 25% inorganic N, P and K can be replaced by using native PGPR consortium without affecting plant growth, yield, plant nutrient content and soil nutrient content. Considering the above parameters, two best consortia (Consortium 2 and Consortium 3) were selected for further field evaluation.

Field evaluation was carried out to assess the efficiency of two selected native PGPR consortia at RARS, Ambalavayal. Five treatments included were, consortium 2 + 75% RDF, consortium 3 + 75% RDF, reference biofertilizer PGPR mix-1 + 75% RDF, 100% RDF and farmer's practice (farm yard manure 5t ha^{-1}). Results suggested that root colonization of total bacteria, N fixers, P, K and Zn solubilizers was higher in all treatments of combined application of biofertilizers with 75% inorganic fertilizer than 100% RDF alone. Growth and yield parameters suggested that combined application of Consortium 2 with 75 % RDF was statistically on par with 100% RDF. Therefore, it can be concluded native PGPR strains in consortium 2 (*Bacillus* sp. strain AkNF3, *Pseudomonas putida* strain KgNF1, *Bacillus megaterium* strain PkPS1, *Acinetobacter calcoaceticus* strain MvKS3 and *Cytobacillus kochii* PkZnS3)

successfully colonized the rice rhizosphere, increased nutrient availability to the plants and produced higher yield. The results also emphasized on the importance of exploiting native, location specific microorganisms as biofertilizer consortium, rather than a common consortium for the entire State. Native PGPR based consortia 2 reduced the 25% of inorganic fertilizer (N, P and K) without affecting the growth and yield of rice. This would be more cost effective and ecofriendly when compared with the use of chemical fertilizers alone. Further multi-locational field trials are required to validate the results before commercialization of this consortium, as a biofertilizer.