EXPLORATION OF NATIVE MINERAL PHOSPHATE SOLUBILIZING MICROORGANISMS AS BIOFERTILIZER FOR THE ACIDIC SOILS OF KERALA

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled **"Exploration of native mineral phosphate solubilizing microorganisms as biofertilizer for the acidic soils of Kerala"** is a bonafide record of research done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled **"Exploration of native mineral phosphate solubilizing microorganisms as biofertilizer for the acidic soils of Kerala"** is a record of research work done independently by **Saranya, K.S. (2011-11-134)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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CONTENTS

LIST OF TABLES

LIST OF TABLES

LIST OF PLATES

LIST OF PLATES

LIST OF FIGURES

ABBREVIATIONS

A -	Absorbance
AFLP-	Amplified Fragment Length Polymorphism
AMF-	Arbuscular Mycorrhizal Fungi
ANSA-	l-Amino-2-naphthol-4-sulfonic acid
ARDRA-	Amplified Ribosomal DNA Restriction Analysis
bp-	Base pair
cm-	Centimeter
cfu-	Colony forming units
^{0}C-	Degree Celsius
DNA-	Deoxyribo Nucleic Acid
EEO-	Electroendosmosis
et al.-	and others
FYM-	Farm Yard Manure
g-	Gram
GA-	Gibberellic Acid
ha -	Hectare
h-	Hour
HCN-	Hydrogen Cyanide
ICAR-	Indian Council of Agricultural Research

ABBREVIATIONS

IAA-	Indole Acetic Acid
kb-	Kilobyte
l-	Litre
M-	Mole
min-	Minutes
ml-	Millilitre
mg-	Milli gram
MRP-	Mussorie rock phosphate
µg-	Micro gram
µl-	Microlitre
µM-	Micromolar
N-	Normality
nm-	Nanometer
OD-	Optical Density
PCR-	Polymerase Chain Reaction
PDA-	Potato Dextrose Agar
PGPR-	Plant Growth Promoting Rhizobacteria
PGPS-	Plant Growth Promoting Substances
pH-	Hydrogen ion concentration

ABBREVIATIONS

Pi-	Inorganic phosphate
ppm-	Parts per million
PSE-	Phosphate Solubilizing Efficiency
PSB-	Phosphate Solubilizing Bacteria
PSF-	Phosphate Solubilizing Fungi
PSM-	Phosphate Solubilizing Microorganisms
PVK-	Pikovskaya
RAPD-	Random Amplified Polymorphic DNA
RP-	Rock phosphate
rpm-	Revolutions per minute
RNA-	Ribonucleic acid
SDS-	Sodium Dodecyl Sulfate
SSP-	Single Super Phosphate
TAE-	Tris-acetate-EDTA
TCP-	Tri Calcium Phosphate
U-	Unit
UV-	Ultraviolet
V-	Voltage
VAM-	Vesicular Arbuscular Mycorrhiza

Introduction

1. INTRODUCTION

Phosphorus, a key element in soil plant system, is one of the major essential macronutrients for plants and is applied to soil in the form of phosphatic fertilizers. Phosphorus (P) is known to play important physiological and bio-chemical roles in crop plants. As a component of every living cell, phosphorus controls all living processes including heredity and energy transport system. Therefore, it regulates the crop growth and yield to the greater extent. Plants take P in the form of soluble orthophosphate ions, $H_2PO^-_4$ and HPO_4^{2-}. Phosphorus exists in the combined form in soil with Al, Fe, Ca etc. Hence its availability in the soil to the crops is very low. A large portion of soluble inorganic phosphate applied to the soil as chemical fertilizer is immobilized rapidly and becomes unavailable to plants. The availability of these ions to the plant depends mainly on the soil pH (Nath and Borah, 1983). In acidic soils, P occurs in various forms of Al and Fe phosphates where as in neutral and alkaline soils, it is more likely to occur as Ca and Mg phosphates and adsorbed on surface of Ca and Mg carbonates. Low phosphorus availability of many tropical and subtropical soils in combination with insufficient P fertilizer application has been identified as one of the major factors responsible for the low yields on small farms (Kretzschmar *et al.*, 1991).

Microorganisms are involved in a range of processes that affect the transformation of soil P and are thus an integral part of the soil P cycle. Various species of soil bacteria, fungi and mycorrhizae have been reported to be involved in this bioconversion. Certain microorganisms are known to convert insoluble inorganic phosphorus into soluble form that can be utilized by the plants. Many bacterial, fungal, yeast, and actinomycetes species are capable of solubilizing sparingly soluble phosphorus in pure culture have been isolated and studied (Halder *et al.*, 1990; Abd-Alla, 1994; Whitelaw, 2000).

Phosphate solubilizing microorganisms (PSM) play important role in supplementing phosphorus to the plants, allowing a sustainable and efficient use

of phosphate fertilizers. Several mechanisms like lowering of pH by acid production, ion chelation and exchange reactions in the growth environment have been reported to play a role in phosphate solubilization by PSM. Plant root-associated phosphate solubilizing bacteria (PSB) have been considered as one of the possible alternatives for inorganic phosphate fertilizers for promoting plant growth and yield. However, their root colonization, persistence and performance in the rhizosphere are severely affected by environmental factors, especially under stressful soil conditions.

Application of PSM in the field has been reported to increase crop yield. Seed or soil inoculation with PSB is known to improve the solubilization of fixed soil phosphorus and applied phosphates, resulting in higher crop yield (Yahya and Al-Azawi, 1989). The interest in PSB has increased due to the prospective use of efficient strains as bio-inoculant (biofertilizer) components in organic agriculture, which is emerging as an alternative to chemical inputs in intensive agriculture (Ryder *et al.,* 1994; Bashan and Holguin, 1998). In fact, PSB render more phosphates into the soluble form than required for their growth and metabolism by secreting organic acids or enzymes (e.g. phosphates), the surplus is made available to the plants (Vessey, 2003).

Several studies have been conducted in India using various phosphate solubilizing microorganisms (PSM) as bioinoculants in wheat, rice, potato, bengal gram and other crops and emphasized the need for evolving efficient strains adapted to the local conditions to exploit the full potential of the technology for crop production. Soils of Kerala having high P fixing capacity and the P solubilizing organisms have a significant role in solubilizing the insoluble P in the soil and hence P nutrition of plants. Efficient P solubilizers have been obtained from the soil of Kerala through extensive isolation programmes (Sivaprasad and Meenakumari, 2005). In the above scenario, the present study was undertaken with the following objectives.

- To exploit native microorganisms with mineral phosphate solubilization
- To assess their plant growth promoting activities
- Develop native P-solubilizing microorganisms as biofertilizer, for the acidic soils of Kerala

Review of Literature

2. REVIEW OF LITERATURE

Phosphorus (P) is one of the essential macronutrients for plant growth and reproduction. Plants acquire P from the soil solution as phosphate anions. However, phosphate anions are extremely reactive and may be immobilized through precipitation with cations such as Ca^{2+}, Mg^{2+}, Fe^{3+} and Al^{3+} depending on the particular properties of the soil and as a result, the phosphate is highly insoluble and unavailable to plants. Phosphorous fixation predominates in both acidic and alkaline soils, resulting in its low availability.

Many soil microorganisms are able to solubilize or release this unavailable P through their metabolic activities exudating organic acids, or chelating calcium ions that release P to the solution. Production of microbial metabolites results in a decrease in soil pH, which probably plays an important role in the solubilization (Abd-Alla, 1994).

Phosphorous biofertilizers could help increase the availability of accumulated phosphate, efficiency of biological nitrogen fixation and increase the availabilty of Fe, Zn etc., through production of plant growth promoting substances (Kucey *et al.,* 1989). Several bacterial species are referred to as phosphate solubilizing bacteria and have been considered to have potential use as biofertilizer to improve the plant growth and yield (Vessey, 2003). Plant growth promotion by phosphate solubilizing microorganisms is an added advantage, and occurs through plant hormones, suppression of plant pathogens through antibiosis or siderophores (Kang *et al.*, 2009).

Species of genera *Pseudomonas, Bacillus, Rhizobium, Aspergillus* and *Cephalosporium* are the phosphate solubilizers. Use of phosphate solubilizing bacteria to soils increase phosphorus uptake, crop yield, induces resistance against salinity and pathogens (Mahdi *et al.*, 2011).

2.1 IMPORTANCE OF PHOSPHOROUS IN PLANT NUTRITION

Pierre (1938) referred to phosphorous as the 'Master key' element in crop production. It acts as an important factor in plants in many physiological activities such as cell division, cell organization, photosynthesis, development of good root system, nucleus formation and also in carbohydrate utilization (Arnon, 1956; McVicker *et al.*, 1963). It is an essential constituent of both the structural components of cells, such as nucleic acids and membrane phospholipids, and of mobile storage units of metabolic energy, such as ATP (Almeida *et al.*, 2009).

Phosphorous enhances seed germination and early growth, stimulates blooming, enhances pod set and seed formation, hastens maturity, and provides winter hardiness to crops planted in late fall and early spring. The meristem region of growing plants is high in phosphorus. It stimulates root growth, initiation of flower primordial, helps in early maturity and occurs in large quantities in plant, seed and fruit (Gaur, 1990). Plant height, tillers, shoot and root dry weight, shoot to root ratio, P concentration and P uptake in root and shoot and P use efficiency are significantly influenced by levels of phosphorus in soil. Phosphorous has been shown to reduce disease incidence in some plants and has been found to improve the quality of certain crops (Uchida, 2000).

In leguminous crops, phosphorus promotes root nodulation, nitrogen fixation, increases nutrient-use efficiency, efficient partitioning of photosynthates between source and sink, and biomass production (Gitari and Mureithi, 2003). Phosphorous deficiency in soil can severely limit plant growth productivity, particularly in legumes, where both the plants and their symbiotic bacteria are affected, and this may have a deleterious effect on nodule formation, development and function (Alikhani *et al.*, 2006). Phosphorous deficiency may result in reduced plant size and usually deep green or reddish-purple colour.

2.2 AVAILABILITY OF PHOSPHORUS IN SOIL

Phosphorous can naturally be found in diverse forms in soil solution. Plants derive P from soil in the form of $H_2PO^-_4$ and HPO_4^{2-}. Although the total amount of P in the soil may be high, 95-99 per cent of it may be in fixed /grid forms. In the soil, a large portion of applied fertilizer P also becomes immobile and unavailable for plant uptake because of adsorption, precipitation and fixation (Omar, 1998).

Most of the fertilizer P applied (often as much as 90%) is retained in fixed form which is rendered unavailable for crop uptake (Stevenson, 1986). As a consequence of continuous application of phosphatic fertilizers at high doses, most of the agricultural soils generally contain large reserves of accumulated phosphorous (Richardson, 2004).

Chemical fixation of P takes place in soils depending on pH. In acidic soils, Fe, Al and Mn ions combine with phosphate to form insoluble compounds. In alkaline soils also P fixation occurs resulting in the formation of insoluble Ca compounds. Tropical and subtropical soils are predominantly acidic, and often extremely phosphorous deficient (Gaume, 2000) with high phosphorous sorption (fixation) capacities. Therefore, phosphorous is often regarded as a limiting nutrient in agricultural soils (Guinazu *et al.*, 2010).

The concentration of soluble P in soil is usually very low, normally at levels of 1ppm or less (Goldstein, 1944). Koshy and Varghese (1972) reported that laterite soils in general were poor in available phosphorous and had high P fixing capacity. P fixation capacity of Kerala soils is very high due to the high acidity, dominance of kaolinitic fractions in the mineral composition as well as due to the presence of excess quantities of hydrous oxides of Fe, Al and Mn. Sajnanath (2000) also reported that the avialable P content in the soil of the main campus of Kerala Agricultural University (laterite) was generally low, due to high P fixing capacity.

A study by the Rajasekharan *et al.* (2013) involving a comprehensive analysis of soils from all the Panchayats across all districts of the Kerala state showed that acidity at a whopping 91% of the samples tested, with 54% of the samples testing for strong to extremely acid reaction (pH < 5.5). Thus P availability should have been seriously hampered in these soils, making it unavailable to crops.

2.3 PHOSPHATE SOLUBILIZING MICROORGANISMS (PSM)

Soil microorganisms play an important role in making the phosphorus available to plants by mineralizing the organic phosphorus in the soil. These microorganisms have been isolated from a number of different soils in India (Vikram *et al.*, 2007). Microorganisms which are capable of solubilizing insoluble phosphate are called phosphate solubilizing microorganisms (PSM). Several varieties of phosphate-solubilizing microorganisms (PSM) have been isolated from the rhizospheric soils of crops. Of these, 20% to 40% are culturable soil microorganisms. A majority of the isolated organisms are bacteria, although several fungi are also known to solubilize phosphates. These bacteria and fungi have the potential to be used as biofertilizers. Their role in increasing the soil nutrient value is of utmost importance. PSM are known to be abundant in rhizospheric soils of various plants. They can be divided into two groups: phosphate solubilizing bacteria (PSB) and phosphate solubilizing fungi (PSF).

Among the whole microbial population in soil, phosphate solubilizing bacteria (PSB) constitute upto 50 %, while phosphate solubilizing fungi (PSF) are only 0.1 to 0.5 % in P- solubilization potential. High proportion of PSM is concentrated in the rhizosphere, and they are metabolically more active than from other sources (Vazquez *et al.*, 2000). PSM have been well studied and their isolation and role in crop production have been assessed. Studies on the distribution of these organisms have been conducted in several soils. The information available is insufficient for the study of the diversity of these

organisms; many of these organisms' exhibit stress tolerance and can adapt themselves to varying environments (Kirk *et al.*, 2004).

There are several microorganisms including fungi, bacteria and actinomycetes that solubilize insoluble P (Goldstein, 1986). Several soil fungi, particularly those belonging to the genera *Penicillium*, *Aspergillus*, *Trichoderma*, *Mucor*, *Candida*, yeast, *Discosia*, *Eupenicillium* and *Gliocladium* possess ability to bring insoluble soil phosphates into soluble form (Xiao *et al.*, 2008; Rahi *et al.*, 2009).

Singal *et al.* (1994) reported that *A. japonicus* and *A. foetidus* solubilized five types of Indian rock phosphate. Many fungal strains can solubilize rockphosphate, aluminium phosphate and tricalcium phosphate (Isbelia *et al.*, 1999; Sparks, 1999; Whitelaw *et al.*, 1999; Didiek *et al.*, 2000; Helen *et al.*, 2002; Reddy *et al.*, 2002; Achala *et al.*, 2007).

A nematofungus *Arthrobotrys oligospora* has the ability to solubilize phosphate rocks (Duponnos *et al.*, 2006). Strains from the genera *Aspergillus* and *Penicillium* are among the most powerful phosphate solubilizers. Filamentous fungi are widely used as producers of organic acids, particularly black *Aspergillus* sp. and some species of *Penicillium.* These species have been tested for solubilization of rock phosphate and have been reported for various properties of biotechnological importance, such as, biocontrol, biodegradation, phosphate solubilization and P fertilizer (Richa *et al.*, 2007; Pandey *et al.*, 2008).

A number of soil bacteria possess mineral phosphate solubilizing activity (Yahya and Al-Azawi, 1989; Mikanova and Kubat, 1994). *Bacillus megaterium* var. *phosphaticum* was used to create a bio-preparation called Phosphobucterin with the purpose of enhancing mineral phosphorus solubilization. If phosphorus is present in the complex structures of the soil and, at the same time, readily decomposable carbon sources, such as manure, are incorporated in the soil, phosphorus solubilization can be increased due to biological activity stimulation.

This organic carbon increase may aid to complexing the soil aluminum in acids, thus reducing the aluminum phosphate (Sylvia *et al.*, 1999).

Plant root-associated phosphate solubilizing bacteria (PSB) have been considered as one of the possible alternatives for inorganic phosphate fertilizers for promoting plant growth and yield (De-Freitas *et al.*, 1997; Rodriguez and Fraga, 1999; Richardson, 2001; Vessey, 2003; Thakuria *et al.*, 2004).

B. megaterium, *B. circulans*, *B. subtilis*, *B. polymyxa*, *B. sircalmous*, *Pseudomonas striata* and *Enterobacter* could be referred as the most important strains (Subbarao, 1988; Kucey *et al.*, 1989). Strains from the genera *Pseudomonas*, *Bacillus* and *Rhizobium* are the most powerful phosphate solubilizers (Rodriguez and Fraga, 1999). Apart from those species, the other bacteria reported as phosphate solubilizers include *Azotobacter*, *Pantoea* and *Klebsiella* (Chung *et al.*, 2005), *Rhodococcus*, *Arthrobacter*, *Serratia*, *Chryseobacterium*, *Gordonia*, *Phyllobacterium* and *Delftia* sp. (Chen *et al.*, 2005).

Symbiotic nitrogenous rhizobia, have also shown phosphate solubilization activity. For instance, *Rhizobium leguminosarum* bv. *trifolii* (Abril *et al.*, 2007), *R. leguminosarum* bv. *Viciae* (Alikhani *et al.*, 2007) and *Rhizobium* species nodulating *Crotalaria* species (Sridevi *et al.*, 2007) improved solubilization of phosphates by mobilizing inorganic and organic phosphorus.

In an experiment conducted at Kottarakkara, maximum P solubilization was observed in soil + mussorie rock phosphate + farm yard manure + P solubilizing organisms. This was attributed to the beneficial effect of PSM applied along with MRP and FYM (Shehana, 2001). Sivaprasad and Meenakumari, 2005 reported that soils of Kerala are highly P fixing and the p- solubilizing microorgnisms have a significant role.

2.3.1 **Acid tolerant phosphate solubilizers**

Phosphate solubilizing microorganisms are commonly found in most soils (Chonker and Taraedar, 1984; Venkateswarlu *et al.*, 1984). However, their establishment and performances are severely affected by environmental factors such as temperature, pH and salt concentration of the soils especially under stress conditions (Gupta *et al.*, 1986; Tilak, 1991). Kerala soils are highly acidic in nature. Among various soil productivity constraints, phosphorous play a very crucial role in crop production in acidic soils. Phosphate solubilizing microorganisms with the genetic potential for increasing tolerance to low pH are considered to be important for the establishment, multiplication and production of environmentally friendly bio-inoculants. Pal (1998) reported that a phosphate solubilizing bacterial strain (PAS-2) isolated from the acidic soils of Himalayan regions of Uttarpradesh recorded highest phosphate solubilization and highest acid tolerance rating 42. This strain was later identified as *Bacillus* sp. There are some information regarding the stress tolerant PSMs such as *Burkholderia vietnamiensis* M6 is tolerant to temperature, pH and salt conditions (Park *et al.*, 2010), *Rhodotorula* sp. PS4 is highly tolerant to temperature, pH and salt variations (Mundra *et al.*, 2011).

A study was conducted by Panhwar *et al.* (2014) to determine the total microbial population, the occurrence of growth promoting bacteria and their beneficial traits in acid sulfate soils. Three potential PSB strains *B. thailandensis*, *Sphingomonas pituitosa* and *B. seminalis* were isolated from the acid sulfate soil and they were able to grow well under low pH conditions. Walpola *et al.* (2014) found that the *K. oxytoca* is a potent thermo, acid, alkali and salt tolerant bacteria which retain its phosphate solubilizing capacity over a wide range of pH.

2.4 MECHANISM OF PHOSPHATE SOLUBILIZATION

Several mechanisms have been proposed to explain the phosphate solubilization by Phosphate solubilizing microorganisms.

2.4.1 **Release of organic acids and fall in pH**

It is generally accepted that the major mechanism of mineral phosphate solubilization is the action of acids synthesized by soil microorganisms. Production of acids results in acidification of the microbial cell and its surroundings. The acids convert tricalcium phosphate to di and mono basic phosphates with the net result of an enhanced availability of the element to the plant. The type of organic acid produced and their amounts differ with different organisms. Gluconic acid seems to be the most frequent agent of mineral phosphate solubilization. 2-ketogluconic acid is another organic acid identified in strains with phosphate solubilizing ability (Song *et al.,* 2008). Organic acids, such as glycolic, oxalic, malonic, and succinic acid, have also been identified among phosphate solubilizers (Ahmed and Shahab, 2011).

Besides organic acids, inorganic acids such as nitric and sulphuric acids are also produced by nitrifying bacteria and *Thiobacillus* during the oxidation of nitrogenous or inorganic compounds of sulphur which react with calcium phosphate and convert them into soluble forms (Khan *et al.,* 2007). Efficiency of solubilization is dependent upon the strength and nature of acids. Tri and di-carboxylic acids are more effective as compared to mono basic and aromatic acids. Aliphatic acids are also found to more effective in phosphate solubilization compared to phenolic, citric and fumaric acids (Mahidi *et al.,* 2011). Some of the reports on production of organic acids by various PSM are given in table 1.

Venkateswarlu *et al.* (1984) reported that during the solubilization of rock phosphate by fungi, the pH of the culture was lowered from 7 to 3. *A. niger* is known to produce citric, oxalic and gluconic acids (Formina *et al.,* 2004), thus lowering the pH of the medium and facilitating solubilization. The rise in available phosphorous concentration was accompanied by decrease in pH of the medium. This drop in pH indicate the production of organic acids (Pradhan and Sukla, 2005; Jain *et al.,* 2011).

Table 1. Production of organic acids by various PSM

Sl no.	Organism	Predominant acid produced	Reference
1	*Escherichia freundii*	Lactic	Sperber (1958)
2	*Aspergillus niger* *Penicillium* sp.	Citric,glycolic, succinic, gluconic oxalic, lactic	Sperber (1958)
3	*Bacillus megaterium* *Pseudomonas* sp. *Bacillus subtilis*	Lactic , malic	Taha *et al.* (1969)
4	*Bacillus megaterium* *Escherichia freundii*	Citric , gluconic	Taha *et al.* (1969)
5	*Arthrobacter* sp. *Bacillus* sp. *Bacillus firmis* B-7650	Lactic , citric	Bajpai and Sundara Rao (1971)
6	*Aspergillus fumigatus* *Aspergillus candidus*	Oxalic, tartaric, citric	Banik and Dey (1982)
7	*Pseudomonas aerogenosa*	Gluconic	Banik and Dey (1982)
8	*Rhizobium* sp.	Gluconic	Van Schie *et al.* (1984)
9	*Pseudomonas striata*	Oxalic , tartaric	Van Schie *et al.* (1984)
10	*Pseudomonas striata*	Tartaric , citric	Krishnamurthy (1989)

2.4.2 **Role of phosphatase enzymes**

The release of phosphatase enzymes that mineralize organic P compounds has been also suggested as another mechanism (Stevenson, 1986). The production of phosphatase is controlled by complex regulating mechanisms and the enzyme is detected only under specific conditions. The principal mechanism for the regulation of phosphatase production is the regulation of inorganic phosphate (Pi) concentration.

Activity of various phosphatases in the rhizosphere of maize, barley and wheat showed that phosphatases activity was considerable in the inner rhizosphere of acidic and neutral soil pH (Bums, 1983). Soil bacteria expressing a significant level of acid phosphatases include strains from the genus *Pseudomonas* (Gugi *et al.*, 1991), *Rhizobium* (Abd-Alla, 1994), *Enterobacter, Serratia, Citrobacter, Proteus, Klebsiella* (Thaller *et al.*, 1995) and *Bacillus* (Skrary and Cameron, 1998).

2.4.3 **Other mechanisms of phosphate solubilization**

Another mechanism of phosphate solubilization is the production of H_2S which reacts with ferric phosphate to yield ferrous sulphate with concomitant release of phosphate (Swaby and Sperber, 1958).

Chien (1979) reported that the dissolution of P from North Carolina phosphate rock was increased by the addition of urea in acid soils. Urea hydrolyses the organic matter of the soil and the products of hydrolysis chelate Ca^{2+} ions thereby releasing the phosphorous from rock. Ammonium sulphate and potassium chloride, on the other hand, increased the phosphorous–sorption capacity of the soil thereby reducing the water soluble phosphorous in the soil.

Production of chelating substances, H_2S, CO_2, mineral acids, siderophores and proton extrusion mechanism are also involved in phosphate solubilization (Kapoor *et al.*, 1989; Kucey *et al.*, 1989; Gaur, 1990; Illmer *et al.*, 1995).

2.5. *IN VITRO* SCREENING OF MICROBES FOR PHOSPHATE SOLUBILIZATION

Tricalcium phosphate (TCP) is regarded as a model compound for measuring the potential or relative rates of microbial solubilization of insoluble inorganic phosphate compounds. In addition, the insoluble calcium phosphate forms a major portion of insoluble phosphate in soil (Devi and Narasimhan, 1978).

Pikovskaya (1948) suggested a medium containing tricalcium phosphate with glucose, yeast extract and other salts for growth and isolation of phosphate solubilizing microorganisms. This medium has been widely used for isolation, enumeration, and maintanance of phosphate dissolving microorganisms. Phosphate solubilizing microorganisms produce clearing zones around the microbial colonies in the Pikovskaya's medium due to the ability of microbes to solubilize insoluble mineral phosphates such as tricalcium phosphate or hydroxyapatite present in the medium.

According to De Freitas *et al.* (1997), good phosphate-solubilizers produce halos around their colonies with diameters higher than 15 mm. Out of all bacteria and fungi isolated from the rice field soil of Bhubaneswar, two fungi *A. fumigatus* and *Penicillium* sp. showed significant zone of phosphate solubilization. A clear halo zone was formed around colonies after 5 days of incubation on solidified Pikovskaya's medium supplemented with calcium phosphate, indicating phosphate-solubilizing ability of the fungal isolates (Pradhan and Sukla, 2005).

A total of 28 isolates of phosphate solubilizing bacteria were isolated from soil by Shahab *et al.* (2009). Three strains namely, CMG860, CMG854 and CMG857 were selected on the basis of largest halos, of approximately 20 - 40 mm within 4 days of incubation.

Kaviyarasi *et al.* (2011) isolated actinomycetes including *Actinobispora yunnanensis, Streptomyces albus, Micromonospora echinospora, Saccharopolyspora hirsute, Streptomycetes cyaneus, Actinomadura citrea, Saccharomonospora viridis, Thermomonospora mesophila, Streptoverticillium album, Microtetrospora fastidiosa* from Manora. They were screened for phosphate solubilization using Pikovskaya's agar medium and *A. yunnanensis* was found to be dominant in phosphate solubilization.

Thirty bacterial isolates isolated from rhizospheric soil samples of French bean were screened for phosphate solubilization on modified PVK agar, of which twelve isolates showed the development of sharp phosphate solubilization zones, ranging from 4 mm to 20 mm. Other isolates showed the development of hazy zones (Kumar *et al.*, 2012).

2.5.1 **Phosphorous solubilization efficiency**

Phosphrous solubilizing ability of PSM is determined by observing halo/clear zone on plate due to the production of organic acids into the surrounding medium (Katznelson *et al.*, 1962). The comparative potential of various PSM is routinely determined by screening through this qualitative assay of measuring the size of halo zone arround the colony on the plate (Nautiyal, 1999).

The ability of PSM to solubilize the insoluble phosphate was studied by the determination of solubilization index: the ratio of the total diameter (colony + halozone) and the colony diameter (Edi-Premono *et al.*, 1996).

On the bases of diameter of clearing halo zones, solubilization efficiency (SE) and solubilization index (SI) (Gaur, 1990; Nguyen *et al.*, 1992; Vazquez *et al.*, 2000) were calculated using the following formulas.

$$SE = \left(\frac{\text{Colony diameter + halo zone diameter}}{\text{Colony diameter}} \right) \text{ X } 100$$

$$SI = \frac{\text{Colony diameter + halo zone diameter}}{\text{Colony diameter}}$$

Kannapiran and Ramkumar (2011) reported that *Pseudomonas* sp. was the most efficient phosphate solubilizer on Pikovskaya's agar plates with solubilization index 228 ± 6.12 at 7th day incubation. Measurements of SI ranged from 96.24 ± 4.32 to 228.26 ± 6.12.

Tripti *et al.* (2012) reported that among 10 potent isolates, *Bacillus* sp. and *Pseudomonas* sp. showed the maximum phosphate solubilization index of 3.1 and 3.0 in agar plates. Ghosh *et al.* (2012) found that *Bacillus circulans* was effective in phosphate solubilization with phosphate solubilization efficiency 375 ± 8.54.

2.6. QUANTITATIVE ESTIMATION OF EFFICIENCY OF P-SOLUBILIZATION BY MICROORGANISMS

In contrast to the precipitated phosphate agar plate assays, the liquid method is considered more sensitive for detecting P-solubilization by microorganisms as measurable Pi concentration can be detected from more microorganisms.

Zhu *et al.* (2011) reported that phosphate solubilizing bacterium was inoculated into liquid media supplemented with $Ca_3(PO_4)_2$ and cultured at 28°C for 12 days with continuous agitation and the phosphorus availability was determined with Mo blue method. The bacterium solubilized 283.16 µg/ml phosphorus in 11 days, and the growth of the bacterium was concomitant with a significant decrease of acidity of the medium. Yadav *et al.* (2011) tested phosphate solubilizing potential of three fungal strains *A. niger* strain BHUAS01, *Trichoderma harzianum* and *P. citrinum* strain BHUPC01 using Pikovskaya's broth containing tricalcium phosphate. *Aspergillus niger* showed maximum amount of soluble phosphate (328 µg L-1) after 6 days of incubation.

Gnanachitra and Govindarajan (2002) screened *Azospirillum* cultures isolated from acid soils of tea grown areas in South India for P-solubilizing

ability and found four out of 27 isolates to solubilize 2.00 to 3.25 percent TCP against *B. megaterium* which solubilized 5.65 percent TCP. Five fungi, namely *Penicillium* sp. 22, *Penicillium* sp.2, *Penicillium* sp.21, unidentified fungus 2 and *Aspergillus* sp. MNF2 were able to solubilize rock phosphate in liquid culture and produced 0.14 to 4.8725 µg PO_4/ml into the medium (Gupta *et al.*, 2007).

Nuraini *et al.* (2011) found that the isolate $K_2P_2$9.1 identified as *Bacillus subtilis* strain BS501a solubilized 5.24 ppm phosphate per ml of liquid broth. Nath *et al.* (2012) found that the tricalcium phosphate solubilizing activity of endophytic *Penicillium* species 1 ranged between 39.22 $\pm$1.17 to 86.10 $\pm$ 1.20 µg/ml while that of species 2 varied between 32.57 $\pm$ 1.41 to 84.25 $\pm$ 1.5 µg/ml within a span of 2 to 10 days. *Pseudomonas fluorescens* K - 34 solubilized tricalcium phosphate and produced substantial amount of soluble phosphorus (968.5 mg / l) in Pikovskaya 's (PVK) broth (Parani& Saha, 2012).

2.7. IDENTIFICATION AND CHARACTERISATION OF PSM

The classical microbiological methods for identification and classification of microorganisms include morphological, physiological and biochemical tests. Each of these methods helps to determine the taxonomic position of microbes to a certain extent.

2.7.1 **Morphological characteristics**

Bacterial colony morphology includes colony shape, dimension, pigmentation, and others. Cell morphology, as observed under the microscope, includes gram reaction (positive or negative), shape (e.g., coccus or rod), organization (e.g., single or chain), presence and properties of endospore e.g (central or terminal), flagellation and others (Zourob *et al.*, 2008).

Different concepts have been used by the mycologists to characterize the fungal species, out of which morphological (phenetic or phenotypic) and reproductive stages are the classic approaches and baseline of fungal taxonomy

and nomenclature that are still valid (Davis, 1995; Guarro *et al.,* 1999; Diba *et al.,* 2007; Zain *et al.,* 2009).

2.7.2 Biochemical and physiological traits

A large number of biochemical and physiological characteristics have been used in bacterial identification. Results of these tests may be used in bacterial identification on the basis of numerical taxonomy (Sneath, 2005).

2.7.2.1 *Rapid biochemical reactions*

These indicate the presence of a single enzyme or enzyme complex. Representing this category are reactions catalyzed by catalase, oxidase, nitrate reductase, amylase, β-galactosidase, thermonuclease and urease.

2.7.2.2 *Carbohydrate fermentation*

Analysts may assess the ability of the test organism to utilize a certain carbohydrate as the only carbon source. The carbohydrate utilization profile could be useful in identifying a bacterial isolate. Fermentation of glucose, sorbitol and mannitol are examples of tests under this category. As heterotrophic organisms, phosphate solubilizers need a carbon source and energy for both the synthesis of new cell material and the oxidation of carbon compounds (Moat and Foster, 1988).

Sowmya *et al.* (2013) reported that the phospahte solubilizing uranium tolerant bacterium *Acinetobacter* sp. was able to grow on arabinose, xylose, melibiose and glucose as sole carbon source but could not hydrolyze esculin nor utilize adonitol, rhamnose, cellobiose, saccharose, rafinose, trehalose and lactose.

Three *Bacillus* species utilized the monosaccharides and produce acid / gas, where as sucrose is negative i.e. disaccharides are not utilized by *Bacillus* species (Sultana *et al.,* 2010).

2.7.2.3 ***Physiological features***

Growth at different temperatures, pH values, salt concentrations and gaseous environments are some of the physiological properties used in identifying bacteria. Woyessa and Assefa (2011) reported that all isolates of the genus *Pseudomonas* were found to grow in the pH range of 4.5 to 10.0, except two isolates that failed at pH value of 4.5. All isolates of four species (*B. megaterium* 1, *B. coagulans*, *B. cereus* 1 & *B. cereus* 2 and *B. pumilus*) tolerated and grew from the pH range of 4.5 to 10.0.

2.7.3 **Molecular characterization**

The traditional method of classification ad identification of microbes is based on phenotypic approach which includes morphological, physiological and biochemical characterization (Prakash *et al.,* 2007). The methods are now employed for bacterial systematics include, the complete 16S rRNA gene sequencing and its comparative analysis by phylogenetic trees, DNA-DNA hybridization studies with related organisms, analyses of molecular markers (AFLP, RAPD, rep-PCR) and signature pattern.

2.7.3.1 ***16S rDNA sequencing***

The 16S rDNA gene has been used as a trust worthy molecular marker for phylogenetic identification of organisms. It contains a conserved region, a unique array of sequences that are relative among species or different species (Moyer *et al.,* 1994). Polyphasic taxonomical studies, which include phenotypic, genetic and phylogenetic information, have been used in microbial diversity studies (Vandamme *et al.,* 1996). The sequence of the 16S rRNA gene has been widely used as phylogenetic marker in microbial ecology (Ludwig *et al.,* 1998). Since the extent of divergence in the sequence of this gene provides an estimate of the phylogenetic distance existing between different species (Igual *et al.,* 2001).

So far, the determination of total genomic DNA-DNA homology values has been considered as a dominant component of taxonomic analyses (Wayne *et al.,*

1987; Murray *et al.*, 1990). In fact it has been referred to as the 'gold standard' for the definition of bacterial species (Stackebrandt and Goebel, 1994).

Seventy per cent DNA-DNA homology values are considered to be the 'species limit'. Based on polyphasic taxonomical studies, new PSB species have been identified, such as *P. rhizospharae* (Perix *et al.*, 2003), *P. lutea* (Perix *et al.*, 2004) and *Microbacterium ulmi* (Rivas *et al.*, 2004).

Victoria *et al.* (2009) amplified the 16S rRNA gene of the BUAP29, BUAP36 and CPO8 strains, cloned and sequenced for taxonomic identification. The analysis of ARDRA patterns as well as the dendrogram exhibited a large genetic diversity among the 36 PSB analyzed with BUAP 36 and BUAP15 strains showing 100% similarity,the analysis of the 16S rDNA gene partial sequences at gene bank showed 99% similarity.

2.8 PLANT GROWTH PROMOTING AND ANTAGONISTIC ACTIVITIES OF PHOSPHATE SOLUBILIZING MICROBES

Phosphate solubilizing microorganisms are also known to produce plant growth promoting substances (PGPS). Plant growth promotion by PSM is an added advantage, and occurs through Indole Acetic Acid, Gibberellic acid and suppression of plant pathogens through ammonia or HCN or siderophores (Barea *et al.*, 1976; Baya *et al.*, 1981; Leinhos and Vacek, 1994; Kadapati, 2001; Srinivasan, 2002).

2.8.1 **Production of plant growth regulators**

Organic substances capable of regulating plant growth produced either endogeneously or applied exogeneously are called plant growth regulators. Several microorganisms are capable of producing auxins, cytokinins, gibbereillins, ethylene or abscisic acid (Lebuhn *et al.*, 1997). They regulate growth by affecting physiological and morphological processes at very low concentrations (Arshad and Frankenberger, 1998).

2.8.1.1 ***Indole Acetic acid***

Indole acetic acid is one of the most physiologically active auxins. IAA is a common product of L-tryptophan metabolism by several microorganisms (Frankenberger and Brunner, 1983; Lynch, 1985). IAA produced by bacteria improves plant growth by increasing the number of root hairs & lateral roots (Okon and Kapulnik, 1986). Microbial biosynthesis of IAA in soil is enhanced by tryptophan from root exudates or decaying cells (Frankenberger and Arshad, 1991; Benizri *et al.,* 1998).

Sharma *et al.* (2012) reported that several naturally occurring PSB isolates from tea rhizospheres of Darjeeling hills are capable of producing plant growth promoting substance, IAA. Substantial production of IAA was observed by *Pseudomonas* strains when it was grown in PVK broth without tryptophan (Parani and Saha, 2012). Production of IAA was10 and 7.5 µg/ml respectively for P solubilizing *P. agglomerans* and *B. anthina* was reported by Walpola and Yoon (2013).

Ng *et al.* (2012) evaluated the efficacy of *Corynebacterium agropyri* (UPMP7), *Enterobacter gergoviae* (UPMP9) and *B. amyloliquefaciens* (UPMS3) in promoting rice seed germination and seedling growth through phosphate solubilization and production of indole-3-acetic acid (IAA). *In vitro* bioassay indicated all the rhizobacteria tested were able to produce IAA and solubilize phosphate through the production of organic acids. Kumar *et al.* (2012) reported that most of the bacteria isolated from the rhizospheric soil of French bean produced IAA. Isolate FBJ6, most efficient P-solubilizer was found to produced 198.08 µg/ml of IAA.

Two stress tolerant phosphate solubilizing rhizobacteria *Arthrobacter* sp. and *Bacillus* sp. have been isolated from tomato rhizosphere and these strains also exhibited various plant growth promoting and biocontrol activities including indole acetic acid (IAA) production. These two strains have the potential to be used as plant growth promoting rhizobacteria (Banerjee *et al.,* 2010). IAA

production and p-solubilization were high in *Klebsiella* sp. (Chaiharan and Lumyong, 2011). The phosphate solubilizers, *Pseudomonas aeruginosa* and *Bacillus* sp. showed maximum IAA production of 26.5 and 19.8 µg /ml of IAA in Luria Bertani broth (Kannapiran and Ramkumar, 2011).

2.8.1.2 ***Gibberellic acid***

Gibberellic acids are a class of phytohormones most commonly associated with modifying plant morphology by the extension of plant tissue, particularly stem tissue (Salisbury, 1994).

Ponmurugan and Gopi (2006) reported that phosphate solubilizing bacteria isolated from rhizosphere soil samples collected from different agroclimatic zones of Namakal district were able to produce GA. Vikram *et al.* (2007) isolated phosphate solubilizing bacteria from the crops grown in vertisols and tested for plant growth promoting substances. All the 30 isolates of PSB were able to produce GA and the amount ranged from 0.6 µg to 9.8 µg/25 mL of broth. Kang *et al.* (2009) reported the production of gibberellic acid (GA) and growth promotion in cucumber, Chinese cabbage and crown daisy by a p-solubilizing strain of *Acinetobacter calcoaceticus*.

Ramkumar *et al.* (2011) reported that all the twelve isolates of phosphate solubilizing bacteria were able to produce phytohormone GA_3 under *in vitro* condition. The production of GA_3 by the strain KPB4 (16.55 ppm) had reached higher amount. Sivasakthi *et al.* (2013) found that *P. fluorescens* showed maximum gibberellic acid production (5.96 µg/ml).

2.8.2 **Production of siderophores**

Iron is an essential nutrient for all living organisms. In the soil it is unavailable for direct assimilation by microorganisms because ferric iron (Fe III) which predominates in nature is only sparingly soluble and too low in concentration to support microbial growth. Some bacteria have developed iron uptake systems (Neilands and Nakamura, 1997). These systems involved

siderophore an iron binding legand and an uptake protein needed to transport iron into the cell.

Siderophores are low molecular weight iron-binding ligands which can bind to ferric ion and make it available to the producer microorganism. Under Fe deficiency conditions, many microorganisms produce siderophores for Fe acquisition (Neilands, 1981). The role of siderophores in P- solubilization appears to be important in acid soils where ferric phosphates are one of the major forms of insoluble phosphates. Siderophores also increase P availability in acid soils by chelating iron (Bossier *et al.*, 1988).

Endophytic bacteria of banana in Kenya were isolated and identified as *R. aquatilis* and *F. oryzihabitans* were the most efficient in phosphate solubilization and siderophore production (Ngamau *et al.*, 2012). Kayasth *et al.* (2013) found that *Bacillus licheniformis* exhibited positive siderophore production and also able to solubilize tricalcium phosphate efficiently.

Walpola and Yoon (2013) were reported that two phosphate solubilizing bacterial (PSB) isolates (*Pantoea agglomerans* and *Burkholderia anthina*) produced more than 80% siderophore and they were considered as efficient siderophore producers.

Microbial siderophores play an important role in the biocontrol of some soil-borne plant diseases and in plant iron nutrition (Loper and Buyer, 1991). *Bacillus* species are potent siderophore producer and thus may promote plant growth and act as plant pathogen antagonist (Yu *et al.*, 2011; Zhao *et al.*, 2011). Strains of *Pseudomonas* were found to be siderophores producers. These siderophores bind to the available form of iron (Fe^{3+}) in the rhizosphere, thus making it unavailable to the phytopathogens and protecting the plant growth (Parani and Saha, 2012). Siderophore producing bacteria are good candidates for plant growth promotion, especially in neutral to alkaline soil.

2.8.3 **Production of HCN**

Volatile compounds Such as hydrocyanic acid (HCN) is produced by many bacterial strains and has been considered as important metabolites in biocontrol (Walsh *et al.*, 2001; Bano and Musarrat, 2003; Fernando *et al.*, 2005).

HCN inhibits the electron transport thereby the energy supply to the cell is disrupted leading to the death of the organism. It inhibits proper functioning of enzymes and natural receptors by reversible mechanism of inhibition (Corbett, 1974). It is also known to inhibit the action of cytochrome oxidase (Gebring *et al.*, 1993).

Fluorescent *Pseudomonas* strain RRS, isolated from Rajanigandha, a flowering plant produced HCN and the strain improved seed germination and root length (Saxena *et al.*, 1996). Production of HCN by *Pseudomonas* strain EM 85 was reported by Anith *et al.* (1999).

Kremer and Souissi (2001) reported that approximately 32 percent of bacteria from a collection of over 2000 isolate were cyanogenic, evolving HCN from trace concentration to >30 moles/mg cellular protein. They also suggested that cyanogenesis was predominantly associated with *Pseudomonas* and was enhanced when glycine was provided in the culture medium. Ramette *et al.* (2003) reported that HCN synthase is encoded by three biosynthetic genes (hcn A, hcn B and hcn C) in fluorescent Pseudomonads.

Paul *et al.* (2005) studied the antagonistic mechanisms of fluorescent pseudomonads against *P. capsici* in black pepper and reported many isolates were able to produce HCN which limited the growth of pathogen.

2.8.4 **Production of Ammonia**

Ammonia is considering one of the plant growth promoting substances produced by various microbes inhabiting rhizosphere. It has been reported that ammonia production indirectly influences the plant growth. Some authors

consider the production of ammonia to be involved in antagonistic interactions that result in disease control (Saraf *et al.*, 2008).

B. subtilis strain MA-2 and *P. fluorescence* strain MA-4 was efficient in ammonia production and significantly increased biomass of medicinal and aromatic plant such as Geranium (Mishra *et al.*, 2010).

Ammonia production was detected in 95 % of the isolates from the rhizosphere of rice, mangrove and effluent contaminated soil influencing plant growth promotion (Joseph *et al.*, 2007; Samuel and Muthukkaruppan, 2011). Kayasth *et al.* (2013) found that the isolate *Bacillus licheniformis* produced ammonia in the range of 0.66 µg/ml.

2.8.5 **Antagonistic activity of phosphate solubilizing microorganisms**

In addition to providing P to plants, PSM also act as a biocontrol agent and promote the growth of plants by suppressing the soil borne plant pathogens. Microbial antagonism is an ecological association between organisms where one or more of the participants is harmed or its activities curtailed. Microbial antagonism contributes much to the biological control of plant pathogens. Several *in vitro* studies showed the potential of PSM for the simultaneous synthesis and release of pathogen suppressing metabolites, mainly siderophores, and lytic enzymes (Pandey *et al.*, 2006; Rane *et al.*, 2008).

2.8.5.1 ***Effect of PSB on fungal pathogens***

Fungal genera like *Sclerotium rolfsii, Macrophomina phaseolina, Fusarium solani* and *Fusarium oxysporum* etc. have been recognized to play a major role in the root disease complex causing seed decay, damping off, root rot, seedling blight, collar rot, crown rot, foot rots and wilt (Cook and Baker, 1983).

Bacillus subtilis has shown antagonistic activity towards *F. solani* (Kendrick, 1963) and *F. oxysporum* f sp. *ciceris* (Kumar, 1999). Czaczyk *et al.* (2000) isolated 4 strains of *Bacillus* from cowdung compost. All the strains

possessed strong inhibition properties against the mycelial growth of *R. solani*, *Bipolaris sorokiniaria*, *S. sclerotium*, *Trichothecium roseum*, *F. solani* and *F. oxysporum* and recorded 56.2 - 71.0 per cent inhibition of these pathogens.

Trivedi and Pandey (2008) reported that, *B. megaterium* strain, B388, inhibited the growth of two phytopathogens such as *Alteraria alterata* and *F. oxysporum*. Inoculation of pepper with PSB significantly reduces the *Phytophthora* blight of peppers caused by *P. capsici* and increased the yields (Akgiil and Mirik, 2008). Several phosphate solubilizing bacteria with lytic enzyme activity were obtained from vermiwash which exhibited antifungal properties against soil borne pathogens (Zambare *et al.*, 2008).

Application of phosphate solubilizing strains in carnation protected the plants systemically against *Fusarium* wilt caused by *F. oxysporum* f sp. *dianthi* (Van peer *et al.*, 1991). Rini and Sulochana (2007) found that *Pseudomonas fluorescens* isolates P28 and P51 showed the greatest inhibition against *R. solani* whereas against *F. oxysporum*, P20 and P28 were most effective. Production of antifungal compounds and compounds inducing systemic resistance were also detected in the p-solubilizing *Pseudomonas putida* by Pandey *et al.*, 2008. A native isolate, *P. fluorescens* PN026R was found to be effective in suppressing the pathogen *R. Solani* and promoting plant growth of Amaranthus (Nair and Anith, 2009). A few PSBs isolated from the Western Ghats of Kerala exhibited antagonistic activity against soil borne plant pathogens like *P. capsici*, *R. solani* and *S. rolfsii* (Girija, 2010).

Both *Bacillus* and *Paenibacillus* species expressed antagonistic activities by suppressing the pathogens under *in vitro* and *in vivo* conditions (Joshi *et al.*, 2006; Chen *et al.*, 2009; Arkebola *et al.*, 2010). Stephen and Jisha (2011) reported that *Burkholderia* sp. recorded 48 % growth inhibition against *F. oxysporum* and 50 % inhibition against *Phytophthora* sp. Amalraj *et al.* (2012) studied the antagonistic activity of *B. megaterium* var. *phosphaticum* agianst *R. solani* (41 %), *M. phaseolina* (42%), *S. rolfsii* (27%) and *F. oxysporum* (40%).

Strains of *Pseudomonas* spp. exhibited antimicrobial activity against *F. oxysporum, A. alternata, Collectotrichum* sp., *Erwinia* sp. and *Xanthomonas* sp. (Parani and Saha, 2012). Manivannan *et al.* (2012) reported that Phosphate solubilizers isolated from the different areas of Cuddalore district in Tamil Nadu, India shown antifungal activity against *F. oxysporum, R. solani* and *S. rolfsii.*

2.8.5.2 ***Effect of PSB on Ralstonia solanacearum***

Bacterial wilt caused by *R. solanareum* is deemed to be one of the most important plant diseases in tropical agriculture. *P. brassicacerum* isolated from the rhizosphere soil of tomato plants strongly inhibited the growth of phytopathogenic bacteria (Zhou *et al.*, 2012).

Algam *et al.* (2010) found that two *Paenibacillus* strains, in particular *Paenibacillus polymyxa* MB02-1007, were found to have strong *in vitro* antibacterial activities against *R. solanacearum.* Nguyen and Ranamukhaarachchi (2010) found that three antagonists, *B. megaterium, Enterobacter cloacae* and *Pichia guillermondii* suppressed the *R. solanacearum* up to 56 per cent in tomato and capsicum in pot experiments. Organic compost mixed with *B. amyloliquefaciens* strain QL-5 effectively decreased *R. solanacearum* incidence in green house and field conditions upto 75 per cent (Zhong *et al.*, 2011).

2.8.5.3 ***Effect of PSF on fungal pathogens***

Among the fungal p-solubilizers, *Trichoderma* sp. are the most commonly studied biocontrol microorganisms which also exhibit plant growth promoting activity (Harman and Bjorkman, 1998). Rajan (2003) reported that foot rot of balck pepper (*Piper nigrum* L.) caused by *P. capsici* is a serious disease of the crop. The use of several antagonists like *T. harzianum, T. virens* and VAM fungi were studied and evaluated the effective biological disease suppession. Gopinath *et al.* (2005) also reported the antagonistic activity of *Trichoderma* sp. against *P. capsici.*

Biocontrol effect of phosphate solubilizing filamentous fungi against *Fusarium* wilt in tomato (*Fusarium oxysporum* f sp. *lycopersici*) was reported by Khan and Khan, 2001. Rini and Sulochana (2007) screened twenty six local isolates of *Trichoderma* spp. from Kerala for their antagonistic activity against *R. solani* and *F. oxysporum* under *in vitro* conditions. The most antagonistic isolates against *R. solani* were *T. pseudokoningii* TR17 and *T. harzianum* TR20. Likewise, *T. viride* TR19 and TR22 were most effective against *F. oxysporum*. *P. oxalicum* showed strong antibiotic activity against pathogenic fungi, including *Sclerotinia sclerotium*, a wide spread pathogenic fungus that severely attacks rape seed (*Brassica napus*) (Lipping *et al.*, 2008). Zeid *et al.* (2008) reported that *Gliocladium* fungus and *Trichoderma* fungi significantly inhibited *A. alterata*, *Cephalosporium madurae*, *Cladosporium herbarum*, *F. oxysporum*, *Humicola grisea*, *Penicillium chrysogenum* and *Ulocladium botrytis*. Application of *T. harzianum* IMI 392432 significantly suppressed *Alternaria* fruit rot disease in chilli (Begum *et al.*, 2010).

2.8.5.4 ***Effect of PSF on R. solanacearum***

A significant disease reduction of 57.3% against *R. solanacearum* was observed in tomato plants pretreated with phosphate solubilizing fungi TriHJSB27 (Jogaiah *et al.*, 2013). Murthy *et al.* (2013) reported the high antagonistic activity of *Trichoderma asperellum* against *R. solanacearum*.

2.8.6 **Effect of phosphate solubilizers on germination and seedling vigour**

Plant growth promoting rhizobacteria (PGPR) stimulate plant growth by producing phytohormone which enhances the growth and physiological activities of the host plant. Holl *et al.* (1988) reported that seed inoculation with phosphate solubilizing *Bacillus* strain L6 has shown to stimulate seedling emergence of crested wheat grass (*Agropyron cristatum* L.). According to Kleifed and Chet (1992), *T. harzianum* application increased germination of pepper seeds, emergence of seedlings and bean, radish, tomato and cucumber.

Trials conducted in cardamom seedling by Thomas *et al.* (2000) confirmed that *G. mossae*, *G. microcarpum* or *G. fasciculatum* promoted early growth and seedling vigour when inoculation was done at the germination stage. Phosphate solubilizing bacteria enhanced the seedling length of *Cicer arietinum* (Sharma *et al.*, 2007).

Seeds inoculated with the phosphate solubilizing bacteria BJ5, isolated from beejamrutha registered significantly higher seedling length and seedling vigour index of soybean (Sreenivasa *et al.*, 2009). Aipova *et al.* (2010) studied the influence of phosphate solubilizing bacteria on seed germination and reported that application of PSB in seed treatment increases wheat germination capacity and also stimulated root growth. Ravikumar *et al.* (2010) found that the PSB has beneficial effect on raising vigorous seedlings of *Rhizophora mucoronata* under nursery and field conditions.

Vishal *et al.* (2011) reported that *Pseudomonas* PMV-14 bacterized rice seeds enhanced shoot length and rootlength compared to non-bacterized seeds. The rootlength, shootlegth and the total biomass of rice and yard-long bean were significantly increased by the application of PSB (Duarah *et al.*, 2011). Chookietwattana and Maneewan (2012) reported that tomato seeds which were inoculated with halotolerant PSB *B. megaterium* A12ag significantly increased the germination percentage and germination index.

Bhakthavatchalu *et al.* (2013) reported that inoculation of cowpea seeds with the phosphate solubilizing *P. aeruginosa* FP6 significantly enhanced seed germination, seedling vigor index, plant height, and also fresh and dry weight in comparison with the control. Demissie *et al.* (2013) conducted a study to evaluate effect of phosphate solubilizing bacteria on seed germination & seedling growth of faba bean. The co-inoculants (JURB48+JURMB69) increased the percentage of germination, vigour index, and radical & plumule lengths of germinated seeds of faba bean. Yadav *et al.* (2013) reported that coinoculated biopriming with

Trichoderma, *Pseudomonas* and *Rhizobium* improved seed germination and seedling growth in chickpea and rajma.

2.9 EFFECT OF PHOSPHATE SOLUBILIZING MICROORGANISMS ON P UPTAKE

The availability of P for plant uptake is determined by the amount of bioactivity in the soil. Use of phosphorus solubilizing bacteria as inoculants increases P uptake. The bacterial species *Pseudomonas* has a considerable potential in phosphorous uptake efficiency (Tilak *et al.*, 1995). Growth and phosphorous content in two alpine carex species increased by inoculation with *Pseudomonas fotinii* (Bartholdy *et al.*, 2001).

Sundara Rao *et al.* (1963) reported that the seed inoculation with *Bacillus megaterium* increased the uptake of PO_4 from both soil and fertilizer P sources. Raj *et al.* (1981) observed that increased plant height and P uptake by finger millet and also increased P availability in soil by inoculating the crop with *Bacillus circulans* and applying ^{32}P labelled super phosphate and tricalcium phosphate. Seed inoculation with *P. putida* GR 12-2 increased the uptake of labelled P (^{32}P) by seedlings grown in growth pouches (Lifshitz *et al.*, 1987).

Asea *et al.* (1988) found that *Penicillium billai* and *P. fuscum* increased total plant phosphorus uptake by 14% and wheat dry matter yield by 16%. Highest P content in the tissue of *Laecaena leucocephala* was found with plant colonization by p-solubilizing fungi, *Mortirella* sp. and mycorrhizal fungi (Osorio and Habte, 2001). Barea *et al.* (2002) reported that the combined inoculation with phosphate solubilizing rhizobacteria, mycorrhizal fungi and *Rhizobium* increased the phosphate uptake in several legumes fertilized with rockphosphate.

Beneficial microbes like p-solubilizers and N fixers in the vermicompost induced solubilization of rockphosphate in enriched vemicompost and helped in N fixation which leads to increase in yield and maximum p uptake in cowpea (Sailajakumari and Ushakumari, 2002).

Babana and Antoun (2006) found that inoculation of wheat tetra fertilized with Tilemsi phosphate rock with a combination of *Glomus intraradices* and *Aspergillus awamori* produced best grain yield with highest P concentration. Mohammadi *et al.* (2011) reported that combined application of phosphorous solubilizing bacteria and *T. harzianum* produced highest leaf P content and grain P content. Surapat *et al.* (2013) studied the effects of PSB on the growth of chilli in a pot trial and found that KS04 significantly increased shoot height and P uptake, to a greater extent than the other PSB isolates.

The highest P content in pods was found in treatment receiving Nitrogen fixing bacteria + Phosphate solubilizing bacteria (Shabani *et al.*, 2011).

2.10 EFFECT OF PHOSPHATE SOLUBILIZING MICROORGANISMS ON NODULATION

Inoculation with phosphate solubilizing bacteria was found to cause a significant increase in nodule number, dry weight of shoot, root and nodule, shoot nitrogen and phosphorus content and yield (Abdalla *et al.*, 2011). Hossain *et al.* (2013) reported that inoculation of phosphate solubilizing isolates considerably enhanced the nodulation and yield of mungbean while the effect was more pronounced when they were applied with phosphatic fertilizer compared to un-inoculated with no phosphatic fertilizer.

Significant increase in nodule number and weight per plant, nitrogen and dry matter accumulation in groundnut plant was observed in treatment receiving combination of organic amendments and p-solubilizers plus Mussorie rockphosphate (Manjaiah *et al.*, 1996). Sturz *et al.* (1997) found that nodulation by *R. leguminosarum* b.v. *trifolii* of red clover was promoted when it was coinoculated with *Bacillus insolitus* and *B.brevis*.

Kopler *et al.* (1988) indicated that more legume nodulation was obtained with concurrent inoculation of *Rhizobium* and *Pseudomonas* spp. Tiwari *et al.* (1989) recorded increase in nodulation of chickpea due to inoculation of *A.*

awamori in combination with application of rockphosphate and superphosphate as P sources. PSB inoculation increased nodulation, nodule dry weight, nitrogen and phosphorus content in shoot and dry fodder (Hassen and Abdelgani, 2009). *Pseudomonas* spp. enhanced the number of nodules, dry weight of nodules, yield components, grain yield, nutrient availability and uptake in soybean crop (Son *et al.*, 2006). Dutta (2007) reported that root nodulation, growth, yield parameters and uptake of phosphorous were high in pigeon pea which received a treatment of 50% RP + 50 % SSP along with FYM and PSB. *Rhizobium* + PSB yielded maximum number of nodules (67.13) and nodule dry weight (107.73 mg). *Rhizobium* alone showed maximum production of dry matter (3.63 gm) in soy bean (Wasule *et al.*, 2007). Linu *et al.* (2009) reported that inoculation of PSB improved nodulation, root and shoot biomass, straw and grain yield of the crop.

2.11 EFFECT OF PHOSPHATE SOLUBILIZERS ON AVAILABLE PHOSPHOROUS IN SOIL

Release of P by PSM from insoluble and fixed / adsorbed forms is an import aspect regarding P availability in soils. The introduction of efficient microbes (P-solubilizers) in the rhizosphere has been found to increase the availability of phosphorus from both applied and native soil phosphorus (Mahdi *et al.*, 2011).

Nair and Subbarao (1977) reported that the incidence of PSM and available P in different rhizosphere soils were directly related. This was reported in mixed cropping of coconut and cocoa. Tiwari *et al.* (1989) have found a higher effect of the inoculation of plants by p-solubilizing bacteria in soils deficient in available phosphorous. The increased available P content of soil due to inoculation of PSM were also reported (Banik and Dey, 1989; Jisha and Alagawadi, 1996).

Banik and Dey (1981) obtained higher levels of available P in soil when rockphosphate was applied along with FYM and culture of *Bacillus* and *Penicillium* sp. Khalil (1995) reported that phosphate availability from rockphosphate was increased in soil from 0.67 ppm in control to 17.78 ppm with

PSM inoculation in 20 days. Direct application of phosphate rock is often ineffective in the short time period of most annual crops (Goenadi *et al.*, 2000). Acid producing microorganisms are able to enhance the solubilization of phosphatic rock (Gyaneshwar *et al.*, 2002).

The PSB in conjunction with single super phosphate and rock phosphate reduce the P dose by 25 and 50 %, respectively (Sundara *et al.*, 2002). Stephen (2002) found that the highest available p content was recorded by the treatment 50% N as FYM + *Azospirillum* + phosphate solubilizing microorganisms + AMF + 100 percent K as wood ash in pepper.

The PSB solubilize the fixed soil P and applied phosphates resulting in higher crop yields (Gull *et al.*, 2004). Supanjani *et al.* (2006) reported that integration of P and K rocks with inoculation of phosphorus and potassium solubilizing bacteria increased P availability from 12 to 21 per cent. Phosphorus solubilizing bacteria mainly *Bacillus, Pseudomonas* and *Enterobacter* are very effective for increasing the plant available P in soil as well as the growth and yield of crops. So, exploitation of phosphate solubilizing bacteria through biofertilization has enormous potential for making use of ever increasing fixed p in the soil, and natural reserves of phosphate rocks (Khan *et al.*, 2009).

2.12 EFFECT OF PHOSPHATE SOLUBILIZERS ON CROP YIELD

Phosphorous is known to play an important physiological and biochemical role in crop plants. Therefore, it regulates the crop growth and yield to the greater extent. The added P fertilizer P gets fixed in soil and it is not available to the crops. P-ion concentration in most soils varies from 0.1 to 10 µM while p required for optimal growth ranges from 1 to 5 µM for grasses and from 5 to 60 µM for high P demanding crops, such as, tomato (*Lycopersicon esculentum*) and pea (*Pisum sativum*) (Ragothama, 1999). Suboptimal levels of P can however, lead to a 5- 15 % loss in the yield of plants (Hinsinger, 2001).

Phosphate solubilizing bacteria are used as biofertilizer since 1950's (Kudashev, 1956; Krasilinikov, 1957). Increased yield response of crop plants have been observed in seed inoculation with phosphate solubilizing bacteria (Gaur *et al.*, 1980). Strains of *P. putida* and *P. fluorescens* have increased crop yields in potato, radishes, rice, sugar beet, tomato, lettuce, apple, citrus, beans, ornamental plants and wheat (Suslov, 1982; Kloepper, 1988; Lemanceau, 1992). Seed or soil inoculation with PSM is known to improve solubilization of fixed soil phosphorous and applied phosphates resulting in higher crop yields (Jones *et al.*, 1994). Phosphate solubilizing *Agrobacterium radiobacter* combined with nitrogen fixer *Azospirilum lipoferum* produced improved grain yield of barley compared with single inoculation in pot and fixed experiments (Belimov *et al.*, 1995).

Combined inoculation of PSB and *Azospirillum* increased available N, P and K in soil and also resulted in highest oil yield in palmarosa (Thomas, 1996). Fares (1997) reported that inoculation with *Azotobacter* + *Rhizobium*+VAM gave the highest increase in straw and grain yield of wheat plants with rockphosphate as a P fertilizer. Sendukumaran *et al.* (1998) found that combination of FYM+150:100:50 kg NPK ha^{-1} + *Azospirillum*+Phosphobacteria produced high number of fruits per plant and mean fruit weight of tomato as compared to other treatments.

In nutmeg, 100 kg FYM, 400 g N, 300 g P_2O_5 and 1200 g K2O $tree^{-1}$ $year^{-1}$ along with 50 g each of *Azospirillum* and Phosphobacterium increased the yield through other growth parameters like tree girth, fruit weight, and fruit number. In clove also same dose of organic manure and biofertilizers alone with 400:200 g NPK $tree^{-1}ha^{-1}$ resulted in the highest yield (AICRPS, 2000). Dual inoculation of AMF +PSM at 30 kg P_2O_5 ha^{-1} registered the highest green pod yield (4451 $kgha^{-1}$) and haulm yield (8915 kg/ha) in vegetable cowpea (Mathew and Hameed, 2002). Binisha (2003) reported that maximum number of pseudobulbs was obtained for the treatment receiving NPK 10:5:10 inoculated with *Azospirillum* and phosphobacteria at the time of planting in dendrobium. Increase in growth

and yield of blackgram under rice fallow conditions was reported by Sundari and Sureshkumar (2004) due to inoculation of *B. megaterium* var. *phosphaticum.*

Han and Lee (2005) found that co-inoculation of PSB and KSB in combination with direct application of rockphosphate and K materials into the soil resulted in increased N, P and K uptake, photosynthesis and yield of brinjal grown on P and K limited soil. Increased yield to the tone of 12-15 % and replacement of 25-28 % phosphate fertilizer was observed in cereals, legumes, potatoes and other field crops on the addition of rockphosphate and inoculation with PSM (Arun, 2007). Single and dual inoculation along with P fertilizer was 30-40 % better than P fertilizer alone for improving grain yield of wheat (Afzal and Bano, 2008).

Application of *Rhizobium* and PSB produced higher yield of cowpea and its effect was higher when integrated with high quantities of organic manure (KAU, 2009). Sherene *et al.* (2012) found that the yield attributes such as kapas yield, lint yield and seed yield recorded highest in the treatment that received 50 percent Jhabua rock phosphate + 50 percent SSP along with phosphobacteria and compost in cotton MW12. Singh *et al.* (2013) reported that maximum curd yield was recorded with 50% P (RP) + P solubilizer + VAM in cauliflower.

Materials and Methods

3. MATERIALS AND METHODS

The present study entitled, "Exploration of native mineral phosphate solubilizing microorganisms as biofertilizer for the acidic soils of Kerala" was carried out at the Department of Agricultural Microbiology, College of Horticulture, Vellanikkara during the period from 2011 to 2013. The materials used and the methodologies adopted are briefly described in this chapter.

3.1 MATERIALS

3.1.1 **Chemicals, glassware and plastic ware**

The chemicals and media used for this study were procured from agencies like MERCK India Ltd., Sisco Research Laboratory (SRL), HIMEDIA Laboratories Pvt. Ltd., MEDILISE Chemicals and LOBA CHEMIE Pvt. Ltd. Composition of media used for various tests are given in Appendix I. All the plastic ware used was obtained from Genaxy Scientific Pvt. Ltd and Tarson India Ltd. The primers were synthesized from Integrated DNA Technologies, USA and buffers were supplied by Banglore Genei Ltd.

3.1.2 **Equipment and machinery**

The equipment items available at the Department of Agricultural Microbiology and Radio tracer Laboratory, College of Horticulture, Vellanikkara were used for present study. Microbial cultures were incubated in incubator shaker (Genei, Banglore). Absorbance of the solutions was recorded using Spectrophotometer, Spectroquant® Pharo 300 (Merck Millipore, Germany). pH was measured using pH meter (EUTECH Instruments, Europe). Centrifugation was carried out in a high speed refrigerated centrifuge (Eppendorf centrifuge 5804R, Germany). Sterilization of media and glass wares was carried out using Autoclave, EQUITRON® (Media Instrument MFG. Co, Mumbai). PCR was carried out in Eppendorf Master Cycler, Gradient (Eppendorf, Germany) and documentation was done using UVP Gel Doc-It ™ imaging system (UVP, UK).

Plant samples were digested in microwave digester, Mars (CEM Corporation, USA).

3.1.3 **Microbial isolates**

Two hundred isolates (bacteria and fungi) available at the Department of Agricultural Microbiology, obtained from the Western Ghats of Kerala under the ICAR funded net work project entitled "Application of Microorganisms in Agriculture and Allied Sectors" were used for screening of efficiency of mineral P-solubilization. The details of the location from where the isolates were obtained are given in table 2. Commercial formulation of phospahte solubilizing microorganism obtained from the College of Agriculture, Vellayani was used as reference culture for seedling vigour and pot culture studies.

3.2 QUALITATIVE SCREENING OF MICROBES FOR PHOSPHATE SOLUBILIZING EFFICIENCY

3.2.1 **Screening of phosphate solubilizing bacteria (PSB) and phosphate solubilizing fungi (PSF)**

Bacterial suspensions were prepared by dispensing a single colony in 1ml of sterile water. Twenty microlitres of bacterial suspension was spot inoculated at the centre of the Pikovskaya's agar plate and incubated at room temperature. Diameter of colony and clear zone were measured every day, upto seven days after inoculation.

One potato dextrose agar (PDA) disc of 6 mm size of actively growing mycelium of fungal cultures was placed at the centre of Pikovskaya's agar plate and incubated at room temperature. Diameter of colony and clear zone were measured every day, upto five days.

Table 2. Details of PSM used for screening of phosphate solubilization

Sl no.	Bacteria	Location		
		District	Place	Latitude , Longitude and Elevation
1	PSB-1	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
2	PSB-2	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
3	PSB-3	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
4	PSB-4	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
5	PSB-5	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
6	PSB-6	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
7	PSB-7	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
8	PSB-8	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
9	PSB-9	Thrissur	Peechi	N-10°53511' E-76°3772' Ele-584
10	PSB-10	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
11	PSB-11	Kollam	Check dam	N-8° 57.259' E-77° 9.191' Ele-2534
12	PSB-12	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
13	PSB-13	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
14	PSB-14	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
15	PSB-15	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
16	PSB-16	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
17	PSB-17	Kollam	Check dam	N-8° 57.259' E-77° 9.191' Ele-2534
18	PSB-18	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele- 1088
19	PSB-19	Palakkad	Nenmara	N-10° 31.595 E-76° 38.270' Ele- 1088
20	PSB-20	Kasargod	Veetty	N-12°53521' E-75°20895' Ele-772
21	PSB-21	Palakkad	Nenmara	N-10° 31.595' E-76°38.270' Ele-1088
N: North E: East Ele: Elevation in feet				

Table 2 contd. Details of PSM used for screening of phosphate solubilization

Sl no.	Bacteria	Location		
		District	Place	Latitude, Longitude and Elevation
22	PSB-22	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
23	PSB-23	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
24	PSB-24	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
25	PSB-25	Thrissur	Peechi	N-10°53511' E-76 ° 3772' Ele-584
26	PSB-26	Palakkad	Silent Valley	N-11°05.555' E-76° 26.779' Ele-1147
27	PSB-27	Palakkad	Eliveli	N-10° 52.058' E-38° 359' Ele-368
28	PSB-28	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
29	PSB-29	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
30	PSB-30	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
31	PSB-31	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
32	PSB-32	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
33	PSB-33	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
34	PSB-34	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
35	PSB-35	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
36	PSB-36	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
37	PSB-37	Palakkad	Silent valley	N-11°05.555' E-76° 26.779' Ele-1147
38	PSB-38	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
39	PSB-39	Palakkad	Silent valley	N-11°05.555' E-76° 26.779' Ele-1147
40	PSB-40	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
41	PSB-41	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
42	PSB-42	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
43	PSB-43	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
44	PSB-44	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
45	PSB-45	Kasaragod	Veetty	N-12 °53509' E-75° 2089' Ele-663
N: North E: East Ele: Elevation in feet				

Table 2 contd. Details of PSM used for screening of phosphate solubilization

Sl no.	Bacteria	Location		
		District	Place	Latitude, Longitude and Elevation
46	PSB-46	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
47	PSB-47	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
48	PSB-48	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
49	PSB-49	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
50	PSB-50	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
51	PSB-51	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
52	PSB-52	Kasaragod	Kanhangadu	N-75°406250' E-12° 281280'Ele-2396
53	PSB-53	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
54	PSB-54	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
55	PSB-55	Kasaragod	Kanhangadu	N-75°406250' E-12° 281280'Ele-2396
56	PSB-56	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
57	PSB-57	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
58	PSB-58	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
59	PSB-59	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
60	PSB-60	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
61	PSB-61	Palakkad	Silent valley	N-11°05.555' E-76° 26.779' Ele-1147
62	PSB-62	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
63	PSB-63	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
64	PSB-64	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
65	PSB-65	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
66	PSB-66	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
67	PSB-67	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
N: North E: East Ele: Elevation in feet				

Table 2 contd. Details of PSM used for screening of phosphate solubilization

Sl no.	Bacteria	Location		
		District	Place	Latitude, Longitude and Elevation
68	PSB-68	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
69	PSB-69	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
70	PSB-70	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
71	PSB-71	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
72	PSB-72	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
73	PSB-73	Kasaragod	Kanhangadu	N-75°406250' E-12° 281280'Ele-2396
74	PSB-74	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
75	PSB-75	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
76	PSB-76	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
77	PSB-77	Kasaragod	Kanhangadu	N-75°406250' E-12° 281280'Ele-2396
78	PSB-78	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
79	PSB-79	Palakkad	Silent valley	N-11°05.555' E-76° 26.779' Ele-1147
80	PSB-80	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
81	PSB-81	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
82	PSB-82	Palakkad	Silent valley	N-11°05.555' E-76° 26.779' Ele-1147
83	PSB-83	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
84	PSB-84	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
85	PSB-85	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
86	PSB-86	Palakkad	Silent valley	N-11°05.555' E-76° 26.779' Ele-1147
87	PSB-87	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
88	PSB-88	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
89	PSB-89	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
90	PSB-90	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
91	PSB-91	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
92	PSB-92	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
93	PSB-93	Palakkad	Nenmara	N-10° 31.595' E-76° 38.270' Ele-1088
N: North E: East Ele: Elevation in feet				

Table 2 contd. Details of PSM used for screening of phosphate solubilization

Sl no.	Bacteria	Location		
		District	Place	Latitude, Longitude and Elevation
94	PSB-94	Palakkad	Nenmara	N-10°31.595'E-76°38.270'Ele-1088
95	PSB-95	Palakkad	Nenmara	N-10°31.595'E-76°38.270'Ele-1088
96	PSB-96	Palakkad	Nenmara	N-10°31.595'E-76°38.270'Ele-1088
97	PSB-97	Palakkad	Nenmara	N-10°31.595'E-76°38.270'Ele-1088
98	PSB-98	Palakkad	Nenmara	N-10°31.595'E-76°38.270'Ele-1088
99	PSB-99	Palakkad	Nenmara	N-10°31.595'E-76°38.270'Ele-1088
100	PSB-100	Palakkad	Nenmara	N-10°31.595'E-76°38.270'Ele-1088
101	PSB-101	Palakkad	Silent valley	N-11°05.555'E-76°26.779'Ele-1147
102	PSB-102	Malappuram	Nilambur	N-11°30264' E-76° 42048' Ele-844
103	PSB-103	Malappuram	Nilambur	N-11°30264' E-76° 42048' Ele-844
104	PSB-104	Malappuram	Nilambur	N-11°30264' E-76° 42048' Ele-844
105	PSB-105	Malappuram	Nilambur	N-11°30264' E-76° 42048' Ele-844
106	PSB-106	Idukki	Mayiladumpara	N-9° 53389' E-77° 14884' Ele-2810
107	PSB-107	Idukki	Thattekkad	N-10°6.822' E-76° 42.568' Ele-121
108	PSB-108	Idukki	Thattekkad	N-10°6.822' E-76° 42.568' Ele-121
109	PSB-109	Idukki	Thattekkad	N-10°6.822' E-76° 42.568' Ele-121
110	PSB-110	Thrissur	Peechi	N-10°53511' E-76 ° 3772' Ele-584
111	PSB-111	Idukki	Thattekkad	N-10°6.822' E-76° 42.568' Ele-121
112	PSB-112	Idukki	Mayiladumpara	N-9° 53389' E-77° 14884' Ele-2810
113	PSB-113	Idukki	Mayiladumpara	N-9° 53389' E-77° 14884' Ele-2810
N: North E: East Ele: Elevation in feet				

Table 2 contd. Details of PSM used for screening of phosphate solubilization

Sl no.	Bacteria	Location		
		District	Place	Latitude,Longitude and Elevation
114	PSB-114	Thrissur	Peechi	N-10°53511'E-76°3772' Ele-584
115	PSB-115	Malappuram	Nilambur	N-11°30264'E 76° 42048' Ele-844
116	PSB-116	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8°35.099' Ele-794
117	PSB-117	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8°35.099' Ele-794
118	PSB-118	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8°35.099' Ele-794
119	PSB-119	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8°35.099' Ele-794
120	PSB-120	Thrissur	Peechi	N-10°53511'E-76°3772' Ele-584
121	PSB-121	Malappuram	Nilambur	N-11°30264'E-76°42048' Ele-844
122	PSB-122	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8°35.099' Ele-794
123	PSB-123	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8°35.099' Ele-794
124	PSB-124	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8°35.099' Ele-794
125	PSB-125	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8°35.099' Ele-794
N: North E: East Ele: Elevation in feet				

Table 2 contd. Details of PSM used for screening of phosphate solubilization

Sl no.	Bacteria	Location		
		District	Place	Latitude,Longitude and Elevation
126	PSB-126	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8°35.099' Ele-794
127	PSB-127	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8°35.099' Ele-794
128	PSB-128	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8°35.099' Ele-794
129	PSB-129	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8°35.099' Ele-794
130	PSB-130	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8°35.099' Ele-794
131	PSB-131	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8°35.099' Ele-794
132	PSB-132	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8°35.099' Ele-794
133	PSB-133	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8°35.099' Ele-794
134	PSB-134	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8°35.099' Ele-794
135	PSB-135	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8°35.099' Ele-794
136	PSB-136	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8°35.099' Ele-794
137	PSB-137	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8°35.099' Ele-794
N: North E: East Ele: Elevation in feet				

Table 2 contd. Details of PSM used for screening of phosphate solubilization

Sl no.	Bacteria	Location		
		District	Place	Latitude,Longitude and Elevation
138	PSB-138	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8°35.099' Ele-794
139	PSB-139	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8°35.099' Ele-794
140	PSB-140	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8°35.099' Ele-794
141	PSB-141	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8°35.099' Ele-794
142	PSB-142	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8°35.099' Ele-794
143	PSB-143	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8°35.099' Ele-794
144	PSB-144	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8°35.099' Ele-794
145	PSB-145	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8°35.099' Ele-794
146	PSB-146	Palakkad	Nenmara	N-10°31.595'E-76° 38.270' Ele-1088
147	PSB-147	Palakkad	Nenmara	N-10°31.595'E-76° 38.270'Ele-1088
148	PSB-148	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8° 35.099'Ele-794
149	PSB-149	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8° 35.099'Ele-794
N: North E: East Ele: Elevation in feet				

Table 2contd. Details of PSM used for screening of phosphate solubilization

Sl no.	Bacteria	Location		
		District	Place	Latitude, Longitude and Elevation
150	PSB-150	Malappuram	Nilambur	N-11°30264'E-76°420.48' Ele-844
151	PSB-151	Palakkad	Nenmara	N-10°31.595'E-76° 38.270' Ele-1088
152	PSB-152	Palakkad	Nenmara	N-10°31.595'E-76° 38.270' Ele-1088
153	PSB-153	Palakkad	Nenmara	N-10°31.595'E-76° 38.270' Ele-1088
154	PSB-154	Palakkad	Nenmara	N-10°31.595'E-76° 38.270' Ele-1088
155	PSB-155	Palakkad	Nenmara	N-10°31.595'E-76° 38.270' Ele-1088
156	PSB-156	Malappuram	Nilambur	N-11°30264'E-76°420.48' Ele-844
157	PSB-157	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8° 35.099' Ele-794
158	PSB-158	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8° 35.099' Ele-794
159	PSB-159	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8° 35.099' Ele-794
160	PSB-160	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8° 35.099' Ele-794
161	PSB-161	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8° 35.099' Ele-794
N: North E: East Ele: Elevation in feet				

Table 2 contd. Details of PSM used for screening of phosphate solubilization

Sl no.	Bacteria	Location		
		District	Place	Latitude, Longitude and Elevation
162	PSB-162	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8° 35.099' Ele-794
163	PSB-163	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8° 35.099' Ele-794
164	PSB-164	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8° 35.099' Ele-794
165	PSB-165	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8° 35.099' Ele-794
166	PSB-166	Idukki	Mayiladumpara	N-9°53389'E-77°14884' Ele-2810
167	PSB-167	Malappuram	Nilambur	N-11°30264'E-76°420.48' Ele-844
168	PSB-168	Malappuram	Nilambur	N-11°30264'E-76°42048'Ele-844
169	PSB-169	Kozhikkode	Jeerakappara	N-11°28.528'E-76°3.705' Ele-2183
170	PSB-170	Kozhikkode	Jeerakappara	N-11°28.528'E-76°3.705' Ele-2183
171	PSB-171	Wayanadu	Begur	N-11°53.344'E-76°5.324' Ele-2634
172	PSB-172	Malappuram	Nilambur	N-11°30264'E-76°420.48' Ele-844

N: North E: East Ele: Elevation in feet

Table 2 contd. Details of PSM used for screening of phosphate solubilization

Sl no.	Fungi	Location		
		District	Place	Latitude, Longitude and Elevation
173	PSF-173	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8° 35.099' Ele-794
174	PSF-174	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8° 35.099' Ele-794
175	PSF-175	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8° 35.099' Ele-794
176	PSF-176	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8° 35.099' Ele-794
177	PSF-177	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8° 35.099' Ele-794
178	PSF-178	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8° 35.099' Ele-794
179	PSF-179	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8° 35.099' Ele-794
180	PSF-180	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8° 35.099' Ele-794
181	PSF-181	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8° 35.099' Ele-794
182	PSF-182	Kozhikkode	Jeerakappara	N-11°28.528'E-76°3.705' Ele-2183
183	PSF-183	Idukki	Thattekkad	N-10°6.822'E-76°42.568' Ele-121
N: North E: East Ele: Elevation in feet				

Table 2 contd. Details of PSM used for screening of phosphate solubilization

Sl no.	Fungi	Location		
		District	Place	Latitude, Longitude and Elevation
184	PSF-184	Idukki	Thattekkad	N-10°6.822'E-76°42.568' Ele-121
185	PSF-185	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8°35.099' Ele-794
186	PSF-186	Idukki	Thattekkad	N10°6.822'E76°42.568' Ele-121
187	PSF-187	Idukki	Thattekkad	N10°6.822'E76°42.568' Ele-121
188	PSF-188	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8° 35.099 ' Ele-794
189	PSF-189	Kasaragod	Kanhangadu	N-75°406250'E-12° 281280' Ele-2396
190	PSF-190	Kasaragod	Kanhangadu	N-75°406250'E-12° 281280'Ele-2396
191	PSF-191	Kozhikkode	Jeerakappara	N-11°28.528'E-76° 3.705' Ele-2183
192	PSF-192	Idukki	Thattekkad	N-10°6.822'E-76°42.568' Ele-121
193	PSF-193	Kasaragod	Kanhangadu	N-75°406250'E12° 281280 'Ele-2396
194	PSF-194	Kozhikkode	Thusharagiri	N-11°28.315'E-76° 03.352' Ele-1588
195	PSF-195	Wayanadu	Begur	N-11°53.344'E-76° 5.324' Ele-2634
N: North E: East Ele: Elevation in feet				

Table 2 contd. Details of PSM used for screening of phosphate solubilization

Sl no.	Fungi	Location		
		District	Place	Latitude, Longitude and Elevation
196	PSF-196	Wayanadu	Begur	N-11°53.344'E-76° 5.324' Ele-2634
197	PSF-197	Palakkad	Eliveli	N-10°52.058'E-38°'359' Ele-368
198	PSF-198	Idukki	Thattekkad	N-10°6.822'E-76°42.568' Ele-121
199	PSF-199	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8° 35.099' Ele-794
200	PSF-200	Thiruvananthapuram	Kulathoopuzha	N-77°8.758'E-8° 35.099' Ele-794
N: North E: East Ele: Elevation in feet				

3.2.2 **Phosphate Solubilizing Efficiency (PSE)**

The ability of isolates to solubilize the insoluble phosphorous was studied by the determination of solubilizing efficiency. Phosphate solubilizing efficiency was determined by the formula as suggested by Maheswar and Sathiyavani, 2012.

$$\text{PSE (\%)} = \left[\frac{\text{Zone diameter (cm)} + \text{Colony diameter (cm)}}{\text{Colony diameter (cm)}}\right] \times 100$$

Based on the phosphate solubilizing efficiency, twenty (ten bacteria and ten fungi) most efficient phosphate solubilizers were selected. These twenty isolates were used for further studies.

3.3 QUANTITATIVE ESTIMATION OF P-SOLUBILIZING EFFICIENCY

Twenty most efficient isolates were used for the quantitative estimation of P-solubilized using Mo-blue method (Olsen *et al.*, 1954). Hundred millilitres of Pikovskaya's broth medium amended with 0.5 g of tricalcium phosphate was prepared in 250 ml flask and sterilized. One millilitre of suspension of each bacterial isolates was inoculated in the Pikovskaya's broth. One PDA disc of actively growing mycelium was used for inoculation of PSF (Yadav *et al.*, 2011). Three replications were maintained for each culture. Pikovskaya's broth without inoculum served as control. The inoculated cultures were incubated for 14 days on rotary shaker at room temperature. pH of the broth was recorded 14 days after incubation using pH meter.

The cultures were centrifuged at 10,000 rpm for 10 minutes. One millilitre of the supernatant was pipetted out into a test tube and the volume made up to 8.6 ml with distilled water. One millilitre of ammonium molybdate reagent (Anexure II) (2.5% ammonium molybdate in 3N H_2SO_4) followed by 0.4 ml of ANSA (1-Amino-2-naphthol-4-sulfonic acid) reagent (To prepare 0.25% ANSA, 0.5g ANSA was added to 195 ml of 15% sodium bisulphate and 5ml of 20% sodium sulphite was added and stored in a brown bottle in cold) were added to the test tube.

Mixed thoroughly and allowed to stand for 10 min. The colour intensity was read in a spectrophotometer at 660 nm. Concentration of available phosphorous present in the broth was calculated by referring to the standard graph.

The standard curve was prepared by diluting 5 ml of stock standard 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 contained 8µg of P) to prepare working standard. Transferred 0, 1, 2, 3, 4 and 5 ml of working standard solution to test tubes to get 0, 8, 16, 24, 32, 40 µg of P and the volume made up to 8.6 ml with distilled water. One ml of ammonium molybdate follwed by 0.4 ml of ANSA reagent were added to test tubes. Mixed thoroughly and allowed to stand for 10 min. The colour intensity was read in a spectrophotometer at 660 nm. Absorbance was plotted against concentration on a graph.

3.4 IDENTIFICATION AND CHARACTERISATION OF SELECTED ISOLATES

An attempt was made to identify the selected twenty phosphate solubilizing microorganisms.

3.4.1 **Identification of PSB**

Ten PSB isolates were identified based on colony morphology, biochemical characters, physiological characters and 16S rDNA sequencing.

3.4.1.1 ***Colony characters***

Colony characteristics of bacteria such as size, shape, margin, elevation, surface, texture and colour of the colonies on nutrient agar were studied.

3.4.1.2 **Staining reactions**

3.4.1.2.1 ***Gram staining***

All bacterial isolates were subjected to Gram staining. Gram staining was done as described by Gephardt *et al.*, 1981.

1. A thin smear of bacterial isolate was prepared on clean glass slide

2. Smear was air dried and heat fixed by passing over the flame
3. The smear was flooded with crystal violet for one minute and washed under running tap water
4. The smear was flooded with Gram's iodine solution for one minute and washed under running tap water
5. Decolourized with 95% ethyl alcohol for 30 seconds
6. The smear was treated with counter stain safranin for one minute
7. Finally the slide was washed; air dried and observed under 100X (oil immersion) objective of a compound binocular microscope for shape and arrangement of cells

3.4.1.2.2 ***Solubility in KOH***

Test on solubility in KOH was carried out to confirm Gram reaction. A drop of 3% freshly prepared KOH was placed on a clean glass slide. A loopful of growth from a single, well-isolated colony was mixed for one minute with the drop and the loop was gently pulled from the suspension. If the mixture becomes viscous and forms strings then the bacterium was considered as Gram-negative.

3.4.1.2.3 ***Endospore staining***

Endospore staining was done for gram positive bacteria as described by Schaeffer and Fulton, 1993.

1. A thin smear of bacterial isolates were prepared on clean and dried glass slides, air dried and heat fixed
2. The smears were flooded with malachite green
3. The slides were heated in steam for 5 min. More stain was added to the smears from time to time
4. The slides were washed under slowly running tap water
5. Counterstained with safranin for 30 seconds and washed
6. The slides were blot dried with blotting paper

7. The slide was observed under 100 X (oil immersion) objective of a compound binocular microscope

3.4.1.3. **Biochemical characters**

3.4.1.3.1 ***Production of oxidase enzyme***

Ready-to-use oxidase discs from Himedia ®, Mumbai were used for this test. A disc was placed on a clean glass slide and a loopful of 24 h old inoculum was rubbed on the disc (Kovacs, 1956). Development of purple colour indicated production of oxidase enzyme.

3.4.1.3.2 ***Production of catalase***

A smear of 24 h old broth culture was prepared on a clean glass slide and immediately 2-3 drops of 3% hydrogen peroxide were added. Formation of effervescence indicated positive reaction (Cappuccino and Sherman, 1992).

3.4.1.3.3 ***Utilization of sugars***

Phenol red medium was dispensed into test tubes at the rate of 5ml per tube. Durham's tubes were carefully inserted into test tubes in an inverted position without formation of bubbles. These were then sterilized at 0.84 $kgcm^2$ for 15 min. One percent solution of sugars such as glucose, lactose, maltose, fructose, mannitol, dulcitol and sorbitol were sterilized by tyndallization. Five hundred microlitres of each sugar solution was added into medium contained in test tubes. One loopful of 24 h old bacterial culture was inoculated into the medium and kept one uninoculated tube as a comparative control. The colour change from red to yellow indicated acid production and formation of bubbles indicated gas production. Observations were recorded after 24 h of incubation.

3.4.1.3 Physiological characters

3.4.1.3.1 *Tolerance to low pH*

Assessment of acid tolerance of ten bacterial isolates were done in tubes containing sterile Luria Bertani broth adjusted to pH 4.0, 5.0, 6.0 and 7.0 (Pal, 1998). pH was adjusted before and after sterilization using either filter sterilized 0.1 N NaOH or HCl. The 48h old culture was inoculated @ 0.1ml into nutrient broth with pH values 4.0, 5.0, 6.0 and 7.0 respectively. The tubes were incubated at 32^0C and the turbidity was recorded at 12h, 24h, 36h, 48h, 60h, 72h, after incubation using Spectroquant®pharo 300 spectrophotometer at 530nm. Growth in pH 4.0, 5.0 and 6.0 were compared with pH 7.0 and per cent change in acid tolerance was calculated as per the following formula.

Per cent change in acid-tolerance over control

$$= \left(\frac{\text{(O.D. at pH 7.0 - O.D. at pH x)}}{\text{O.D. at pH 7.0}} \right) \text{ X } 100$$

Where, pH x = pH 4.0, 5.0 or 6.0

Those cultures having less per cent change in acid-tolerance were rated as most acid-tolerant strains.

3.4.1.4 Molecular characterization by 16S rDNA sequencing

The ten phosphate solubilizing bacterial isolates selected for the study were identified by 16S rDNA sequencing.

3.4.1.4.1 *Amplification of 16S rDNA gene*

A single colony was taken with micropipette tip, mixed with 10 µl sterile water in a microcentrifuge tube. Two microlitres of this culture suspension was

used as template for amplification of 16S rDNA gene. Universal primers 8F and 152 R (Thomas *et al.*, 2008) were used to amplify the 1500 bp 16S rDNA.

Table 3. Sequence of primers used

Primer details	Sequence 5'-3'	Base pair
8F	AGA GTT TGA TCC TGG CTC AG	20
152R	AAG GAG GTG ATC CAG CG CA	20

Polymerase chain reaction was carried out in Eppendorf Master Cycler (Gradient, Germany) using PCR master mix 'Emerald Amp GT PCR'. The composition of the reaction mixture for PCR is as follows:

Table 4. Components of PCR reaction mix

Component	Quantity (µl)
Master mix	12.5
Template	2.0
Forward primer	0.5
Reverse primer	0.5
dH2O	9.5
Total	25.0

The reaction was set in 200 µl microfuge tube chilled over ice flakes. A momentary spin was given to mix completely all reagents and set in master cycler for amplification. The details of thermal cycler are given below.

Table 5. Details of thermal Cycler Programme

No.	Step	Temperature (°C)	Time (min)
1	Initial denaturation	95	3.00
2	Denaturation	94	1.30
3	Annealing	55	0.40
4	Primer extension	72	1.30
5	Steps 2-4	34 cycles	-
6	Final extension	72	20.00
7	Final hold	4	10.00

3.4.1.4.2 ***Agarose gel electrophoresis***

The amplification of 16S rDNA was confirmed by agarose gel electrophoresis (Sambrook *et al.*, 1989). 1X TAE buffer was prepared from the 50 X TAE (pH 8.0) stock solutions. Agarose (Genei, Low EEO) (1%) was weighed and dissolved in TAE buffer by boiling. Ethidium bromide prepared from a stock of 10 mg/ml was added to it at a concentration of 0.5 µg/ml and mixed well. The comb was placed properly and dissolved agarose was poured into the tray. The gel was placed in the electrophoresis unit after 30 min with well side directed towards the cathode. 1X TAE buffer was added to the buffer tank (Genei, Banglore) so as to cover the well with a few mm of buffer. 2 µl of the PCR product was carefully loaded into the wells using a micropipette. The

Gene Ruler 1 kb DNA ladder was used as the molecular weight marker. The cathode and anode of the electrophoresis unit were connected to the power pack and the gel was run at constant voltage of 100V. The power was turned off when the tracking dye reached at about 3 cm from the anode end.

3.4.1.4.3 ***Gel documentation***

The DNA bands separated by electrophoresis were viewed and photographed using UVP Gel Doc-It ^{TM} imaging system.

3.4.1.4.4 ***Purification and sequencing of PCR product***

The PCR product was purified and sequenced at Scigenome Pvt.Ltd.Cochin using the primers 8F and 1522R.

3.4.1.4.5 ***Nucleotide sequence analysis***

DNA baser was used for merging the sequences and the contig was subjected to blastn programme (http://blast.ncbi.nlm.gov/Blast) to find out the homology of the nucleotide sequences.

3.4.2 **Identification of PSF**

Cultural and morphological characters were used for the fungal identification.

3.4.2.1 ***Colony characters***

Colony characters of fungus such as colony colour, shape, texture, growth rate and sporulation on PDA medium were studied.

3.4.2.2 ***Morphological characters***

A drop of lactophenol was placed on a glass slide. The growth of the fungus was taken on the slide and observed under low objective microscope and identified based the hypahae, conidiophores and conidial characters. For further

confirmation, fungal cultures were sent to National Centre of Fungal Taxonomy (NCFT), New Delhi.

3.5 PGPR/ANTAGONISTIC ACTIVITIES OF SELECTED ISOLATES

Twenty phosphate solubilizing microorganisms were tested for their PGPR/antagonistic activities such as production of IAA, GA, siderophores, HCN and NH_3 under *in vitro* conditions.

3.5.1 **Production of Indole Acetic Acid (IAA)**

Production of IAA by selected isolates was tested as described by Bric *et al.* (1991). Luria agar supplemented with 0.06 per cent sodium dodecyl sulphate (SDS) and one per cent glycerol was prepared and plated. The surface area of the agar medium was divided into squares of 2 cm × 2 cm by marking on the bottom of each plate.

The overnight culture of each bacterial isolates grown on Luria agar was spotted with sterile tooth pick in each square. The spotted plates were overlaid immediately with sterile disc of Whatman No. 1 filter paper discs. Plates were incubated until the colonies reached the size of 0.5 to 2.0 mm in diameter. After the incubation period, the filter paper discs were removed from the plates and treated with Salkowski's reagent (2 % of 0.5 M $FeCl_3$ in 35 % perchloric acid) by soaking in a petridish containing the reagent. The reaction was allowed to proceed until adequate colour was developed. The filter paper discs were observed for the development of pink colour and such isolates were taken as positive for IAA.

3.5.2 **Production of gibberellic acid (GA)**

Production of GA by selected isolates was tested as described by Brown and Burlingham, 1968. The paper discs after treatment with Salkowski's reagent in IAA production test were dried and viewed under UV light. The spots giving typical green fluorescence were taken as positive for GA production.

3.5.3 **Production of siderophores**

The selected PSM were tested for production of iron chelating siderophores by CAS assay.

First step was the preparation of CAS indicator solution. For this, initially 60.5 mg of Chrome Azurol S was dissolved in 50 ml of distilled water. After that, 10 ml of Fe III solution (27 mg $FeCl_3.6H_2O$ and 83.3 µl concentrated HCl in 100 ml distilled water) was added along with 72.9 mg hexadecyl trimethyl ammonium bromide (HDTMA) dissolved in 40 ml distilled water. The HDTMA solution was added slowly while stirring, resulting in a dark blue solution (100 ml total volume), which was then autoclaved.

Second step involved the preparation of succinate medium. The pH of the solution was adjusted to 7.0 using 6 M NaOH. The solution was then autoclaved.

The third step was the preparation of CAS agar plate. Here, the autoclaved succinate medium was cooled to 50 °C. The CAS indicator solution was added to the succinate medium with constant stirring, which was added carefully and slowly along with the walls of the flask with constant stirring, but at a speed so as not to generate bubbles. Once mixed thoroughly, the resulting solution 100 ml was poured into sterile petriplates, each plate receiving approximately 25 ml of blue agar. The isolates were streaked on this in a zig zag manner and the plates were incubated at room temperature. Siderphore was detected by the presence of an orange halo around the inoculated area.

3.5.4 **Production of hydrogen cyanide**

Production of hydrogen cyanide (HCN) by the selected twenty isolates was tested as described by Kumar *et al.*, 2012.

Nutrient Agar medium amended with glycine (4.4 g/l) was sterilized and poured into the sterile plates. Each isolate was streaked in one petri dish. Sterilized Whatman No.1 filter paper padding soaked with 2 ml sterile picric acid

solution (2.5 g picric acid + 12.5 g Na_2CO_3 in 1000 ml of water) were placed inside the lid of each petri dish and were sealed with parafilm in order to contain gaseous metabolites produced by the antagonists and to allow for chemical reaction with picric acid present in the filter padding. These were incubated for 4 days at 30 $\pm$0.1^{0}C, the colour change of the filter paper was noted and the HCN production potential of the antagonists was assessed as shown below:

a. No change of yellow colour - No HCN production
b. Colour change from yellow to brown - Weak HCN production
c. Colour change from yellow to brownish orange - Moderate HCN production
d. Colour change from yellow to complete orange - Strong HCN production

3.5.5 **Ammonia production**

Selected isolates were tested for the production of ammonia (NH_3). The cultures were inoculated in 10 ml peptone broth and incubated at 30 ± 0.1°C for 48 h in Incubator Shaker. Control without inoculation was also maintained. After incubation 0.5 ml of Nessler's reagent was added to inoculated broth. The development of faint yellow to dark brown color indicated the production of ammonia (Cappuccino and Sherman, 1992). The reaction was scored on a 1-4 scale depending on the colour generation as follows:

a. No color-no ammonia -

b. Pale Orange color-small amount of ammonia +

c. Orange-more ammonia ++

d. Brown precipitate-large amount of ammonia +++

3.5.6 Effect of selected isolates on vigour index of cowpea seedlings

The isolates which produced IAA/ GA were screened for enhancing the germination and growth of cowpea. Seeds of cowpea variety Bhagyalakshmi obtained from Department of Olericulture, College of Horticulture, Vellanikkara was used for seedling vigour index study.

The seeds were treated with bioinoculants at a rate of 500 ml/5 kg seeds for 2h. Bacterial and fungal inoculants were adjusted to 10^{8} cfu/ml and 10^{7} cfu/ml respectively and used for seed treatment. An equal amount of uninoculated media was used for treating seeds in control. Rice water was used as an adhesive material. Culture of *Pseudomonas fluorescens* and commercial culture of phosphate solubilizers of KAU were used as reference cultures. Seeds were sown in paper cups containing sterile sand at the rate of 3 seeds per cup. The cups were watered with equal amount of sterile water and incubated for 10 days. Per cent germination and the shoot and root length of cowpea were assessed 10 days after germination (Bakthavatchalu *et al.*, 2012).

$$\text{Per cent germination} = \left(\frac{\text{No. of seeds germinated}}{\text{No. of seeds sown}} \right) \text{X } 100$$

The seedling vigour index was calculated by using the formula as suggested by Abdul-Baki and Anderson (1973).

$$\text{Seedling vigour index} = \left[\text{Root length (cm)} + \text{Shoot length (cm)}\right] \text{X Per cent germination}$$

3.5.7 Inhibitory effect of selected isolates on soil borne plant pathogens

The antagonistic activity of selected isolates were tested under *in vitro* conditions using dual culture technique (Saktivel and Gnanamanikam, 1987) against four soil borne fungal pathogens, such as *Rhizoctonia solani* causing root and web blight, *Pythium aphanidermatum* causing collar rot, *Fusarium*

oxysporum causing fusarial wilt, *Sclerotium rolfsii* causing collar rot and bacterial pathogen *Ralstonia solanacerum* causing bacterial wilt in cowpea obtained from the Department of Plant Pathology, College of Horticulture, Vellanikkara.

3.5.7.1 ***In vitro evaluation of antagonistic activity of phosphate soubilizing bacteria against fungal plant pathogens by dual culture method***

A mycelial disc of pathogen of 6 mm size was inoculated at a distance of 2 cm away from the periphery of the Petri dish with PDA medium. The bacterial isolate was inoculated 2 cm away from the opposite side of the Petri dish as a line of streak which was perpendicular to the pathogen. Plates with pathogen alone served as control. The Petri dishes were incubated at room temperature ($28\pm2\ ^{0}C$) and observations were recorded till the control plate was fully covered with the pathogen.

The per cent inhibition of the pathogen was calculated by the formula suggested by Vincent (1927).

Per cent inhibition (PI) = (C-T/ C) X 100

Where,

C= Radial growth of fungus in control (cm)

T= Radial growth of fungus in treatment (cm)

3.5.7.2 ***In vitro evaluation of antagonistic activity of phosphate solubilizing fungi against fungal plant pathogens by dual culture method***

A mycelial disc of 6mm diameter was cut from actively growing culture of PSF and placed at a distance of 2 cm away from the periphery of the Petri dish, opposite the disc of fungal pathogen on PDA medium. Observations were recorded till the full growth of the pathogen in control was obtained. The per cent inhibition with pathogen was calculated as mentioned in 3.5.7.1.

3.5.7.3 ***In vitro evaluation of antagonistic activity phosphate solubilizing bacteria against Ralstonia solanacearum***

In vitro antagonistic activity of PSB antagonists against *Ralstonia solanacearum* was tested by point inoculation and cross streaking methods.

3.5.7.3.1 ***Point inoculation method***

In this method, the PSB isolates were point inoculated at four corners of nutrient agar plate, seeded with *R. solanacearum* prepared by spreading 100 µl from a suspension of one colony in 5 ml sterile water. Each treatment was replicated twice. The plates were incubated at room temperature for 48 h, and the observation on inhibition zone was noted. The plate with pathogen alone served as control.

3.5.7.3.2 ***Cross streaking method***

Both the pathogen and the test isolates of PSB were streaked perpendicular to each other simultaneously on plates having nutrient agar. Lysis of the pathogen at the juncture was noted after 48 h of incubation.

3.5.7.4 ***In vitro evaluation of antagonistic activity of phosphate solubilizing fungi against Ralstonia solanacearum***

A mycelial disc of 6 mm diameter was cut from actively growing culture of PSF antagonists and were placed at four corners of nutrient agar plate seeded with pathogen. The plates were incubated at room temperature for 48 h and observed for the inhibition zone. The plate with pathogen alone served as control.

3.6 EVALUATION OF SELECTED ISOLATES UNDER POT CULTURE

Based on P-solubilization and antagonistic activities, three most efficient isolates from the earlier experiments were evaluated for plant growth promotion in pot culture condition. Of the three selected isolates, two were bacteria (PSB-14, PSB-149) and one fungus (PSF-174). The experiment was carried out during

June-August, 2013 at College of Horticulture, Vellanikkara. The isolates were evaluated in presence and absence of applied phosphorus. Two sources of phosphorus were tested viz. Mussoorie rock phosphate and factamphos. The treatment details of the experiment are as follows:

Crop	: Cowpea
Variety	: Bhagyalakshmi
Design	: CRD
Treatments	: 15
Replications	: 3 (8 plants/ replication)
No. of plants / treatment	: 24

Method of application of bioinoculants: Seed and soil application

Treatment details of experiment were as follows:

T_1 : No P + *Providencia* sp. (PSB-14)
T_2 : Rock phosphate + *Providencia* sp. (PSB-14)
T_3 : Factamphos + *Providencia* sp. (PSB-14)
T_4 : No P + *Pseudomonas* sp. (PSB-149)
T_5 : Rock phosphate + *Pseudomonas* sp. (PSB-149)
T_6 : Factamphos + *Pseudomonas* sp. (PSB-149)
T_7 : No P + *Trichoderma* sp. (PSF-174)
T_8 : Rock phosphate + *Trichoderma* sp. (PSF-174)
T_9 : Factamphos + *Trichoderma* sp. (PSF-174)
T_{10} : No P + Reference bioinoculant
T_{11} : Rock phosphate + Reference bioinoculant
T_{12} : Factamphos+ Reference bioinoculant
T_{13} : Control (Rock phosphate +No bioinoculant)
T_{14} : Control (Factamphos + No bioinoculant)

T_{15} : Absolute control (No P+ No bioinoculant)

Bioinoculants *Providencia* sp. (PSB-14), *Pseudomonas* sp. (PSB-149) and *Trichoderma* sp. (PSF-174) were selected native P-solubilizing microorganisms and commercial formulation under the label 'Phosphate solubilizers' of KAU (*Bacillus* sp.) was used as reference bioinoculant.

3.6.1 **Weather data during the cropping period**

The daily weather data (Appendix-III) for the period from June, 2013 to August, 2013 was collected from Department of Agricultural Meteorology, College of Horticulture, Thrissur.

3.6.2 **Preparation of potting mixture**

Sand, soil and farmyard manure in the ratio 1:1:1 were thoroughly mixed and individual pots of size 12"X12" were filled with 5 kg of the potting mixture.

3.6.3 **Seed treatment and sowing**

Cowpea seeds were treated with bioinoculants @ 500 ml/ 5 kg seeds. Rice gruel was used as adhesive material. The seeds were dried under shade for 2 h and used for sowing @ 3 seeds/ pot. The pots were kept in open condition. After the establishment of plants, thinning was carried out, retaining two healthy seedlings per pot.

3.6.4 **Fertilizer application**

Soil test was conducted in order to study the nutrient status of the soil which is given in Table 6. Fertilizers were applied as per soil test based on modified package of practices by KAU @ urea-100.04 mg/pot, Muriate of Potash-39.43 mg/pot, Rock phosphate-308.77 mg/pot and factamphos-239 mg/pot. Lime was applied @ 960 mg/pot two weeks before fertilizer application. Half quantity of nitrogen, full phosphorous and potash were applied as basal dose. The remaining nitrogen was applied 15 days after sowing.

3.6.5 **Soil application of bioinoculants**

Soil application of bioinoculants was carried out at the time of flower initiation. Bacterial inoculants were prepared by inoculating 1 ml of 10^8 cfu/ml culture to 50 ml nutrient broth and fungal culture was prepared by inoculating 1 ml of 10^8 cfu/ ml culture to 50 ml potato dextrose broth. The cultures were incubated for 48 h at 200 rpm. This was diluted with sterile water to get 10^8 cfu/ ml and applied @ 30 ml/ plant at the time of flower initiation. An equal amount of uninoculated media was used for treating plants in control treatments.

Table 6. Results of analysis of soil sample

Parameters	Quantity	Remarks
pH	5.20	Strongly acidic
Electrical Conductivity (ds/m)	0.32	Normal
Organic Carbon (%)	0.82	Medium
Available Phosphorous (kg/ha)	17.00	Medium
Available Potassium (kg/ha)	77.30	Low
Available Calcium (mg/kg)	273.30	Deficient
Available Magnesium (mg/kg)	120.00	Medium
Available Sulphur (mg/kg)	6.50	Sufficient

3.7 OBSERVATIONS

3.7.1 **Biometric observations**

Without disturbing the root system, cowpea plants were pulled out at 20 DAS, 40 DAS and at harvest. Their root and shoot system were separated and subjected for following observations.

3.7.1.1 ***Shoot and root length***

The length of the shoot and root were recorded after separating it and expressed in centimetres.

3.7.1.2 ***Biomass***

Fresh weight of the shoot and root were recorded and expressed in grams per plant.

3.7.1.3 ***Number of nodules***

The number of nodules from each plant was counted and expressed as number of nodules per plant.

3.7.1.4 ***Days to flowering***

Number of days taken from sowing to opening of first flower was recorded and mean was found out.

3.7.1.5 ***Days to fruiting***

Number of days taken from sowing to appearance of pods was recorded.

3.7.1.6 ***Number of pods***

The number of pods from each plant were counted and expressed as number of pods per plant.

3.7.1.7 ***Length of pod***

Length of pods were measured and expressed in centimeters.

3.7.1.8 ***Number of grains***

The number of grains in each pod was counted and expressed as number of grains per pod.

3.7.1.9 ***Yield per plant***

Mean weight of pod was multiplied with the total number of pods and expressed in gram.

3.7.2 **Chemical analysis**

Chemical analyses of soil and plant samples were carried out at the time of sowing, 20DAS, 40 DAS and at harvest.

3.7.2.1 ***Chemical analysis of plant sample***

3.7.2.1.1 ***Collection and preparation of plant samples***

Plants were collected for phosphorous analysis at 20 DAS, 40 DAS and at harvest. Samples from individual treatment were dried in an oven at 60^0C till constant weight was observed and further ground to fine powder using mortar and pestle. The powdered samples were used for nutrient analysis.

3.7.2.1.2 **Phosphorous content in shoot, root and grain**

Phosphorus content in plant sample was estimated by using vanadomolybdate reagent. 0.5 g of powdered plant sample was digested using 8 ml of conc. HNO_3 in microwave digester. Five ml of digested sample was transferred to 25 ml volumetric flask and added 5 ml of vanadomolybdate reagent. Volume was made up with distilled water and the contents were mixed thoroughly. Absorbance of the solution was read after 30 min at 420 nm on

spectrophotometer. The concentration (ppm) of P was found out using standard curve.

The standard curve was prepared by transferring 0, 1, 2, 3, 4 and 5 ml of standard P solution to 25 ml volumetric flask to get 0, 2, 4, 6, 8 and 10 ppm of P respectively. Five ml of vanadomolybdate reagent was added to each flask. Volume was made up and mixed it thoroughly. Absorbance was read at 420 nm after 30 min using spectrophotometer. Absorbance was plotted against concentration on a graph.

$$\text{Per cent P in the sample} = \left(\frac{\text{Concentration from the graph ppm X vol. of digested sample X vol.made}}{\text{Wt. of sample X aliquot taken X}10^6} \right) \text{X } 100$$

3.7.2.2 **Chemical analysis of soil sample**

The soil samples were analysed for available phosphorous at the time of sowing, 20 DAS, 40 DAS and at harvest.

3.7.2.2.1 ***Available phosphorous***

Available 'P' was extracted using Bray No. 1 (Bray and Kurtz, 1945), which consisted of 0.03 N NH_4F and 0.025 N HCl. The combination of HCl and NH_4F was designed to remove easily acid soluble 'P' forms, largely calcium phosphates, and a portion of the aluminium and iron phosphates. The NH_4F dissolves aluminium and iron phosphates by its complex ion formation with these metal ions in acid solution.

5 g of soil was transferred into a 250 ml conical flask and added 50 ml of Bray No.1 reagent and shake for exactly 5 min. Filtering was done though Whatman No. 42 filter paper. To avoid interference of fluoride, 7.5 ml of 0.8 M (10 ml, 4%) boric acid (50 g H_3BO_3 per liter) was added to 5 ml of the extract.

Phosphorus in the extract was estimated by ascorbic acid method (Watanabe and Olsen, 1965).

Estimation was done by reduced molybdate blue colour method. Five ml of the extract was pipette out into a 25 ml volumetric flask and diluted to approximately 20 ml. Four ml of reagent B was added. The volume was made up with distilled water and shakes the contents well. After 10 min the intensity of colour was read at 660 nm. The colour was stable for 24h and the maximum intensity develops with in 10 min. The concentration of P in the sample was computed from the standard curve.

For the preparation of standard curve, different concentrations of P taking 1, 2, 3, 4, 5 and 10 ml of 2 µg/ml P solution was prepared in 25 ml volumetric flasks. Five milli liter of the extracting reagent (Bray No.1) was added and developed colour as described above by adding reagent B. The concentration vs. absorbance curve was plotted on a graph paper.

$$\text{Available P (mg/kg soil)} = \left(\frac{\text{Absorbance for sample}}{\text{Slope of standard curve}} \right) \times \frac{50}{5} \times \frac{25}{5}$$

3.7.3 **Soil microbial analysis**

The enumeration of total P-solubilizers was carried out by serial dilution plate count method at 20 DAS, 40 DAS and harvest.

Ten grams of soil sample was suspended in conical flask containing 90 ml sterile water to obtain 10^{-1} dilution. The flask was shaken for 15 minutes and serial dilutions were made to obtain the final dilution of 10^{-6}. Dilution of 10^{-4} was used for both bacteria and fungi. One ml from the required dilution was poured aseptically into a sterile petri dish to which cooled molten Pikovskaya's agar medium was poured and gently rotated. The inoculated petridishes were incubated at room temperature. The number of colonies with clear zone were

counted and expressed as cfu/g of soil. Different bioinoculants were identified based on colony morphology.

3.8 **Statistical analysis**

Analysis of variance was done on the data collected using the statistical package MSTAT (Freed, 1986). Multiple comparisons among the treatment means were done using DMRT (Duncan's Multiple Range Test).

Results

4. RESULTS

The results of the study on "Exploration of native mineral phosphate solubilizing microorganisms as biofertilizer for the acidic soils of Kerala" undertaken during the period from 2011 to 2013 at the Department of Agricultural Microbiology, College of Horticulture, Vellanikkara, are presented in this chapter.

4.1 SCREENING OF THE REPOSITORY OF Phosphate Solubilizing Microorganisms (PSM)

Two hundred isolates (172 bacteria and 28 fungi) from the repository of PSM available in the Department of Agricultural Microbiology, College of Horticulture were used for *in vitro* evaluation of phosphate solubilizing efficiency on Pikovskaya's medium.

Phosphate solubilization efficiency (PSE) ranged from 106.6% to 555.5% among bacteria and 106.6 % to 291.7 % among fungi (Table 7). Based on per cent PSE, the isolates were categorized into five groups: 100-199, 200-299, 300-399, 400-499 and 500-599 (Table 8). Eighty eight isolates recorded PSE between 100-199 % and eighty eight achieved PSE between 200-299 %. Eighteen isolates recorded PSE between 300-399 % and five isolates recorded PSE between 400-499%. Only one isolate recorded PSE between 500-599. PSE was higher in bacteria but fungi were faster in solubilization of P. Results showed that among bacteria, the isolate PSB-22 and among fungi, PSF-181 recorded maximum phosphate solubilizing efficiency.

The number of days to achieve maximum P-solubilization was also recorded for all isolates. Out of eighty eight isolates that recorded PSE within the range 100-199%, 7 isolates recorded maximum efficiency within two days, 9 isolates took three days, 18 isolates took four days, 20 isolates took five days, 12 isolates took six days and 22 isolates took seven days. Out of eighty eight isolates under the category 200-299 % PSE, one isolate took two days, 2 isolates took three days, 10 isolates took four days, 31 isolates took five days, 18 isolates took six

Table 7. Phosphate solubilizing efficiency of microbial isolates

Sl no.	Bacteria	Solubilization zone at 7th day after inoculation			Days taken to achieve maximum PSE
		Solubilization zone (cm) + Colony diameter (cm)	Colony diameter (cm)	PSE (%)	
1	PSB-1	1.2	0.9	133.3	4
2	PSB-2	1.3	0.8	162.5	6
3	PSB-3	3.0	0.9	333.3	2
4	PSB-4	1.3	0.8	162.5	5
5	PSB-5	1.6	1.0	160.0	7
6	PSB-6	1.5	0.9	166.6	5
7	PSB-7	3.0	1.6	187.5	6
8	PSB-8	2.2	1.0	220.0	5
9	PSB-9	1.6	0.9	177.7	7
10	PSB-10	1.3	0.9	144.4	4
11	PSB-11	2.3	1.0	230.0	6
12	PSB-12	2.4	1.2	200.0	7
13	PSB-13	3.6	0.9	400.0	6
14	PSB-14	3.6	0.8	450.0	7
15	PSB-15	2.6	1.0	260.0	5
16	PSB-16	2.1	1.0	210.0	5
17	PSB-17	2.4	0.9	266.6	6
18	PSB-18	4.9	1.0	490.0	7
19	PSB-19	3.6	1.0	360.0	5
20	PSB-20	1.4	1.0	140.0	4
21	PSB-21	1.4	1.0	140.0	7
22	PSB-22	5.0	0.9	555.5	7
23	PSB-23	2.0	0.9	222.2	6
24	PSB-24	2.7	1.3	207.7	2
25	PSB-25	2.2	0.9	244.4	5
26	PSB-26	2.3	0.8	287.5	6

Table 7 contd. Phosphate solubilizing efficiency of microbial isolates

Sl no.	Bacteria	Solubilization zone at 7th day after inoculation			Days taken to achieve maximum PSE
		Solubilization zone (cm)+Colony diameter (cm)	Colony diameter(cm)	PSE (%)	
27	PSB-27	2.2	1.2	183.3	4
28	PSB-28	3.0	1.1	272.7	5
29	PSB-29	2.7	1.2	225.0	5
30	PSB-30	2.0	1.0	200.0	3
31	PSB-31	2.0	0.9	222.2	5
32	PSB-32	2.0	0.9	222.2	6
33	PSB-33	2.5	0.8	312.5	7
34	PSB-34	1.7	1.0	170.0	4
35	PSB-35	2.4	0.9	266.6	5
36	PSB-36	2.2	1.0	220.0	7
37	PSB-37	1.7	0.9	188.8	2
38	PSB-38	3.0	1.0	300.0	6
39	PSB-39	1.2	0.9	133.3	7
40	PSB-40	3.5	1.4	250.0	5
41	PSB-41	2.4	1.4	171.4	7
42	PSB-42	2.2	0.8	275.0	6
43	PSB-43	3.5	1.0	350.0	4
44	PSB-44	3.0	1.0	300.0	7
45	PSB-45	1.1	0.9	122.2	4
46	PSB-46	1.8	1.1	163.6	5
47	PSB-47	3.0	0.9	333.3	5
48	PSB-48	2.5	1.3	192.3	7
49	PSB-49	2.0	0.9	222.2	3
50	PSB-50	1.1	0.9	122.2	5
51	PSB-51	2.3	1.0	230.0	6
52	PSB-52	1.5	1.0	150.0	4

Table 7 contd. Phosphate solubilizing efficiency of microbial isolates

Sl no.	Bacteria	Solubilization zone at 7th day after inoculation			Days taken to achieve maximum PSE
		Solubilization zone (cm) + Colony diameter(cm)	Colony diameter(cm)	PSE (%)	
53	PSB-53	2.2	0.9	244.4	6
54	PSB-54	1.4	0.9	155.5	5
55	PSB-55	2.7	1.2	225.0	6
56	PSB-56	2.3	1.0	230.0	6
57	PSB-57	2.0	0.9	222.2	5
58	PSB-58	2.7	1.2	225.0	7
59	PSB-59	2.0	1.0	200.0	4
60	PSB-60	2.7	1.2	225.0	7
61	PSB-61	2.0	1.0	200.0	4
62	PSB-62	1.7	0.8	212.5	7
63	PSB-63	2.3	0.9	255.6	6
64	PSB-64	1.9	0.9	211.1	3
65	PSB-65	3.7	1.4	264.3	5
66	PSB-66	2.1	1.1	190.9	7
67	PSB-67	2.0	0.9	222.2	6
68	PSB-68	3.1	0.9	344.4	5
69	PSB-69	2.6	1.5	173.3	7
70	PSB-70	1.4	0.9	155.5	6
71	PSB-71	2.2	1.5	146.6	5
72	PSB-72	3.6	1.0	360.0	4
73	PSB-73	2.8	1.2	233.3	6
74	PSB-74	3.3	1.5	220.0	7
75	PSB-75	2.7	1.2	225.0	5
76	PSB-76	2.1	0.9	233.3	7
77	PSB-77	1.6	1.5	106.6	4
78	PSB-78	2.0	1.0	200.0	6

Table 7 contd. Phosphate solubilizing efficiency of microbial isolates

Sl no.	Bacteria	Solubilization zone 7th day after inoculation			Days taken to achieve maximum PSE
		Solubilization zone (cm)+Colony diameter (cm)	Colony diameter (cm)	PSE (%)	
79	PSB-79	3.0	1.3	230.7	5
80	PSB-80	2.9	0.9	322.2	6
81	PSB-81	2.0	0.9	222.2	5
82	PSB-82	0.9	0.8	112.5	6
83	PSB-83	2.7	1.2	225.0	7
84	PSB-84	1.7	0.7	242.8	4
85	PSB-85	2.7	0.9	300.0	5
86	PSB-86	1.2	0.9	133.3	7
87	PSB-87	1.4	1.1	127.2	5
88	PSB-88	2.0	0.8	250.0	6
89	PSB-89	2.6	1.3	200.0	5
90	PSB-90	2.4	1.5	160.0	4
91	PSB-91	1.9	0.8	237.5	7
92	PSB-92	2.0	1.3	153.8	5
93	PSB-93	3.1	1.1	281.8	6
94	PSB-94	1.6	1.4	114.2	3
95	PSB-95	3.1	1.1	281.8	7
96	PSB-96	1.6	1.0	160.0	7
97	PSB-97	2.0	1.0	200.0	5
98	PSB-98	2.2	1.5	146.6	6
99	PSB-99	2.1	1.0	210.0	7
100	PSB-100	2.9	1.0	290.0	5
101	PSB-101	2.9	1.4	207.1	7
102	PSB-102	3.6	0.9	400.0	4
103	PSB-103	2.9	1.0	290.0	5
104	PSB-104	3.7	1.3	284.6	6

Table 7 contd. Phosphate solubilizing efficiency of microbial isolates

Sl no.	Bacteria	Solubilization zone 7th day after inoculation			Days taken to achieve maximum PSE
		Solubilization zone (cm)+Colony diameter (cm)	Colony diameter (cm)	PSE (%)	
105	PSB-105	2.2	1.0	220.0	5
106	PSB-106	2.8	1.5	186.6	5
107	PSB-107	2.4	1.0	240.0	6
108	PSB-108	2.0	1.3	153.8	7
109	PSB-109	2.5	0.8	312.5	4
110	PSB-110	2.1	1.0	210.0	7
111	PSB-111	2.1	1.4	150.0	5
112	PSB-112	2.7	1.0	270.0	7
113	PSB-113	2.1	1.2	175.0	6
114	PSB-114	3.9	1.0	390.0	3
115	PSB-115	2.8	1.4	200.0	7
116	PSB-116	1.8	1.3	138.4	7
117	PSB-117	2.5	1.2	208.3	7
118	PSB-118	2.5	0.9	277.3	6
119	PSB-119	3.1	1.1	281.8	7
120	PSB-120	1.5	1.1	136.4	4
121	PSB-121	2.7	1.5	180.0	5
122	PSB-122	2.0	1.0	200.0	7
123	PSB-123	2.5	1.6	156.2	5
124	PSB-124	2.0	0.8	250.0	6
125	PSB-125	2.3	1.5	153.3	4
126	PSB-126	3.0	1.3	230.8	5
127	PSB-127	2.9	1.3	223.0	7
128	PSB-128	3.4	1.4	242.9	7
129	PSB-129	3.5	1.5	233.3	5
130	PSB-130	2.1	1.3	161.5	7

Table 7 contd. Phosphate solubilizing efficiency of microbial isolates

Sl no.	Bacteria	Solubilization zone 7th day after inoculation			Days taken to achieve maximum PSE
		Solubilization zone (cm)+Colony diameter (cm)	Colony diameter (cm)	PSE (%)	
131	PSB-131	2.1	1.3	161.5	7
132	PSB-132	2.1	1.3	161.5	5
133	PSB-133	2.7	1.7	158.8	7
134	PSB-134	3.5	0.9	388.8	7
135	PSB-135	2.1	1.4	150.0	5
136	PSB-136	2.9	1.5	193.3	7
137	PSB-137	3.3	1.2	275.0	5
138	PSB-138	3.4	1.5	226.7	7
139	PSB-139	2.9	1.5	193.3	5
140	PSB-140	3.4	1.2	283.3	4
141	PSB-141	3.4	1.3	261.5	7
142	PSB-142	3.0	0.9	333.3	7
143	PSB-143	1.8	1.0	180.0	4
144	PSB-144	1.8	1.0	180.0	5
145	PSB-145	2.7	1.5	180.0	5
146	PSB-146	2.8	1.4	200.0	4
147	PSB-147	2.9	1.0	290.0	7
148	PSB-148	3.4	0.9	377.7	7
149	PSB-149	3.5	0.8	437.5	6
150	PSB-150	2.0	0.9	222.2	5
151	PSB-151	4.0	1.1	363.6	7
152	PSB-152	3.5	0.9	388.8	7
153	PSB-153	2.7	1.2	225.0	7
154	PSB-154	2.8	1.0	280.0	7
155	PSB-155	3.0	1.4	214.3	5
156	PSB-156	3.3	1.3	253.8	7

Table 7 contd. Phosphate solubilizing efficiency of microbial isolates

Sl no.	Bacteria	solubilization zone at 7th day after inoculation			Days taken to achieve maximum PSE
		solubilization zone (cm)+ Colony diameter (cm)	Colony diameter (cm)	PSE (%)	
157	PSB-157	2.9	1.4	207.1	7
158	PSB-158	2.0	1.3	153.8	7
159	PSB-159	3.4	1.5	226.7	7
160	PSB-160	3.2	1.4	228.6	5
161	PSB-161	2.0	1.3	153.8	7
162	PSB-162	2.6	1.5	173.3	7
163	PSB-163	2.3	1.3	176.9	7
164	PSB-164	3.0	1.2	250.0	7
165	PSB-165	1.7	1.5	113.3	5
166	PSB-166	2.1	0.9	233.3	7
167	PSB-167	1.9	1.0	190.0	7
168	PSB-168	3.2	1.3	246.2	5
169	PSB-169	3.0	1.4	214.3	7
170	PSB-170	2.7	1.6	168.8	7
171	PSB-171	2.0	1.3	153.8	5
172	PSB-172	2.0	1.1	181.8	7

Table 7 contd. Phosphate solubilizing efficiency of microbial isolates

Sl no.	Fungi	Solubilization zone at 5th day after inoculation			Days taken to achieve maximum PSE
		Solubilization zone (cm)+colony diameter (cm)	Colony diameter (cm)	PSE (%)	
173	PSF-173	6.2	4.2	147.6	3
174	PSF-174	7.6	4.9	155.1	3
175	PSF-175	6.2	4.2	147.6	3
176	PSF-176	7.9	6.1	129.5	2
177	PSF-177	8.0	7.2	111.1	4
178	PSF-178	7.0	6.2	112.9	2
179	PSF-179	7.0	5.0	140.0	4
180	PSF-180	8.5	4.1	207.3	3
181	PSF-181	7.9	5.6	141.0	4
182	PSF-182	8.3	5.1	162.7	4
183	PSF-183	8.4	5.4	155.5	4
184	PSF-184	7.4	5.6	132.1	3
185	PSF-185	8.5	6.5	130.7	5
186	PSF-186	7.9	6.4	291.7	5
187	PSF-187	7.0	6.5	107.6	5
188	PSF-188	8.0	7.0	114.3	2
189	PSF-189	7.5	6.5	115.3	5
190	PSF-190	9.6	8.0	120.0	3
191	PSF-191	5.5	4.0	137.5	4
192	PSF-192	6.2	4.2	147.6	5
193	PSF-193	8.6	7.6	113.1	2
194	PSF-194	8.0	7.5	106.6	5
195	PSF-195	4.0	3.6	111.1	4
196	PSF-196	6.2	4.2	147.6	3
197	PSF-197	7.5	6.1	122.9	4
198	PSF-198	8.2	4.6	178.2	2
199	PSF-199	8.5	4.9	165.3	2
200	PSF-200	3.6	0.9	400.0	5

days and 26 isolates took seven days to achieve maximum PSE. Out of eighteen those ranked 300-399 % PSE, one isolate achieved maximum PSE in 3 days and that of 3 isolates took five days and 14 isolates took seven days. Among five isolates those fell in 400-499 % PSE category, one isolate took 5 days, two isolates took six days and 2 took seven days to achieve maximum PSE. One isolate which recorded 500-599 % PSE took seven days to achieve maximum PSE.

Table 8. Grouping of phosphate solubilizing microorganisms based on efficiency

P - solubilization efficiency (%)	No. of isolates	No. of isolates that recorded maximum PSE					
		Days of incubation					
		2	3	4	5	6	7
100-199	88	7	9	18	20	12	22
200-299	88	1	2	10	31	18	26
300-399	18	-	1	-	3	-	14
400-499	5	-	-	-	1	2	2
500-599	1	-	-	-	-	-	1
Total	200	8	12	28	55	32	65

4.1.1. **Selection of PSM based on screening**

Based on PSE, twenty most efficient isolates (ten bacteria and ten fungi) namely, PSB-13, PSB-14, PSB-18, PSB-22, PSB-114, PSB-134, PSB-148, PSB-149, PSB-151, PSB-152, PSF-173, PSF-174, PSF-175, PSF-180, PSF-182, PSF-

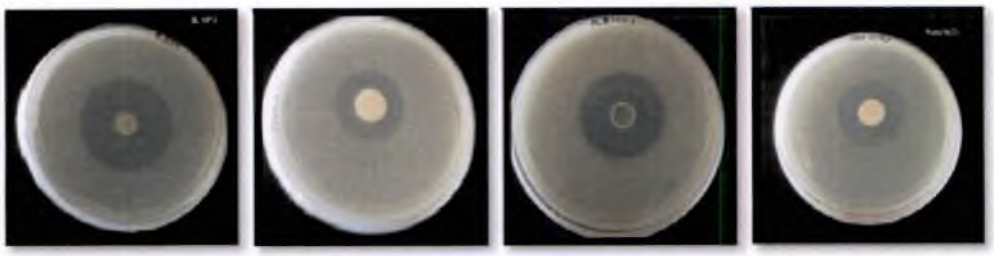
183, PSF-186, PSF-198, PSF-199 and PSF -200 were selected for further studies (Table 9, Plate 1 and Plate 2).

Among the bacterial isolates, PSB-13 and PSB-149 took 6 days to achieve maximum PSE of 400.0 % and 437.5 % respectively. PSB-114 took 3 days to achieve maximum PSE of 390.0 %. All other bacterial isolates such as PSB-14 (450.0 %), PSB-18 (490.0 %), PSB-22 (555.5 %), PSB-134 (388.8 %), PSB-148(377.7 %), PSB-151 (363.6 %) and PSB-152 (388.8 %) took seven days to achieve maximum PSE.

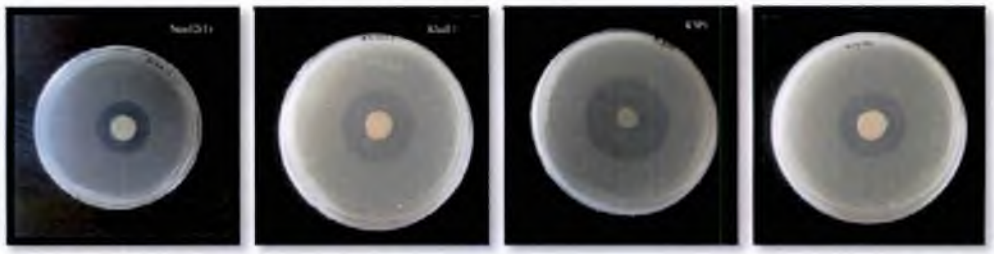
Among fungi, PSF-173, PSF-174, PSF-175 and PSF-180 took 3 days to achieve PSE of 147.6, 155.1, 147.6, 207.3 % respectively. Four days were taken by PSF-182 and PSF-183 to achieve PSE of 162.7 % and 155.5 %. PSF-186 and PSF-200 achieved maximum PSE of 291.7 and 400.0 % respectively in 5 days. PSE of 178.2% and 165.3 % was achieved by PSF-198 and PSF-199 respectively in two days.

4.2. QUANTITATIVE ESTIMATION OF PHOSPHATE SOLUBILIZING EFFICIENCY

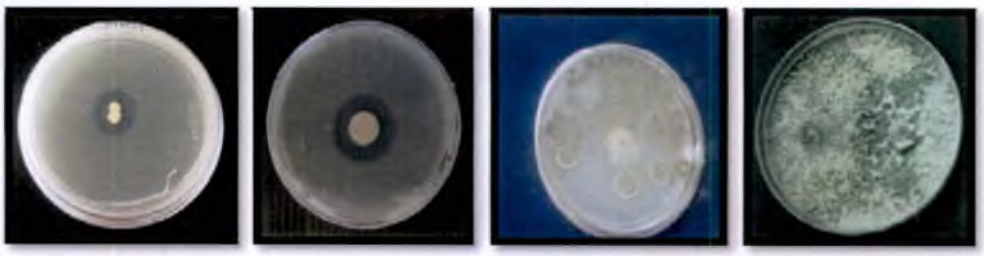
Twenty most efficient isolates selected from qualitative screening were used for quantitative estimation of P-solubilization (Plate 3). Results indicated that P-solubilization in Pikovskaya's broth after 14 days of incubation ranged from 3.5µg/ml to 87µg/ml among bacteria and 18µg/ml to 103.5µg/ml among fungi (Table 9). Among the twenty isolates, PSF-183 solubilized maximum amount of P (103.5 µg/ml) followed by PSF-182 (102.5 µg/ml) and these two were on par with PSF-175 (88.0µg/ml), PSB-149 (87.0µg/ml) and PSB-14 (77.0 µg/ml) and statistically superior to all other isolates. Among the bacterial isolates, PSB-134 was least efficient in P-solubilization and among fungi PSF-199 was the least efficient P solubilizer. Soluble P in all inoculated treatments was higher than uninoculated control.



PSB-13 PSB-14 PSB-18 PSB-22

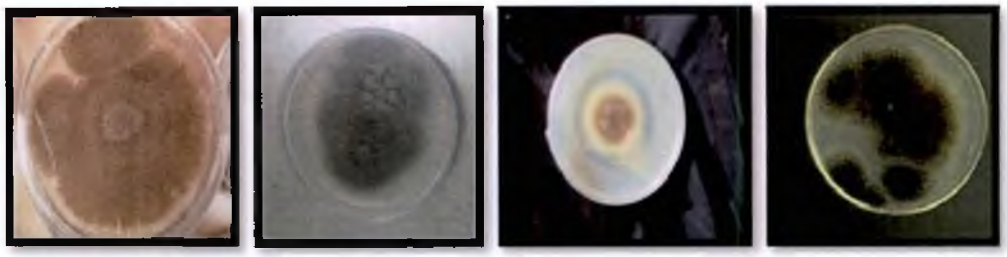


PSB-114 PSB-134 PSB-148 PSB-149

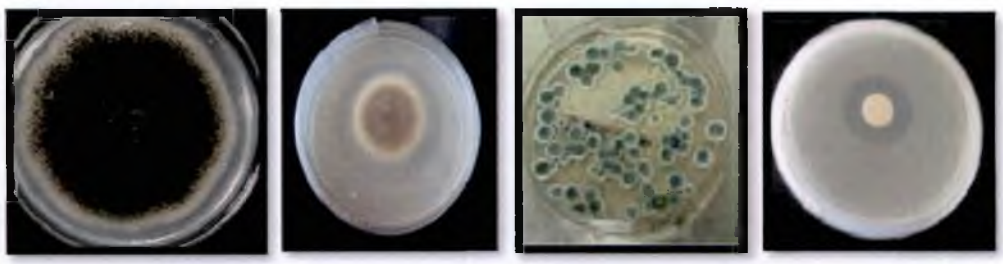


PSB-151 PSB-152 PSF-173 PSF-174

Plate 1: Phosphate solubilizers selected from qualitative screening



PSF-175 PSF-180 PSF-182 PSF-183



PSF-186 PSF-198 PSF-199 PSF-200

Plate 2: Phosphate solubilizers selected from qualitative screening

Control PSF-17 PSF-1: PSF-183 Control PSF-175 PSF-182 PSF-183

Plate 3. Quantitative estimation of phosphate solubilization

Table 9. P-solubilization efficiency of selected isolates of PSM under *in vitro* conditions

Microorganism	Isolate	In solid medium		In broth	pH of the broth after 14 days of incubation (Initial pH of medium-7.2)
		PSE (%)	No. of days required to achieve maximum PSE	Amount of P solubilized at 14 days of incubation (µg/ml)	
Bacteria	PSB-13	400.0	6	63.5^{bcd}	5.4
	PSB-14	450.0	7	77.0^{ab}	5.1
	PSB-18	490.0	7	61.5^{bcd}	5.4
	PSB-22	555.5	7	6.0^{g}	6.5
	PSB-114	390.0	3	6.0^{g}	5.9
	PSB-134	388.8	7	3.5^{g}	5.3
	PSB-148	377.7	7	66.0^{bc}	5.2
	PSB-149	437.5	6	87.0^{ab}	4.6
	PSB-151	363.6	7	22.0^{fg}	5.7
	PSB-152	388.8	7	57.0^{bcdef}	5.5
Fungi	PSF-173	147.6	3	28.5^{defg}	3.7
	PSF-174	155.1	3	24.0^{efg}	3.9
	PSF-175	147.6	3	88.0^{ab}	3.4
	PSF-180	207.3	3	39.5^{cdefg}	3.6
	PSF-182	162.7	4	102.5^{a}	3.3
	PSF-183	155.5	4	103.5^{a}	3.2
	PSF-186	291.7	5	59.5^{bcde}	3.5
	PSF-198	178.2	2	20.5^{fg}	5.2
	PSF-199	165.3	2	18.0^{g}	5.6
	PSF-200	400.0	5	18.5^{g}	5.8

4.2.1 **Drop in pH**

Bacterial and fungal isolates were found to lower the pH of the broth considerably. Fungal isolates were generally found to lower the pH considerably than bacteria and the isolate PSF-183 brought down the pH from 7.2 to 3.2 (Table 9). This was followed by PSF-182 that lowered the pH to 3.3. Among the bacterial isolates, PSB-149 recorded minimum pH of 4.6 after 14 days of incubation. Results indicated that decrease in the pH of the broth was associated with increased amount of available phosphorous in the broth (Fig 1). Among the fungal isolates, PSF-183 solubilized maximum P with a maximum reduction in pH of 3.2. Similarly among bacterial isolates, PSB-149 recorded maximum solubilization of P, which also recorded the lowest pH of 4.6 at the end of incubation.

4.3 IDENTIFICATION AND CHARACTERIZATION OF SELECTED POTENTIAL ISOLATES

4.3.1 **Identification of PSB**

4.3.1.1 ***Colony characters***

The cultural characteristics of ten PSB isolates were studied on nutrient agar medium (Table 10). PSB-13, PSB-22, PSB-114, PSB-134, PSB -149, PSB-151 and PSB-152 produced small sized colonies and the other isolates i.e., PSB-14, PSB-18 and PSB-148 produced medium sized colonies (Plate 4). All the colonies were circular in shape.

PSB-13, PSB-14, PSB-18, PSB-114 and PSB-151 formed white colonies. Translucent type colonies were formed by PSB-148. PSB-149 produced bluish green coloured colonies. Yellow coloured colonies were formed by PSB-134 and PSB- 152. PSB-22 produced creamy white colonies.

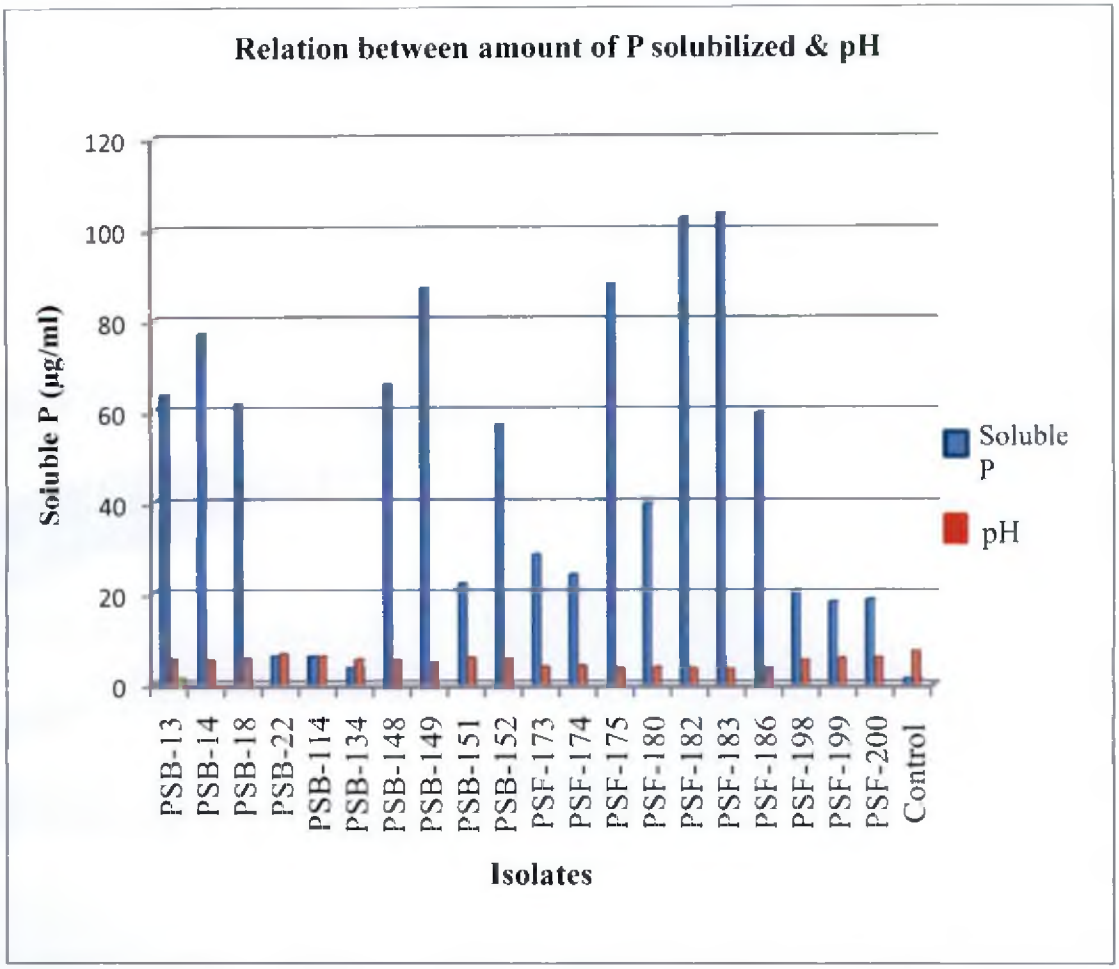
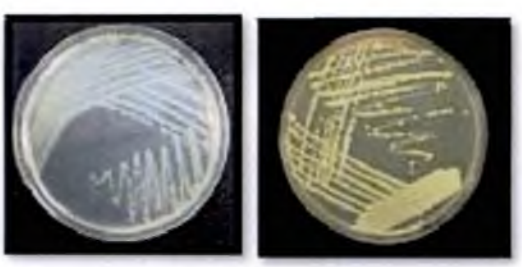


Fig. 1. Relation between amount of P solubilized & pH

PSB-13 PSB-14 PSB-18 PSB-22

PSB-114 PSB-134 PSB-148 PSB-149



PSB-151 PSB-152

Plate 4: Colony characters of selected phosphate solubilizing bacteria

Colonies of isolate PSB-149 showed lobate margins and all the other isolates possessed entire margins. PSB-13, PSB-14, PSB-18, PSB-22, PSB-151 and PSB-152 produced raised colonies and all the other isolates produced flat colonies.

The colony surface of the isolates PSB-148 and PSB-149 exhibited glistening appearance. Rough colonies were produced by PSB-13, PSB-14, PSB-18, PSB-114, PSB-151 and PSB- 152. Other bacterial isolates formed dull colonies.

Mucoid type colonies were formed by PSB-134, PSB-148 and PSB-149. Other isolates were formed dry colonies.

4.3.1.2 **Staining reactions**

4.3.1.2.1 ***Gram staining***

Cells of all isolates except PSB-134 were rod shaped and appeared red after Gram staining reaction indicating Gram negative nature (Table 10 and Plate 5). PSB-134 appeared violet in colour after staining reaction indicating its Gram positive nature. This was further confirmed by solubility in KOH. Gram negative cultures became viscous and gave rise to threads when pulled up with inoculation loop out in presence of KOH.

4.3.1.2.2 ***Endospore staining***

When tested for endospore production, the Gram positive bacterial isolate PSB-134 revealed presence of spores that appeared green in colour, whereas the vegetative cells appeared red (Plate 5).

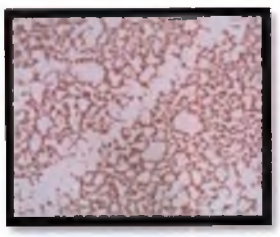
4.3.1.3 **Biochemical reactions**

4.3.1.3.1 ***Production of oxidase***

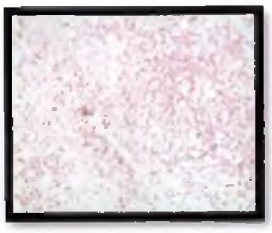
All isolates developed purple colour in Kovac's test, indicating the presence of the enzyme oxidase (Table 11 and Plate 6).

Table 10. Colony morphology and Gram reaction of bacterial isolates

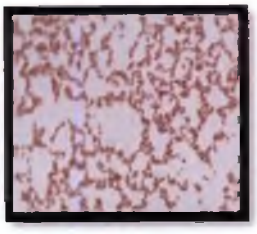
Bacteria	Size	Colour	Shape	Margin	Elevation	Surface	Texture	Gram reaction	Shape of cells
PSB -13	Small	White	Circular	Entire	Raised	Rough	Dry	Negative	Rod
PSB-14	Medium	White	Circular	Entire	Raised	Rough	Dry	Negative	Rod
PSB -18	Medium	White	Circular	Entire	Raised	Rough	Dry	Negative	Rod
PSB-22	Small	Creamy white	Circular	Entire	Raised	Dull	Dry	Negative	Rod
PSB-114	Small	White	Circular	Entire	Flat	Rough	Dry	Negative	Rod
PSB- 134	Small	Yellow	Circular	Entire	Flat	Dull	Mucoid	Positive	Rod
PSB -148	Medium	Translucent	Circular	Entire	Flat	Glistening	Mucoid	Negative	Rod
PSB-149	Small	Bluish green	Circular	Lobate	Flat	Glistening	Mucoid	Negative	Rod
PSB -151	Small	White	Circular	Entire	Raised	Rough	Dry	Negative	Rod
PSB-152	Small	Yellow	Circular	Entire	Raised	Rough	Dry	Negative	Rod



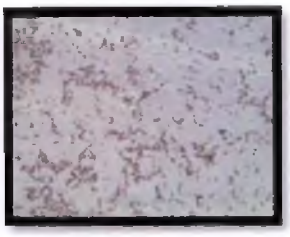
PSB-13



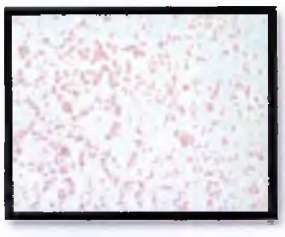
PSB-14



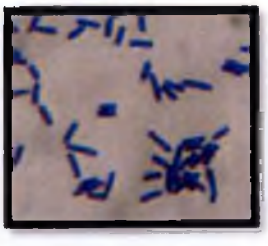
PSB-18



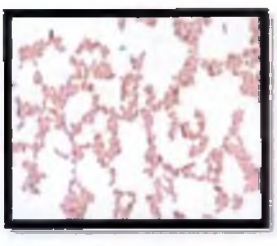
PSB-22



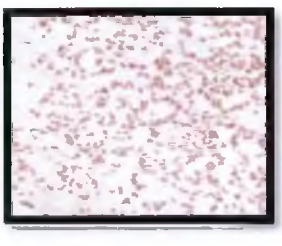
PSB-114



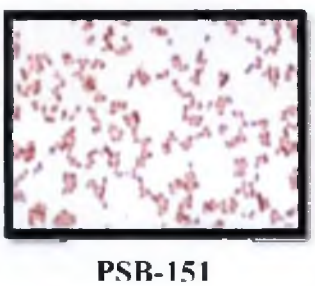
PSB-134



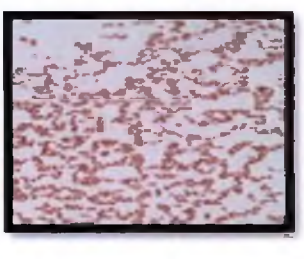
PSB-148



PSB-149



PSB-151



PSB-152

Gram reaction of PSB isolates

Endospore production by PSB-134

Plate 5: Staining reactions of selected PSB isolates

4.3.1.3.2 ***Production of catalase***

All the ten bacterial isolates were observed to be catalase positive (Table 11). Effervescence was observed immediately after adding 2 to 3 drops of hydrogen peroxide (Plate 6).

Table 11. Production of oxidase and catalase enzymes by phosphate solubilizing bacteria

Bacterial isolates	Oxidase	Catalase
PSB -13	+	+
PSB-14	+	+
PSB -18	+	+
PSB-22	+	+
PSB-114	+	+
PSB- 134	+	+
PSB -148	+	+
PSB-149	+	+
PSB -151	+	+
PSB-152	+	+

+ Positive - Negative

4.3.1.3.3 ***Utilization of carbohydrates***

Utilization of carbohydrates by bacterial isolates was indicated by a colour change from red to yellow because of acid production (Plate 6). Glucose and

maltose were the most preferred carbohydrate (Table 12). Five isolates utilized glucose (PSB-13, PSB-14, PSB18, PSB-22 and PSB-152) and five isolates utilized maltose (PSB-22, PSB-134, PSB-149, PSB-151 and PSB-152) Fructose was utilized by four isolates (PSB-13, PSB-18, PSB-22 and PSB-149). Only two isolates were capable of utilizing the sugar alcohol, mannitol (PSB-149 and PSB-152). Dulcitol and lactose were utilized by a single isolate each (PSB-134 and PSB-152 respectively). None of the phosphate solubilizing bacteria were able to utilize sorbitol. Among the different isolates, PSB-152 was found to be highly versatile in carbohydrate utilization, since it was capable of using five different carbohydrates tested (glucose, lactose, maltose and mannitol. PSB-114 and PSB-148 did not utilize any of the carbohydrates tested.

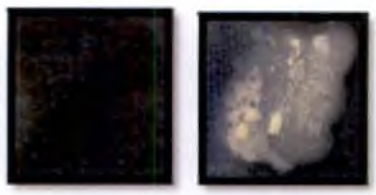
Table 12. Utilization of carbohydrates by PSB

Isolates	Glucose	Lactose	Maltose	Fructose	Mannitol	Dulcitol	Sorbitol
PSB -13	+	-	-	+	-	-	-
PSB-14	+	-	-	-	-	-	-
PSB -18	+	-	-	+	-	-	-
PSB-22	+	-	+	+	-	-	-
PSB-114	-	-	-	-	-	-	-
PSB- 134	-	-	+	-	-	+	-
PSB -148	-	-	-	-	-	-	-
PSB-149	-	-	+	+	+	-	-
PSB -151	-	-	+	-	-	-	-
PSB-152	+	+	+	-	+	-	-

+ Positive - Negative

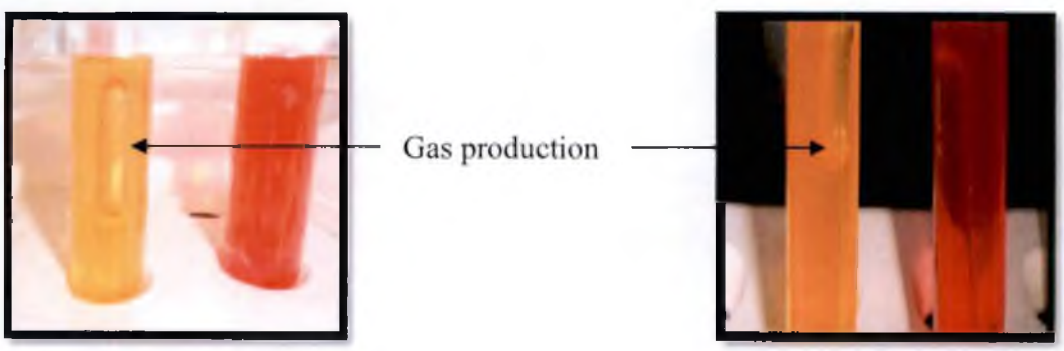
Control **PSB-13**

a. Oxidase reaction by PSB isolate



Control **PSB-13**

b. Catalase reaction by PSB isolate



c Sugar utilization by PSB isolates

Plate 6. Biochemical characterisation of PSB isolates

4.3.1.4 Physiological characterization

4.3.1.4.1 *Tolerance to low pH*

Tolerance of phosphate solubilizing bacteria to low pH was studied by finding out the per cent change in population over neutral pH, which was calculated using standard formula (Table 13). The isolates were ranked on the basis of the sum of per cent change in population at pH 4.0, 5.0 and 6.0 as measured by OD at 530 nm. The minimum value for sum of per cent change in population was recorded by the isolate PSB-14 (28.50), and this isolate was ranked first in tolerance to acidity. This was followed by PSB-22 and PSB-13, which recorded a sum of 52.15 and 53.09 respectively and these were ranked at second and third places. Minimum tolerance to acidity was exhibited by PSB-18, with a sum of per cent change in population 108.36.

Table 13. Tolerance of bacterial isolates to low pH levels

Bacteria	Per cent change in population over neutral pH				Ranking based on acid-tolerance
	pH 4.0	pH 5.0	pH 6.0	Sum of per cent change	
PSB-13	26.79	22.48	3.82	53.09	3
PSB-14	17.02	9.78	1.70	28.50	1
PSB -18	64.85	22.59	20.92	108.36	10
PSB-22	25.21	14.34	12.60	52.15	2
PSB-114	45.90	31.67	28.11	105.68	8
PSB- 134	74.34	27.82	6.08	108.24	9
PSB -148	21.34	20.94	15.01	57.29	4
PSB-149	49.54	14.22	2.29	66.05	6
PSB -151	75.21	23.93	4.70	103.84	7
PSB-152	39.02	15.04	10.16	64.22	5

4.3.1.5 **Molecular characterization**

16S rDNA sequencing of ten bacterial isolates was carried out to identify them at the molecular level. The NCBI accession having maximum score was considered for identifying the bacterial isolates. Details of five blast hits for each bacterial isolate are provided in Table 14 and Table 15.

PSB-13 showed 97 per cent homology with *Acinetobacter calcoaceticus* and PSB-14 recorded 97 per cent homology with *Providencia alcalifaciens.* PSB-18 and PSB-151 recorded maximum homology with *Achromobacter* sp. PSB-22 showed 97 per cent homology with *Providencia alcalifaciens*. PSB-PSB-114 and PSB-149 recorded maximum homology with *Pseudomonas* sp. showed 99 per cent homology with *Stenotrophomonas maltophilia.* PSB-134 showed 97 per cent homology with *Bacillus cereus*. PSB-148 showed 77 % homology with *Ochrobactrum* sp. PSB-151 shared 93 per cent homology with *Achromobacter* sp. and PSB-152 showed 99 per cent homology with *Burkholderia* sp (Plate 7 to Plate 16).

Table 14. Bacterial isolates identified by 16S rDNA sequencing

Bacteria	Organisms
PSB -13	*Acinetobacter calcoaceticus*
PSB-14	*Providencia alcalifaciens*
PSB -18	*Achromobacter* sp.
PSB-22	*Providencia alcalifaciens*
PSB-114	*Pseudomonas* sp.
PSB- 134	*Bacillus cereus*
PSB -148	*Ochrobactrum* sp.
PSB-149	*Pseudomonas* sp.
PSB -151	*Achromobacter* sp.
PSB-152	*Burkholderia* sp.

Table15. Details of sequence analysis of phosphate solubilizing bacteria

Isolate	NCBI accessions showing maximum homology		Maximum score	Query coverage %	Identity %	e-value
	Accession no.	Name				
PSB-13	JF681294	*Acinetobacter calcoaceticus*	2346	96	97	0.0
	KJ920201	*Acinetobacter* sp.	2340	96	96	0.0
	KF887017	*Acinetobacter* sp.	2430	96	96	0.0
	KC257019	*Acinetobacter* sp.	2430	96	96	0.0
	KC541050	Uncultured bacterium	2430	96	96	0.0
PSB-14	JQ624323	Uncultured *Providencia* sp.	2097	98	97	0.0
	HQ407255	*Providencia alcalifaciens*	2091	98	97	0.0
	HQ407254	*Providencia alcalifaciens*	2091	98	97	0.0
	HQ407253	*Providencia alcalifaciens*	2091	98	97	0.0
	JQ799026	Uncultured *Providencia* sp.	2087	98	97	0.0
PSB-18	KC853552	Bacterium enriched culture	272	27	94	5e-69
	EF655647	Uncultured bacteria	270	23	91	2e-68
	JN043371	*Achromobacter* sp.	268	21	93	6e-68
	KJ716673	*Achromobacter* sp.	267	20	94	2e-67
	KJ716671	*Achromobacter* sp.	267	20	94	2e-67

Table15 contd. Details of sequence analysis of phosphate solubilizing bacteria

Isolate	NCBI accessions showing maximum homology		Maximum score	Query coverage %	Identity %	e-value
	Accession no.	Name				
PSB-22	HQ407246	*Providencia alcalifaciens*	1945	98	98	0.0
	JQ624323	Uncultured *Providencia*	1940	98	97	0.0
	JN236237	Uncultured bacterium	1940	98	97	0.0
	GU166183	Uncultured bacterium	1940	98	97	0.0
	GU166182	Uncultured bacterium	1940	98	97	0.0
PSB-114	KM108530	*Pseudomonas* sp.	1208	96	99	0.0
	KF135436	*Stenotrophomonas* sp.	1208	96	99	0.0
	JX548232	*Pseudomonas* sp.	1208	96	99	0.0
	NR040804	*Stenotrophomona maltophilia*	1208	96	99	0.0
	DQ359944	*Stenotrophomonas maltophilia*	1208	96	99	0.0
PSB-134	JF758862	*Bacillus cereus*	1838	87	97	0.0
	JX544748	*Bacillus cereus*	1832	87	97	0.0
	KC428750	*Bacillus cereus*	1831	87	97	0.0
	FJ863099	Uncultured *Bacillus*	1831	90	97	0.0
	FJ627946	*Bacillus* sp.	1831	87	97	0.0

Table15 contd. Details of sequence analysis of phosphate solubilizing bacteria

Isolate	NCBI accessions showing maximum homology		Maximum score	Query coverage %	Identity %	e-value
	Accession no.	Name				
PSB-148	JX910140	*Ochrobactrum* sp.	339	53	77	6e-89
	JQ743667	*Ochrobactrum intermedium*	339	53	77	6e-89
	JN416564	*Achromobacter* sp.	339	53	77	6e-89
	GQ417012	Uncultured *Ochrobactrum*	339	53	77	6e-89
	EU281633	*Bacillus* sp.	339	53	77	6e-89
PSB-149	FM995952	Uncultured bacterium	1062	97	99	0.0
	HQ232955	*Pseudomonas* sp.	1062	97	99	0.0
	HG531868	Uncultured bacterium	1059	96	99	0.0
	KC962412	*Pseudomonas* sp.	1059	96	99	0.0
	KC139461	*Pseudomona* sp.	1059	96	99	0.0
PSB-151	KC853552	Bacterium enriched culture	272	21	94	5e-69
	EF655647	Uncultured bacterium	270	23	91	2e-68
	JN043371	*Achromobacter* sp.	268	21	93	6e-68
	KJ716673	*Achromobacter* sp.	267	20	94	2e-67
	KJ716671	*Achromobacter* sp.	267	20	94	2e-67

Table15 contd. Details of sequence analysis of phosphate solubilizing bacteria

Isolate	NCBI accessions showing maximum homology		Maximum score	Query coverage %	Identity %	e-value
	Accession no.	Name				
PSB-152	KM054695	*Burkholderia* sp.	1125	97	99	0.0
	KM054694	*Burkholderia* sp.	1125	97	99	0.0
	AB975358	*Burkholderia cepacia*	1125	97	99	0.0
	HG938371	*Burkholderia cenocepacia*	1125	97	99	0.0
	HG938372	*Burkholderia cenocepacia*	1125	97	99	0.0

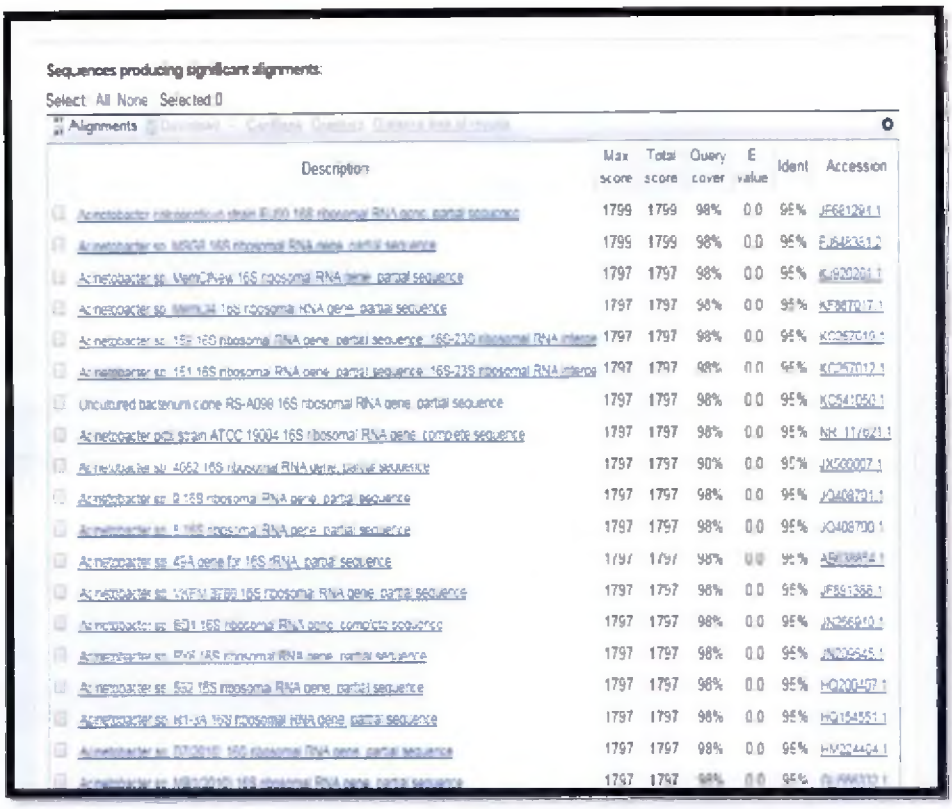
4.3.2. **Identification of fungal isolates**

Ten fungal isolates were identified based on cultural and morphological characters.

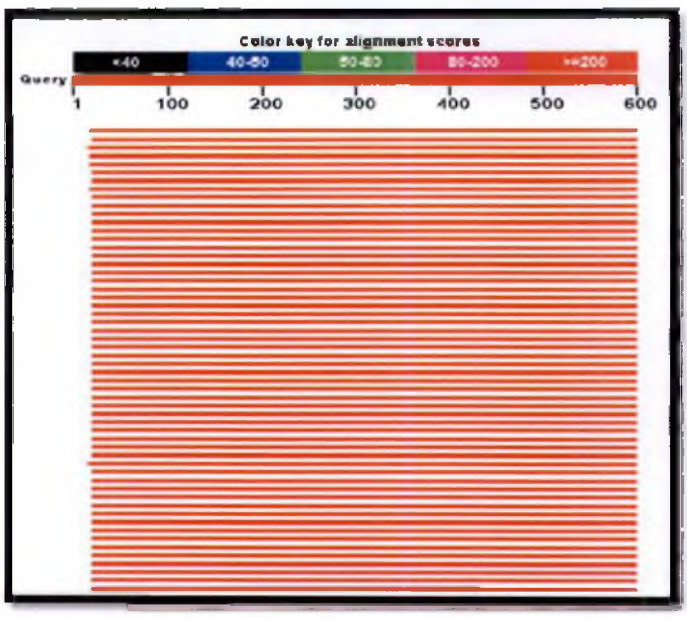
4.3.2.1 *Colony characters*

The cultural characters of phosphate solubilizing fungi were studied on potato dextrose agar (Table 16). The isolates PSF-173 and PSF-199 were fast growing and colonies appeared green in colour. Growth consisted of a dense felt of conidiophores. PSF-174 was fast growing; colonies were initially white and downy, later turning yellowish- green to deep green compact tuffs.

PSF-175, PSF-180, PSF-182, PSF-183 and PSF-186 were fast growers. These isolates produced yellow basal felt and dark brown large spores with high sporulation. PSF-198 was fast growing and produced red-brown basal felt and

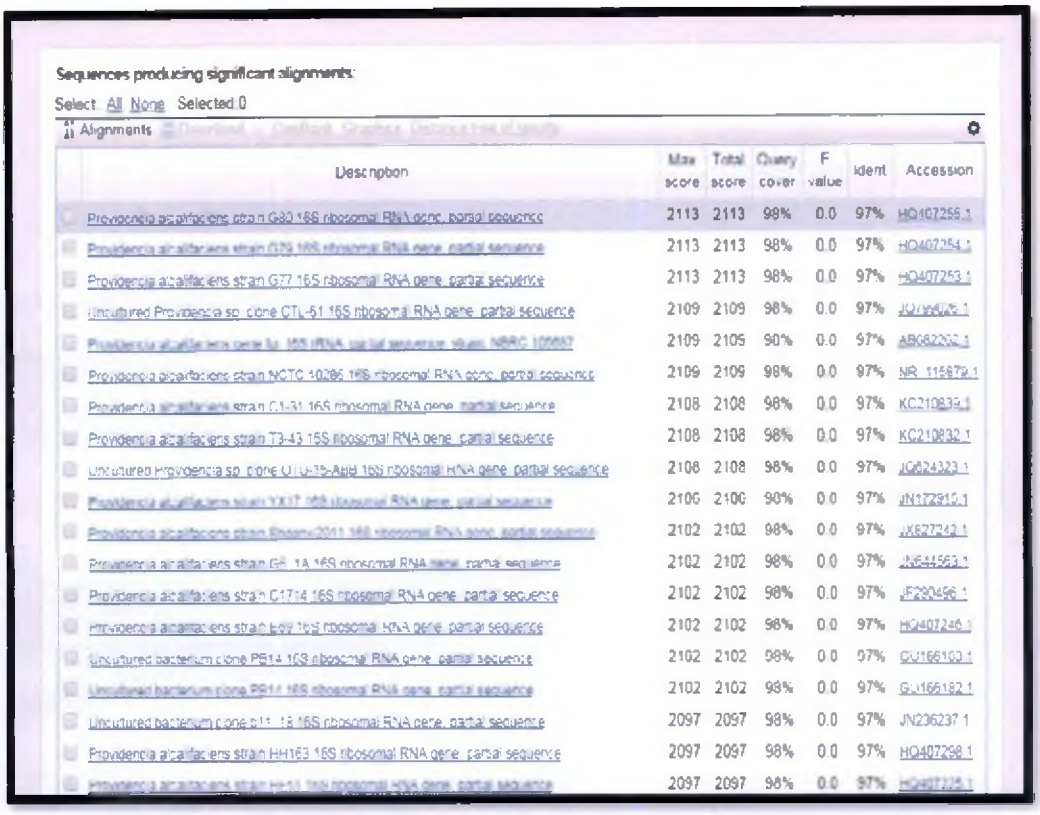


A. Sequences showing homology



B. Blastn output

Plate 7. Sequence analysis of PSB-13



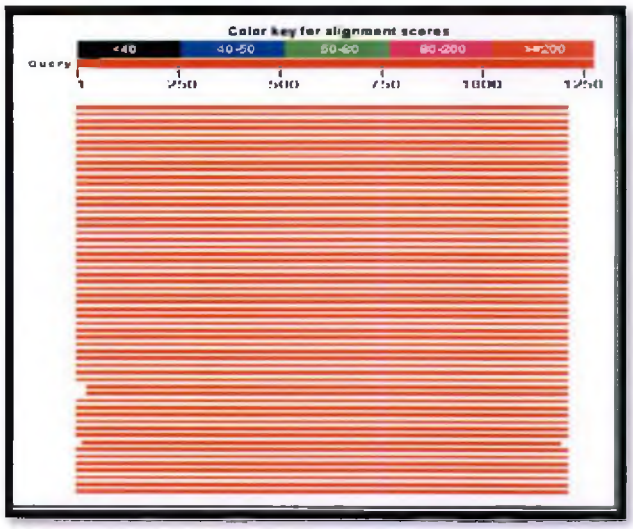
Sequences producing significant alignments:

Select All None Selected 0

Alignments

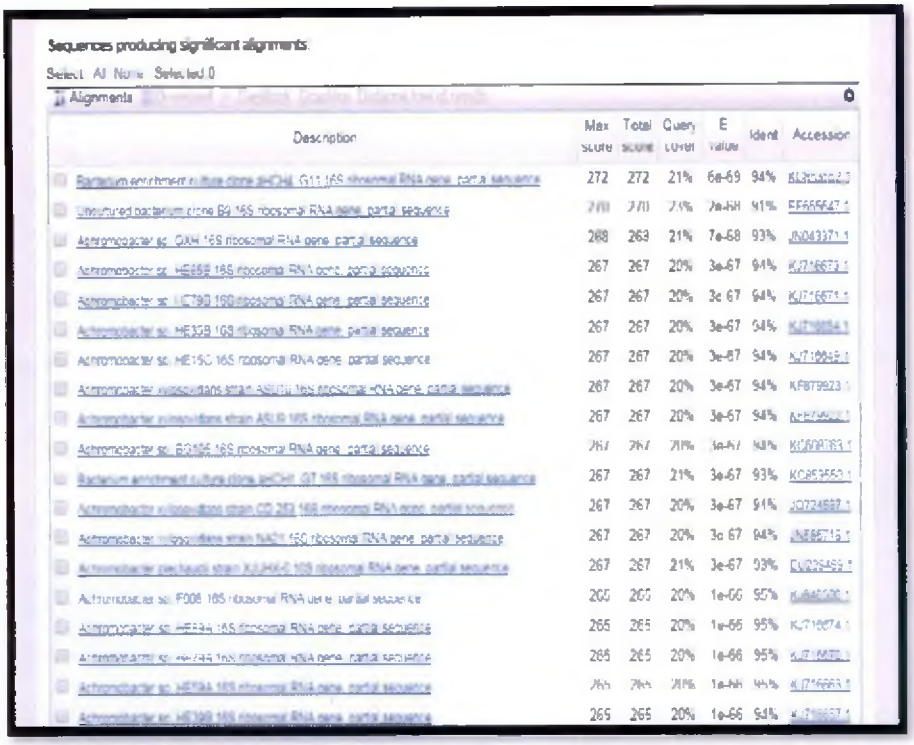
Description	Max score	Total score	Query cover	E value	Ident	Accession
Providencia alcalifaciens strain G80 16S ribosomal RNA, partial sequence	2113	2113	98%	0.0	97%	HQ407255.1
Providencia alcalifaciens strain G79 16S ribosomal RNA gene, partial sequence	2113	2113	98%	0.0	97%	HQ407254.1
Providencia alcalifaciens strain G77 16S ribosomal RNA gene, partial sequence	2113	2113	98%	0.0	97%	HQ407253.1
Uncultured Providencia sp. clone CTL-51 16S ribosomal RNA gene, partial sequence	2109	2109	98%	0.0	97%	JQ776045.1
Providencia alcalifaciens 16S rRNA, partial sequence, strain: NRRC 100667	2109	2105	90%	0.0	97%	AB682202.1
Providencia alcalifaciens strain NCTC 10286 16S ribosomal RNA, partial sequence	2109	2109	98%	0.0	97%	NR_115672.1
Providencia alcalifaciens strain C1-31 16S ribosomal RNA gene, partial sequence	2108	2108	98%	0.0	97%	KC210833.1
Providencia alcalifaciens strain T3-43 16S ribosomal RNA gene, partial sequence	2108	2108	98%	0.0	97%	KC210832.1
Uncultured Providencia sp. clone QHJ-35-A6H 16S ribosomal RNA gene, partial sequence	2108	2108	98%	0.0	97%	JQ624323.1
Providencia alcalifaciens strain YX17 16S ribosomal RNA gene, partial sequence	2106	2106	98%	0.0	97%	JN372915.1
Providencia alcalifaciens strain Rhizo2011 16S ribosomal RNA gene, partial sequence	2102	2102	98%	0.0	97%	JX627242.1
Providencia alcalifaciens strain GR_1A 16S ribosomal RNA gene, partial sequence	2102	2102	98%	0.0	97%	JX644563.1
Providencia alcalifaciens strain C1714 16S ribosomal RNA gene, partial sequence	2102	2102	98%	0.0	97%	JF290456.1
Providencia alcalifaciens strain key 16S ribosomal RNA gene, partial sequence	2102	2102	98%	0.0	97%	HQ407246.1
Uncultured bacterium clone PS14 16S ribosomal RNA gene, partial sequence	2102	2102	98%	0.0	97%	GU166163.1
Uncultured bacterium clone PS11 16S ribosomal RNA gene, partial sequence	2102	2102	98%	0.0	97%	GU166162.1
Uncultured bacterium clone p11_18 16S ribosomal RNA gene, partial sequence	2097	2097	98%	0.0	97%	JN236237.1
Providencia alcalifaciens strain HH163 16S ribosomal RNA gene, partial sequence	2097	2097	98%	0.0	97%	HQ407298.1
Providencia alcalifaciens strain HH31 16S ribosomal RNA gene, partial sequence	2097	2097	98%	0.0	97%	HQ407275.1

A. Sequences showing homology

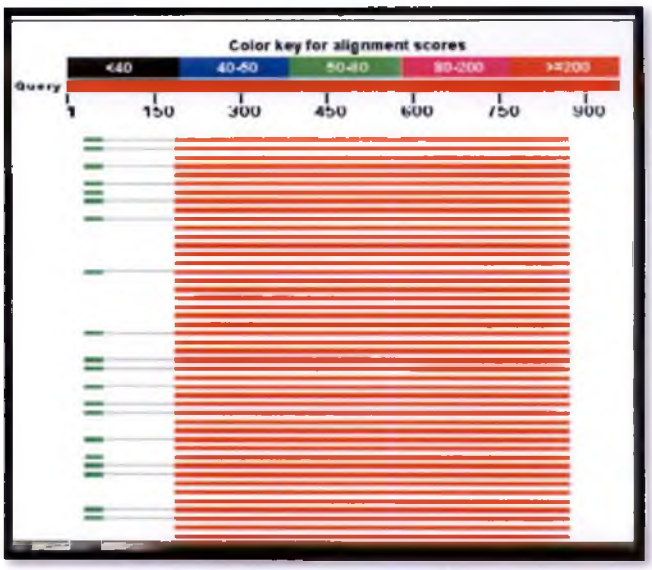


B. Blastn output

Plate 8. Sequence analysis of PSB-14

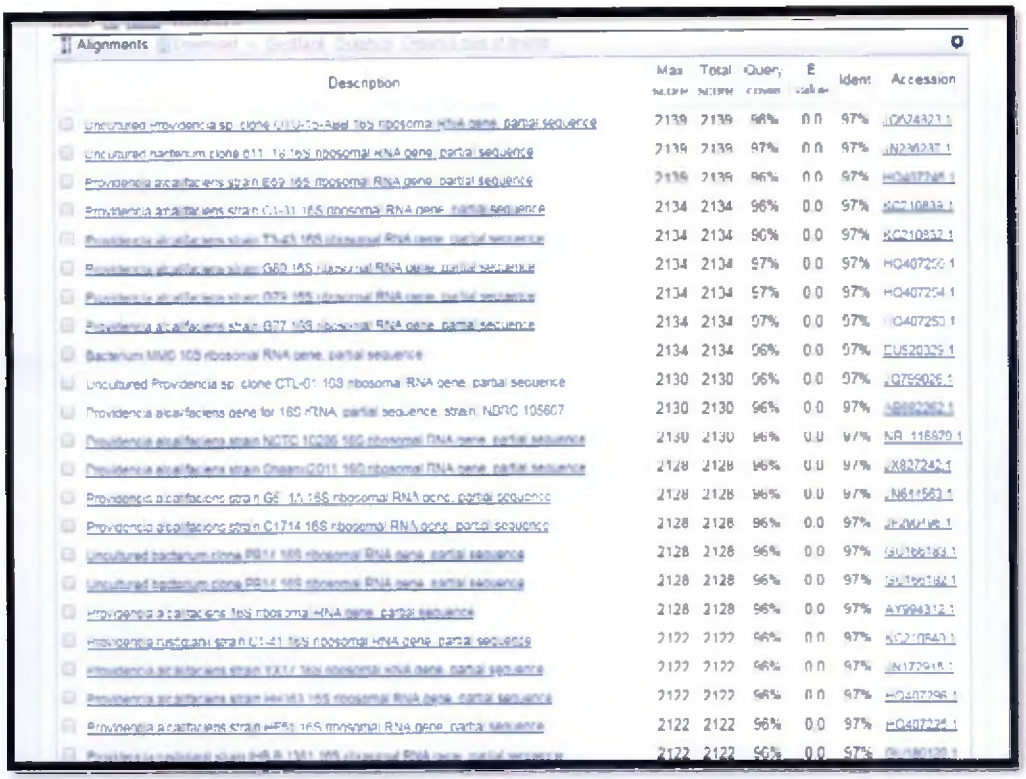


A. Sequences showing homology

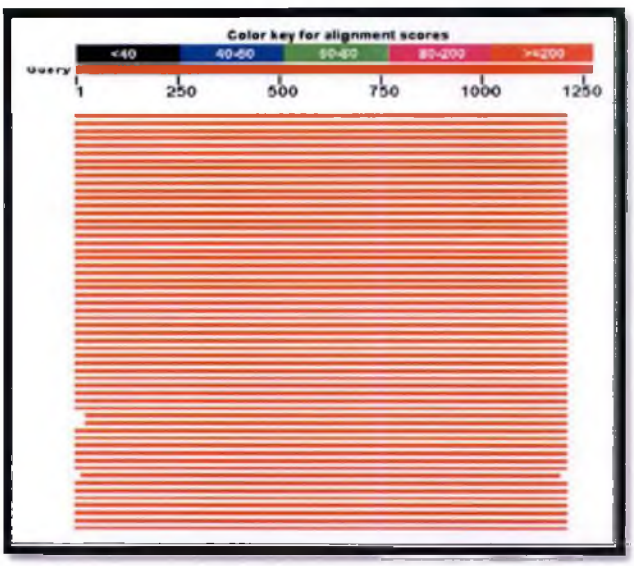


B. Blastn output

Plate 9. Sequence analysis of PSB-18

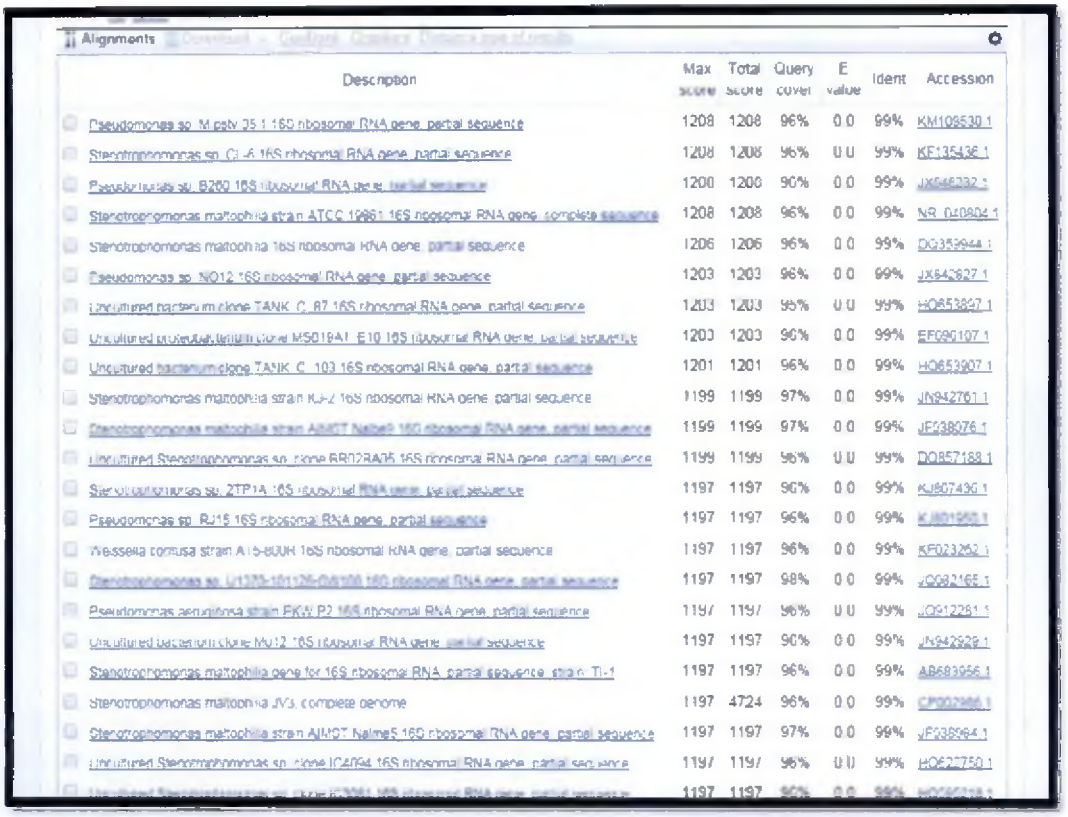


A. Sequences showing homology



B. Blastn output

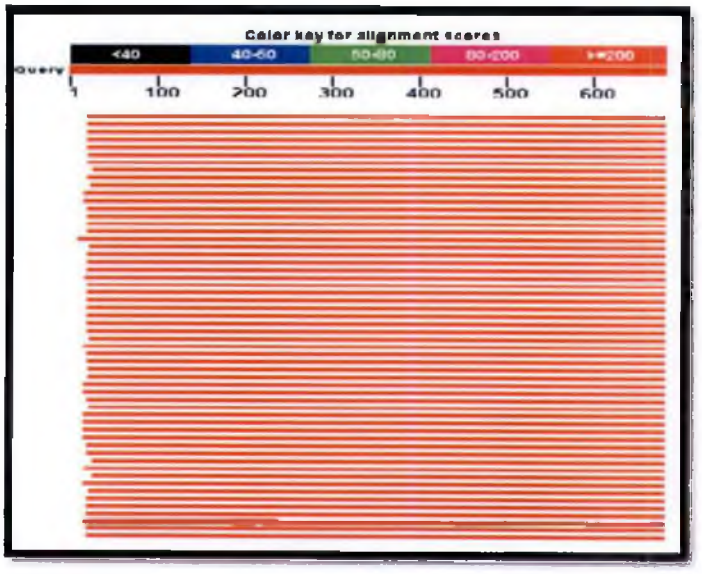
Plate 10. Sequence analysis of PSB-22



Alignments

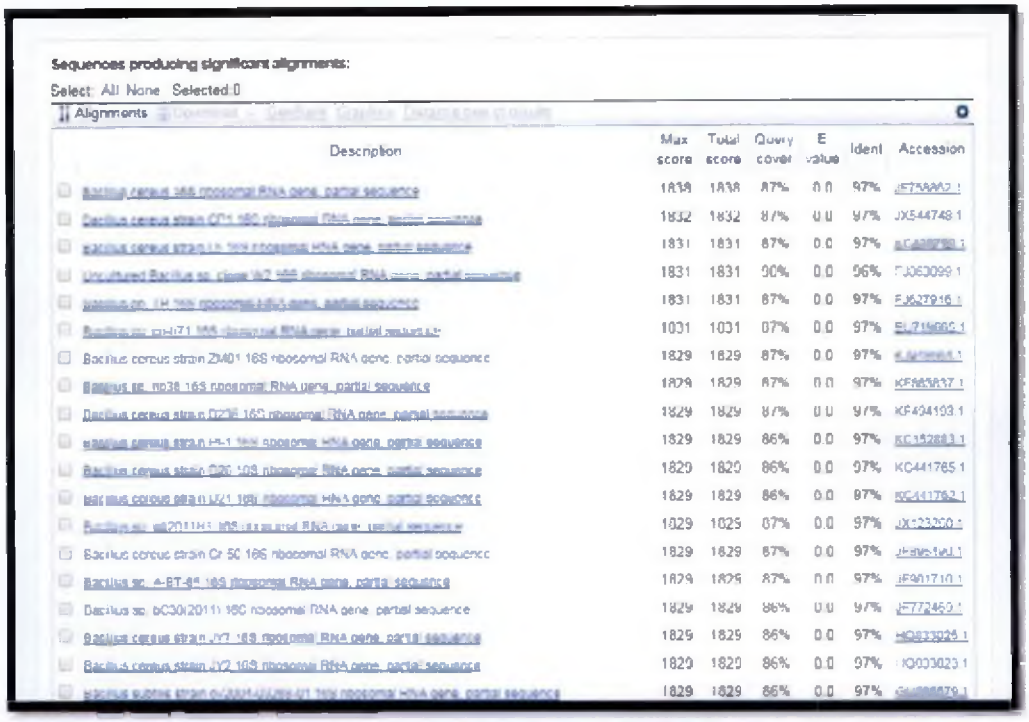
Description	Max score	Total score	Query cover	E value	Ident	Accession
Pseudomonas sp. M pstv 35.1 16S ribosomal RNA gene, partial sequence	1208	1208	96%	0.0	99%	KM109530.1
Stenotrophomonas sp. CJ-6 16S ribosomal RNA gene, partial sequence	1208	1208	96%	0.0	99%	KF135436.1
Pseudomonas sp. B260 16S ribosomal RNA gene, partial sequence	1208	1208	96%	0.0	99%	JX646332.1
Stenotrophomonas maltophilia strain ATCC 13637 16S ribosomal RNA gene, complete sequence	1208	1208	96%	0.0	99%	NR_040804.1
Stenotrophomonas maltophilia 16S ribosomal RNA gene, partial sequence	1206	1206	96%	0.0	99%	DQ359944.1
Pseudomonas sp. NO12 16S ribosomal RNA gene, partial sequence	1203	1203	96%	0.0	99%	JX642627.1
Uncultured bacterium clone TANK_C_87 16S ribosomal RNA gene, partial sequence	1203	1203	95%	0.0	99%	HQ653837.1
Uncultured [illegible] clone MS01BA1_E10 16S ribosomal RNA gene, partial sequence	1203	1203	96%	0.0	99%	EF090107.1
Uncultured bacterium clone TANK_C_103 16S ribosomal RNA gene, partial sequence	1201	1201	96%	0.0	99%	HQ653907.1
Stenotrophomonas maltophilia strain KJ-2 16S ribosomal RNA gene, partial sequence	1199	1199	97%	0.0	99%	JN942761.1
Stenotrophomonas maltophilia strain AIMST Nalme3 16S ribosomal RNA gene, partial sequence	1199	1199	97%	0.0	99%	JF938076.1
Uncultured Stenotrophomonas sp. clone BR07BA35 16S ribosomal RNA gene, partial sequence	1199	1199	96%	0.0	99%	DQ857188.1
Stenotrophomonas sp. 2TP1A 16S ribosomal RNA gene, partial sequence	1197	1197	96%	0.0	99%	KJ807430.1
Pseudomonas sp. RJ15 16S ribosomal RNA gene, partial sequence	1197	1197	96%	0.0	99%	KJ801565.1
Weissella cornusa strain A15-6UUH 16S ribosomal RNA gene, partial sequence	1197	1197	96%	0.0	99%	KF023252.1
Stenotrophomonas sp. U1375-101126-0A108 16S ribosomal RNA gene, partial sequence	1197	1197	98%	0.0	99%	JQ082165.1
Pseudomonas aeruginosa strain PKW P2 16S ribosomal RNA gene, partial sequence	1197	1197	96%	0.0	99%	JQ912281.1
Uncultured bacterium clone MU12 16S ribosomal RNA gene, partial sequence	1197	1197	96%	0.0	99%	JN942929.1
Stenotrophomonas maltophilia gene for 16S ribosomal RNA, partial sequence, strain: TI-1	1197	1197	96%	0.0	99%	AB683956.1
Stenotrophomonas maltophilia JV3, complete genome	1197	4724	96%	0.0	99%	CP002986.1
Stenotrophomonas maltophilia strain AIMST Nalme5 16S ribosomal RNA gene, partial sequence	1197	1197	97%	0.0	99%	JF938064.1
Uncultured Stenotrophomonas sp. clone IC4764 16S ribosomal RNA gene, partial sequence	1197	1197	96%	0.0	99%	HQ622750.1
[illegible]	1197	1197	96%	0.0	99%	[illegible]

A. Sequences showing homology

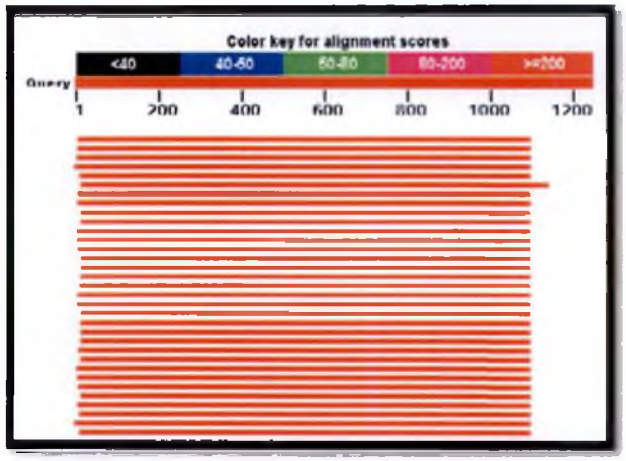


B. Blastn output

Plate 11. Sequence analysis of PSB-114

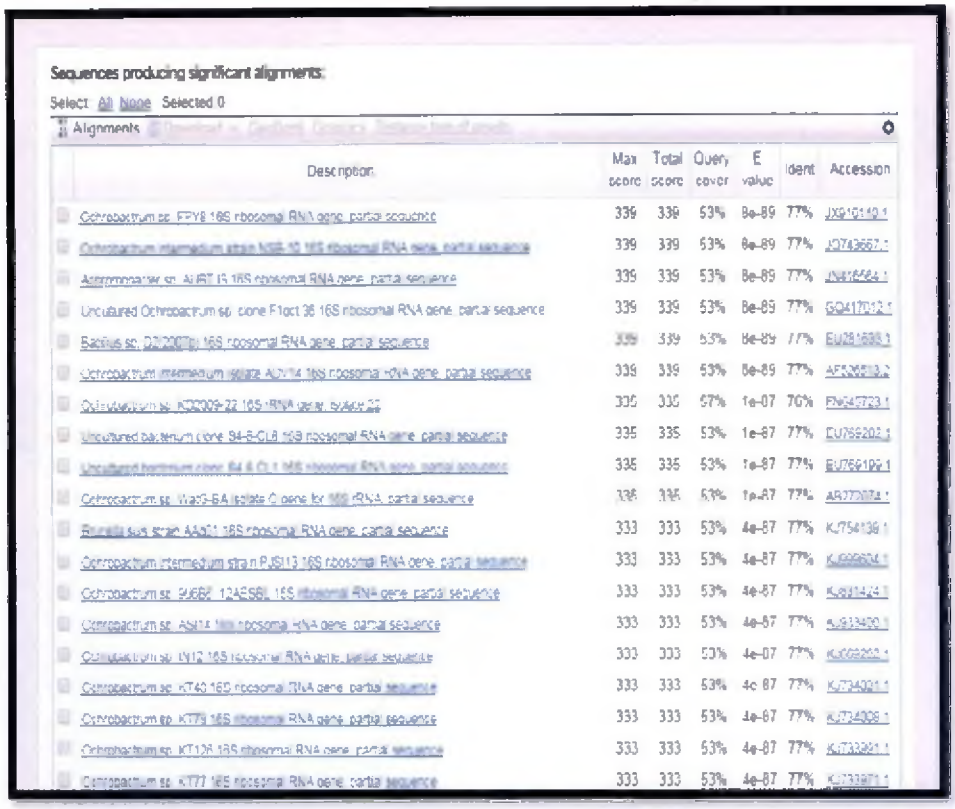


A. Sequences showing homology

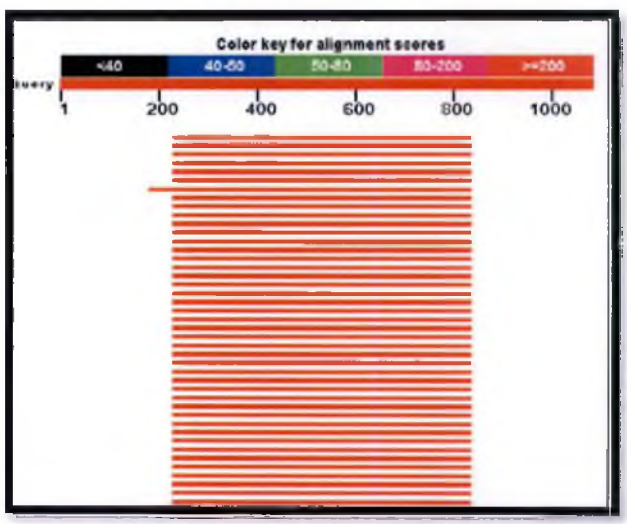


B. Blastn output

Plate 12. Sequence analysis of PSB-134

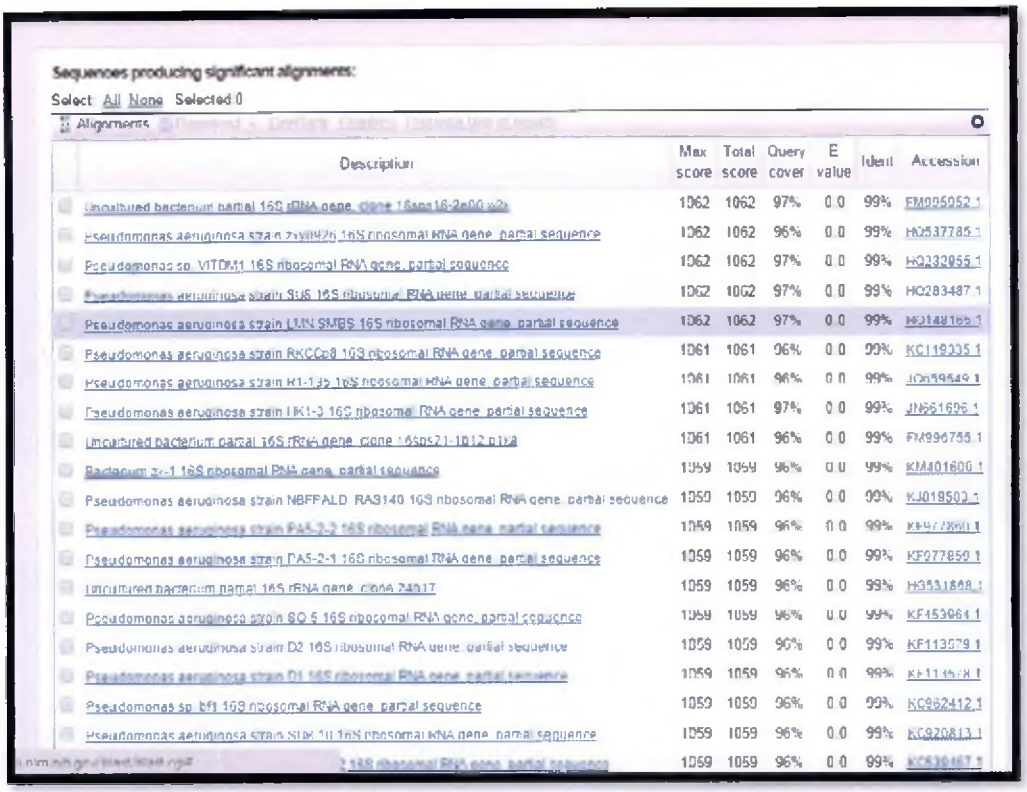


A. Sequences showing homology



A. Blastn output

Plate 13. Sequence analysis of PSB-148



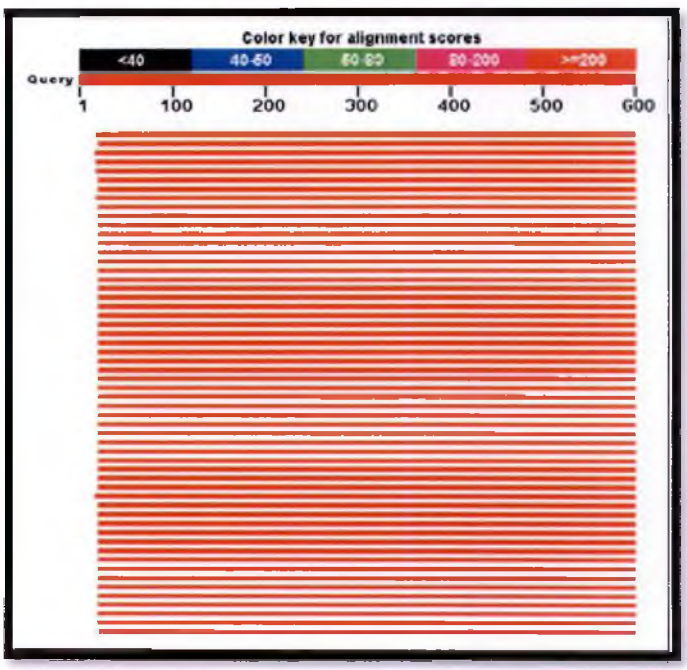
Sequences producing significant alignments:

Select All None Selected 0

Alignments

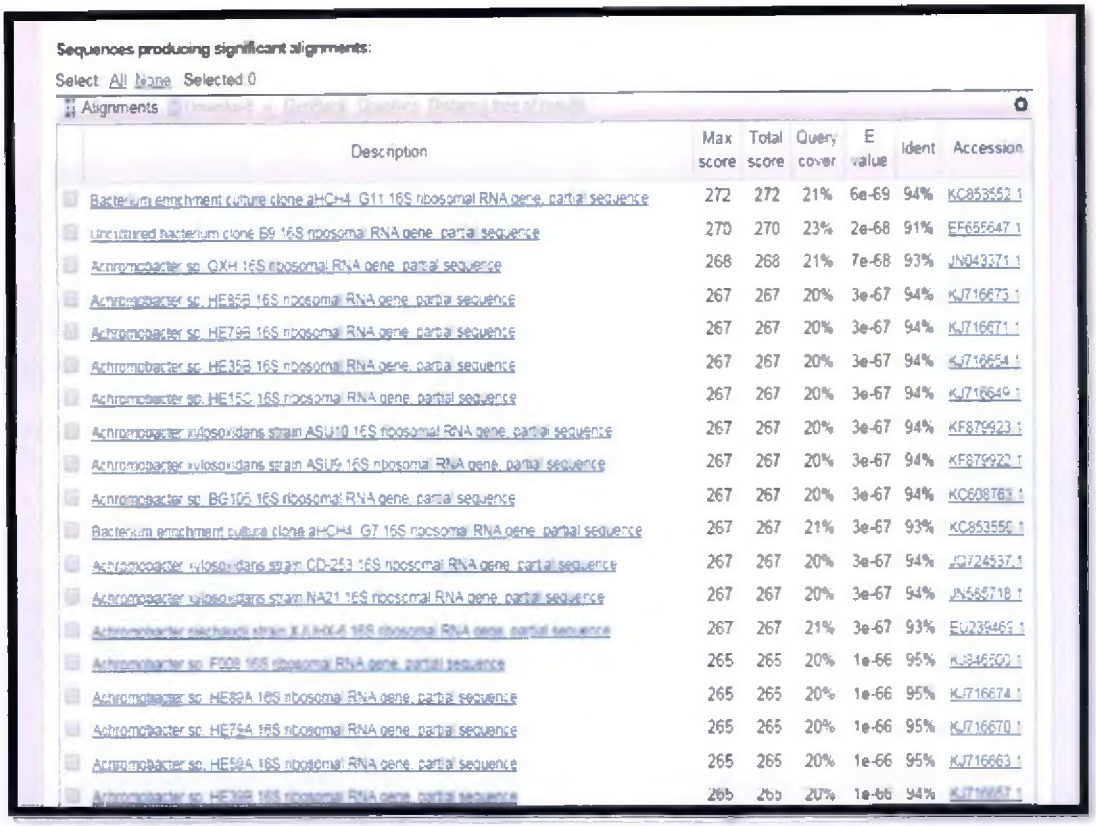
Description	Max score	Total score	Query cover	E value	Ident	Accession
Uncultured bacterium partial 16S rRNA gene, clone 16sps16-2p00 w2h	1062	1062	97%	0.0	99%	FM995052.1
Pseudomonas aeruginosa strain 2xWH2h 16S ribosomal RNA gene, partial sequence	1062	1062	96%	0.0	99%	HQ537785.1
Pseudomonas sp. VITDM1 16S ribosomal RNA gene, partial sequence	1062	1062	97%	0.0	99%	HQ332955.1
Pseudomonas aeruginosa strain St6 16S ribosomal RNA gene, partial sequence	1062	1062	97%	0.0	99%	HQ283487.1
Pseudomonas aeruginosa strain UMN SMBS 16S ribosomal RNA gene, partial sequence	1062	1062	97%	0.0	99%	HQ148166.1
Pseudomonas aeruginosa strain RKCCp8 16S ribosomal RNA gene, partial sequence	1061	1061	96%	0.0	99%	KC119335.1
Pseudomonas aeruginosa strain R1-1b5 16S ribosomal RNA gene, partial sequence	1061	1061	96%	0.0	99%	JQ599649.1
Pseudomonas aeruginosa strain UK1-3 16S ribosomal RNA gene, partial sequence	1061	1061	97%	0.0	99%	JN661696.1
Uncultured bacterium partial 16S rRNA gene, clone 16sp521-1D12.p1k9	1061	1061	96%	0.0	99%	FM996755.1
Bacterium zc-1 16S ribosomal RNA gene, partial sequence	1059	1059	96%	0.0	99%	KM401600.1
Pseudomonas aeruginosa strain NBFFALD_RA3140 16S ribosomal RNA gene, partial sequence	1059	1059	96%	0.0	99%	KJ019502.1
Pseudomonas aeruginosa strain PA5-2-2 16S ribosomal RNA gene, partial sequence	1059	1059	96%	0.0	99%	KF977851.1
Pseudomonas aeruginosa strain PA5-2-1 16S ribosomal RNA gene, partial sequence	1059	1059	96%	0.0	99%	KF977850.1
Uncultured bacterium partial 16S rRNA gene, clone 24017	1059	1059	96%	0.0	99%	HG531868.1
Pseudomonas aeruginosa strain SQ 5 16S ribosomal RNA gene, partial sequence	1059	1059	96%	0.0	99%	KF153064.1
Pseudomonas aeruginosa strain D2 16S ribosomal RNA gene, partial sequence	1059	1059	96%	0.0	99%	KF113079.1
Pseudomonas aeruginosa strain D1 16S ribosomal RNA gene, partial sequence	1059	1059	96%	0.0	99%	KF113078.1
Pseudomonas sp. bf1 16S ribosomal RNA gene, partial sequence	1059	1059	96%	0.0	99%	KC982412.1
Pseudomonas aeruginosa strain SIBK 1U 16S ribosomal RNA gene, partial sequence	1059	1059	96%	0.0	99%	KC920813.1
[illegible] 16S ribosomal RNA gene, partial sequence	1059	1059	96%	0.0	99%	KC636467.1

A. Sequences showing homology



B. Blastn output

Plate 14. Sequence analysis of PSB-149



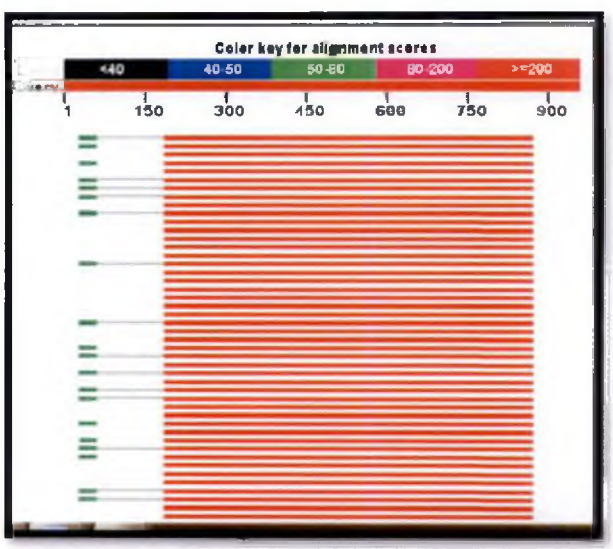
Sequences producing significant alignments:

Select All None Selected 0

Alignments

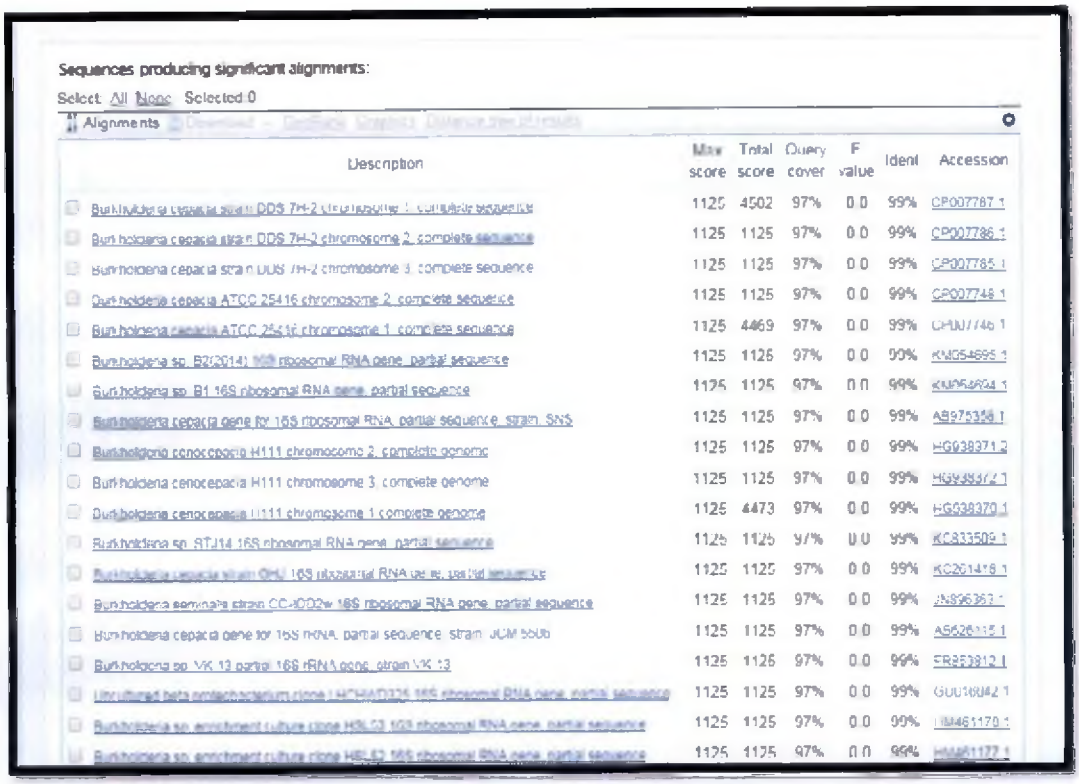
Description	Max score	Total score	Query cover	E value	Ident	Accession
Bacterium enrichment culture clone aHCH4_G11 16S ribosomal RNA gene, partial sequence	272	272	21%	6e-69	94%	KC853552.1
Uncultured bacterium clone B9 16S ribosomal RNA gene, partial sequence	270	270	23%	2e-68	91%	EF655647.1
Achromobacter sp. QXH 16S ribosomal RNA gene, partial sequence	268	268	21%	7e-68	93%	JN043371.1
Achromobacter sp. HE85B 16S ribosomal RNA gene, partial sequence	267	267	20%	3e-67	94%	KJ716675.1
Achromobacter sp. HE79B 16S ribosomal RNA gene, partial sequence	267	267	20%	3e-67	94%	KJ716671.1
Achromobacter sp. HE35B 16S ribosomal RNA gene, partial sequence	267	267	20%	3e-67	94%	KJ716654.1
Achromobacter sp. HE15C 16S ribosomal RNA gene, partial sequence	267	267	20%	3e-67	94%	KJ716649.1
Achromobacter xylosoxidans strain ASU10 16S ribosomal RNA gene, partial sequence	267	267	20%	3e-67	94%	KF879923.1
Achromobacter xylosoxidans strain ASU5 16S ribosomal RNA gene, partial sequence	267	267	20%	3e-67	94%	KF879922.1
Achromobacter sp. BG105 16S ribosomal RNA gene, partial sequence	267	267	20%	3e-67	94%	KC608763.1
Bacterium enrichment culture clone aHCH4_G7 16S ribosomal RNA gene, partial sequence	267	267	21%	3e-67	93%	KC853550.1
Achromobacter xylosoxidans strain CD-253 16S ribosomal RNA gene, partial sequence	267	267	20%	3e-67	94%	JQ724537.1
Achromobacter xylosoxidans strain NA21 16S ribosomal RNA gene, partial sequence	267	267	20%	3e-67	94%	JN565718.1
Achromobacter piechaudii strain KUHKA 16S ribosomal RNA gene, partial sequence	267	267	21%	3e-67	93%	EU239465.1
Achromobacter sp. F008 16S ribosomal RNA gene, partial sequence	265	265	20%	1e-66	95%	KJ946502.1
Achromobacter sp. HE89A 16S ribosomal RNA gene, partial sequence	265	265	20%	1e-66	95%	KJ716674.1
Achromobacter sp. HE75A 16S ribosomal RNA gene, partial sequence	265	265	20%	1e-66	95%	KJ716670.1
Achromobacter sp. HE59A 16S ribosomal RNA gene, partial sequence	265	265	20%	1e-66	95%	KJ716663.1
Achromobacter sp. HE70B 16S ribosomal RNA gene, partial sequence	265	265	20%	1e-66	94%	KJ716667.1

A. Sequences showing homology

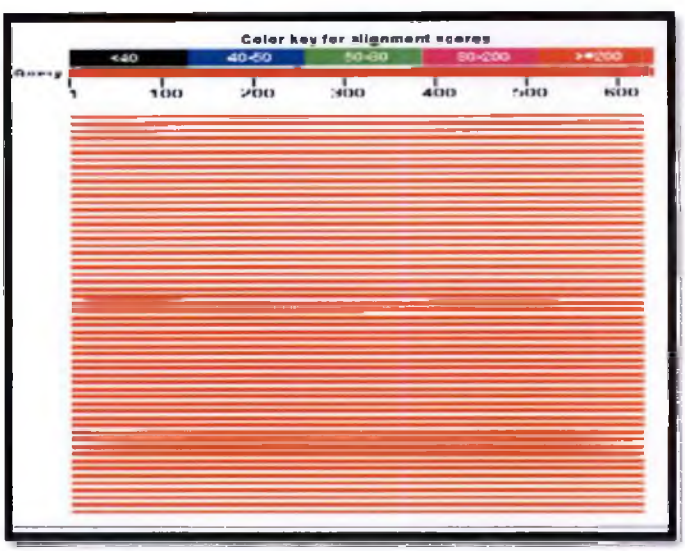


B. Blast n output

Plate 15. Sequence analysis of PSB-151



A. Sequences showing homology



B. Blastn output

Plate 16. Sequence analysis of PSB-152

green coloured large spores. PSF-200 appeared as creamy white colonies on nutrient agar media.

4.3.2.2 ***Morphological characters***

All the 10 phosphate solubilizing fungal isolates were tentatively identified based on morphological characters (Table 16 and Plate 17).

PSF-173 and PSF-199 produced hyaline conidiophores and chains of single celled, globose shaped conidia. PSF-174 produced hyaline, smooth and septate mycelium and rough walled phialospores. Conidiophores were branched. PSF-175, PSF-180, PSF-182, PSF-183 and PSF-186 produced large conidial heads with biseriate phialides. globose and dark brown to black spores. PSF -198 appeared with biseriate vesicles, globose shaped and smooth, finely roughened conidial surface. PSF-200 produced ellipsoidal cells.

Based on cultural and morphological characterization, the ten phosphate solubilizing fungal isolates under the study were identified (Table 16). PSF-173 and PSF-199 were tentatively identified as *Penicillium* sp. PSF-174 was identified as *Trichoderma* sp. The isolates PSF-175, PSF-180, PSF-182, PSF-183 and PSF-186 were identified as *Aspergillus* sp. and PSF-198 as *Aspergillus* sp. The fungal isolate PSF-200 was classified as yeast. Six isolates of *Aspergillus* sp. were discarded and the remaining four isolates including two of *Penicillium* sp. (PSF-173 and PSF-199), one *Trichoderma* sp. (PSF-174) and yeast (PSF-200) were used for further experiments. For further confirmation, PSF-173, PSF-199 and PSF-174 were sent to NCFT, New Delhi. PSF-173 and PSF-199 were identified as *Penicillium chrysogenum* (Id No. 6106.14) and PSF-174 as *Trichoderma viride* (Id No.6108.14).

Table 16. Cultural and morphological characters of phosphate solubilizing fungi

Isolate	Colony characters	Morphological characters	Identified as
PSF- 173	Green colour, fast growing and consisting a dense felt of conidiophores.	Chains of single celled and hyaline conidia produced hyaline conidiophores and globose conidia	*Penicillium* sp.
PSF-174	Fast growing, at first white and downy, later developing yellowish- green to deep green compact tuffs.	Branched conidiophores, hyaline, smooth and septate mycelium. Phialospores are rough walled	*Trichoderma* sp.
PSF-175	Dark brown spores with high sporulation. Fast growing and yellow basal felt.	Large conidial heads with biseriate phialides. Conidiospores globose and dark brown to black colour	*Aspergillus* sp.
PSF-180	Dark brown spores with high sporulation. Fast growing and yellow basal felt.	Large conidial heads with biseriate phialides. Conidiospores globose and dark brown to black colour	*Aspergillus* sp.
PSF-182	Dark brown spores with high sporulation. Fast growing and yellow basal felt.	Large conidial heads with biseriate phialides. Conidiospores globose and dark brown to black colour	*Aspergillus* sp.
PSF-183	Dark brown spores with high sporulation. Fast growing and yellow basal felt.	Large conidial heads with biseriate phialides. Conidiospores globose and dark brown to black colour	*Aspergillus* sp.
PSF-186	Dark brown spores with high sporulation. Fast growing and yellow basal felt.	Large conidial heads with biseriate phialides. Conidiospores globose and dark brown to black colour	*Aspergillus* sp.
PSF-198	Pale brown coloured large spores with high sporulation. Fast growing and red-brown basal felt.	Biseriate vesicles, globose shaped and smooth finely roughened conidial surface	*Aspergillus* sp.
PSF -199	Green colour, fast growing and consisting a dense felt of conidiophores.	Chains of single celled conidia produced hyaline conidiophores and globose conidia	*Penicillium* sp.
PSF-200	Creamy white colonies	Ellipsoidal shaped cells	Yeast

4.4 PGPR/ ANTAGONISTIC ACTIVITIES OF SELECTED ISOLATES

4.4.1 **IAA / GA Production**

All the selected twenty isolates were examined for IAA and GA production on Luria agar supplemented with SDS (0.06 %) and glycerol (1 %). Based on the development of red colour on the filter paper four isolates: *Providencia alcalifaciens* (PSB-22), *Pseudomonas* sp. (PSB-149), *Penicillium* sp. (PSF-173) and *Trichoderma* sp. (PSF-174) were found to produce IAA. None of the isolates produced GA (Table 17 and Plate 18).

4.4.2. **Production of HCN**

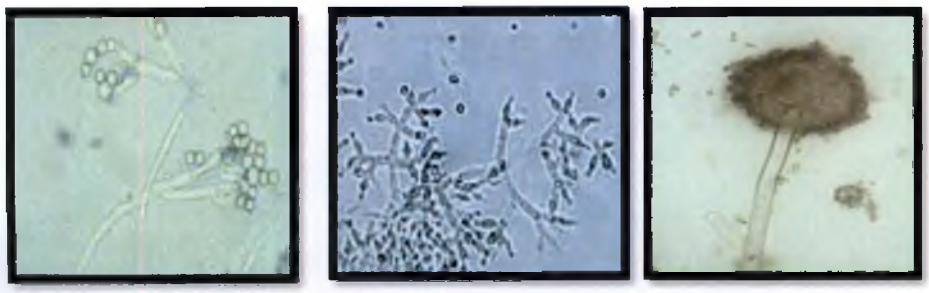
Hydrogen cyanide was produced by three of the bacterial isolates namely *Providencia* sp. (PSB-), and two isolates of *Pseudomonas* sp. (PSB-114 and PSB-149) (Table 17). The colour change of the filter paper containing picric acid from yellow to brownish orange indicated moderate HCN production potential of the isolates within 4 days. None of the other isolates produced HCN (Plate 19).

4.4.3 **Production of NH_3**

Six out of twenty phosphate solubilizers were able to produce ammonia (Table 17). Bacterial isolates *Providencia alcalifaciens* (PSB-22) and *Pseudomonas* sp. (PSB-149) produced high amount of ammonia. *Achromobacter* sp. (PSB-151), *Ochrobactrum* sp. (PSB-148) and *Achromobacter* sp. (PSB-18) produced medium amount of ammonia and the phosphate solubilizing fungi *Trichoderma* sp. (PSF-174) produced low amount of ammonia (Plate 20).

4.4.4 **Production of siderophores**

Siderophore production by 20 isolates was tested by CAS plate assay. Chrome azurol S blue agar medium (CAS) was used to detect siderophore production. Three of the bacterial isolates namely *Achromobacter* sp. (PSB-151), *Burkholderia* sp. (PSB-152) and *Pseudomonas* sp. (PSB-114) produced yellow to orange coloured zone around the colonies after 24 hours of incubation, which

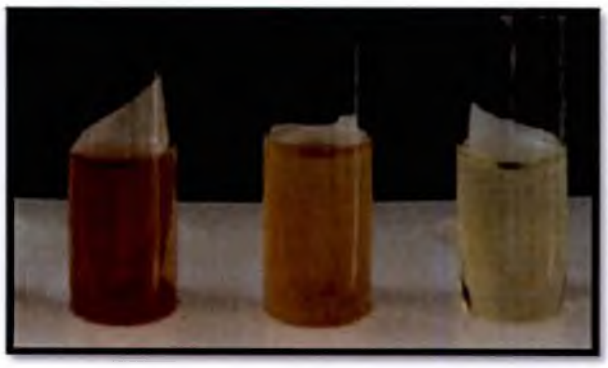


PSF-173 PSF-174 PSF-175

Plate 17. Morphological characters of phosphate solubilizing fungi

Control PSB-14 PSB-149

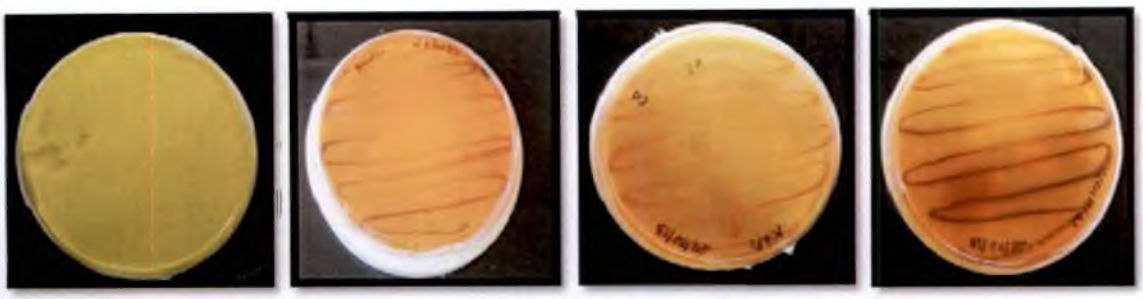
A. IAA production by PSB



PSF-173 PSF-174 Control

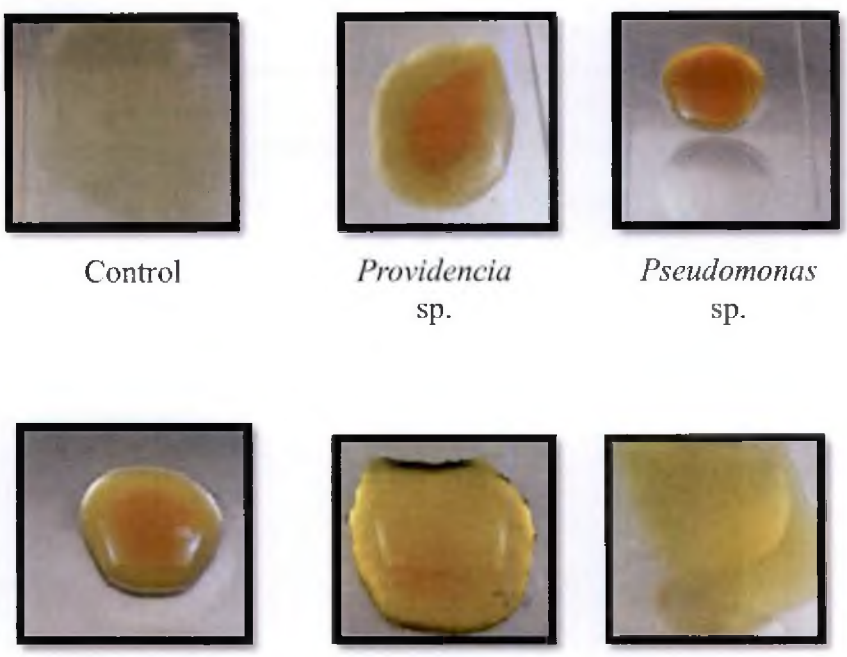
B. IAA production by PSF

Plate 18. IAA production by selected phosphate solubilizing microorganisms



Control | *Providencia* sp. (PSB-14) | *Pseudomonas* sp. (PSB-114) | *Pseudomonas* sp. (PSB-149)

Plate 19.HCN production by PSB isolates



Control | *Providencia* sp. | *Pseudomonas* sp.

***Ochrobactrum* sp.** | ***Achromobacter* sp. (PSB-18)** | ***Achromobacter* sp.(PSB-151)**

Plate 20. Ammonia production by PSB isolates

primarily indicated the ability of the isolates to produce siderophores (Table 17). None of the fungal isolates produced siderophores (Plate 21).

Table 17. PGPR and antagonistic activities of phosphate solubilizing microorganisms

Sl. no.	Isolates	Plant growth promoting activity		Mechanism for antagonistic activity		
		IAA	GA	Siderophore	HCN	Ammonia
1	*Acinetobacter calcoaceticus* (PSB-13)	-	-	-	-	-
2	*Providencia* sp. (PSB-14)	+	-	-	+	+++
3	*Achromobacter* sp. (PSB-18)	-	-	-	-	++
4	*Providencia alcalifaciens* (PSB-22)	-	-	-	-	-
5	*Pseudomonas* sp. (PSB-114)	-	-	+	+	-
6	*Bacillus cereus* (PSB-134)	-	-	-	-	-
7	*Ochrobactrum* sp. (PSB-148)	-	-	-	-	++
8	*Pseudomonas* sp. (PSB-149)	+	-	-	+	+++
9	*Achromobacter* sp. (PSB-151)	-	-	+	-	++
10	*Burkholderia* sp.	-	-	+	-	-
11	Yeast	-	-	-	-	-
12	*Penicillium* sp.(PSF-173)	+	-	-	-	-
13	*Penicillium* sp. (PSF-199)	-	-	-	-	-
14	*Trichoderma* sp. (PSF-174)	+	-	-	-	+

- Negative; + positive; + Low; ++ Medium; +++ High

Control | *Achromobacter* sp. (PSB-151) | *Burkholderia* sp. | *Pseudomonas* sp.

Plate 21. Siderophore production by PSB isolates

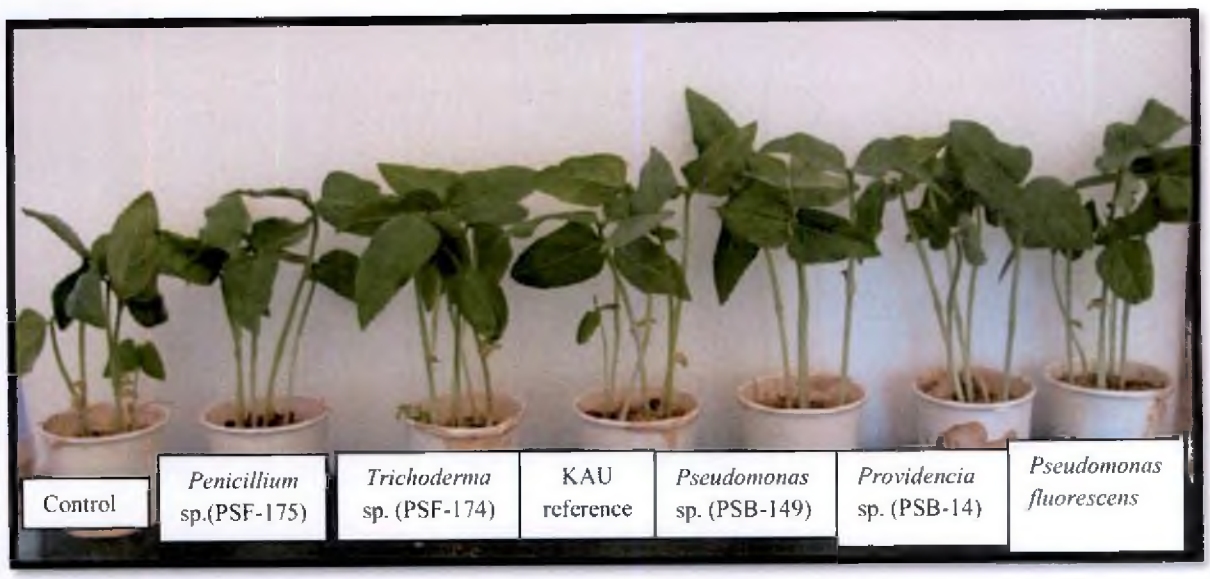


Plate 22: Seedling vigour index study of cowpea seedlings

Based on the PGPR activities, four isolates were selected for testing their effect on vigour index of cowpea seedlings. These included *Pseudomonas* sp. PSB-149), *Providencia* sp. (PSB-14), *Trichoderma* sp. (PSF-174) and *Penicillium* sp. (PSF-175).

4.5. EFFECT OF SELECTED ISOLATES OF PHOSPHATE SOLUBILIZERS ON VIGOUR INDEX OF COWPEA SEEDLINGS

4.5.1 **Germination percentage**

Hundred per cent germination was observed in treatments with *Pseudomonas* sp. (PSB-149), *Providencia* sp. (PSB-14), *Trichoderma* sp. (PSF-174), *Pseudomonas fluorescens* and KAU reference culture as bioinoculants (Table 18). Lowest percent of germination (86.67 %) was observed in control. *Penicillium* sp. (PSF-175) recorded 93.33 per cent germination which was higher than control.

4.5.2 **Seedling vigour index**

All the bioinoculants except *Penicillium* sp. (PSF-175) recorded superior seedling vigour index than uninoculated control (Table 18 and Plate 22).

Among the bioinoculants *Pseudomonas* sp. (PSB-149) recorded higher seedling vigour index (3058.66) which was statistically superior to *Penicillium* sp. (PSF-175). This was followed by *Providencia* sp. (PSB-14), which recorded a vigour index of 2954.33. All bioinoculants, except *Penicillium* sp. (PSF-175) recorded significantly higher vigour index of seedlings when compared to control.

4.6. ANTAGONISTIC ACTIVITY OF PHOSPHATE SOLUBILIZERS AGAINST SOIL BORNE PLANT PATHOGENS

The results of the experiment on antagonistic activity of selected phosphate solubilizers against soil borne plant pathogens are given in Table 19. It is observed from the table that all isolates showed antagonistic activity against *R. solani* which ranged from 13.67 -43.67. Among the isolates, *Achromobacter* sp.

(PSB-18) recorded highest antagonistic activity against *Rhizoctonia solani* with 43.67 per cent inhibition. It is also noted that among the 14 isolates tested, six isolates showed more than 40 per cent inhibition against this pathogen. In case of *Pythium aphanidermatum*, both *Bacillus cereus* (PSB-134) and *Ochrobactrum sp.* (PSB-148) recorded highest antagonistic activity with 35.22 per cent inhibition. *Pseudomonas* sp. (PSB-149) showed maximum inhibition (36.04 per cent) against *Sclerotium rolfsii*. All isolates except, *Achromobacter* sp. (PSB-151) and *Penicillium* sp. (PSF-173) showed inhibitory effect on *Fusarium oxysporum* and maximum inhibition was recorded by *B.cereus* (PSB-134) with 34.88 per cent. None of the isolates showed antagonistic activity against the bacterial pathogen *Ralstonia solanacearum* (Plate 23).

Table 18. Effect of selected isolates of phosphate solubilizers on vigour index of cowpea seedlings

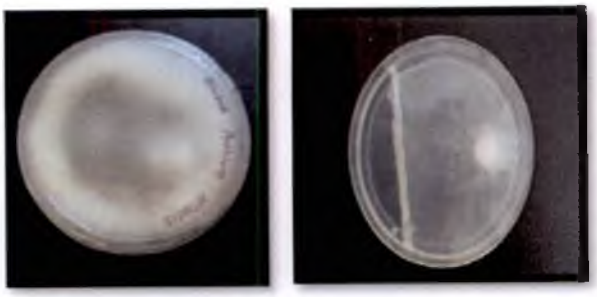
Sl no.	Bioinoculants	Germination %	Seedling vigour index
1	*Providencia* sp. (PSB-14)	100	2954.33^{ab}
2	*Pseudomonas* sp. (PSB-149)	100	3058.66^{a}
3	*Trichoderma* sp. (PSF-174)	100	2750.33^{ab}
3	*Penicillium* sp. (PSF-175)	93.33	2440.46^{bc}
4	*Pseudomonas fluorescens*	100	2833.33^{ab}
5	KAU reference culture (Phosphate solubilizers)	100	2742.00^{ab}
6	Control	86.67	1991.80^{c}

Table 19. Antagonistic activity of selected phosphate solubilizers

Sl no.	Isolates of phosphate solubilizers	Per cent inhibition				
		Rhizoctonia solani	*Pythium aphanidermatum*	*Sclerotium rolfsi*	*Fusarium oxysporum*	*Ralstonia solanancerum*
1	*Acinetobacter calcoaceticus* (PSB-13)	26.43	0.00	0.00	13.95	0.00
2	*Providencia* sp. (PSB-14)	22.98	0.00	0.00	15.11	0.00
3	*Achromobacter* sp. (PSB-18)	43.67	0.00	19.76	24.41	0.00
4	*Providencia alcalifaciens* (PSB-22)	13.79	0.00	0.00	11.62	0.00
5	*Pseudomonas* sp. (PSB-114)	42.52	25.00	26.74	32.55	0.00
6	*Bacillus cereus* (PSB-134)	41.37	35.22	26.74	34.88	0.00
7	*Ochrobactrum* sp. (PSB-148)	21.83	35.22	34.88	31.39	0.00
8	*Pseudomonas* sp. (PSB-149)	35.63	23.86	36.04	31.39	0.00
9	*Achromobacter* sp. (PSB-151)	41.37	0.00	0.00	0.00	0.00
10	*Burkholderia* sp.	27.58	0.00	0.00	19.76	0.00
11	*Penicillium* sp. (PSF-173)	38.41	15.23	0.00	0.00	0.00
12	*Trichoderma* sp.	40.11	17.80	18.60	19.00	0.00
13	*Penicillium* sp. (PSF-199)	41.86	10.36	12.79	18.25	0.00
14	Yeast (PSF-200)	18.38	28.40	23.25	15.11	0.00

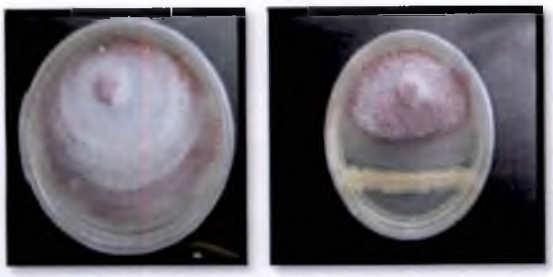
Control *Pseudomonas* sp. *X*

Sclerotium rolfsii



Control *Bacilus cereus X*

Pythium aphanidermatum

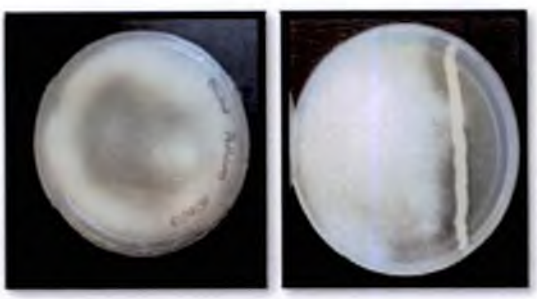


Control *Bacillus cereus* X

Fusarium oxysporum

Control *Achromobacter* sp. X

Rhizoctonia solani



Control *Ochrobactrum* sp. X

Pythium aphanidermatum

Plate 23: Antagonistic activities of selected isolates against plant pathogens

4.7 EVALUATION OF SELECTED PHOSPHATE SOLUBILIZERS UNDER POT CULTURE

Based on the efficiency of P-solubilization and plant growth promoting activities, three native isolates *Providencia alcalifaciens* (PSB-14), *Pseudomonas* sp. (PSB-149) and *Trichoderma viride* (PSF-174) were selected for evaluation in pot culture experiment, with cowpea as the test crop (Plate 17). The recommended dose of P was provided as rock phosphate and factamphos as detailed under 3.6.3 in the chapter on Materials and Methods. The biometric observations were recorded at 20 DAS, 40 DAS and at harvest. Chemical analyses of soil and plant samples were carried out at the time of sowing, 20DAS, 40 DAS and at harvest. The results of the experiment are detailed below:

4.7.1 **Biometric observations**

4.7.1.1 ***Effect of P-solubilizers on shoot length of plants***

4.7.1.1.1 ***In the absence of applied P***

At 20 DAS in the absence of applied P, KAU reference culture recorded maximum shoot length (15.20 cm), followed by *Pseudomonas* sp. (PSB-149) which recorded a shoot length of 14.38 cm and these two treatments were statistically on par. These treatments were statistically superior to all other treatments (Table 20).

All the bioinoculants recorded higher shoot length than control at 40 DAS. Among the different bioinoculants, *Pseudomonas* sp. (PSB-149) recorded maximum shoot length (34.07 cm) which was on par with *Providencia* sp. (PSB-14) and *Trichoderma* sp. (PSF-174). These three treatments were statistically superior to KAU reference culture (Table 20).

Providencia sp. (PSB-14) recorded maximum shoot length (56.18 cm) at harvest, which was on par with *Pseudomonas* sp. (PSB-149). These two treatments were statistically superior to other bioinoculants. *Pseudomonas* sp.,

Plate 24: Over view of pot culture experiment

Trichoderma sp. (PSF-174) and KAU reference culture were on par. However, all the bioinoculants recorded significantly higher shoot length than control (Table 20).

4.7.1.1.2 ***In presence of rock phosphate***

At 20 DAS in presence of rock phosphate, all the bioinoculants recorded significantly higher shoot length than control. Among the bioinoculants, KAU reference culture recorded maximum shoot length (17.75 cm) which was statistically superior to all other bioinoculants and this was followed by *Pseudomonas* sp. (PSB-149) (16.45 cm). All other bioinoculants were on par (Table 20).

All bioinoculants recorded significantly higher shoot length compared to uninoculated control at 40 DAS (Table 22). Maximum shoot length (39.59 cm) was recorded by *Trichoderma* sp. (PSF-174), and this was superior to all other bioinoculants.

At harvest, all the bioinoculants recorded significantly higher shoot length than control (Table 20). *Providencia* sp. (PSB-14) recorded maximum shoot length (60.67 cm) which was on par with *Pseudomonas* sp. (PSB-149) (59.00 cm) and KAU reference culture (58.77cm). These three treatments were significantly superior to *Trichoderma* sp. (PSF-174) (52.73 cm).

4.7.1.1.3 ***In presence of factamphos***

Among treatments which received factamphos, *Trichoderma* sp. (PSF-174) recorded maximum shoot length (18.63 cm) at 20 DAS which was statistically superior to all other treatments (Table 20). *Pseudomonas* sp. (PSB-149) and *Providencia* sp. (PSB-14) recorded significantly higher shoot length (16.75 cm and 16.50 cm respectively) than KAU reference culture and uninoculated control.

At 40 DAS, *Trichoderma* sp. (PSF-174) in combination with factamphos recorded maximum shoot length (43.00 cm) which was statistically superior to all other treatments. *Providencia* sp. (PSB-14) and *Pseudomonas* sp. (PSB-149) recorded shoot length of 38.76 cm and 38.05 cm respectively, which were significantly superior to KAU reference culture and control (Table 20).

At harvest, *Trichoderma* sp. (PSF-174) in presence of factamphos recorded maximum shoot length (67.23 cm) and this was on par with *Pseudomonas* sp. (PSB-149) which recorded 65.70 cm and KAU reference culture that recorded 64.50 cm. These treatments were significantly superior to *Providencia* sp. (PSB-14) and control (Table 20).

4.7.1.2 **Effect of P solubilizers on root length of plants**

4.7.1.2.1 ***In absence of applied phosphorous***

All bioinoculants recorded significantly higher root length than uninoculated control at 20 DAS (Table 21). However, KAU reference culture recorded maximum root length of 8.87 cm.

At 40 DAS, all the bioinoculants recorded significantly higher root length as compared to control (Table 21). However, among the bioinoculants, *Pseudomonas* sp. (PSB-149) recorded maximum root length (21.00 cm) and this was statistically superior to all bioinoculants.

Pseudomonas sp. (PSB-149) recorded maximum root length (24.00 cm) at harvest and this was statistically superior to all other treatments. *Trichoderma* sp. (PSF-174) and KAU reference culture, which recorded root length of 21.25 cm and 20.00 cm respectively, were on par (Table 21).

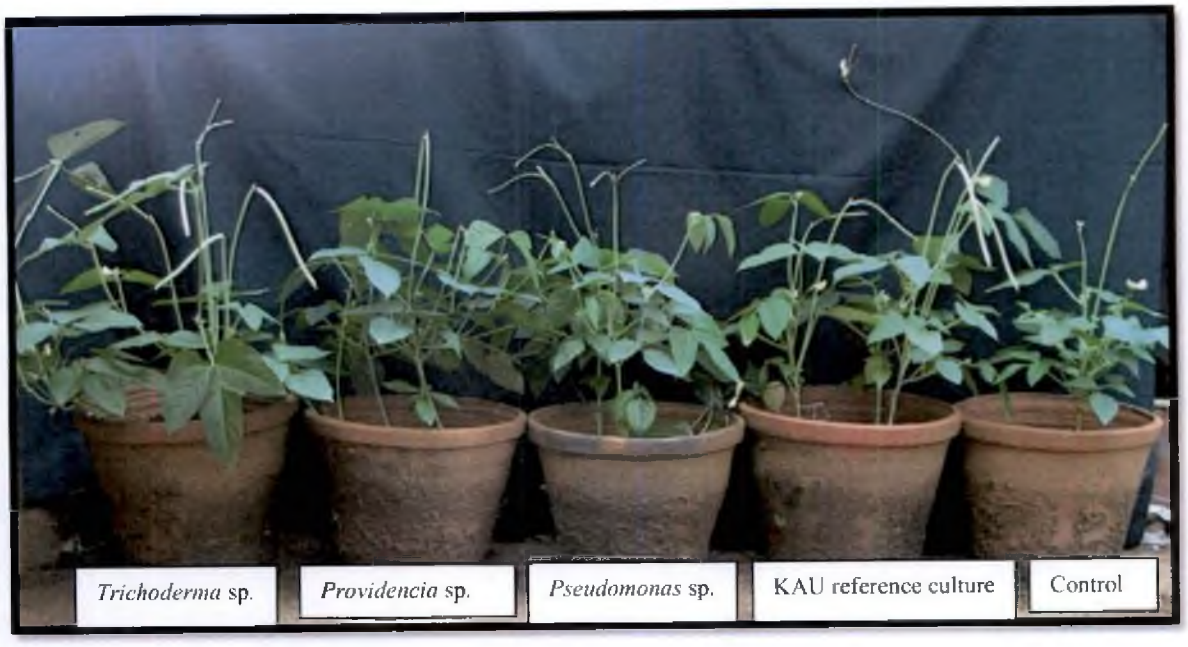


Plate 25 : Effect of bioinoculants in the absence of applied phosphorous

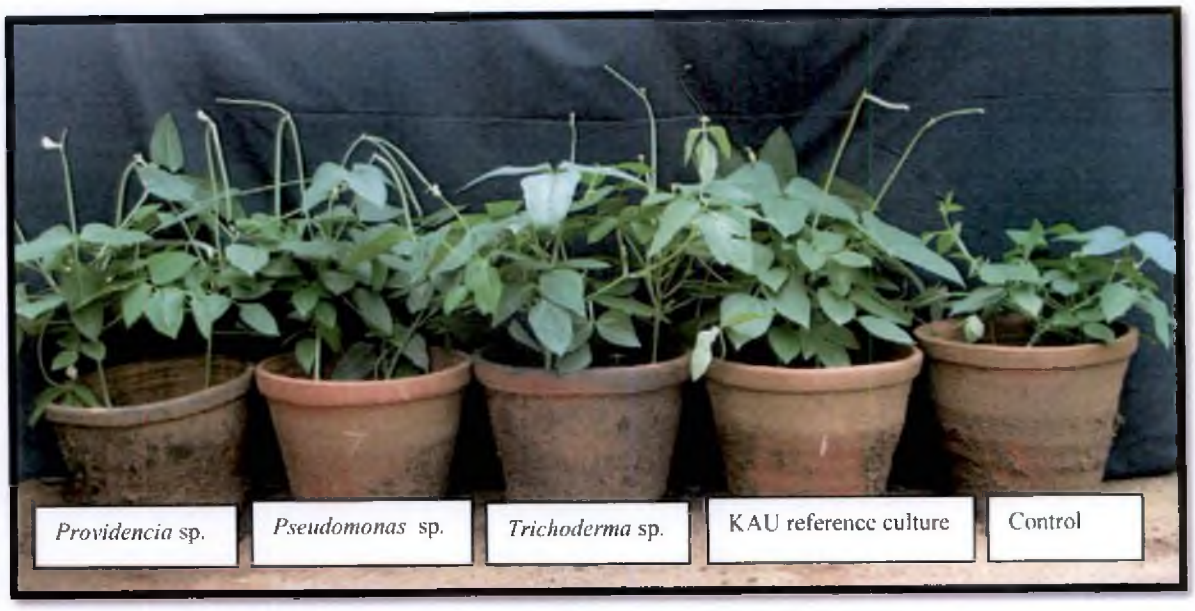


Plate 26 :Effect bioinoculants in the presence of rock phosphate

4.7.1.2.2 ***In presence of rock phosphate***

At 20 DAS, all the bioinoculants except *Trichoderma* sp. (PSF-174) recorded significantly higher root length than control (Table 21). *Providencia* sp. (PSB-14) recorded maximum root length (9.77 cm) which was on par with KAU reference culture (9.26 cm). These two treatments were statistically superior to all other treatments. *Pseudomonas* sp. (PSB-149) and KAU reference culture were on par and both of them were superior to uninoculated control.

All the bioinoculants in combination with rock phosphate recorded significantly higher root length than control at 40 DAS (Table 21). Among the bioinoculants, *Trichoderma* sp. (PSF-174) recorded maximum root length (25.16 cm) which was statistically superior to all other bioinoculants. *Pseudomonas* sp. (PSB-149) and KAU reference culture were on par and superior to *Providencia* sp. (PSB-14).

At harvest, *Trichoderma* sp. (PSF-174) in presence of rock phosphate recorded maximum root length (25.67 cm) which was on par with *Pseudomonas* sp. (PSB-149) that recorded 24.17 cm and KAU reference culture with 23.43 cm. these treatments were significantly superior to all other treatments (Table 21).

4.7.1.2.3 ***In presence of factamphos***

Among treatments which received factamphos, *Providencia* sp. (PSB-14) recorded maximum root length (9.53 cm) at 20 DAS which was on par with *Pseudomonas* sp. (PSB-149) (8.77 cm). These two treatments were significantly superior to all other treatments. *Trichoderma* sp. (PSF-174), KAU reference culture and uninoculated control were on par (Table 21).

At 40 DAS, root length did not show much difference with different P-solubilizers. However, *Trichoderma* sp. (PSF-174) recorded maximum root

length (26.02 cm) followed by KAU reference culture (24.72 cm). All the bioinoculants recorded statistically superior root length compared to control (Table 21).

All the bioinoculants recorded significantly higher root length than control at harvest. KAU reference culture in combination with factamphos recorded maximum root length (28.33 cm) and this was on par with *Trichoderma* sp. (PSF-174) with a corresponding value of 26.33 cm. These treatments were significantly superior to all other treatments (Table 21).

4.7.1.3 **Effect of P solubilizers on shoot weight of plants**

4.7.1.3.1 ***In absence of applied phosphorous***

At 20 DAS, all the bioinoculants recorded higher shoot weight than control (Table 22). Among the different bioinoculants, *Trichoderma* sp. (PSF-174) recorded maximum shoot weight (1.19 g) which was statistically superior to other bioinoculants. KAU reference culture recorded significantlly higher shoot weight (0.54 g) than *Pseudomonas* sp. (PSB-149) and *Providencia* sp. (PSB-14).

Pseudomonas sp. (PSB-149) recorded maximum shoot weight (10.11 g) at 40 DAS, followed by *Providencia* sp. (PSB-14) which recorded a shoot weight of 8.50 g and these two treatments were statistically on par. KAU reference culture and *Providencia* sp. (PSB-14) were on par and both of them were superior to *Trichoderma* sp (PSF-174) and uninoculated control (Table 22).

At harvest, *Providencia* sp. (PSB-14) recorded maximum shoot weight (46.32 g), which were on par with *Pseudomonas* sp. (PSB-149). These two treatments were statistically superior to other bioinoculants. *Trichoderma* sp. (PSF-174)and KAU reference culture were on par. However, all the bioinoculants recorded significantly higher shoot weight than control (Table 22).

Table 20 Effect of p-solubilizers on shoot length of cowpea

Shoot length of cowpea plant (cm)									
Bioinoculants	In the absence of applied P			In the presence of rock phosphate			In the presence of factamphos		
	At 20 DAS	At 40 DAS	At harvest	At 20 DAS	At 40 DAS	At harvest	At 20 DAS	At 40 DAS	At harvest
Providencia sp. (PSB-14)	12.66^{b}	33.22^{a}	56.18^{a}	15.43^{b}	34.50^{b}	60.67^{a}	16.50^{b}	38.76^{b}	63.55^{b}
Pseudomonas sp. (PSB-149)	14.38^{a}	34.07^{a}	52.66^{ab}	16.45^{b}	34.53^{b}	59.00^{a}	16.72^{b}	38.05^{b}	65.70^{ab}
Trichoderma sp. (PSF-174)	12.45^{b}	32.08^{a}	49.00^{b}	16.08^{b}	39.59^{a}	52.73^{b}	18.63^{a}	43.00^{a}	67.23^{a}
KAU reference culture	15.20^{a}	29.33^{b}	50.33^{b}	17.75^{a}	33.07^{b}	58.77^{a}	14.47^{c}	33.58^{c}	64.50^{ab}
Control	12.16^{b}	22.44^{c}	41.00^{c}	12.50^{c}	26.27^{c}	45.17^{c}	14.17^{c}	28.11^{d}	62.44^{b}

Table 21 Effect of p-solubilizers on root length of cowpea

Root length of cowpea plant (cm)									
Bioinoculants	In the absence of applied P			In the presence of rock phosphate			In the presence of factamphos		
	At 20 DAS	At 40 DAS	At harvest	At 20 DAS	At 40 DAS	At harvest	At 20 DAS	At 40 DAS	At harvest
Providencia sp. (PSB-14)	8.46^{ab}	18.26^{b}	17.67^{c}	9.77^{a}	18.55^{c}	20.50^{b}	9.53^{a}	22.72^{a}	24.39^{b}
Pseudomonas sp. (PSB-149)	8.03^{ab}	21.00^{a}	24.00^{a}	8.35^{bc}	22.83^{b}	24.17^{a}	8.77^{ab}	23.33^{a}	24.60^{b}
Trichoderma sp. (PSF-174)	7.18^{ab}	18.10^{b}	21.25^{b}	7.58^{cd}	25.16^{a}	25.67^{a}	7.91^{b}	26.02^{a}	26.33^{ab}
KAU reference culture	8.87^{a}	18.61^{b}	20.00^{b}	9.26^{ab}	21.88^{b}	23.43^{a}	7.83^{b}	24.72^{a}	28.33^{a}
Control	6.71^{b}	14.03^{c}	16.47^{c}	7.25^{d}	15.33^{d}	20.42^{b}	7.33^{b}	15.91^{b}	19.00^{c}

4.7.1.3.2 ***In presence of rock phosphate***

At 20 DAS, *Trichoderma* sp. (PSF-174) in presence of rock phosphate recorded maximum shoot weight (1.32 g) which was statistically superior to all other bioinoculants and this was followed by KAU reference culture (1.02g) (Table 22). KAU reference culture was superior to *Providencia* sp. (PSB-14) and *Pseudomonas* sp. (PSB-149).

Providencia sp. (PSB-14) recorded maximum shoot weight (11.36 g) at 40 DAS, which was on par with *Pseudomonas* sp. (PSB-149). These two treatments were staistically superior to other bioinoculants. *Trichoderma* sp. (PSF-174) and KAU reference culture were on par (Table 22).

At harvest, *Pseudomonas* sp.(PSB-149) in combination with rock phosphate recorded maximum shoot weight (52.07g) which was statistically superior to other bioinoculants (Table 22). *Providencia* sp. (PSB-14) recorded significantly higher shoot weight (44.45g) than uninoculated control. This treatment was on par with *Trichoderma* sp. (PSF-174)and KAU reference culture.

4.7.1.3.3 ***In the presence of factamphos***

All the bioinoculats recorded significaly higher shoot weight compared to uninoculated control at 20 DAS (Table 22). Among treatments which received factamphos, *Providencia* sp. (PSB-14) recorded maximum shoot weight (1.73g) which was statistically superior to all other treatments. *Trichoderma* sp. (PSF-174) recorded significantly higher shoot weight (1.38 g) than *Pseudomonas* sp. (PSB-149) and on par with KAU reference culture.

At 40 DAS, KAU reference culture in combination with factamphos recorded maximum shoot weight (15.96g), which was on par with *Pseudomonas* sp. (PSB-149) (14.16g). These two treatments were statistically superior to other

bioinoculants. *Providencia* sp. (PSB-14) recorded significantly higher shoot weight than *Trichoderma* sp. (PSF-174) and uninoculated control (Table 22).

Among treatments with factamphos *Pseudomonas* sp. (PSB-149) recorded maximum shoot weight (49.78 g) at harvest, followed by *Providencia* sp. (PSB-14) which recorded a shoot weight of 49.55 g and these two treatments were statistically on par (Table 22). These treatments were statistically superior to all other treatments. All other bioinoculants were on par.

4.7.1.4 **Effect of P solubilizers on root weight of plants**

4.7.1.4.1 ***In absence of applied phosphorous***

In the absence of applied P, KAU reference culture recorded maximum root weight (0.79g) at 20 DAS. This treatment was staistically superior to all other treatments. *Pseudomonas* sp. (PSB-149) and *Trichoderma* sp. (PSF-174) were on par. These two treatments were significantly superior to *Providencia* sp. (PSB-14) and control (Table 23).

At 40 DAS, *Pseudomonas* sp. (PSB-149) recorded maximum root weight (1.95 g) which was on par with *Providencia* sp. (PSB-14) (Table 23). This treatment was statistically superior to all other bioinoculants. *Providencia* sp. (PSB-14), *Trichoderma* sp. (PSF-174) and KAU reference culture were on par. However, all the bioinoculants recorded significantly higher root weight than control.

Providencia sp. (PSB-14) recorded maximum root weight (4.52g) at harvest which was statistically superior to all other treatments (Table 23). *Pseudomonas* sp. (PSB-149) recorded significantly higher root weight (3.82g) than *Trichoderma* sp. (PSF-174) and KAU reference culture. All other bioinoculants were on par.

Plate 27 : Effect of bioinoculants in the presence of factamphos

4.7.1.4.2 ***In presence of rock phosphate***

At 20 DAS, KAU reference culture in presence of rock phosphate recorded maximum root weight (0.63g) which was on par with *Pseudomonas* sp. (PSB-149) (0.62 g). These two treatments were statistically superior to all other treatments. All other bioinoculants were on par (Table 23).

All the bioinoculants in combination with rock phosphate recorded significantly higher root weight than control (Table 23) at 40 DAS. Among the bioinoculants, *Pseudomonas* sp. (PSB-149) recorded maximum root weight (2.19g) which was on par with KAU reference culture. This treatment was statistically superior to all other bioinoculants. KAU reference culture was significantly superior to *Providencia* sp. (PSB-14) and on par with *Trichoderma* sp. (PSF-174).

At harvest , all bioinoculants recorded significantly higher root weight than uninoculated control (Table 23). However, *Pseudomonas* sp. (PSB-149) recorded maximum root weight of 4.30 g.

4.7.1.4.3 ***In presence of factamphos***

At 20 DAS, *Pseudomonas* sp. (PSB-149) in presence of factamphos recorded maximum root weight (0.90 g) which was on par with KAU reference culture (0.80) and *Trichoderma* sp. (PSF-174) (0.72g). These three treatments were significantly superior to all other treatments (Table 23).

At 40 DAS, *Pseudomonas* sp. (PSB-149) recorded maximum root weight (2.62 g) which was significantly superior to all other bioinoculants (Table 23). All other bioinoculants were on par.

Table 22 Effect of p-solubilizers on shoot weight of cowpea

Bioinoculants	Shoot weight of cowpea plant (g)								
	In the absence of applied P			In the presence of rock phosphate			In the presence of factamphos		
	At 20 DAS	At 40 DAS	At harvest	At 20 DAS	At 40 DAS	At harvest	At 20 DAS	At 40 DAS	At harvest
Providencia sp. (PSB-14)	0.17^{c}	8.50^{ab}	46.32^{a}	0.22^{cd}	11.36^{a}	44.45^{b}	1.73^{a}	11.63^{b}	49.55^{a}
Pseudomonas sp. (PSB-149)	0.24^{c}	10.11^{a}	44.25^{a}	0.41^{c}	10.62^{a}	52.07^{a}	1.08^{c}	14.16^{a}	49.78^{a}
Trichoderma sp. (PSF-174)	1.19^{a}	5.31^{c}	37.06^{b}	1.32^{a}	6.93^{b}	39.51^{bc}	1.38^{b}	9.66b^{c}	43.71^{b}
KAU reference culture	0.54^{b}	8.05^{b}	36.26^{b}	1.02^{b}	6.37^{b}	39.83^{bc}	1.31^{bc}	15.96^{a}	43.48^{b}
Control	0.03^{d}	2.68^{d}	28.69^{c}	0.13^{d}	6.26^{b}	35.10^{c}	0.25^{d}	7.35^{c}	39.13^{b}

Table 23 Effect of p-solubilizers on root weight of cowpea

Bioinoculants	Root weight of cowpea plant (g)								
	In the absence of applied P			In the presence of rock phosphate			In the presence of factamphos		
	At 20 DAS	At 40 DAS	At harvest	At 20 DAS	At 40 DAS	At harvest	At 20 DAS	At 40 DAS	At harvest
Providencia sp. (PSB-14)	0.25^{c}	1.76^{ab}	4.52^{a}	0.38^{b}	1.46^{c}	4.28^{a}	0.49^{b}	1.69^{b}	4.32^{b}
Pseudomonas sp. (PSB-149)	0.43^{b}	1.95^{a}	3.82^{b}	0.62^{a}	2.19^{a}	4.30^{a}	0.90^{a}	2.62^{a}	5.62^{a}
Trichoderma sp. (PSF-174)	0.49^{b}	1.60^{b}	3.12^{c}	0.39^{b}	1.67^{bc}	3.82^{a}	0.72^{a}	1.88^{b}	4.02^{b}
KAU reference culture	0.79^{a}	1.52^{b}	2.92^{c}	0.63^{a}	1.96^{ab}	3.70^{a}	0.80^{a}	1.85^{b}	4.52^{b}
Control	0.22^{c}	1.17^{c}	2.60^{c}	0.30^{b}	0.94^{d}	2.62^{b}	0.43^{b}	1.12^{b}	2.64^{c}

All the bioinoculants recorded significantly higher root weight than control (Table 23) at harvest. *Pseudomonas* sp. (PSB-149) in combination with factamphos recorded maximum root weight (5.62 g) which was statistically superior to all other bioinoculants. *Providencia* sp. (PSB-14), *Trichoderma* sp. (PSF-174) and KAU reference culture were on par.

4.7.1.5 Effect of P solubilizers on number of nodules

4.7.1.5.1 *In absence of applied phosphorous*

At 20 DAS, all the bioinoculants except *Trichoderma* sp. (PSF-174) recorded significantly higher no. of nodules than control (Table 24). In the absence of applied P, KAU reference culture recorded maximum no. of nodules (14.33), followed by *Pseudomonas* sp. (PSB-149) which recorded 13.67 no. of nodules and these two treatments were statistically on par. *Providencia* sp. (PSB-14) and *Pseudomonas* sp. (PSB-149) were on par and both of them were superior to uninoculated control.

All the bioinoculants in the absence of applied P at 40 DAS recorded significantly higher no. of nodules than control (Table 24). Among the bioinoculants, *Pseudomonas* sp. (PSB-149) recorded maximum no. of nodules (32.00) which was on par with KAU reference culture (28.33). This treatment was statistically superior to *Providencia* sp. (PSB-14) and *Trichoderma* sp. (PSF-174) All other bioinoculants were on par.

At harvest, *Pseudomonas* sp. (PSB-149) recorded maximum no. of nodules (41.00) which was statistically superior to other bioinoculants. KAU reference culture was recorded significantly higher no. of nodules (32.33) than *Trichoderma* sp. (PSF-174), and this was on par with *Providencia* sp. (PSB-14) However, all the bioinoculants recorded significantly higher no of nodules than control (Table 24).

4.7.1.5.2 ***In presence of rock phosphate***

At 20 DAS in the presence of rock phosphate, *Pseudomonas* sp. (PSB-149) recorded maximum no. of nodules (14.67) which was on par with KAU reference culture and *Providencia* sp. (PSB-14) (13.00). This treatment was statistically superior to *Trichoderma* sp. (PSF-174) and control. KAU reference culture and *Providencia* sp. (PSB-14) were on par and both of them were superior to uninoculated control (Table 24).

All bioinoculants recorded significantly higher no. of nodules than control (Table 24) at 40 DAS. *Pseudomonas* sp. (PSB-149) recorded maximum no. of nodules (42.00), and this was superior to all other bioinoculants. *Providencia* sp. (PSB-14) was on par with KAU reference culture. This treatment was statistically superior to *Trichoderma* sp. (PSF-174). KAU reference culture and *Trichoderma* sp. (PSF-174) were on par.

At harvest, *Pseudomonas* sp. (PSB-149) in presence of rock phosphate recorded maximum no. of nodules (45.00) which was on par with *Providencia* sp. (PSB-14) that recorded (40.00) and KAU reference culture with 37.67 (Table 24). This treatment was statistically superior to *Trichoderma* sp. (PSF-174). All other bioinoculants were on par. However, all the bioinoculants recorded significantly higher no of nodules than control.

4.7.1.5.3 ***In presence of factamphos***

At 20 DAS, KAU reference culture in presence of factamphos recorded maximum no. of nodules 16.67, followed by *Providencia* sp. (PSB-14) (40.00). These two treatments were on par with *Pseudomonas* sp. (PSB-149) and significantly superior to *Trichoderma* sp. (PSF-174) (Table 24).

Among treatments with factamphos, *Providencia* sp. (PSB-14) recorded maximum no. of nodules (40.33) at 40 DAS. This was on par with KAU reference culture and significantly superior to other treatments (Table 24). KAU reference culture, *Trichoderma* sp. (PSF-174) and *Pseudomonas* sp. (PSB-149) were on par. However, all the bioinoculants recorded statistically superior no of nodules than control.

All the bioinoculants recorded significantly higher no of nodules than control (Table 24) at harvest. *Pseudomonas* sp. (PSB-149) in combination with factamphos recorded maximum no. of nodules (55.33) which was significantly superior to all other treatments. KAU reference cultue recorded significantly higher no. of nodules than *Trichoderma* sp. (PSF-174) and *Providencia* sp. (PSB-14).

4.7.1.6. **Days taken for flowering**

4.7.1.6.1 **In the absence of applied phosphorous**

In the absence of applied phosphorous, days taken to flowering did not show much difference with different treatments (Table 25). However, all the bioinoculants taken 60 days for flowering compared to control.

4.7.1.6.2 **In presence of rock phosphate**

In the presence of rock phosphate, days taken to flowering did not show much difference with different treatments (Table 25). All treatments were on par (60.00).

4.7.1.6.3 **In presence of factamphos**

Bioinoculants, in general, induced early flowering and the minimum time was redorded in KAU reference culture (58 days). This was followed by *Pseudomonas* sp. (PSB-149) (Table.25).

4.7.1.7 **Days taken for fruiting**

4.7.1.7.1 **In the absence of applied phosphorous**

In the absence of applied phosphorous, days taken to flowering did not show much difference with different treatments (Table 25). However all the bioinoculants taken minimum number of days for fruiting (65.00) than uninoculated control (70.33).

4.7.1.7.2 **In presence of rock phosphate**

In the presence of rock phosphate, days taken for fruiting did not show much difference with different treatments (Table 25). All treatments were on par (65.00).

4.7.1.7.3 **In presence of factamphos**

Bioinoculants in combination with factamphos did not show much difference with different treatments (Table 25). However, KAU reference culture taken minimum number of days for fruiting (63.00) followed by *Pseudomonas* sp. (PSB-149) (64.00). All other treatments were on par.

Table 24 Effect of p-solubilizers on no. of nodules

Bioinoculants	No. of nodules of cowpea plant								
	In the absence of applied P			In the presence of rock phosphate			In the presence of factamphos		
	At 20 DAS	At 40 DAS	At harvest	At 20 DAS	At 40 DAS	At harvest	At 20 DAS	At 40 DAS	At harvest
Providencia sp. (PSB-14)	11.00^{bc}	25.33^{b}	29.00^{bc}	13.00^{ab}	36.00^{b}	40.00^{ab}	16.33^{a}	40.33^{a}	35.00^{c}
Pseudomonas sp. (PSB-149)	13.67^{ab}	32.00^{a}	41.00^{a}	14.67^{a}	42.00^{a}	45.00^{a}	15.00^{ab}	30.33^{b}	55.33^{a}
Trichoderma sp. (PSF-174)	9.33^{cd}	24.00^{b}	28.00^{c}	11.67^{bc}	29.33^{c}	35.00^{b}	11.67^{b}	31.00^{b}	36.00^{c}
KAU reference culture	14.33^{a}	28.33^{ab}	32.33^{b}	14.33^{ab}	33.33^{bc}	37.67^{ab}	16.67^{a}	34.33^{ab}	43.00^{b}
Control	8.00^{d}	14.33^{c}	17.00^{d}	9.67^{c}	16.33^{d}	24.00^{c}	11.00^{b}	22.00^{c}	23.33^{d}

Table 25 Effet of p solubilizers on flowering and fruiting

Bioinoculants	In the absence of applied P		In the presence of rock phosphate		In the presence of factamphos	
	Days to flowering	Days to fruiting	Days to flowering	Days to fruiting	Days to flowering	Days to fruiting
Providencia sp. (PSB-14)	60.00	65.00	60.00	65.00	60.00	65.00
Pseudomonas sp. (PSB-149)	60.00	65.00	60.00	65.00	58.67	64.00
Trichoderma sp. (PSF-174)	60.00	65.00	60.00	65.00	60.00	65.00
KAU reference culture	60.00	65.00	60.00	65.00	58.00	63.00
Control	67.00	70.33	60.00	65.00	63.67	65.00

4.7.1.8 **Effect of P solubilizers on number of pods /plant**

4.7.1.8.1 ***In absence of applied phosphorous***

In the absence of applied P, KAU reference culture recorded maximum no. of pods/plants (38.33) which was statistically superior to all other treatments (Table 26). *Pseudomonas* sp. (PSB-149) and *Trichoderma* sp. (PSF-174) recorded significantly higher no. of pods (31.67 and 38.33 respectively) than *Providencia* sp. (PSB-14) and uninoculated control.

4.7.1.8.2 ***In presence of rock phosphate***

In presence of rock phosphate, all the bioinoculants recorded significantly higher no of pods/plant than control (Table 26). Among the bioinoculants, *Providencia* sp. (PSB-14) recorded maximum no. of pods (41.67) followed by KAU refrence culture (41.33). These two treatments were on par with *Pseudomonas* sp. (PSB-149) and statistically superior to all other bioinoculants. *Pseudomonas* sp. (PSB-149) and *Trichoderma* sp. (PSF-174) were on par.

4.7.1.8.3 ***In presence of factamphos***

All the bioinoculants recorded significantly higher no of pods compared to control (Table 26). Among treatments which received factamphos, *Psedomonas* sp. (PSB-149) recorded maximum no. of pods (62.33) which was statistically superior to all other treatments. KAU reference culture was on par with *Trichoderma* sp. (PSF-174) and this was superior to *Providencia* sp. (PSB-14)

4.7.1.9 **Effect of P solubilizers on pod length**

4.7.1.9.1 ***In absence of applied phosphorous***

In the absence of applied P, KAU reference culture recorded maximum pod length (19.67 cm) which was on par with *Pseudomonas* sp. (PSB-149) (17.67 cm)

and *Trichoderma* sp.(PSF-174).(17.33 cm) (Table 26). This treatment was significantly superior than *Providencia* sp. (PSB-14) and uninoculated control. All other bioinoculants were on par.

4.7.1.9.2 ***In presence of rock phosphate***

Pseudomonas sp. (PSB-149) in combination with rock phosphate recorded maximum pod length (23 cm) followed by *Trichoderma* sp. (PSF-174) (22 cm) and KAU reference culture (22 cm). These three treatments were significantly superior than *Providencia* sp. (PSB-14) and uninoculated control (Table 26).

4.7.1.9.3 ***In presence of factamphos***

Among treatments with factamphos, KAU reference culture recorded maximum pod length (23.33cm) followed by *Pseudomonas* sp. (PSB-149) (23.00 cm). These two treatments were on par with *Providencia* sp. (PSB-14) and *Trichoderma* sp. (PSF-174) and superior than control. All other treatments were on par (Table 26).

4.7.1.10 **Effect of P solubilizers on number of grains per pod**

4.7.1.10.1 ***In absence of applied phosphorous***

In the absence of applied P, *Trichoderma* sp. (PSF-174) recorded maximum no. of grains per pod (14.67g) which was on par with *Providencia* sp. (PSB-14) and *Pseudomonas* sp. (PSB-149) (Table 26). This treatment was statistically superior to KAU reference culture and uninoculated control. All other treatments were on par.

4.7.1.10.2 ***In presence of rock phosphate***

Among treatments with rock phosphate, no. of grains per pod did not show much difference with different p-solubilizers (Table 26). However , *Pseudomonas* sp. (PSB-149) recorded maximum no. of grains per pod (15.00g) followed by *Providencia* sp. (PSB-14) (14.00g) and KAU reference culture (14.00g).

4.7.1.10.3 ***In presence of factamphos***

In the presence of factamphos, no. of grains per pod did not show much difference with different p-solubilizers (Table 26). However, *Providencia* sp.(PSB-14), *Pseudomonas* sp. (PSB-149) and *Trichoderma* sp. (PSF-174) recorded maximum no. of grains per pod (16.00g) followed by KAU reference culture (15.00g). All the bioinoculants recorded statistically superior no. of grains per pod compared to control.

4.7.1.11 **Effect of P solubilizers on pod yield**

4.7.1.11.1 ***In absence of applied phosphorous***

All the bioinoculants recorded significantly higher pod yield as compared to control (Table 26). Among the bioinoculants, KAU reference culture in the absence of applied P recorded maximum pod yield (216.33g) followed by *Pseudomonas* sp. (PSB-149) (214.00g). These two treatments were statistically superior to other bioinoculants. *Providencia* sp. (PSB-14) and *Trichoderma* sp. (PSF-174) were on par (Fig 2).

4.7.1.11.2 ***In absence of rock phosphate***

Pseuodomonas sp. (PSB-149) in combination with rock phosphate recorded maximum pod yield (344.00 g) and this was statistically superior to all other treatments. KAU reference culture recorded significantly higher pod yield (272.67g) than *Trichoderma* sp. (PSF-174) and on par with *Providencia* sp.

(235.00g). *Providencia* sp. (PSB-14) and *Trichoderma* sp. (PSF-174) were on par (Table 26). However, all the bioinoculants recorded significantly superior pod yield compared to control.

4.7.1.11.3 ***In presence of factamphos***

Among treatments with factamphos, pod yield did not show much difference with different p-solubilizers. However, *Providencia* sp. (PSB-14) recorded maximum pod yield (349.00g) followed by *Pseudomonas* sp. (PSB-149) (343.67g). All the bioinoculants recorded statistically superior pod yield compared to uninoculated control (Table 26).

4.7.2 **Chemical Analysis**

4.7.2.1 ***Chemical analysis of plant sample***

4.7.2.1.1 **Effect of P solubilizers on phosphorous content in shoot of plant**

4.7.2.1.1.1 ***Absence of applied phosphorous***

At 20 DAS, *Pseudomonas* sp. (PSB-149) recorded maximum phosphorous content in shoot (0.20%) followed by KAU reference culture (0.15%) and both of them were significantly higher than control (Table 27). These two treatments were on par with *Providencia* sp. (PSB-14) and *Trichoderma* sp. (PSF-174) All other treatments were on par.

At 40 DAS, *Pseudomonas* sp. (PSB-149) in the absence of applied P recorded maximum phosphorous content (0.30%) which was on par with all other bioinoculants (Table 27) . This treatment was statistically superior to uninoculated control. All other treatments were on par.

Table 26 Effect of p-solubilizers on no. of pods/plant, pod length, no. of grains /pod and pod yield

Bioinoculants	In the absence of applied P				In the presence of rock phosphate				In the presence of factamphos			
	No.of pods/plant	Pod length (cm)	No.of grains/pod	Pod yield (g)	No.of pods/plant	Pod length (cm)	No.of grains/pod	Pod yield (g)	No.of pods/plant	Pod length (cm)	No.of grains/pod	Pod yield (g)
Providencia sp. (PSB-14)	25.33^{c}	16.00^{b}	13.33^{ab}	150.67^{b}	41.67^{a}	16.00^{b}	14.00^{a}	235.00^{bc}	42.33^{c}	22.00^{ab}	16.00^{a}	349.00^{a}
Pseudomonas sp. (PSB-149)	31.67^{b}	17.67^{ab}	13.33^{ab}	214.00^{a}	37.00^{ab}	23.00^{a}	15.00^{a}	344.00^{a}	62.33^{a}	23.00^{a}	16.00^{a}	343.67^{a}
Trichoderma sp. (PSF-174)	31.33^{b}	17.33^{ab}	14.67^{a}	126.00^{b}	36.33^{b}	22.00^{a}	13.00^{a}	199.00^{c}	45.67^{bc}	21.67^{ab}	16.00^{a}	309.00^{a}
KAU reference culture	38.33^{a}	19.67^{a}	11.33^{b}	216.33^{a}	41.33^{a}	22.00^{a}	14.00^{a}	272.67^{b}	49.67^{b}	23.33^{a}	15.00^{a}	335.33^{a}
Control	13.33^{d}	15.33^{b}	10.67^{b}	59.67^{c}	14.33^{c}	15.67^{b}	11.67^{a}	101.67^{d}	34.33^{d}	17.67^{b}	11.67^{b}	217.33^{b}

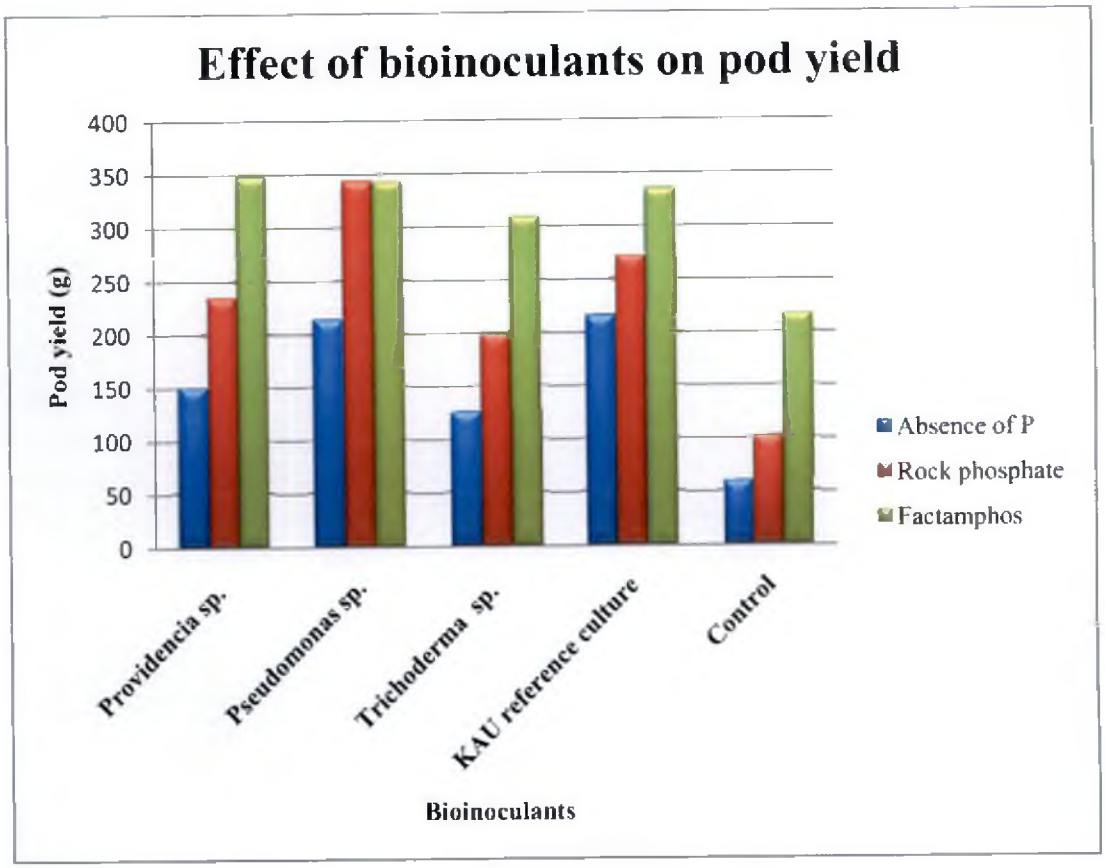


Fig 2. Effect of bioinoculants on pod yield

Pseudomonas sp. (PSB-149) recorded maximum phosphorous content in shoot (0.36%) at harvest followed by *Providencia* sp. (PSB-14) (0.32%) and both of them were statistically superior compared to control. These two treatments were on par with KAU reference culture and *Trichoderma* sp. (PSF-174)(Table 27).

4.7.2.1.1.2 ***In presence of rock phosphate***

At 20 DAS, phosphorous content in shoot did not show much difference with different p-solubilizers (Table 27). However, among the bioinoculants , *Pseudomonas* sp. (PSB-149) recorded maximum phosphorous content (0.21%) followed by *Trichoderma* sp. (PSF-174) (0.18) and KAU reference culture .

Pseudomonas sp. (PSB-149) at 40 DAS, in combination with rock phosphate recorded maximum phosphorous content (0.31%) which was on par with all other bioinoculants. This treatment was significantly superior than control (Table 27). *Providencia* sp. (PSB-14), *Trichoderma* sp. (PSF-174) and KAU reference culture were on par

In the presence of rock phosphate, *Pseudomonas* sp. (PSB-149) recorded maximum phosphorous content in shoot (0.41%) at harvest which was statistically superior to uninoculated control (Table 27). This treatment was on par with *Providencia* sp. (PSB-14), *Trichoderma* sp. (PSF-174) and KAU reference culture. All other treatments were on par.

4.7.2.1.1.3 ***In presence of factamphos***

At 20 DAS, *Pseudomonas* sp. (PSB-149) in presence of factamphos recorded maximum phosphorous content in shoot (0.26%) which was significantly higher compared to control. This treatment was on par with *Providencia* sp. (PSB-14), *Trichoderma* sp. (PSF-174) and KAU reference culture (Table 27). All other treatments were on par.

At 40 DAS, in presence of factamphos, phosphorous content in shoot did not show much difference with different p-solubilizers (Table 27). Among the bioinoculants, *Pseudomonas* sp. (PSB-149) recorded maximum phosphorous content in shoot (0.35%) followed by KAU reference culture (0.29%). However, all the bioinoculants recorded significantly higher phosphorous content compared to uninoculated control.

All the bioinoculants at harvest in combination with factamphos recorded significantly higher phosphorous content in shoot than control. *Pseudomonas* sp. (PSB-149) recorded maximum phosphorous content (0.46%) followed by KAU reference culture (0.45%). These two treatments were statistically superior to *Providencia* sp. (PSB-14) and *Trichoderma* sp. (PSF-174) All other bioinoculants were on par (Table 27).

4.7.2.1.2 **Effect of P solubilizers on phosphorous content in roots of plants**

4.7.2.1.2.1 ***In absence of applied phosphorous***

At 20 DAS, KAU reference culture recorded maximum phosphorous content in root (0.027%), which was statistically superior than all other treatments (Table 28). This treatment was on par with *Pseudomonas* sp. (PSB-149). *Pseudomonas* sp. (PSB-149) was on par with *Providencia* sp. (PSB-14) and *Trichoderma* sp. (PSF-174) and this was statistically superior than control. All other bioinoculants were on par.

KAU reference culture at 40 DAS recorded maximum phosphorous content in root (0.066%) which was significantly higher compared to all other treatments (Table 28). *Trichoderma* sp. (PSF-174) and *Providencia* sp. (PSB-14) were on par with *Pseudomonas* sp. (PSB-149) These two treatments were statistically superior to control. All other treatments were on par.

At harvest, KAU reference culture recorded maximum phosphorous content in root (0.096%) which was on par with *Trichoderma* sp. (PSF-174) and *Pseudomonas* sp. (PSB-149) . This treatment was significantly superior compared to *Providencia* sp. and uninoculated control (Table 28). *Trichoderma* sp. (PSF-174) (0.066%) was on par with *Pseudmonas* sp. (PSB-149) and *Providencia* sp. (PSB-14) This treatment was significantly superior to control. All other treatments were on par.

4.7.2.1.2.2 ***In presence of rock phosphate***

At 20 DAS, *Trichoderma* sp. (PSF-174) in presence of rock phosphate recorded maximum phosphorous content in root (0.053%) followed by KAU reference culture (0.050 %). These two treatments were significantly higher compared to other treatments (Table 28) . All other treatments were on par.

KAU reference culture at 40 DAS in combination with rock phosphate recorded maximum phosphorous content in root (0.130 %) which was statistically superior than all other treatments (Table 28). *Pseudomonas* sp. (PSB-149) recorded phosphorous content of 0.080% which was significantly higher than other bioinoculants. *Providencia* sp. (PSB-14) and *Trichoderma* sp. (PSF-174) were on par.

At harvest, KAU reference culture recorded maximum phosphorous content (0.673%) which was significantly higher than all other bioinoculants (Table 28). *Providencia* sp. (PSB-14), *Pseudomonas* sp. (PSB-149) and *Trichoderma* sp. (PSF-174) were on par.

4.7.2.1.2.3 ***In presence of factamphos***

Phosphorous content in root at 20 DAS did not show much difference with different p-solubilizers (Table 28). However, among the bioinoculants,

Pseudomonas sp. (PSB-149) and KAU reference culture recorded maximum phosphorous content (0.06%). All the bioinoculants recorded significantly higher phosphorous content than control.

KAU reference culture at 40 DAS in presence of factamphos recorded maximum phosphorous content (0.14%) which was on par with *Pseudomonas* sp. (PSB-149). This treatment was statistically superior compared to other treatments. *Pseudomonas* sp. (PSB-149) was on par with *Providencia* sp. (PSB-14) and these two treatments were significantly superior than *Trichoderma* sp. (PSF-174) and uninoculated control. All other treatments were on par (Table 30).

All the bioinoculants recorded significantly higher phosphorous content than control at harvest. KAU reference culture in presence of factamphos recorded maximum phosphorous content (0.23%) which was statistically superior than all other bioinoculants. *Pseudomonas* sp. (PSB-149) recorded 0.16 % of phosphorous content which was significantly superior to *Trichoderma* sp. (PSF-174) and *Providencia* sp. (PSB-14) *Trichoderma* sp. (PSF-174) recorded a statistically superior phosphorous content in root than *Providencia* sp. (PSB-14) (Table 28).

4.7.2.1.3 **Effect of P solubilizers on phosphorous content in grains**

4.7.2.1.3.1 ***In the absence of applied phosphorous***

All the bioinoculants in the absence of applied P recorded statistically superior phosphorous content in grain compared to control (Table 29). There was no significant difference in phosphorous content in grains among different bioinoculants. However, KAU reference culture recorded maximum P content in grains (0.34 %) followed by *Providencia* sp. (0.30 %) and *Pseudomonas* sp. (0.30%).

Table 27 Effect of p-solubilizers on P content in shoot of cowpea

Bioinoculants	P content in shoot (%)								
	In the absence of applied P			In the presence of rock phosphate			In the presence of factamphos		
	At 20 DAS	At 40 DAS	At harvest	At 20 DAS	At 40 DAS	At harvest	At 20 DAS	At 40 DAS	At harvest
Providencia sp. (PSB-14)	0.13^{ab}	0.22^{ab}	0.32^{a}	0.14^{a}	0.24^{ab}	0.36^{ab}	0.18^{ab}	0.28^{a}	0.37^{b}
Pseudomonas sp. (PSB-149)	0.20^{a}	0.30^{a}	0.36^{a}	0.21^{a}	0.31^{a}	0.41^{a}	0.26^{a}	0.35^{a}	0.46^{a}
Trichoderma sp. (PSF-174)	0.12^{ab}	0.20^{ab}	0.25^{ab}	0.18^{a}	0.27^{ab}	0.36^{ab}	0.20^{ab}	0.28^{a}	0.37^{b}
KAU reference culture	0.15^{a}	0.25^{ab}	0.28^{ab}	0.18^{a}	0.27^{ab}	0.35^{ab}	0.19^{ab}	0.29^{a}	0.45^{a}
Control	0.02^{b}	0.13^{b}	0.17^{b}	0.07^{a}	0.14^{b}	0.22^{b}	0.12^{b}	0.16^{b}	0.24^{c}

Table 28 Effect of p-solubilizers on P content in root of cowpea

Bioinoculants	P content in root (%)								
	In the absence of applied P			In the presence of rock phosphate			In the presence of factamphos		
	At 20 DAS	At 40 DAS	At harvest	At 20 DAS	At 40 DAS	At harvest	At 20 DAS	At 40 DAS	At harvest
Providencia sp. (PSB-14)	0.01^{bc}	0.040^{b}	0.033^{bc}	0.016^{b}	0.040^{c}	0.070^{b}	0.04^{a}	0.09^{b}	0.12^{d}
Pseudomonas sp. (PSB-149)	0.020^{ab}	0.036^{bc}	0.060^{abc}	0.023^{b}	0.080^{b}	0.050^{b}	0.06^{a}	0.12^{ab}	0.16^{b}
Trichoderma sp. (PSF-174)	0.013^{bc}	0.043^{b}	0.066^{ab}	0.053^{a}	0.050^{c}	0.050^{b}	0.05^{a}	0.04^{c}	0.13^{c}
KAU reference culture	0.027^{a}	0.066^{a}	0.096^{a}	0.050^{a}	0.130^{a}	0.673^{a}	0.06^{a}	0.14^{a}	0.23^{a}
Control	0.003^{c}	0.016^{c}	0.020^{c}	0.013^{b}	0.026^{c}	0.026^{b}	0.01^{b}	0.03^{c}	0.03^{e}

4.7.2.1.3.2 ***In presence of rock phosphate***

There was no significant difference in phosphorous content in grains among different bioinoculants in the presence of rock phosphate (Table 29). *Pseudomonas* sp. and KAU reference culture recorded maximum P content in grain (0.33 %) which was on par with all other treatments. *Trichoderma* sp. and uninoculated control were on par however, *Trichoderma* sp. recorded maximum P content in grains (0.31 %).

4.7.2.1.3.3 ***In presence of factamphos***

All the bioinoculants in combination with factamphos recorded statistically superior phosphorous content in grains compared to control (Table 29). However, *Pseudomonas* sp. recorded maximum P content (0.39 %) which was statistically significant than all other treatments. *Providencia* sp., *Trichoderma* sp. and KAU reference culture were on par.

4.7.3 **Chemical analysis of soil**

4.7.3.1 **Effect of P solubilizers on available phosphorous content in soil**

4.7.3.1.1 ***In absence of applied phosphorous***

In the absence of applied P, *Pseudomonas* sp. (PSB-149) recorded maximum available phosphorous (32.67 mg/kg of soil) at 20 DAS, which was statistically superior than KAU reference culture and control (Table 30). This treatment was on par with *Providencia* sp. (PSB-14) (28.00 mg/kg) and *Trichoderma* sp. (PSF-174) (27.33 mg/kg). All other treatments were on par.

At 40 DAS, in the absence of applied P, available phosphorous did not show much difference with different p-solubilizers (Table 30). However , KAU reference culture recorded maximum available P (18.33 mg/kg) followed by *Providencia* sp. (PSB-14) (17.00 mg/kg).

Table 29. Effect of P-solubilizers on P content in grains

Bioinoculants	In the absence of applied P	In presence of rock phospahte	In presence of factamphos
Providencia sp. (PSB-14)	0.30[a]	0.32[a]	0.35[b]
Pseudomonas sp. (PSB149)	0.30[a]	0.33[a]	0.39[a]
Trichoderma sp. (PSF-174)	0.28[a]	0.31[ab]	0.35[b]
KAU reference culture	0.34[a]	0.33[a]	0.32[b]
Control	0.19[b]	0.25[b]	0.28[c]

At harvest, available phosphorous did not show much difference with different p-solubilizers. Among the bioinoculants, *Providencia* sp. (PSB-14) and KAU reference culture recorded maximum available phosphorous (13.67 mg/kg). However, all bioinoculants recorded significantly superior available phosphorous than control (Table 30).

4.7.3.1.2 ***In presence of rock phosphate***

At 20 DAS, *Pseudomonas* sp. (PSB-149) recorded maximum available phosphorous (35.67 mg/kg) which was statistically superior to uninoculated control and on par with other bioinoculants (Table 30). All other treatments were on par.

Providencia sp. (PSB-14) at 40 DAS, in combination with rock phosphate recorded maximum available phosphorous (27.00 mg/kg), which was on par with KAU reference culture (23.33 mg/kg). This treatment was significantly higher than all other bioinoculants (Table 30). KAU reference culture was on par with *Pseudomonas* sp. (PSB-149) and significantly higher than *Trichoderma* sp. (PSF-174) and control.

At harvest, *Pseudomonas* sp. (PSB-149) recorded maximum available phosphorous (17.67 mg/kg) which was on par with *Providencia* sp. (PSB-14) (15.33mg/kg). This two treatments were significantly superior than control. *Providencia* sp. (PSB-14), KAU reference culture and *Trichoderma* sp. (PSF-174) were on par (Table 30).

4.7.3 .1.3. ***In presence of factamphos***

At 20 DAS, among treatments which received factamphos, *Pseudomonas* sp. (PSB-149) recorded maximum available phosphorous (47.33 mg/kg) followed by KAU reference culture (46.33 mg/kg). These two treatments were on par with

Providencia sp. (PSB-14) and significantly higher than *Trichoderma* sp. (PSF-174) (Table 30) and uninoculated control. *Providencia* sp. was on par with *Trichoderma* sp. (PSF-174) and significantly superior than uninoculated control.

Pseudomonas sp. (PSB-149) in combination with factamphos at 40 DAS recorded maximum available phosphorous (28.67 mg/kg), which was on par with *Trichoderma* sp. (PSF-174), *Providencia* sp. (PSB-14) and KAU reference culture (Table 30). This treatment was statistically superior compared to uninoculated control. All other treatments were on par.

At harvest, *Pseudomonas* sp. (PSB-149) recorded maximum available phosphorous (22.67 mg/kg) which was on par with *Providencia* sp. (PSB-14), *Trichoderma* sp. (PSF-174) and KAU reference culture (Table 30). This treatment was statistically superior to control. All other bioinoculants were on par.

4.7.4 **Population of p-solubilizers in soil**

4.7.4.1 ***In the absence of applied phosphorous***

All bioinoculants at 20 DAS recorded significantly higher population than control (Table 31). In the absence of applied phosphorous, *Providencia* sp. (PSB-14) recorded maximum population (29.67 X 10^4 cfu/g) followed by *Pseudomonas* sp. (PSB-149) (29.00 X10^4 cfu/g). These two treatments were statistically superior to other bioinoulants. KAU reference culture recorded significantly higher population than *Trichoderma* sp. (PSF-174).

At 40 DAS, all bioinoculants recorded significantly higher population as compared to control (Table 31). Among the bioinoculants, *Providencia* sp. (PSB-14) recorded maximum population (28.00 X10^4 cfu/g) followed by *Pseudomonas* sp. (PSB-149) (26.33 X10^4 cfu/g). These two treatments were statistically superior to other bioinoculants. KAU reference culture recorded significantly higher population than *Trichoderma* sp. (PSF-174).

At harvest, *Providencia* sp. (PSB-14) recorded maximum population (20.67 $X10^4$ cfu/g) which was on par with *Pseudomonas* sp. (PSB-149) and *Trichoderma* sp. (PSF-174) (19.33 $X10^4$ cfu/g and 18.67 $X10^4$ cfu/g respectively) (Table 31). This treatment was significantly superior than KAU reference culture and control. *Pseudomonas* sp., (PSB-149) *Trichoderma* sp. (PSF-174) and KAU reference culture were on par. However, all bioinoculants recorded significantly higher population than control.

4.7.4.2 ***In presence of rock phosphate***

All the bioinoculants recorded significantly higher population than control (Table 31) at 20 DAS. Among treatments with rock phosphate, *Pseudomonas* sp. (PSB-149) recorded maximum population (66.00 $X10^4$ cfu/g) which was significantly higher than all other treatments. KAU reference culture recorded significantly higher population (44.00 $X10^4$ cfu/g) than *Providencia* sp. (PSB-14) and *Trichoderma* sp. (PSF-174). *Providencia* sp. (PSB-14) recorded significantly higher population than *Trichoderma* sp.(PSF-174).

At 40 DAS, *Pseudomonas* sp. (PSB-149) in presence of rock phosphate recorded maximum population(55.00 $X10^4$ cfu/g) than all other treatments (Table 31). This treatment was significantly higher than all other bioinoculants. KAU reference culture recorded significantly higher population (33.33 $X10^4$ cfu/g) than *Providencia* sp. (PSB-14) and *Trichoderma* sp. (PSF-174).

All bioinoculants recorded significantly higher population than control at harvest. Among bioinoculants, *Pseudomonas* sp. (PSB-149) recorded maximum population (33.00 $X10^4$ cfu/g) which was significantly superior to all other bioinoculants. *Providencia* sp. (PSB-14) and *Trichoderma* sp. and KAU reference culture were on par (Table 31).

4.7.4.3 ***In presence of factamphos***

Among treatments which received factamphos at 20 DAS, *Trichoderma* sp. (PSF-174) recorded maximum population, 62.33 $X10^4$ cfu/g which was significantly superior than all other treatments (Table 31). KAU reference culture and *Providencia* sp. (PSB-14) were on par and these two treatments were significantly higher than *Pseudomonas* sp. (PSB-149) However, all the bioinoculants recorded significantly higher population than uninoculated control.

At 40 DAS, all the bioinoculants recorded significantly higher population than control (Table 31). *Trichoderma* sp. (PSF-174) in the presence of factamphos, recorded maximum population (52.33 $X10^4$ cfu/g) which was significantly superior to all other treatments. KAU reference culture recorded a population of 40.33 X 10^4 cfu/g which was significantly superior to *Providencia* sp. (PSB-14) This treatment was on par with *Pseudomonas* sp. (PSB-149) *Providencia* sp. (PSB-14) and *Pseudomonas* sp. (PSB-149) were on par.

At harvest, *Trichoderma* sp. (PSF-174) in presence of factamphos recorded maximum population (28.00 X 10^4 cfu/g) which was on par with *Providencia* sp. (PSB-14) and KAU reference culture. This treatment was significantly higher than *Pseudomonas* sp. (PSB-149) and uninoculated control. *Providencia* sp. (PSB-14), KAU reference culture and *Pseudomonas* sp. (PSB-149) were on par. These three treatments were significantly superior to control (Table 31).

Table 30 Effect of p-solubilizers on available P content of soil

Bioinoculants	Available P content of soil (mg/kg of soil)								
	In the absence of applied P			In the presence of rock phosphate			In the presence of factamphos		
	At 20 DAS	At 40 DAS	At harvest	At 20 DAS	At 40 DAS	At harvest	At 20 DAS	At 40 DAS	At harvest
Providencia sp. (PSB-14)	28.00^{ab}	17.00^{a}	13.67^{a}	31.00^{ab}	27.00^{a}	15.33^{ab}	43.00^{ab}	27.67^{ab}	18.00^{ab}
Pseudomonas sp. (PSB-149)	32.67^{a}	16.33^{a}	13.33^{a}	35.67^{a}	19.00^{bc}	17.67^{a}	47.33^{a}	28.67^{a}	22.67^{a}
Trichoderma sp. (PSF-174)	27.33^{ab}	14.33^{a}	13.00^{a}	33.33^{ab}	17.67^{c}	13.33^{bc}	30.67^{bc}	28.33^{ab}	16.67^{ab}
KAU reference culture	22.00^{bc}	18.33^{a}	13.67^{a}	30.67^{ab}	23.33^{ab}	13.67^{bc}	46.33^{a}	26.67^{ab}	16.67^{ab}
Control	18.00^{c}	11.00^{a}	10.67^{b}	21.33^{b}	13.67^{c}	11.33^{c}	23.67^{c}	19.33^{b}	13.00^{b}

Table 31. Population of p-solubilizers in soil

Bioinoculants	Population of PSM (10^4 cfu/g)								
	In the absence of applied P			In the presence of rock phosphate			In the presence of factamphos		
	At 20 DAS	At 40 DAS	At harvest	At 20 DAS	At 40 DAS	At harvest	At 20 DAS	At 40 DAS	At harvest
Providencia sp. (PSB-14)	29.67^{a}	28.00^{a}	20.67^{a}	38.67^{c}	29.00^{c}	20.67^{b}	48.33^{b}	37.00^{c}	22.67^{ab}
Pseudomonas sp. (PSB-149)	29.00^{a}	26.33^{a}	19.33^{ab}	66.00^{a}	55.00^{a}	33.00^{a}	42.00^{c}	38.33^{bc}	21.67^{b}
Trichoderma sp. (PSF-174)	20.33^{c}	18.33^{c}	18.67^{ab}	24.33^{d}	22.33^{d}	19.67^{b}	62.33^{a}	52.33^{a}	28.00^{a}
KAU reference culture	25.33^{b}	21.67^{b}	15.33^{b}	44.00^{b}	33.33^{b}	19.00^{b}	50.33^{b}	40.33^{b}	22.67^{ab}
Control	14.00^{d}	11.00^{d}	10.67^{c}	17.00^{e}	15.00^{e}	11.00^{c}	19^{d}	16.00^{d}	14.00^{c}

Discussion

5. DISCUSSION

Phosphorous, is a major nutrient required for root formation, plant growth, synthesis of starch, protein and fat. Being a constituent of nucleic acids, it is closely concerned with vital plant growth processes. Like any other nutrient, P availability to crops is largely influenced by soil pH. The ideal soil pH is close to neutral and neutral soils are considered to fall within a range from a slightly acidic pH of 6.5 to slightly alkaline pH of 7.5. It has been determined that most plant nutrients are optimally available to plants within this pH range 6.5–7.5. This pH range is generally favourable to plant root growth also.

It is estimated that about 98 per cent of Indian soils contain insufficient amounts of available phosphorus, which is necessary to support maximum plant growth. The uptake of phosphorus by the plant is only a small fraction of what is actually added as phosphate fertilizer (Vassilev and Vassileva, 2003). In Kerala, characterized by heavy rainfall and extreme humid conditions, 90 per cent of the geographic area is covered by laterite soils, which are inherently acidic (Dinesh *et al.*, 2014). At a low pH below 6.5, phosphorus ions react with aluminium (Al) and iron (Fe) and form less soluble compounds. Hence these soils are considered as high P fixing soils. The commonly applied P fertilizer is Mussoorie rock phosphate (MRP), which is slowly available to crop as it contains insoluble tricalcium phosphates.

Soil microorganisms play an important role in making the chemically fixed, insoluble phosphorus available to plants. This process is called phosphate solubilization and the microorganisms responsible, as phosphate solubilizing microorganisms (PSMs). A large number of PSMs have been isolated from different soils in India (Vikram *et al.*, 2007). A majority of the isolated organisms are bacteria, although several fungi are also known to solubilize phosphates. These bacteria and fungi have the potential to be used as biofertilizers. Their role in increasing the soil nutrient value is of utmost importance. Their application to

crop fields has resulted in an increased yield of several crops, such as cereals, legumes, fibres, vegetables, oils, and other crop plants (Kundu *et al.*, 2009).

PSMs not only provide plants with phosphorus, but also facilitate the growth of plants through (a) fixing atmospheric nitrogen (Dobbelaere *et al.*, 2002; Sahin *et al.*, 2004); (b) accelerating the accessibility of other trace elements (Mittal *et al.*, 2008); (c) producing plant hormones such as auxins (Jeon *et al.*, 2003; Egamberdiyeva, 2005), cytokinins (Gracia de Salamone *et al.*, 2001), and gibberellins (Gutierrez-Manero *et al.*, 2001); (d) releasing siderophores (Wani *et al.*, 2007), hydrogen cyanide (Kang *et al.*, 2010), enzymes and/or fungicidal compounds such as chitinase, cellulose, protease (Dey *et al.*, 2004; Lucy *et al.*, 2004; Hamdali *et al.*, 2008) which ensure antagonism against phytopathogenic microorganisms. Therefore, it is worth to believe that production of plant growth promoting substances by PSMs may effectively contribute to their effect on the enhancement of the plant performance (Hameeda *et al.*, 2006).

With this background information, a study was undertaken at the Department of Agricultural Microbiology, College of Horticulture, on 'Exploration of native mineral phosphate solubilizing microorganisms as biofertilizer for the acidic soils of Kerala'. The main objective was to exploit native microorganisms with mineral phosphate solubilization and plant growth promoting activities for the acidic soils of Kerala.

Two hundred microbial isolates (172 bacteria and 28 fungi) from the repository maintained at Dept. of Agricultural Microbiology were screened on Pikovskaya's agar medium. This medium acts as specific medium for PSMs due to the presence of tricalcium phosphate (TCP) which is known for halo zone formation (Sharma, 2005). Solubilization of precipitated TCP in unbuffered solid agar medium plates has been widely used as the initial criterion for the isolation of phosphate solubilizing microorganisms (Pikovskaya, 1948). Microorganisms on precipitated calcium phosphate agar plates produce clear zones around their colonies if they are capable of solubilizing calcium phosphate.

In the present investigation, phosphate solubilization efficiency (PSE) was calculated based on the size of clear zone produced on Pikovskaya's agar. In general, the halo zone and colony diameter were larger in fungi, than bacteria (Table 8 and plate 1 and plate 2). Earlier reports also indicate that fungi form bigger zones on agar plates (Alam *et al.*, 2002). The zone ranged from 2 to 9.7cm for bacteria and 4.07 to 8.84 cm for fungi. Babana *et al.* (2013) reported that halo zones formed by rock phosphate solubilizing microorganisms ranged from 0-5mm for bacteria and 12-17 mm for fungi.

PSE ranged from 106.6% to 555.5% among bacteria and 106.6 % to 291.7 % among fungi. Among the bacterial isolates, PSB-22 (*Providencia alcalifaciens*) recorded maximum PSE of 555.5 per cent and among the fungi; PSF-186 (*Aspergillus niger*) recorded maximum value of 291.7 per cent. The solubilization index of the most efficient bacteria was greater than that of fungi although fungi produced large halo zones compared to bacteria. Antarikanonda *et al.* (1991) also found that fungi were more active in solubilizing phosphate than bacteria. Srivastav *et al.* (2004) reported P-solubilization efficiency in the range of 9.0 to 75.0 per cent for bacterial isolates on solid medium. Ramachandran *et al.* (2007) reported that the PSE of bacteria obtained from the rhizosphere soil of black pepper ranged from 125 to 412.5 per cent.

In the present study, the period of time required to achieve maximum P-solubilization was also recorded for the isolates. The period ranged from 2 to 7 days. Eight isolates including two bacteria and six fungi, reached maximum PSE within two days. In general, fungi required less time to achieve maximum P-solubilization efficiency than bacteria. This could be because of the higher biomass production by fungi, as compared to bacteria. Earlier reports indicated that fungi solubilized more P because they formed larger colonies and their biomass was greater than bacteria (Illmer & Schinner, 1995).

Based on the preliminary screening for phosphate solubilisation on agar medium, twenty most efficient isolates (consisting of ten bacteria and ten fungi)

were subjected to quantitative estimation of the amount of P solubilised in liquid medium containing insoluble P. Mo-blue method (Olsen *et al.*, 1954) was used for the assay. In contrast to the qualitative assays, the liquid method is considered more sensitive for detecting P-solubilization by microorganisms because a measurable Pi concentration can be detected. Results of the experiment indicated that the amount of P solubilised by the selected microbes in Pikovskaya's broth after 14 days of incubation ranged from 3.5µg ml^{-1} to 87µg ml^{-1} among bacteria and 18 µg ml^{-1} to 103.5 µg ml^{-1} among fungi. Among the twenty isolates, PSF-183 solubilized maximum amount of P (103.5 µg ml^{-1}) followed by PSF-182 (102.5 µg/ml). These were also on par with PSF-175 (88.0µg/ml), PSB-149 (87.0µg/ml) and PSB-14 (77.0 µg/ml). PSF-183, PSF-182 and PSF-175 were later identified as *Aspergillus niger*. The bacterial isolates PSB-149 and PSB-14 were identified as *Pseudomonas* sp. and *Providencia alcalifaciens* respectively.

The P-solubilization efficiency on agar medium was highest for bacteria, though the clear zone was bigger for fungi. However, in the quantitative estimation using liquid medium, fungi were more efficient. This could be attributed to the better biomass production by fungi in liquid medium. A similar trend has been reported by earlier workers who noted that in general, fungal isolates exhibited greater P- solubilizing ability than bacteria in both liquid and solid culture (Banik & Dey 1982; Venkateswarlu *et al.*, 1984; Kapoor *et al.*, 1989; Sanjotha *et al.*, 2011). Further, phosphate solubilizing ability in bacteria was lost upon repeated sub culturing but no such loss was observed in the case of phosphate solubilizing fungi (Kucey, 1983). Poonguzhali *et al.* (2008) observed that solubilization of TCP in liquid medium by *Pseudomonas* spp. varied in the range of 24.7 to 44.0 mg per 100 ml. Yadav *et al.* (2011) also tested phosphate solubilizing potential of three fungal strains *A. niger* strain BHUAS01, *T. harzianum* and *P. citrinum* strain BHUPC01 using Pikovskaya's broth containing TCP. *A. niger* solubilized maximum amount of phosphate (328 µg L^{-1}) after 6 days of incubation.

The major mechanism of mineral phosphate solubilization is through the production of acids by microorganisms. The fall in pH by phosphate solubilizing

microorganisms could be due to production of either organic/ inorganic acids in the media. Earlier reports indicate that bacteria having the ability to solubilise P also produced organic acids which act like chelates and solubilized insoluble phosphorus (Zaidi *et al.*, 2004; Khan *et al.*, 2006). Phosphate solubilizing microorganisms convert insoluble phosphates into soluble forms generally through the process of acidification, chelation and exchange reactions.

In the present investigation, a drop in pH of the Pikovskaya's broth was observed. The initial pH of the medium was 7.2, which was brought down by all isolates of bacteria and fungi. Fungi were found to be more active in lowering the pH. Minimum pH was observed in PSF-183, which reduced the pH to 3.2. Bacterial isolates lowered the pH to a minimum of 4.6 (PSB-149). The extent of soluble phosphate was negatively correlated with pH of the culture filtrate. Among bacterial isolates, PSB-149 recorded maximum soluble P (87.0µg/ml) and a minimum pH of 4.6.

A fall in pH of the liquid culture during solubilization of inorganic phosphatic compounds has also been reported by various workers (Gerretsen 1948; Ahmad and Jha, 1968 and Pandey *et al.*, 2006). Venkateswarlu *et al.* (1984) reported that during the solubilization of rock phosphate by fungi, the pH of the culture was lowered from 7 to 3. Reports indicate that several organic and inorganic acids were produced by PSMs. Chen *et al.* (2005) reported that *Serratia marcescens* synthesized citric acid in combination with other acids resulting in a maximum drop in the pH and higher levels of soluble phosphorus in the culture medium. Among 33 isolates that secreted organic acids in the medium, propionic acid was produced by only four *B. megaterium* isolates. There was a significant negative correlation between number of acids produced. Pradhan and Sukla (2005) also reported that *Aspergillus* sp. solubilized 480 g/ml of phosphorus from 0.5% tricalcium phosphate with decrease in pH from 7.0 to 4.0 in 4 days. *Penicillium* sp. solubilized 275 g/ml of phosphorus in 3 days with pH falling to 4.7 from initial PH of 7.0. They also reported that *Aspergillus* sp. show much higher drop in pH and simultaneous higher P solubilization when compared to

Penicillium sp. Pandey *et al.* (2006) reported that a bacterial strain solubilized 247 µg mL−1 TCP under *in vitro* conditions and the maximum phosphate solubilizing activity coincided with the concomitant decrease in pH of the medium. In another study, nineteen phosphate-solubilizing fluorescent *Pseudomonas* strains of *P. fluorescens*, *P. poae*, *P. trivialis*, and *Pseudomonas* spp. produced gluconic acid, oxalic acid, 2-ketogluconic acid, lactic acid, succinic acid, formic acid, citric acid and malic acid in the culture filtrates during the solubilization of tricalcium phosphate (Vyas and Gulati, 2009).

Colony morphology of ten PSB isolates such as size, shape, margin, elevation, surface, texture and colour on the nutrient agar medium was studied. Most of the isolates produced circular, raised colonies with entire margin. All the isolates except PSB-134 were rod shaped, Gram negative bacteria. Gram reaction was confirmed by KOH test, which depends on the lysis of Gram negative cell in dilute alkali (3 per cent KOH) solution. In Gram negative bacteria, cellular DNA is released and the suspension turns viscous (Suslow *et al.*, 1982). For Gram negative bacteria, the cell wall is a very thin layer of peptidoglycan and they have an outer membrane composed of phospholipids, proteins, lipoproteins and lipopolysaccharides. In the Gram staining technique, crystal violet, which is the primary stain is bound by Gram's iodine (mordant) and an insoluble complex is formed. The decolourising agent, ethyl alcohol washes off the complex in case of Gram negative bacterium. Safranin which is the counter stain gives the red colour. Gram-positive bacteria have cell walls that contain thick layers of peptidoglycan (90% of cell wall). The highly cross-linked and multi-layered peptidoglycan of the gram-positive cell is dehydrated by the addition of ethanol. The multi-layered nature of the peptidoglycan along with the dehydration from the ethanol treatment traps the large CV-I complexes within the cell. After decolorization, the Gram-positive cell remains purple in color (Davies *et al.*, 1983).

Endospore production is a very important characteristic of some Gram positive bacteria, allowing them to resist adverse environmental conditions such

as desiccation, chemical exposure, extreme heat, radiation, etc. In this study the Gram positive bacterial isolate PSB-134 revealed presence of endospores that appeared green in colour, whereas the vegetative cells appeared red. The form of a spore is characteristic to the bacterium and allows it to survive in drastic environmental conditions. In the Schaeffer-Fulton's method, a primary stain-malachite green is forced into the spore by steaming the bacterial emulsion. Malachite green is water soluble and has a low affinity for cellular material, so vegetative cells may be decolourized with water. Vegetative cells are then counterstained with safranin (Schaeffer and Fulton, 1993).

Biochemical tests are used for the identification of bacterial species based on the differences in the biochemical activities of different bacteria. These differences in utilization of sugars, production of certain enzymes like catalase and oxidase, etc. help them to identified by the biochemical tests.

All the bacterial isolates in the present study were able to produce catalase and oxidase. Bacterial strains showing catalase activity must be highly resistant to environmental, mechanical and chemical stress. Catalase is an enzyme which degrades hydrogen peroxide, which is harmful to the cell and releases oxygen which is detected as effervescence. A positive oxidase test indicates the presence of cytochrome oxidase, present in the electron transport chain (Kovac's, 1956). This enzyme catalyses oxidation of reduced cytochrome by oxygen. The oxidase reagent is one per cent solution of tetra methyl-p-phenylene diamine hydrochloride.

All bacteria must utilize the energy sources in their environment in order to produce ATP. Each bacterium has its own collection of enzymes that enable it to use diverse carbohydrates; this is often exploited in the identification of bacterial species. One of the very important attributes for an effective microbial strain is its competitive ability and saprophytic competence. Competitive ability of microorganisms for survival depends on the number of energy sources utilized. Strains exhibiting the ability to utilize diverse substrates can survive more

competitively under different environmental conditions. In order to find out the competitive ability, a test on utilization of carbohydrates was conducted.

Five isolates utilized glucose (PSB-13, PSB-14, PSB18, PSB-22 and PSB-152) and five isolates utilized maltose (PSB-22, PSB-134, PSB-149, PSB-151 and PSB-152). Fructose was utilized by four isolates (PSB-13, PSB-18, PSB-22 and PSB-149). Only two isolates were capable of utilizing the sugar alcohol, mannitol (PSB-149 and PSB-152). Dulcitol and lactose were utilized by a single isolate each (PSB-134 and PSB-152 respectively). None of the phosphate solubilizing bacteria were able to utilize sorbitol. Among the different isolates, PSB-152 was found to be highly versatile in carbohydrate utilization, since it was capable of using four different carbohydrates tested (glucose, lactose, maltose and mannitol). PSB-152 was later identified as *Burkholderia* sp. Same results was reported by song *et al.,* 2008. Utilization of carbohydrates by bacterial isolates was indicated by a colour change from red to yellow because of acid production. The colour change is due to the production of acid. Phenol red acts as the indicator, which is red in colour in neutral pH and turns to yellow under acidic pH (MacFaddin, 2000).

Physiological tests are usually carried out to characterize and identify microorganisms. These tests include tolerance to different levels of pH, temperature and salinity. Since Kerala soils are generally acidic in nature, any biofertilizer intended for these soils must tolerate acidic pH. Therefore all the phosphate solubilizing microorganisms obtained in the present study were subjected to screening for tolerance to low pH.

Tolerance of phosphate solubilizing bacteria to low pH (pH 4.0, 5.0 and 6.0) was studied by finding out the sum of per cent change in population over neutral pH, which was calculated using standard formula. PSB-14 (28.50) recorded the lowest value for sum of per cent change in population and therefore, this isolate was ranked first in tolerance to acidity. This was followed by PSB-22

and PSB-13, which were ranked at second and third places. The ability to tolerate acidic conditions would be an indicator of better colonization in soil.

Microorganisms that are able to grow at low pH levels have different mechanisms to tolerate acidity. The ability of an isolated cell to grow and produce an individual colony in medium of a particular pH is the most rigorous test of acid tolerance. Woyessa and Assefa (2011) reported that all isolates of the genus *Pseudomonas* were found to grow in the pH range of 4.5 to 10.0, except two isolates that failed at pH value of 4.5. All isolates of four species (*B. megaterium* 1, *B. coagulans*, *B. cereus* 1 & *B. cereus* 2 and *B. pumilus*) tolerated and grew from the pH range of 4.5 to 10.0.

Woese and his co-workers found that, apart from phenotypic characteristics, 16S rDNA gene sequence can be used for classifying and identifying bacteria (Fox *et al.*, 1977). This provides the added advantage that it is not affected by environmental factors or nutrient composition of the medium, unlike phenotypic characters. The 16S rRNA gene contains conserved regions, a unique array of sequences that are relatively common among different species. These genes are universally present in all bacteria. The conserved regions are used for designing primers, which will amplify the gene. Then the gene is sequenced and the sequence information used to identify the bacteria, using bioinformatics tools. Genes that encode the rRNA have been used extensively to determine taxonomy, phylogeny, and to estimate rates of species divergence among bacteria. Thus the comparison of 16s rDNA sequence can show evolutionary relatedness among microorganisms.

In the present study, 16S rDNA sequencing of ten selected efficient bacterial isolates was carried out to identify them. Universal primers 8F and 152 R (Zhou *et al.*, 1995) were used to amplify the 1500bp 16S rDNA. Based on the maximum score in the blastn output, with NCBI accessions, the bacterial isolates were identified as *Acinetobacter calcoaceticus* (PSB-13), *Providencia alcalifaciens* (PSB-14 & PSB-22), *Achromobacter* sp. (PSB-18 and PSB-151),

Pseudomonas sp. (PSB-114 and PSB-149), *Stenotrophomonas maltophilia* (PSB-114), *Bacillus cereus*. (PSB-134), *Ochrobactrum* sp. (PSB-148), *Achromobacter* sp.(PSB-151) and *Burkholderia* sp. (PSB-152).

Very few reports are available on *Acinetobacter* as phosphate solubilizing plant-associated bacteria, even though it is a ubiquitous bacterial genus widely distributed in soil and water environments. However, their role in the soil P cycle is less well known. (Kuklinsky-Sobral *et al.,* 2004). Maximum phosphate solubilising activity of *Acinetobacter* sp. BR-25 was recorded by Islam *et al.* (2006). High phosphate solubilisation potential of *Acinetobacter* sp. strains under *in vitro* conditions was reported by Ogut *et al.* (2010). They also found that *Acinetobacter* sp. showed presumptive evidence for PQQ independent P-solubilization. Fan *et al.* (2011) reported that *A. calcoaceticus* YC-5a, isolated from rhizospheric soil of maize in Northwestern China showed strong phosphate solubilizing activity. Ren *et al.* (2013) reported tolerance of a phosphate solubilising *A. calcoaceticus* to high concentrations of lead.

Shovitri *et al.* (2014) isolated *Providencia* sp. as indigenous phosphate solubilizing microorganisms form the Poteran Island. Reports on the role of *Providencia* in bioremediation, especially of pesticides such as chlorpyriphos (Van Hamme *et al.,* 2003; Rani *et al.,* 2008), and in phosphate solubilization are also available (Rodriguez and Fraga, 1999). *Achromobacter* sp. was able to solubilize phosphorous and live in tropical environment (Laheurite and Berthelin, 1988). Venkateswaran and Natarajan (1983) reported *Pseudomonas* sp. and *Bacillus* sp. as dominant inorganic phosphorus compounds solubilizing microbes.

The phosphate solubilizing characteristics of a strain YC, which was isolated from phosphate mines (Hubei, China), were studied in National Botanical Research Institute's phosphate (NBRIP) growth medium containing (TCP) as sole phosphorus source. The strain YC is identified as *Stenotrophomonas maltophilia* (Chun-qiao Xiao *et al.,* 2009).

Ochrobactrum sp. TRS-2, isolated from tea rhizosphere could solubilize phosphate (Chakraborty *et al.*, 2009). Ramani and Patel (2011) concluded that the phosphate solubilization by *B. cepacia* was enhanced in presence of different pesticides, so it can be exploited as a good candidate for microbial inoculant.

In the present investigation, ten fungal isolates were tentatively identified based on cultural and morphological characteristics. The cultural characters of fungi were studied on potato dextrose agar medium. Morphological identification was carried out by microscopic examination. Based on cultural and morphological characteristics, fungal isolates were identified as *Aspergillus* sp., *Penicillium* sp., *Trichoderma* sp. and yeast. Six isolates of *Aspergillus* sp. were discarded because of their nature of pathogenicity (Bennett, 1979) and four isolates were used for further studies. The colonies of *Penicillium* species were fast-growing, flat, and filamentous. The colonies were initially white and later turned dark green. Simple or branched conidiophores, metulae, phialides, and conidia were observed under the microscope. The appearance of the spore head is like that of a brush; and the spore head is called a penicillus, which is Latin for a brush. Pitt (1979) also reported that *Penicillium* spp. are characterized by their branched or simple hyaline brush-like conidiophores that terminate in clusters of ampulliform or acerose phialides that give rise to long, dry chains of conidia. The genus is subdivided into four subgenera based on the branching pattern of the *Penicillium.*

Aspergillus sp. was identified based on the presence of dark brown large spores with high sporulation. Fast growing and yellow basal felt. Large conidial heads with biseriate phialides. Spores are globose and dark brown to black. *Aspergillus sp.* was identified based on the pale brown coloured large spores with high sporulation. Fast growing and red-brown basal felt. Biseriate vesicles, globose shaped and smooth finely roughened conidial surface. Diba *et al.* (2007) identified *A. niger* based on slightly brown stipes with smooth walled biseriate vesicles, globose shaped conidia with irregular surface. *Aspergillus* sp. was identified by pale brown roughened stipes, globose to ellipsoidal conidia, biseriate vesicles.

Trichoderma sp. was fast growing, at first white and downy, later developing yellowish- green to deep green compact tuffs. Mycelium was septate, hyaline and smooth and conidiophores branched. Phialospores are rough walled. Shah *et al.* (2012) also reported that conidia of *T. harzianum* (2.8x2.6 µm) were globose to sub-globose and light green in colour. Phialides were flask shaped and the whole conidiophore system appeared singly rather than pyramidal.

White coloured colonies which were moist and slimy in the solid agar medium was identified as yeast. On morphological examination they appeared as ellipsoidal shaped cells. Halasz, 1991 also reported that differentiation between bacterial colonies and those of yeasts normally needs microscopic investigation. Most young yeast colonies are moist and somewhat slimy, but they may appear mealy; most colonies are whitish but some are cream coloured or pink. Some colonies change little with age, but others become dry and wrinkled. The shape of yeast cells varies from spherical to ovoid, lemon-shaped, pear-shaped, cylindrical or even elongated.

The identification of fungal isolates based on cultural and morphological traits was again confirmed at National Centre for Fungal Taxonomy, New Delhi.

Data on phosphate solubilization *in vitro* by soil yeasts are scarce or lacking. Katznelson *et al.* (1962) studied phosphate solubilization of *Candida* sp. isolated from soil. It has been found that the soil yeast *Williopsis californica* is able to oxidize ammonium sulphate to nitrate via nitrite and it could also solubilize insoluble phosphate (Al-Falith and Wainwright, 1995). Abdullah and Al-Falith (2005) isolated soils yeasts and tested their ability to solubilize insoluble phosphate. These soil yeasts solubilized insoluble phosphate *in vitro* leading to the formation of large amounts of soluble phosphate.

Phosphate solubilizing microorganisms are also known to produce plant growth an added adavantage, and promoting substances. Plant growth promotion by PSM is occurs through the production of IAA, GA and suppresssion of plant pathogens .through ammonia or HCN or siderophores

Indole acetic acid is one of the most physiologically active auxins. IAA produced by bacteria improves plant growth by increasing the number of root hairs and lateral roots (Okon and Kapulnik, 1986). Gibberelic acids are a class of phytohormones most commonly associated with modifying plant morphology by the extension of plant tissue, particularly stem tissue (Salisbury, 1994).

In the present work, all the selected isolates were tested for productiuon of IAA as described by Bric *et al.* (1991). Based on the development of pink colour in the filter paper discs, IAA production was noticed in four isolates: *Providencia* sp., *Pseudomonas* sp., *Penicillium* sp. and *Trichoderma* sp. Production of GA by selected isolates was tested as described by Brown and Burlingham, 1968. However, none of the isolates exhibited typical green fluorescene under the UV light.

Harman (2000) suggested that *Trichoderma* sp. are opportunistic plant colonizers that affect plant growth by promoting abundant and healthy plant roots, possibly via production or control of plant hormones. Joseph *et al.* (2007) showed the high IAA production in all isolates of *Bacillus*, *Pseudomonas* and *Azotobacter* (100%) followed by *Rhizobium* (85.7%). Ashrafuzzaman *et al.* (2009) reported that the IAA production was influenced by cultural conditions, growth stage and substrate availability. IAA production by *Providencia* sp. was also reported by Rana *et al.* (2011) and Manjunath *et al.* (2011). Radhakrishnan *et al.* (2013) reported the IAA production by *Penicillium* sp.

Ammonia is considered as one of the plant growth promoting substances produced by microbes in the rhizosphere. Production of ammonia helps to control plant diseases by way of inhibiting the growth of plant pathogens. In the present investigation an attempt was made to find out the ability of phosphate solubilizers to produce ammonia. Based on the development of faint yellow to dark brown colour of the inoculated broth after addition of Nessler's reagent , bacterial isolates *Providencia* sp. and *Pseudomonas* sp. were identified as high ammonia producers. The role of ammonia production by PGPR has earlier been reported by

several workers. Pavlica *et al.* (1978) concluded that ammonia is the only gas present in sufficient concentration in soil to inhibit soil fungus. The role of ammonia in the control of plant pathogens was reported by Howell *et al.* (1988). Production of ammonia in isolates of *Pseudomonas* sp. was reported by Joseph *et al.* (2007). Similarly, Chacko *et al.* (2009) reported a battery of PGPR activities, including ammonia production in *P. putida* isolated from the rhizosphere of *Pisum sativum.* Rana et al. (2011) also reported ammonia production in *Providencia* sp. Ngoma *et al.* (2013 and 2014) reported that ammonia production by *Achromobacter* sp. and *Ochrobactrum* sp. is considered as an important plant growth promoting trait.

Volatile compounds such as HCN is produced by many bacterial strains and has been considered as important metabolites in disease control. HCN produced by the bacterial isolates inhibits the electron transport system of the pathogens thereby the energy supply to the cell is disrupted to the death of organism. In the present investigation, all the selected isolates were tested for HCN production as described by Kumar *et al.* (2012). HCN was produced by three bacterial isolates viz. *Providencia* sp., and two isolates of *Pseudomonas* sp. (PSB-114 and PSB-149). The colour change of the filter paper containing picric acid from yellow to brownish orange within 3 to 4 days indicated moderate HCN production potential of the isolates. None of the other isolates produced HCN.

Several earlier reports indicate the importance of HCN production by PGPR. Bakker and Schippers (1987) observed that nearly 50% of the pseudomonads from potato and wheat rhizospheres produce HCN which has a primary mechanism in suppression of root fungal pathogens. The production of HCN in excess may play a critical role in the control of fungal diseases in wheat seedlings (Flaishman *et al.,* 1996). Blumer and Hass (2000) reported that HCN production is a defense regulator against phytopathogens. Selvakumar *et al.* (2009) also gave evidence that a psychrotolerant *Pseudomonas fragi*a bacterium produced HCN and seed bacterization with the isolate significantly increased the percent germination and rate of germination, plant biomass and nutrient uptake of

wheat seedlings. HCN production by *Providencia* sp. and *P. aeruginosa* were also reported by Rana *et al.* (2011).

Another important trait of PGPR is the production of siderophore that may indirectly influence plant growth. Iron is an essential nutrient for all living organisms. Siderophores are low molecular weight iron-binding ligands which can bind to ferric iron and make it avaliable to producer organisms. Microbial siderophors play an important role in the biocontrol of some soil borne plant diseases and in plant iron nutrition (Loper and Buyer, 1991). In this study, CAS agar medium was used for detecting siderophore producing microorganisms. Three bacterial isolates viz. *Achromobacter* sp. (PSB-151), *Burkholderia* sp. and *Pseudomonas* sp. produced yellow to orange coloured zone around the colonies in CAS medium. Orange halos develop around colonies of siderophore-producing bacteria as the siderophore removes Fe from the Fe-CAS dye complex which gives the medium its characteristic blue color. These three bacterial isolates have the potential of producing siderophores which help to survive under Fe limited situations and also help to control pathogens.

These results are in agreement with earlier reports of Pandey *et al.* (2005); Moretti *et al.* (2008) and Garcia *et al.* (2012). Strains of *Pseudomonas* were found to be siderophores producers. These siderophores bind to the available form of iron (Fe^{3+}) in the rhizosphere, thus making it unavailable to the phytopathogens and protecting the plant growth (Parani and Saha, 2012).

Microbial antagonism is an ecological association between organisms where one or more of the participants is harmed or its activities curtailed. Biological control of plant pathogens using antagonistic rhizobacteria is an effective and eco-friendly alternative to the use of synthetic chemicals (Emmert and Handelsman, 1999). Microbial antagonism contributes much to the biological control of plant pathogens. In addition to providing phosphorous to plants, PSM also act as biocontrol agent and promote the growth of plants by suppressing the soil borne plant pathogens. The ultimate success of an antagonist depends on the

target pathogen, the crop and the cropping system. There is no single way to search or screen an antagonist against a pathogen (Cook and Baker, 1983).

In the present study, the antagonistic activity of selected isolates were tested under *in vitro* conditions using dual culture technique against major soil borne pathogens such as, *Rhizoctonia solani* causing root rot and web blight, *Pythium aphanidermatum* and *Sclerotium rolfsii* causing collar rot, *Fusarium oxysporum* causing Fusarial wilt, and *Ralstonia solanacerum* causing bacterial wilt in cowpea. All the isolates showed antagonistic activity against *R. solani* with maximum inhibition by *Achromobacter* sp. (PSB-18). In case of *P. aphanidermatum,* both *B. cereus* (PSB-134) and *Ochrobactrum* sp. (PSB-134) recorded highest antagonistic activity with 35.22 per cent inhibition and maximum inhibition against *S. rolfsii* was recorded by *Pseudomonas* sp. (PSB-149). All the isolates except *Achromobacter* sp. (PSB-151) and *Penicillium* sp. (PSF-173), recorded antagonistic activity against *F. oxysporum* with maximum by *B. cereus* (PSB-134). None of the tested PSMs were effective against the bacterial pathogen *R. solanacearum.*

Biocontrol by *Achromobacter* sp. depends on competition for iron (Yuen and Schorth, 1986). The search on literature revealed no information regarding anatagonistic activity of *Achromobacter* sp. against *R. Solani.* Pleban *et al.,* 1995 reported that endophytic *B. cereus*, protected cotton seedlings against *R. solani* in the greenhouse conditions by 51, 46 and 56% respectivley. *Pseudomonas* spp. have received great attention as biocontrol agents because of their catabolic versatility, excellent root-colonizing abilities and production of broad range antifungal metabolites such as 2,4- diacetylphloroglucinoal (DAPG), pyoluteorin,pyrrolnitrin and phenazines (Chin-A-Woeng *et al.,* 2001; Raaijmaker *et al.,* 2002). The antagonistic activity of *T. Viride* and *P. fluorescens* was evaluated against *R. solani* causing root rot of sage (Mallesh *et al.,* 2008). Out of these bioagents, *T. viride* provided maximum inhibition (94.4 %) of *R. solani.*

There are reports on *P.aerogenosa* produced different types of antibiotics that play a major role in suppression of *Pythium*(Pieterse *et al.,* 2001;

Holfte,2001;Perneel *et al.*, 2008). Elazzazy *et al.*(2012) reported the significant reduction of damping off disease of cucumber caused by *P. aphanidrmatum. B. cereus* is a Gram positive bacterium that is very common in rhizosphere. Chiradej *et al.*, 2013 also reported that *T. harzianum* strainNST-02 and *B.cereus* strain, NST-03 produced high levels of crude enzymes, both cellulase and β-1,3-glucanase, which highly inhibited the mycelial growth of *P. aphanidermatum.* Vast search did not reveal any relevant literature on the antagonistic activity of *Ochrobactrum* sp. against *P.aphanideramtum*, perhaps this may be the first attempt.

Kishore *et al.* (2005) reported that *P. aeruginosa* could inhibit mycelial growth of *S. rolfsii* up to 74 per cent using dual culture. The mechanisms through which *Pseudomonas* spp. control plant diseases involve competition for niches and nutrients, antibiosis, predation, and induction of plant defense responses. De Curtis *et al.* (2010) showed that *Pseudomonas* sp. was the most effective anatagonist against *S. rolfsii* and produced a distinct and wide area of pathogen mycelium inhibition.

Rakh (2011) also reported that in dual cultures *P. cf. monteilii* exhibited highest inhibition of 94 per cent against *Sclerotium rolfsi.* Paramageetham and Babu (2012) found that *Pseudomonas* isolates were found to be potential antagonists against *Sclerotium* with almost 73.75 per cent of radial growth inhibition percentage. Babu and Paramageetham (2013) isolated *P. aeroginosa* isolates and their antagonistic activities were screened *in vitro.* The isolate PATPT6 was found to be potential antagonists against *S. rolfsi* with 73.7 per cent growth inhibition. Chanutsa *et al.* (2014) reported 60 per cent inhibition of *S. rolfsi* by *P. aeruginosa.*

Gomez (1981) also reported that *B. cereus* str.C-3 of good result with respect to *Fusarium.* It was noticed by Tjamos *et al.* (1992) that *T. harzianum* controls F. *oxysporum* by competing for both rhizosphere colonization and nutrients. They observed that biocontrol became more effective as the nutrient concentration decreased. Kamilova *et al.*, 2008 reported that biocontrol strain

Pseudomonas fluorescens WCS365 inhibited germination of *Fusarium oxysporum* spores in tomato root exudate. Karkachi *et al.,* 2010 revealed the slight inhibition of *F. oxysporum* f.sp. *lycopersici by B. cereus.* Ramzan *et al.,* 2014 reported that *B. cereus* inhibited *F. oxysporum* and produced zones of inhibition. Sharma , 2011 screened several Trichoderma isolates for their biocontrol activity against *F. oxysporum f.sp. pisi.* Eighteen of these isolates (T1 to T18) showed considerable biocontrol potential and Initial counter inhibition was observed in all the eighteen dual culture sets where *Trichoderma* and *Fusarium* both posed varying degree of inhibition on each other.

R. solanacearum is a relevant and widespread phytopathogenic bacterium that causes a wilt disease with deadly effects on many economically important crops and ornamentals. Greenhouse experiments conducted by Ramesh *et al.,* 2010 reported that the egg plants treated with PSB isolates *Pseudomonas* sp. (EB9, EB67), *Enterobacter* sp. (EB44, EB89) and *Bacillus* sp. (EC4, EC13) reduced more than 70 per cent of the wilt incidence caused by *R. solanacearum.* German *et al.,* 2014, found that two commercial products of *Trichoderma* spp. also showed 100 per cent inhibition of *R. solanacearum* and these results are in contradictory to present findings.

Bacterial inoculants are able to increase plant growth and germination rate, improve seedling emergence, respond to external stress factors and protect plants from disease (Biswas *et al.,* 2000). Vigour index provides additional information on the rate and uniformity of seedling growth under wide range of environmental conditions (Powell and Matthews, 2005). Seedlings with vigorous growth pattern can compete successfully under stress, influencing stand establishment and ultimately grain yield. Germination is a critical stage in the development and life cycle of many plants; it ensures the production and consequently controls the dynamics of the population.

In the present study, hundred per cent germination was observed in treatments with *Pseudomonas* sp. (PSB-149), *Providencia* sp. (PSB-14),

Trichoderma sp. (PSF-174), *Pseudomonas fluorescens* and KAU reference culture as bioinoculants. *Penicillium* sp. (PSF-175) recorded 93.33 per cent germination which was higher than control and significant enhancement of seedling vigour in cowpea was observed with *Pseudomonas* sp. followed by *Providencia* sp. (seedling vigour index of 3058.66 and 2954.33 respectively).

According to Kleifed and Chet (1992), *T. harzianum* application increased germination of pepper seeds, emergence of seedlings and bean, radish,tomato and cucumber. Chickpea seeds bacterized with *Pseudomonas* sp. PGPR 2 showed a significant increase in percentage seed germination (Kaur and Sharma, 2013).

Sayyed *et al.* (2005) reported 10 per cent increase in the rate of germination of wheat seed when inoculated with *P. fluorescens* over the control. Similar finding was also recorded by Ashrafuzzaman *et al.* (2009) who reported the increase in seed germination when seeds were pretreated with PGPR isolates in rice.

Dey *et al.* (2004) also suggested that PGPR enhance growth and seed emergence in peanut. Rana *et al.,* 2011 found that *Providencia* (AW5) also showed a two-fold increase in percentage germination as compared to untreated controls. A similar enhancement of seed germination in wheat crop was also reported by Zarrin *et al.* (2009). Manjunath *et al.* (2011) found out the highest vigour indices of 7361.2 and 7355.6 were recorded in treatments T3 (WRB4 *Providencia* sp.) and T5 (combination of *Providencia* sp. and *Anabaena oscillarioides*; WRC3 + WRB4), respectively. Strain WRB4 (*Providencia* sp.) exhibited the best performance when used individually or in combination.

The significant improvement of seed germination, radical length and vigor index might be due to the production of IAA by the rhizobacteria. It is well known that IAA is an important regulator in shoot and root growth (Kaufman *et al.,* 1995), and seed bacterization with IAA-producing rhizobacteria significantly enhanced early seedling establishment (Noel *et al.,* 1996; Khalid *et al.,* 2004). The capability to increase plant growth parameters in germinating seed bioassay is

highly related to the IAA level, which produced by *Pseudomonas* spp. isolates. Varying results of germinating seed assay had also pointed out that there was complex interaction between bacterial IAA and seedlings, thereby caused different responses of plant growth tendencies, yet there is stimulation of bacterial IAA to the development of the host plant root system (Patten and Glick, 2002).

In the present study, based on the efficiency of P-solubilization and plant growth promoting activities, three native isolates *Providencia* sp., *Pseudomonas* sp. and *Trichoderma* sp. were selected for evaluation in pot culture experiment, with cowpea (variety Bhagyalakshmi) as the test crop. KAU commercial formulation of P-solubilizing microorganisms, sold under the name 'Phosphate solubilizers' was used as reference culture in this study. Recommended dose of P was provided either as rock phosphate or factamphos. Pulse crops are known to respond well to phosphatic fertilizers, since phosphorus plays an important role in root growth, nodulation and nitrogen fixation. Biometric observations like shoot length, root length, shoot weight, root weight, number of nodules, days taken for flowering and fruiting, no of pods, pod length, and yield were analysed to find out the effect of inoculated phosphate solubilizers. Observations were recorded at 20 DAS, 40 DAS and also at harvest of the crop.

With respect to shoot length, *Pseudomonas* sp. performed better than all other treatments, in the absence of applied P. When P was supplied in the form of unavailable form as rock phosphate, all the bioinoculants recorded significantly higher shoot length than uninoculated control. At 20DAS, KAU reference culture recorded maximum shoot length. However, at 40DAS, *Trichoderma* sp. was found to be the best and at harvest, *Providencia* sp. recorded maximum shoot length. In treatments where recommended dose of P was supplied in the available form as factamphos, *Trichoderma* sp. recorded maximum shoot length.

All the bioinoculants increased the root length of the plant in absence of applied P, with respect to control. Among the different bioinoculants, *Pseudomonas* sp. recorded maximum root length. In treatments provided with

rock phosphate, all bioinoculants recorded greater root length than control. Among different bioinoculants, *Trichoderma* sp. recorded the highest value. In presence of factamphos, also all the bioinoculants increased the root length, when compared to control.

Previous reports indicate that PGPM including PSM could increase shoot and root length in several crops. Sabannavar and Lakshman (2009) reported that at 30, 60 and 90 days the length of root and shoot of sesame grown in a soil amended with rock phosphate and *Pseudomonas striata* in TSES 1 variety were significantly higher than those recorded in the control treatment. Prasad *et al.* (2012) found that mycorrhizal inoculation with superphosphate and *P. fluorescens* increased the height of plant in comparison to the control. *P. fluorescens* was found to be common effective bioinoculant which increased the height of the plant in recommended dose of superphosphate. They attributed this to the balanced absorption and solubilization of phosphorus by *P. fluorescens*.

In the absence of applied P, all the bioinoculants increased shoot weight and root weight of plants, when compared to control. Among these, *Providencia* sp. and *Pseudomonas* sp. recorded maximum values. In the presence of applied P in the form of rock phosphate or factomphos, in general, *Pseudomonas* sp. recorded higher shoot weight and root weight followed by *Providencia* sp. Dry matter of cowpea plants did not show very significant variation with or without applied P.

Manjunath *et al.* (2011) observed that the treatment in which *Providencia* sp. was inoculated showed significantly higher shoot weight compared to control. Rana *et al.* (2011) observed that an enhanced root weight was produced by *Providencia* sp. in wheat seedlings. Similarly Prasad *et al.* (2012) observed that after 100 days, the increase in root biomass of chrysanthemum was observed maximum with the combination of *A. laevis* + *P. fluorescens* in medium concentration. Gaind (2013) reported that seed inoculation with *P. striata* and its application to soil fertilized with low-grade

Mussoorie rock phosphate at 60 kg P_2O_5 ha^{-1} under pearl millet (*Pennisetum glaucum*) improved the root and shoot biomass (2.01 and 29.12 g pl^{-1}, respectively). Deshwal and Kumar (2013) also revealed that *P. fluorescens* PW-5 produced maximum shoot and root weight of rice crop by 157.2, 4086 % respectively as compared to control. A study conducted by Reetha *et al.* (2014) reported that the fresh shoot weight of onion plants was significantly high in *Pseudomonas fluorescencs* compare to *Bacillus subtilis*.

In general, bioinoculants did not show much difference in plant growth parameters in absence of P and in presence applied P, either as rock phosphate or as factamphos. This indicates solubilization of native insoluble P in soil, by the bioinoculants.

In the case of root nodules, in general, *Pseudomonas* sp. was found to induce the maximum number of nodules per plant. Regarding the number of pods per plant, all bioinoculants recorded higher number of pods in presence of rock phosphate and factamphos. However, in the absence of any applied P, KAU reference culture was found to be the best. Earlier reports indicate higher number of nodules in leguminous plants inoculated with P-solubilizers. As P makes rhizobia active, their growth and multiplication which in turn increased the nodule mass and weight per plant (Muralidharan and George, 1971). This effect could be attributed to higher release of phosphorous from rock phosphate plus organic amendments and P- solubilizers by the action of organic acids and chelates and improved soil conditions (Tomer *et al.*, 1984; Dhillon and Dev, 1986). Tivari *et al.*, 1989 observed increased nodulation, nitrogen fixation and uptake by chickpea with the addition of P-solubilizers to Mussorie rock phospahte. Manjaiah *et al.* (1996) recorded that the treatments receiving organic amendments and P-solubilizers plus rock phosphate were superior with respect to nodule count in groundnut.

The flower represents a central part of the plant's reproductive growth in which anthesis, pollination, and fertilization occur (Oosterhuis and Jernstedt,

1999). It has been speculated that nutrient imbalances in the flower may cause lower yield and unpredictable yield variability (Oosterhuis et al., 2008). Phosphorous is involved in the biochemical pathway of energy transfer within a plant and that the process of blooming requires a lot of energy. In the present study, early flowering was observed in treatments with factamphos. this earliness was more predominant when KAU reference culture and *Pseudomonas* sp. was used as bioinoculants. Factamphos is a chemical blend of ammonium phosphate and ammonium sulphate.The entire N is in ammonical form and P is completely water soluble. In addition, factamphos also contains sulphur, a secondary plant nutrient which is now attaining great importance in the agricultural sector. The form of nitrogen that is supplied to plants affects the uptake of other cations and anions, cellular pH regulation, and the soil in the rhizosphere (Marschner, 1995). Nitrogen uptake as nitrate or ammonium accounts for about 80% of the total cation or anion import. In general it has been found that plants that prefer acid soil conditions seem to prefer ammonium, while those adapted to higher pH soils prefer nitrate. Gangwar *et al.* (2013) reported that the plants nourished by ammonium sulphate also availed the benefit of sulphur which aids in the synthesis of oils and appears to be associated with chlorophyll synthesis therefore it plays a vital role in the physiology of plants.

Earlier reports indicated that *Pseudomonas* spp. enhanced the number of nodules, dry weight of nodules, yield components, grain yield, nutrient availability and uptake in soybean crop (Son *et al.*, 2006). Dutta, 2007 reported that root nodulation, growth, yield parameters and uptake of phosphorous were high in pigeon pea which received a treatment of 50% RP + 50 % SSP along with FYM and PSB.

Pseudomonas sp. recorded higher pod length in presence of applied P in the form of rock phosphate. However, in absence of applied P, KAU reference culture performed better than other bioinoculants.

No. of grains per pod not show much difference with bioinoculants, in presence of applied P. In the absence of applied P and in presence of factamphos, all bioinoculants recorded significantly higher pod yield as compared to control. *Pseudomonas* sp. recorded maximum pod yield in presence of rock phosphate. Increase in crop yield has been earlier reported by several workers. Strains of *Pseudomonas putida* and *Pseudomonas fluorescens* have increased crop yields in potato, radishes, rice, sugar beet, tomato, lettuce, apple, citrus, beans, ornamental plants and wheat (Suslov, 1982; Kloepper, 1988; Lemanceau, 1992). Dubey (1996) also reported that seed yield and TDMP were increased with the use of *Pseudomonas striata* alone. Manjaiah *et al.* (1996) also reprted the inclusion of P-solubilzers to Mussorie rock phosphate increased the pod yield over Mussorie rock phosphate alone. This might be due to the enhanced root growth and in turn better uptake of nutrients and higher pod yield. Najjar *et al.* (2012) reported that single application of *Pseudomonas* with RP resulted in higher yields for beans in the June experiment. A stuy conducted by Reddy and Singh (2011) showed that inoculation of phosphate solubilizing bacteria along with RP fertilization significantly increased yield and nutrient uptake of wheat and maize plants compared to the control soil. Sherene *et al.* (2012) found that the yield attributes such as kapas yield, lint yield and seed yield recorded highest in the treatment that received 50 percent. Jhabua rock phosphate + 50 percent SSP along with phosphobacteria and compost in cotton MW12. Singh *et al.* (2013) reported that maximum curd yield was recorded with 50% P (RP) + P solubilizer + VAM in cauliflower.

With respect to P content of shoot, *Pseudomonas* sp. (PSB-149) recorded maximum content, in the absence of applied P and also in treatments which received P as rock phosphate and factamphos. This clearly indicated that, the isolate was capable of solubilizing P present in rock phosphate as well as fixed P present in soil.

Swamy *et al.* (2010) reported the total P content of cowpea due to inoculation through bio-activation and seed treatment along with rock phosphate

was increased by 391% and 352% respectively over control without rock phosphate. Reddy and Singh (2011) reported that the P uptake by wheat and maize plants increased significantly in the RP amended soil inoculated with phosphate solubilizing bacteria isolated from mine landfills of rock phosphate as compared to control. Prasad *et al.*, 2012 also reported that in the medium concentration of super phosphate, phosphorus content in shoots of chrysanthemum was found to be maximum in the plants treated with *A. laevis* + *P. fluorescens*. Prasad *et al.* (2012) also reported that in the medium concentration of super phosphate, phosphorus content in shoots of chrysanthemum was found to be maximum in the plants treated with *A. laevis* + *P. fluorescens*. Selvakumar *et al.* (2013) reported that under pot culture conditions, bacterization of lentil seeds with *Pseudomonas* strains when combined with Udaipur rock phosphate as a sole source of phosphorus resulted in significant enhancement in P uptake of the plants, compared to the application of rock phosphate alone. Phosphorous uptake by beans was raised when RP was combined with rockphosphate and *Pseudomonas fluorescens* (Najjar *et al.*, 2012).

In general, the P content of root was maximum in KAU reference culture, in the absence as well as in presence of applied P. No significant effect on P content was observed with the bioinoculants used in the study. The bacterial species *Pseudomonas* has a considerable potential in phosphorous uptake efficiency (Tilak *et al.*, 1995). Growth and phosphorous content in two alpine carex species increased by inoculation with *Pseudomonas fotinii* (Bartholdy *et al.*, 2001). Mohammadi *et al.* (2011) reported that combined application of phosphorous solubilizing bacteria and *T. harzianum* produced highest leaf P content and grain P content.

In the present investigation, available P in soil was maximum in *Pseudomonas* sp. (PSB-149) at 20DAS and at harvest, in the absence of applied P and also in its presence. The introduction of efficient microbes (P-solubilizers) in the rhizosphere has been found to increase the availability of phosphorus from both applied and native soil phosphorus. The microbial property of dissolving

interlocked phosphates appears to have an important implication in Indian agriculture. In general, all the bioinoculants increased the P content in soil, as compared to control without bioinoculants.

Khalil (1995) reported that phosphate availability from rock phosphate was increased in soil from 0.67 ppm in control to 17.78 ppm with PSM inoculation in 20 days. Phosphorus solubilizing bacteria, mainly *Bacillus, Pseudomonas* and *Enterobacter,* have been reported to be very effective for increasing the plant available P in soil as well as the growth and yield of crops. Hence, exploitation of phosphate solubilizing bacteria through biofertilization has enormous potential for making use of ever increasing fixed p in the soil, and natural reserves of phosphate rocks (Khan *et al.*, 2009). Reddy and Singh (2011) also revealed that available P increased significantly in the RP amended soil inoculated with phosphate solubilizing bacteria as compared to control.

Population of P-solubilizers in soil was maximum in *Pseudomonas* sp. (PSB-149) both in absence of applied P and in presence of rock phosphate. However, in the presence of factamphos, *Trichoderma* sp. recorded maximum population of P solubilizers in soil. The contributing factors may include the colonization potential of the inoculum, the nature of maximum population achieved by the introduced strain in the rhizosphere (Bennett and Lynch 1981). There is increasing evidence to show that microbial inoculation of seeds may benefit plant growth through a number of mechanisms (Lynch, 1990). However, recovery of inoculants from roots has been variable, which may be an important factor influencing yield in inoculation experiments (Kloepper *et al.*, 1989). The inoculated microorganism may not always successfully survive and persist in the rhizosphere. This contributes to the inconsistence results of PGPR applications in different geographical regions (Burr *et al.*, 1978; Weller, 1988; Bashan and Levanony, 1990; Nautiyal *et al.*, 2000).

Summary

6. SUMMARY

The present study entitled on "Exploration of native mineral phosphate solubilizing microorganisms as biofertilizer for the acidic soils of Kerala"was carried out in the Dept of Agricultural Microbiology, College of Horticulture, Vellanikkara during 2011-2013. The important findings of the study are summarized below:

- Two hundred microbial isolates (173 bacteria and 27 fungi) from the repository maintained at Dept. of Agrl. Microbiology were screened on Pikovskaya's agar medium.Phosphate Solubilization Efficiency (PSE) ranged from 106.6 % to 555.5%. PSE was higher in bacteria but fugi were faster in solubilization of phosphorous. that among bacteria, the isolate PSB-22 and among fungi, PSF-181 recorded maximum phosphate solubilizing efficiency.
- Twenty most efficient isolates were selected for quantitative estimation of p-solubilization, using Mo-blue method. Two fungal isolates namely PSF-183 and PSF-182 solubilized maximum amount of P (103.5µg/ml and 102.µg/ml respectively)in 14 days. A corresponding drop in pH was recorded in all the isolates
- All the bacterial isolates tested positive for catalase and oxidase production.
- PSB-14 ranked first with respect to tolerance to acidity, followed by PSB-22.
- Bacterial isolates were identified as *Acinetobacter* sp., *Providencia* sp., *Achromobacter* sp., *Providencia alcalifaciens*,*Bacillus cereus*, *Pseudomonas* sp. and *Burkholderia* sp. using 16s rDNA technique.
- Fungal isoaltes were tentatively identified as *Aspergillus* sp., *Penicillum* sp., *Trichoderma* sp., and yeast. Six isolates of *Aspergillus* sp. were discarded and remaining were used for futrther studies.
- *Providencia* sp., *Pseudomonas* sp., *Penicillium* sp. and *Trichoderma* sp. were produced IAA.
- Significant enhancement of seedling vigour in cow pea was observed with *Pseudomonas* sp. followed by *Providencia* sp.

- PSM isolates were effective in inhibiting *R. solani* and was not effective against the bacterial pathogen *R. solanacearum*
- Based on P-solubilization and plant growth promoting activities three native isolates *Providencia* sp., *Pseudomonas* sp. and *Trichoderma* sp. were selected for evaluation in pot culture experiment with cow pea variety Bhagyalakshmi.
- Application of insoluble P source with bacterial inoculation induced more number of nodules.
- *Pseudomonas* sp. recorded maximum shoot weight, root weight, no. of nodules/plant, pod length, no of grains per pod and pod yield per plant, in combination with rock phosphate.
- Chemical analysis of plants after harvest revealed that P content in roots and grains was highest in KAU reference culture, whereas highest P in shoot was observed in *Pseudomonas* sp.
- *Pseudomonas* sp. (PSB-149) was efficient in releasing P from rock phosphate as well as fixed P present in soil.
- *Pseudomonas* sp. proliferated and established in large numbers in the soil.
- The present study highlights the importance of using P-solubilizers as bioinoculants, without applied chemical P fertilizer, to get benefit by solubilization of native, fixed P in soil. This practice will be more remunerative to the farmer, as phosphatic fertilizers can be skipped.

References

REFERENCES

Abd-Alla, M. H. 1994. Phosphates and the utilization of organic phosphorous by *Rhizobium leguminosarum* biovar *viceae*. *Lett. Appl. Microbiol.* 18:294-296.

Abd-Alla, M. H. 1994. Solubilisation of rock phosphates by Rhizobium and Bradyrhizobium. *Folia Microbiologica*. 39:53-56.

Abdalla, A. S., Osman, A. G., Abdelgani, M. E., Rugheim, A. M. E .2011. Effects of Biological and Mineral Fertilization on Nodulation, Nitrogen and Phosphorus Content and Yield of Chick pea (*Cicer arietinum* L.) *Adv. in Environ. Biol.* 5(9): 2886-2894.

Abdul-Baki, A. A. and Anderson, J. D. 1973. Vigour determination in soyabean seed by multiple criteria. *Crop Sci.*13:630-633.

Abdullah, M. and Al-Fatih. 2005. Phosphate Solubilization *in vitro* By Some Soil Yeasts. *Qatar Univ. Sci. J.* 25: ll9-125.

Abril, A., Zurdo-Pineiro, J. L., Peis, A., Rivas, R., Velazquez, E. 2007. Solubilization of phosphate by a strain of *Rhizobium leguminosarium* bv *trifolii* isolated from *Phaseolus vulgaris* in EI Chaco Arido soil (Argentina). *Dev. Plant and Soil Sci.* pp.135-138.

Achala, V., Savantb, V.V and Reddya, M.S. 2007. Phosphate solubilization by a wild type strain and UV-induced mutants of *Aspergillus tubingensis*. Soil *Biol. Biochem.* 39: 695-699.

Afzal, A. and Bano, A. 2008. Rhizobium and phosphate solubilizing bacteria improve the yield and phosphorus uptake in wheat (*Triticum aestivum*). *Int. J. Agric. Biol.* 10 (1): 85-88.

Ahmad, N. and Jha, K.K. 1968. Solubilization of rock phosphate by microorganism isolated from Bihar soil. *J. Can. Appl. Microbiol.* 14: 89–95.

Ahmed, N., Shahab, S. 2011. Phosphate solubilization: Their mechanism genetics and application. *Int. J. Microbiol.* 9: 4408-4412.

AICRPS [All India Coordinated Research Project on Spices].2000. *Annual Report 1999-2000.*Indian Institute of Spices Research, Calicut,101 p.

Aipova, R., Aitkeldiyeva, A., Kurmanbayev, A., Sadanov, K. and Topalova, B.2010. Assessment of biotechnological potential of phosphate solubilizing bacteria isolated from soils of Southern Kazakhstan. *Nat. Sci.* 2(8):841–845.

Akgiil, D.S. and Mirik, M. 2008. Biocontrol of *Phytophthora capsici* on pepper plants by *Bacillus megaterium* strains. *J. Plant Pathol.* 90: 29-34.

Alam, S., Khalil, S., Ayub, N., and Rashid, M. 2002. *In vitro* solubilization of inorganic phosphate by phosphate solubilizing microorganisms (psm) from maize rhizosphere. *Int. J. Agric. Biol.* 4:454-458.

Al-Falith, A. M. and Wainwright, M. 1995. Nitrification, S-oxidation and P-solubilization by the soil yeast *Williopsis colifornica* andby *Saccharomyces cerevisiae. Mycol. Res.* 99:200- 204.

Algam, S.A.E., Xie, G, L., .Li, B., Yu, S.H., Su, T. and Larsen, J. 2010. Effects of *Paenibacillus* strains and chitosan on plant growth promotion and control of *Ralstonia* wilt in tomato. *J. Plant Pathol.* 92: 593-600.

Alikhani, H.A., Saleh-Rastin, N., Antoun, H. 2007. Phosphate solubilization activity of *Rhizobia* native to Iranian soils. *Dev. Plant and Soil Sci.* 135-138 pp.

Alikhani, H.A., Salesh-Rastin, N., and Antoun, H. 2006. Phosphate solubilization activity of *Rhizobia* native to Iranian soil.*Plant soil.*287:35-41.

Almeida, D., Filho, O.K., Felipe, A.F., Almeida, H.C. 2009. Soil microbial biomass under mulch types in an integrated apple orchard from Southern Brazil. *Sci. Agric.* 68(4):1069-1077.

Amalraj, E.L.D, Maiyappan, S. and Peter, A.J .2012. *In vivo* and *in vitro* studies of *Bacillus megaterium* var. *phosphaticum* on nutrient mobilization, antagonism and plant growth promoting traits. *J. Ecobiotech.* 4: 35–42.

Anith, K. N., Tilak, K. V. B. R. and Kanuja, S. P. S. 1999. Molecular basis of antifungal toxin production by fluorescent *Pseudomonas* sp. strain EM 85 – A biological control agent. *Curr. Sci.* 5(77):671-677.

Antarikanonda, P., Sassanarakit, S., Amaret, P., and Waianae, P. 1991. Biological system for improving availability of rock phosphate to plants. *In:* Kinoshita, S. and A. Bhumiratana \eds.). pp: 612–19.

Arkebola, E., Jacobs, R. and Korsten, L. 2010. Iturin A is the principal inhibitor in the biocontrol activity of *Bacillus amyloliquefaciens* PPCB004 against postharvest fungal pathogens. *J. Appl. Microbiol.* 108:386–395.

Arnon, D. I. 1956. Phosphorus metabolism and photosynthesis. *Plant Physiol.*7: 325-354.

Arshad, M.and Frankenberger, W.T. 1998. Plant growth regulati ng substances in the rhizosphere. Microbial production and function. *Adv. Agron.* 62:46 – 51.

Arun, K. S. 2007. *Bio-fertilizers for sustainable agriculture.* Agribios publishers, Jodhpur, India.24pp

Asea, P. E. A., Kucey, R. M. N. and Stewart, J. W. B. 1988. Inorganic phosphate solubilisation by two *Penicillium* sp. in solution culture and soil. *Soil Biol. Biochem.* 20:459-464.

Ashrafuzzaman, M., Hossen, F.A., Ismail, R., M.A., ZahurulIslam, M., Shahidullah, S.M., and Meon, S .2009. Efficiency of plant growth-promoting rhizobacteria (PGPR) for the enhancement of rice growth. *Afr. J. Biotechnol.* 8:1247–1252.

Babana, A. and Antoun, H. 2006. Effect of Tilemsi phosphate rock-solubilizing microorganisms on phosphorus uptake and yield of field grown wheat (*Triticum aestivum* L) in Mali. *Plant Soil* .287:51-58.

Babu, G. P., and Paramageetham, C. 2013. Biocontrol of *Sclerotium rolfsii* - a polyphagous plant pathogen by *Pseudomonas aeruginosa* isolated from forest litter. *Int. J. Res.Plant Sci.* 3 (1):1-4.

Bajpai, P.D. and Sundara Rao, W.V.B.1971. Phosphate solubilizing bacteria. *Plant Nutr* .17: 46-53.

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 Etol. Biothem.* 19(4):198-457.

Bakthavatchalu, S., Shivakumar, S. and Sullia, S.B.2013. Characterization of multiple plant growth promotion traits of *Pseudomonas aeruginosa* FP6, a potential stress tolerant biocontrol agent. *Ann. Biol. Res.*4 (2):214-223.

Bakthavatchalu, S., Shivakumar, S. and Sullia, S.B.2012. Identification of multi-trait PGPR isolates and evaluation of their potential as biocontrol agents. *Acta Biologica Indica.* 1(1):61-67.

Banerjee, S., Palit, R., Sengupta, C. and Standing, D.2010. Stress induced phosphate solubilization by *Arthrobacter* sp. and *Bacillus* sp. isolated from tomato rhizosphere. *AJCS.* 4(6):378-383.

Banik, S and Dey, B.K. 1981. Solubilisation of inorganic phosphate and production of organic acids by micro-organisms isolated in sucrose tricalcium phosphate agar plate. *Hyg.* 11.136:478-486.

Banik, S and Dey, B.K. 1982.Phosphate solubilizing microorganisms of a lateritic soil.III.Effect of inoculation of some tricalcium phosphate –solubilizing microorganisms on available phosphorous content of rhizosphere soils of rice (*Oryza sativa* L.cv. IR-20) plants and their uptake of phosphorous. *Zbl.Bakt.*136, 493-501.

Banik, S. and Dey, B.K. 1982. Available Phosphate Content of an Alluvial Soil as Influenced by Inoculation of Some Isolated Phosphate Solubilizing Microorganisms. *Plant and Soil.* 69(3): 353-364.

Bano, N. and Musarrat, J .2003. Characterization of a new *Pseudomonas aeruginosa* strain NJ-15 as a potential biocontrol agent. *Curr. Microbiol.* 46: 324–328.

Barea, J., Navaro, E. and Montoya, E. 1976. Production of plant growth regulators by rhizosphere phosphate-solubilizing bacteria. *J. Applied Bacteriol.* 40: 129-134.

Barea, J.M., Toro, M., Orozco, M.O., Campos, E. and Azcn, R. 2002. The application of isotopic (^{32}P and ^{15}N) dilution techniques to evaluate the interactive effect of phosphate-solubilizing rhizobacteria, mycorrhizal fungi and Rhizobium to improve the agronomic efficiency of rock phosphate for legume crops. *Nutr. Cycling.* Agroecosyst. 63:35–42.

Bartholdy, B. A., Berreck, M. and Haselwandter, K. 2001. Hydroxamate siderophore synthesis by *Phialocephala fortinii*, a typical dark septate fungal root endophyte. *Biol. Metals.* 14:33-42.

Bashan, Y., Holguin, G. 1998. Proposal for the division of plant growth-promoting Rhizobacteria into two classifications: biocontrol-PGPB (plant growth-promoting bacteria) and PGPB. *Soil. Biol. Biochem.* 30: 1225-1228.

Bashan, Y. and Levanony, H. 1990. Current status of *Azospirillum* inoculation technology: *Azospirillum* as a challenge for agriculture. *Can. J. Microbiol.* 36: 591-608.

Baya, A.M., Robert, S.B. and Ramos, C.A. 1981. Vitamin Production in Relation to Phosphate Solubilization by Soil Bacteria. *Soil Biol. Biochem.* 13(6): 527-532.

Begum, M.F., Rahman, M.A. and Alam, M. F. 2010. Biological control of Alternaria fruit rot of chili by *Trichoderma species* under field conditions. *Mycobiol.*38: 113-117.

Belimov, A.A., Kojemiakov, P.A. and Chuvarliyeva, C.V. 1995. Interaction between barley and mixed cultures of nitrogen fixing and phosphate-solubilizing bacteria. *Plant Soil.* 17: 29–37.

Benizri, E., Courtade, A., Picard, C. and Guckert, A.1998.Role of maize root exudates in the production of auxins by *Pseudomonas fluorescens* M.3.1.*Soil Biol. Biochem.*30:1481-1484.

Bennett, J.E. 1979. Aspergillosis, In P. Beeson, W. McDermott, and J. Wyngaarden, (eds.), Cecil textbook of medicine, 15th edition. W.B Saunders, Philadelphia. pp. 546-547.

Bennett, R.A. and Lynch, J.M.1981.Colonisation potential of bacteria in the rhizosphere. *Curr. Microbiol.* 137-138.

Binisha, S. 2003. Supplementary effect of bio-fertilizers in Dendrobium. M.Sc. (Ag) thesis, Kerala Agricultural University, Thrissur, p.49.

Biswas, J.C., Ladha, J.K., Dazzo, F.B., Yanni, Y.G., and Rolfe, B.G. 2000. Rhizobial inoculation influences seedling vigour and yield of rice. *Agron. J.* 92:880–886.

Blumer, C. and Haas, D. 2000. Mechanism, regulation, and ecological role of bacterial cyanide biosynthesis. *Arch. Microbiol.* 173: 170–177.

Bossier, P., Hofte, M., and Verstraete, W. 1988. Ecological Significance of Siderophores in Soil. *Adv. Microbial Ecol.* 10: 385-414.

Bray R.H. and Kurtz, L.T.1945. Determination of total, organic, and available forms of phosphorus in soils. *Soil Sci.*5 9:39-45.

Bric, J. M., Bostock, R .M. and Silverstone, S. E. 1991. Rapid in situ assay for indoleacetic acid production by bacteria immobilized on a nitrocellulose membrane. *Appl. Environ. Microbiol.* 57:535–538.

Brown, M. E. and Burlingham, S. K. 1968. Production of plant growth substances by *Azotobacter chlorococum. J. Gen. Microbiol.* 53 : 135-144.

Bums, R.G .1983. Extracellular enzyme-substrate interactions in soil. In: Slater, J.H., Wittenbury, R. and Wimpenny, J.W.T. (eds.), *Microbes in Their Natural Environment.* Cambridge University Press,London, pp 249-298.

Burr, T. J., Schroth, M. N., and Suslow, T. 1978. Increased potato yields by treatment of seed pieces with specific strains of *Pseudomonas juorescens* and *P. purida. Phytopathol.* 68:1377-1383.

Cappuccino, J.G. and Sherman, N. 1992. Biochemical activities of microorganisms. In: Microbiology, A Laboratory Manual. The Benjamin / Cummings Publishing Co. California, USA. pp125-128.

Chabot, R., H. Antoun and M.P. Cescas. 1993. Stimulation of growth of maize and lettuce by inorganic phosphorus solubilizing microorganisms. *Canadian J. Microbiol.*, 39: 941–47.

Chaiharan, M. and Lumyong, S. 2011. Screening and optimization of indole-3-acetic acid production and phosphate solubilization from rhizobacteria aimed at improving plant growth. *Curr. Microbiol.* 62(1): 173-81.

Chacko, S., Ramteke, P.W., and John, S.A.2009. Amidase from plant growth promoting rhizobacterium. *J. Bacteriol. Res.* 1(4): 046-050.

Chakraborty, U., Chakraborty, B., Basnet, M., and Chakraborty, A. 2009. Evaluation of *Ochrobactrum anthropi* TRS-2 and its talc based formulation for enhancement of growth of tea plants and management of brown root rot disease. *J. Appl. Microbiol.*107:625–634.

Chanutsa, N., Phonkerd, N., and Bunyatratchata, W.2014. Potential of *Pseudomonas aeruginosa* to Control *Sclerotium rolfsii* Causing Stem Rot and Collar Rot Disease of Tomato. *J. Advan. Agric. Technol.* 1(2):132-135.

Chen, X.H., Koumaoutsi, A., Scholz, R. and Borriss, R. 2009. More than anticipated-production of antibiotics and other secondary metabolites by Bacillus amyloliquefaciens FZB42. *J. Mol. Microbiol. Biotechnol.* 16:14–24.

Chen, Y.P., Rekha, P.D., Arun, A.B., Shen, F.T., Lai, W.A., and Young,C.C. 2005. Phosphate solubilizing bacteria from sub tropical soil and their tri calcium phosphate solubilizing abilities. *Appl. Soil Ecol.* 34: 33-41.

Chien, S.H. 1979. Dissociation of phosphate rock in acid soils as influenced by nitrogen and potassium fertilisers. *Soil Science* .127: 371 -376.

Chiradej, C., Warin, I., and Punnawich, Y.2013. Efficacy of *Trichoderma harzianum, Bacillus cereus* and Their Lytic Enzymes for the Control of Damping-Off Disease of Yard Long Bean Caused by *Pythium aphanidermatum. Philipp. Agric. Scientist.* 96(4): 377–383.

Chin-A-Woeng, T. F. C., Thomas-Oates, J. E., Lugtenberg, B. J. J., and Bloemberg, G. V. 2001. Introduction of the phzH gene of *Pseudomonas chlororaphis* PCL1391 extends the range of biocontrol ability of phenazine-1-carboxylic acidproducing *Pseudomonas* spp. strains. *Mol. Plant–Microbe Interactions.*14: 1006–1015.

Chonkar, P.K. and Tarafdar, J.C. 1984. Accumulation of phosphatase in soils. *J. Indian Soc. Soil Sci.* 32: 266–272.

Chookietwattana, K. and Maneewan, K. 2012. Selection of efficient salt-tolerant bacteria containing ACC deaminase for promotion of tomato growth under salinity stress. *Soil Environ.* 31: 30-36.

Chun-qiao, XIAO., Ru-an, CHI., Huan, HE., Wen-Xue, ZHANG.2009.Characterization of tricalcium phosphate solubilization by *Stenotrophomonas maltophilia* YC isolated from phosphate mines. *J. Cent. South. Univ. Technol.*16:0581-0587.

Chung, H.; Park, M.; Madhaiyan, M.; Seshadri, S.; Song, J.; Cho, H.; Sa, T. 2005. Isolation and characterization of phosphate solubilizing bacteria from the rhizosphere of crop plants of Korea. *Soil Biol. Biochem.* 37: 1970-1974.

Cook, R. J. and Baker, K.F. 1983. The Nature and Practice of Biological Control of Plant Pathogens. The American Phytopathological Society, St. Paul, MN. 539 PP.

Corbett, J.R. 1974. Pesticide design. In: The biochemical mode of action of pesticides, Academic Press,Inc., London, pp.44-86.

Czaczyk, B., Stachowiak, K., Trojanowska, K. and Gulewicz. 2000. Antifungal activity of *Bacillus* sp. isolated from compost. *Folia Microbiol.* 45:552–554.

Davies, J. A., Anderson, G.K., Beveridge, T. J., and Clark, H.C.1983. Chemical Mechanism of the gram stain and synthesis of a new electron-opaque marker for electron microscopy which replaces the iodine mordant of the stain. *J. Bacteriol.* 156 (2):837-845.

Davis, J.I. 1995. Species concepts and phylogenetic analysis. Introduction. *Syst. Bot.* 20: 555-559.

De Curtis, F., Lima, G., Vitullo, D., and De Cicco, V. 2010. Biocontrol of *Rhizoctonia solani* and *Sclerotium rolfsii* on tomato by delivering

antagonistic bacteria through a drip irrigation system. *Crop Prot.* 29: 663-670.

De Freitas, J.R., Banerjee, M.R. and Germida, J.J.1997.Phosphate solubilizing rhizobateria enhance the growth and yield but not phosphorous uptake of canola (*Brassica napus* L.). *Biol. Fert.Soils*.24:358-364.

Demissie, S., Muleta, D. and Berecha, G.2013. Effect of Phosphate Solubilizing Bacteria on Seed Germination and Seedling Growth of Faba Bean (*Vicia faba* L.). *Int. J. Agric .Res.* 8: 123-136.

Deshwal, V.K. and Kumar, P.2013. Production of Plant growth promoting substance by *Pseudomonads. J. Acad. Indust. Res.* 221-225.

Deshwal, V.K., Reena, Sharma, P., Gupta, S., Chakraborty, M., Chatterji, T.2011.Phosphorus solubilizing *Pseudomonas aeruginosa* PMV-14 enhance productivity in Rice crop. *Int. J. Appl. Agri. Res*, 6(1), 2011d, 29-33.

Devi, M .P. and Narasimhan, R. L .1978. Phosphate and lime potentials of some alluvial soils. *J. Indian. Soc. Soil Sci.* 26:33–37.

Dey, R., Pal, K. K., Bhatt, D.M. and Chauhan, S.M. 2004. Growth promotion and yield enhancement of peanut (*Arachis hypogaea L.*) by application of plant growth-promoting rhizobacteria. *Microbiol. Res.* 159:371-394.

Dhillon, N.S. and Dev, G.1986. Effect of applied phosphorous, farm yard manure, and moisture regimeson transformations of inorganis phosphates. *J. Indian. Soc. Soil Sci*.34:605-607.

Diba, K., Kordbacheh, P., Mirhendi, S.H., Rezaie, S. and Mahmoudi, M .2007. Identification of *Aspergillus* species using morphological characteristics. *Pak. J. Med. Sci.* 23(6): 867-872.

Didiek, H.G., Siswanto, K., and Yudho, S. 2000. Bioactivation of Poorly Soluble Phosphate Rocks with a Phosphorus-Solubilizing Fungus. *Soil Sci. Am. J.* 64: 927-932.

Dinesh, R., Srinivasan, V., Hamza, S. and Anandaraj, M. 2014. Massive phosphorus accumulation in soils: Kerala's continuing conundrum. *Correspondence Curr. Sci.* 106(3):343-344.

Dobbelaere, S., Croonenborghs, A., Thys, A., Ptacek, D., Okon, Y. and Vanderleyden, J. 2002. Effects of inoculation with wild type *Azospirillum brasilense* and *A. irakense* strains on development and nitrogen uptake of spring wheat and grain maize. *Biol. Fertil. Soils.* 36:284-297.

Duarah, I., Deka, M., Saikia, N. and Boruah, H.P. 2011. Phosphate solubilizers enhance NPK fertilizer use efficiency in rice and legume cultivation. *Biotech.* doi:10.1007/s13205-011-0028-2.

Dubey, S. K.1996. Response of soybean to rock phosphate applied with *Pseudomonas striata* in a typic chromustert. *J. Indian Soc. Soil Sci.* 44 (2): 252-255.

Duponnois, R., Kisa, M., Plenchette, C .2006. Phosphate solubilizing potential of the nematofungus *Arthrobotrys oligospora*. J. *Plant Nutr. Soil Sci.* 169:280-282.

Dutta, D. 2007. Studies on the efficiency of rock phosphate as a source of phosphorus to pigeonpea in acid laterite soil of West Bengal. *Indian. J. Agric. Sci.* 3: 228-231.

Edi-Premono, M., Moawad, A. and Vleck, P.L.G. 1996. Effect of phosphate solubilizing *Pseudomonas putida* on the growth of maize and its survival in the rhizosphere. *Indo. J. Crop Sci.* 11: 13-23.

Egamberdiyeva, D. 2005. Plant growth promoting rhizobacteria isolated from a Calcisol in a semi-arid region of Uzbekistan: Biochemical characterization and effectiveness. *J. Plant Nutr. Soil Sci.* 168:94-99.

Elazzazy, A.M., Almaghrabi, O.A., Moussa, T.A.A. and Abdel-Moneim, T.S.2012. Evaluation of some plant growth promoting rhizobacteria (PGPR) to control *Pythium aphanidermatum* in cucumber plants. *Life Sci. J.* 9(4):3147-3153.

Emmert, E.A.B. and Handelsman, J. 1999. Biocontrol of plant disease: a (Gram) positive perspective. *FEMS Microbiol. Lett.* 171: 1-9.

Fan , D. D., Ren, Y.X. , Zhu, X. L., Ma, P., and Liang, L.H..2011. Optimization of culture conditions for phosphate solubilizing by *Acinetobacter calcoaceticus* YC-5a using response surface methodology. *Afr. J. Microbiol. Res.* 5(20): 3327-3333.

Fares, C.N. 1997. Growth and yield of wheat plant as affected by biofertilisation with associative, symbiotic N_2-fixers and endomycorrhizae in the presence of the different P-fertilizers. *Ann. Agric. Sci.* 42: 51-60.

Fatima , Z. , Saleemi, M. ,Zia ,M., , Sultan , T., Aslam , M., Rehman, R.U. and Chaudhary, M.F.2009. Antifungal activity of plant growth-promoting rhizobacteria isolates against *Rhizoctonia solani* in wheat. *African J. of Biotechnol.* Vol. 8 (2), pp. 219-225.

Fernando, W.G.D., Ramarathnam, R., Krishnamoorthy, A.S. and Savchuk, S.C. 2005. Identification and use of Potential Bacterial Organic Antifungal Volatiles in Biocontrol. *Soil Biol. Biochem.* 37:955-964.

Flaishman, M.A., Eyal, A., Silberstein, C., Voisardan, H. D. 1996. Suppression of *Septoria tritici* blotch and leaf rust of wheat by recombinant cyanide producing strains of *Pseudomonas putida. Mol. Plant Microbe Interaction* 9: 642-645.

Fomina, M., Alexander, I.J., Hillier, S., and Gadd, G.M. 2004. Zinc phosphate and pyromorphite solubilization by soil plant-symbiotic fungi. *Geomicrobial J.* 21: 351-366.

Fox, G. E., Magrum, L. J., Balch, W. E., Wolfe, R. S., and Woese, C. R. 1977.Classification of methanogenic bacteria by 16S ribosomal RNA characterisation.*Proc.Natl.Acad.Sci.*USA. 74: 4537-4541.

Frankenberger, W.T. and Brunner, W. 1983. Methods of detection of auxinindole acetic acid in soil by high performance liquid chromatography. *Soil Soc. Am. J.* 47: 237-241.

Frankenberger, W.T. Jr. and Arshad, M. 1991. Yield response of *Capsicum annuum* to the auxin precursor, L-trytophan applied to soil. *PGRSA Quart.* 19: 231-240.

Freed, R. 1986. MSTAT Version 4.0. Department of Crop and Soil Science, Michigan State University.

Gaind, S.2013. *Pseudomonas striata* for improving phosphorous availability in soil under pearl millet cultivation. *J. Crop Improv.* 27(3): 255-271.

Gangwar, A.P.S., Singh, J.P., Singh, I.P.2013. Influence of nitrogen and phosphorous fertilizers with nitrogen sources on floral parameters of Tuberose (*Polianthes tuberose* L.).*HortFlora Res. Spectr.* 2(1):40-44.

Garcia de Salamone, I.E., Hynes, R.K. and Nelson, L.M. 2001. Cytokinin production by plant growth promoting rhizobacteria and selected mutants. *Can. J. Microbiol.*47: 04–411.

Garcia, A., Rhoden, S.A., Bernardi-Wenzel, J., Orlandelli, R. C., Azevedo, J.L., and Pamphile, J.A.2012. Antimicrobial Activity of Crude Extracts of Endophytic Fungi Isolated from Medicinal Plant *Sapindus saponaria* L. *J. Appl. Pharma. Sci.* 2 (10):035-040.

Gaume, A. 2000. Low phosphate tolerance of various maize cultivers; the contribution of root exudation. Ph.D. dissertation, Swiss federal institute of Technology, Zurich, Switzerland, 158p.

Gaur, Y. D., Sen, A. N. and Rao, N. S.1980. Improved legume- Rhizobium symbiosis by inoculating preceding cereal crop with Rhizobium. *Plant Soil.* 54: 313–316.

Gaur, A. C. 1990. *Phosphate solubilizing microorganisms as biofertilizers.* Omega Scientific Publisher, New Delhi, 176p.

Gebring, P. J., Nolan, R. J. and Watanabe, P. G. 1993. Solvents, fumigants and related compounds. In : Handbook Pesticide Toxicology, Volume 2, Eds. W. J. Hayes and E. R. Laws, Academic Press Inc., Sand Diego, California, pp. 646-649.

Gephardt, P., Murray, R.G.E., Costilow, R.N., Nester, E.W., Wood, W.A., Krieg, N.R. and Phillips, G.B. 1981. Manual of Methods for General Bacteriology, ASM Press, Washington D.C.78p.

Gerretsen, F. C. 1948. The influence of microorganisms on the phosphate intake by the plant. *Plant Soil* .1:51-81.

Ghosh, U., Subhashini, P., Dilipan, E., Raja, S., Thangaradjou, T. and Kannan, L.2012. Isolation and Characterisation of phospahte solubilizing bacteria from seagrass rhizosphere soil. *J. Oceanor. Uni. China* .11:86-92.

Girija, D. 2010. Diversity of agriculturally important microorganisms in the Western Ghats of Kerala. *AMAAS Annual Report 2009-2010.* NBAIM, Mau. Pp.14-15.

Gitari, J.N and Mureithi, J.G. 2003. Effect of phosphorus fertilization on legume nodule formation and biomass production in Mount Kenya Region. *E. Afr. Agric. J.* 69: 183–187.

Gnanachitra, M. and Govindarajan, K .2002. Solubilization of insoluble phosphates by acid tolerant *Azospirillum* isolates. In : *Natl. Symp. on Mineral Phosphate Solubilization, Univ. Agric. Sci., Dharwad*, p. 96.

Goenadi, D. H., Siswanto and Sugiarto, Y. 2000. Bioactivation of poorly soluble phosphate rocks with a phosphorus-solubilizing fungus. *Soil Sci. Soc. Am. J.* 64:927-932.

Goldstein, A. H.1994. Involvement of the quinoprotein glucosedehydrogenase in the solubilization of exogenous mineral phosphates by Gram negative bacteria. In: Torriani-Gorni A, Yagil E, Silver S (eds) Phospahte in microorganisms: cellular and molecular biology. ASM Press, Washington, pp197-203.

Goldstein, A. H. 1986. Bacterial phosphate solubilization. Historical perspective and future prospects. *Am. J. Altern. Agric.* 1: 57-65.

Gomez, G.R.1981. Inhibition of *Fusarium oxysporum* in rutgers tomato sled by antagonistic bacteria. *Cent. Agricola.*6:65-73.

Gopinath, S.C.B., Anbu, P. and Azariah, H. 2005. Extracelular enzymatic activity profiles in fungi isolated from oil-rich environments. *Mycosci.* 46:19-126.

Guarro, J., Gené, J. and Stchigel, A.M. 1999. Developments in fungal taxonomy. *Clin. Microbiol. Rev.* 12:454–500.

Gugi, B., Orange, N., Hellio, F., Burini, J.F., Guillou, C., Leriche, F. and Guespin-Michel, J.F. 1991. Effect of growth temperature on several exported enzyme activities in the psychrotrophic bacteria *Pseudomonas fluorescens. J. Bacteriol.* 173:3814–3820.

Guinazu, L.B, Andres, J.A., MFDel, P., Pistorio, M., and Rosas, S.B. 2010. Response of alfalfa (*Medicago sativa* L.) to single and mixed inoculation with phosphate-solubilizing bacteria and *Sinorhizobium meliloti. Biol. Fertil. Soils*, 46:185-190.

Gull, M., Hafeez, F.Y., Saleem, M. and Malik, A .2004. Phosphate uptake and growth promotion of chickpea by co-inoculation of mineral phosphate solubilizing bacteria and a mixed rhizobial culture. *Aust. J. Exp. Agric.* 44:623-628.

Gupta, N., Sabat, J., Parida, R. and Kerkatta, D. 2007. Solubilization of tricalcium phosphate and rock phosphate by microbes isolated from chromite, iron and manganese mines. *Acta. Bot. Croat.* 66: 197-204.

Gupta, R.D., Bhardwaj, K.R., Morwan, B.C., Tripathi, B.R. 1986. Occurrence of phosphate dissolving bacteria in soils of North Himalayas under varying biosequence and climosequence. *J. Ind. Soc. Soil. Sci.*34:498–504.

Gutierrez-Manero, F.J., Ramos-Solano, B., Probanza, A., Mehouachi, J., Francisco, R.T. and Manuel, T .2001. The plant-growth-promoting rhizobacteria *Bacillus pumilis* and *Bacillus licheniformis* produce high amounts of physiologically active gibberellins. *Physiolo.Plant.* 111:206-211.

Gyaneshwar, P., Naresh, K.G., Parekh, L.J. and Poole, P.S.2002.Role of soil microorganisms in improving P nutrition of plants. *Plant Soil.*245:83-93.

Halder, A.K., Mishra, A.K., Bhattacharyya, P. and Chakrabartty, P.K. 1990. Solubilization of rock phosphate by *Rhizobium* and *Bradyrhizobium*. *J. Gen. Appl. Microbiol.* 36: 81–92.

Halasz, A.1991. Yeasts. Food quality and Standards. *Encyclopedia of Life Support Syst.* Vol-111.6p.

Hamdali, H., Hafidi, M., Virolle, M.J. and Ouhdouch, Y. 2008. Rock phosphate solubilizing Actinimycetes: Screening for plant growth promoting activities. *World J. Microbiol. Biotechnol.* 24:2565-2575.

Hameeda, B., Harini, G., Rupela, O.P., Wani, S.P. and Reddy, G. 2006. Growth promotion of maize by phosphate solubilizing bacteria isolated from composts and macrofauna. *Microbiol. Res.* 163:234-242.

Han, H.S. and Lee, K.D. 2005. Plant growth promoting rhizobacteria effect on antioxidant status, photosynthesis, mineral uptake and growth of lettuce under soil salinity. *Res. J. Agric. Biol. Sci.* 1: 210-215.

Harman, G.E. and Bjorkman, T. 1998. Potential and existing use of *Trichoderma* and *Gliocladium* for plant disease control and plant growth enhancement. In: G.E. Harman and C.P. Kubicek (Eds.), Trichoderma and Gliocladium. Enzymes, Biological control and Commercial Applications, vol.2. taylor & Francis Ltd, London, United Kingdom, pp.229-265.

Harman, G. E. 2000. Myths and dogmas of biocontrol. Changes in perceptions derived from research on *Trichoderma harzianum* T-22. *Plant Dis.* 84:377-93.

Hassen, M.A. and Abdelgani, M.E. 2009.Effect of microbial biofertilization on nodulation, nitrogen and phosphorous content and forage yield of lablab bean (*Lablab purpureus* L.) *Am.-Eurasian J. Sustain. Agric.* 345-350.

Helen, J., Graeme, B.P., Ritz, K., Fordyce, D.A. and Geoffrey, G.M. 2002. Solubilization of calcium phosphate as a consequence of carbon translocation by *Rhizoctonia solani*. *FEMS Microbiol. Ecol.* 40: 65-71.

Hinsinger, P. 2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root induced chemical changes: a review. *Plant Soil.* 237:173-195.

Holl, F.B., Chanway, C.P., Turkington, R. and Radley, R.A.1988. Response of crested wheatgrass (*Agropyron cristatum* L.), perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) to inoculation with *Bacillus polymyxa*. *Soil Biol. Biochem.* 20: 19-24.

Howell, C. R., Beier, R. C., and Stipanovic, R. D. 1988. Production of ammonia by *Enterobacter cloacae* and its possible role in the biological control of *Pythium* pre- emergence damping-off by the bacterium. *Phytopathol.* 78:1075-1078.

Hussain, M. I., H. N. Asghar, M. J. Akhtar and Arshad, M. 2013. Impact of phosphate solubilizing bacteria on growth and yield of maize. *Soil Environ.* 32(1): 71-78

Igual, J. M., Valverde, A., Cervantes, E. and Velázquez, E. 2001. Phosphate-solubilizing bacteria as inoculants for agriculture: use of updated molecular techniques in their study. *Agron.*24-34.

Illmer, P., Barbato, A. and Schinner, F. 1995. Solubilization of hardly-soluble $AlPO_4$ with P-solubilizing microorganism. *Soil Biol. Biochem.* 27: 265–270.

Illmer, P. and Schinner, F. 1995. Solubilization of inorganic calcium phosphates solubilization mechanisms. *Soil Biol. Biochem.*27:257-263.

Isbelia, R., Bernier, L., Simard, R.R., Tanguay, P. and Antoun, H. 1999. Characteristics of phosphate solubilization by an isolate of a tropical *Penicillium rugulosum* and two UV induced mutants. *FEMS Microbiol. Ecol.* 28: 291-295.

Islam, T., Deora, A., Hashidokoa, Y., Rahmana, A., Itoa, T., and Tahara, S. 2006. Isolation and Identification of Potential Phosphate Solubilizing Bacteria from the Rhizoplane of Oryza sativa L. cv. BR29 of Bangladesh. *Naturforsch.* 103-110.

Jain, R., Saxena, J., Sharma, V. 2011. Determination of the organic acids in fermentation media by HPLC. *Int. J. Biol. Sci. Eng.* 2 (4): 261-265.

Jeon, J.S., Lee, S.S., Kim, H.Y., Ahn, T.S and Song, H.G .2003. Plant growth promotion in soil by some inoculated microorganisms. *J. Microbiol.* 41:271-276.

Jisha, M.S. and Alagawadi, A.R. 1996. Nutrient uptake and yield of sorghum (*Sorghum bicolor* L. Moench) inoculated with phosphate solubilizing bacteria and cellulolytic fungus in a cotton stalk amended vertisol. *Microbiol. Res.* 151: 213-217.

Jogaiah, S., Abdelrahman, M., Tran, L.S.P. and Shin-ichi, I. 2013. Characterization of rhizosphere fungi that mediate resistance in tomato against bacterial wilt disease. *J. Exp. Bot.* 64(12): 3829–3842.

Johnson, L.E., Bond, C.J. and Fribourg, H. 1959. Methods for studying soil microflora-plant disease relationships. Minneapolis: Burgess Publishing Company

Jones, C.G, Lawton, J.H, Shachak, M. 1994. Organisms as ecosystem engineers. *Oikos.* 69: 373–386.

Joseph, B., Ranjan, Patra, R. and Lawrence, R. 2007.Characterization of plant growth promoting rhizobacteria associated with chickpea (*Cicer arietinum* L.). *Int. J. Plant Prod.* 2: 141-152.

Joshi, R., McSpadden and Gardener, B.B. 2006. Identification and characterization of novel genetic Markers Associated with Biological Control Activities in *Bacillus subtilis*. *Phytopathol.* 96(2):145-54

Kadapati, G. S. 2001. Analysis of PGRS mutants of MPS bacteria and their effect on plant growth and nutrient uptake. M. Sc. (Agri) Thesis, Univ. Agril. Sci., Dharwad. 150pp

Kamilova, F., Lamers, G., and Lugtenberg, B. 2008. Biocontrol strain *Pseudomonas fluorescens* WCS365 inhibits germination of *Fusarium*

oxysporum spores in tomato root exudate as well as subsequent formation of new spores. *Environ. Microbiol.* 10(9):2455-2461.

Kang, B.G., Kim,W.T., Yun, H.S. and Chang, S.C. 2010. Use of plant growth promoting rhizobacteria to control stress responses of plant roots. *Plant Biotechnol.* 4:179-183.

Kang, S.M., Joo, G.J., Hamayun, M., Na, C.I., Shin, D.H., Kim,Y.K., Hong J.K., and Lee, I.J. 2009. Gibberellin production and phosphate solubilization by newly isolated strain of *Acinetobacter calcoaceticus* and its effect on plant growth. *Biotechnol. Lett.* 31: 277-281.

Kannapiran, E. and Ramkumar, V. S. 2011.Isolation of phosphate solubilizing bacteria from the sediments coast, Palk Strait, Southeast coast of India. *Ann. Biol. Res.* 5 : 157-163.

Kapoor, K.K., Mishra, M.M., and Kukreja, K.1989. Phosphate solubilization by soil microorganisms. A review. *Indian J. Microbiol.*29:119-127.

Karachi, N.E., Gharbi, S., Kihal, M. and Henni, J.E. 2010. Biological control of *Fusarium oxysporum* f.sp. *lycopersici* isolated from Algerian tomato by *Pseudomonas fluorescens*, *Bacillus cereus*, *Serratia marcescens* and *Trichoderma harzianum*. *Res. J. Agron.* 4(2): 31-34.

Katznelson, H., Petrson, E.A. and Rovat, J.W. 1962. Phosphate disolving microorganism on seed and in the root zone of plants. *Can. J. Bot.* 40:1181-1186.

KAU (Kerala Agricultural University). 2009. *Annual Report 2008-2009.* Kerala Agricultural University, Vellanikkara, Thrissur. Pp.156-157.

Kaufman, P.B., Wu, L., Brock, T.G., and Kim, D. 1995.Hormones and the orientation of growth. In: Davies, P. J., editor. Plant Hormones. Dordrecht, The Netherlands: Kluwer Academic. pp. 547–571.

Kaur, N., and Sharma, P. 2013. Screening and characterisation of native Pseudomonas sp. as plant growth promoting rhizobacteria in chickpea (*Cicer arietinum* L.) rhizosphere. *Afr. J. Microbiol. Res.* 7(16):1465-1474.

Kaviyarasi, K., Kanimozhi, K., Madhanraj, P., Panneerselvam, A. and Ambikapathy, V. 2011. Isolation, Identification and Molecular Characterization of Phosphate Solubilizing Actinomycetes Isolated From The Coastal Region of Manora, Thanjavur (Dt.). *Asian J. Pharma. Tech.* 1(4):119-122.

Kayasth, M., Kumar, V., Gera, R. and Dudeja, S.S. 2013. Isolation and characterization of salt tolerant phosphate solubilizing strain of *Pseudomonas* sp. from rhizosphere soil of weed growing in saline field. *Annal. Biol.* 29(2): 234-237.

Kendrick, W.B. 1963. Fungi associated with breakdown of pine leaf litter in the organic horizon of a podzol. *Mycopath. Appl.* 19:241-245.

Khalid, A., Arshad, M., and Zahir, Z.A. 2004. Screening plant growth promoting rhizobacteria for improving growth and yield of wheat. *J. Appl. Microbiol.* 46: 473-480.

Khalil, S. 1995. Direct application of phosphate rock and appropriate technology fertilizers in Pakistan. In K. Daha nayake et al. (eds.): Direct application of phosphate rock and appropriate technology fertilizers in Asia: what hinders acceptance and growth. Pp. 231236. Kandy, Sri Lanka: Institute of Fundamental Studies.

Khan, M.S, Zaidi, A., Wani, P. 2007. Role of phosphate solubilizing microorganisms in sustainable agriculture-A review. *Agron. Sustain.Develop.* 27:29-43.

Khan, A.A., Jilani, G., Akhtar, M.S., Naqvi, S.M.S. and Rasheed, M. 2009. Phosphorus Solubilizing Bacteria: Occurrence, Mechanisms and their Role in Crop Production. *J. Agric. Biol. Sci.* 1(1): 48-58.

Khan, M.R. and Khan, S.M. 2001. Effect of root-dip treatment with certain phosphate solubilizing microorganisms on Fusarial wilt of tomato. *Biores. Technol.*85:213-215.

Khan, M.S., Zaidi, A., and Wani, P.A. 2006. Role of phosphate-solubilizing microorganisms in sustainable agriculture-A review. *Agron. Sustain. Dev.* 27: 29-43.

Kirk , J.L., Beaudette, L. A., Hart , M., Moutoglis, P., Klironomos, J. N., Lee, H., Trevors, J.T. 2004. Methods of studying soil microbial diversity. *J. Microbiol. Methods.* 58 : 169 – 188.

Kishore, G.K., Pande, S., and Podile, A.R. 2005.Biological control of collar rot disease with broadspectrum antifungal bacteria associated with groundnut. *Can J. Microbiol.* 51: 123-132.

Kleifeld, O. and Chet, I. 1992. *Trichoderma* plant interaction and its effect on increased growth response. *Plant and Soil.*144:267-272.

Kloepper, J.W., Lifshitz, K. and Schroth, M.N .1988. *Pseudomonas* inoculants to benefit plant production. *Atlas Sci. Anim. Plant Sci.* 60–64.

Kloepper, J. W., Lifshitz, R., and Zablotowicz, R. M.1989. Free-living bacterial inocula for enhancing crop productivity. *Trends in Biotechnol.* 7: 39-44.

Koshy, M. M., and Varghese, T. 1972. *Soils of India.* The Fertilizer Association of India, New Delhi, 144-157pp.

Kovacs, N. 1956. Identification of *Pseudomonas pyocyanea* by the oxidase reaction. *Nat.* 178- 703.

Krasilinikov, N.A.1957. On the role of soil micro-organism in plant nutrition. *Microbiologiya*. 26: 659–72.

Kremer, R. J. and Souissi, T. 2001. Cyanide production by rhizobacteria and potential for suppression of weed seedling growth. *Curr. Opinions Microbiol.* 43: 182-186.

Kretzschmar, R.M., Hafner, H., Bationo, A. and Marschner, H. 1991. Long- and short-term effects of crop residues on aluminum toxicity, phosphorus availability and growth of pearl millet in an acid sandy soil. *Plant Soil* 136:215–223.

Krishnamurthy, K.O.1989. Effect of pesticides on phosphate solubilizer microorganisms. MSc (Ag) thesis, University of Agricultural Science, Dharwad,67p.

Kucey, R.M.N. 1983. Phosphate–solubilizing bacteria and fungi in various cultivated and virgin Alberta soils. *Can. J. Soil Sci.* 63: 671–678.

Kucey, R.M.N., Janzen, H.H. and Leggett, M.E.1989. Microbially mediated increases in plant-available phosphorus. *Adv. Agron.* 42: 199–228.

Kudashev, I.S.1956. The effect of phosphobacterin on the yield and protein content in grains of Autumm wheat, maize and soybean. *Doki. Akad. Skh. Nauk.* 8: 20–3.

Kuklinski-Sobral, J., Araujo, W., Mendes, R., Geraldi, I., Pizzirani-Kleiner, A., and Azevedo, J. 2004. Isolation and characterization of soybean-associated bacteria and their potential for plant growth promotion. *Environ. Microbiol.* 6: 1244-1251.

Kumar, D.B.S. 1999. Fusarial wilt supression and crop improvement through two rhizobacteia strains in chickpea growing in soils infested with *Fusarium oxysporum* f. sp. *ciceris*. *Biol. Fertil. Soil.* 29: 87-91.

Kumar, A., Kumar, A., Devi, S., Patil,S., Payal, C. and Sushila Negi, N. 2012. Isolation, screening and characterization of bacteria from rhizospheric soils for different plant growth promotion (PGP) activities: an *in vitro* study. *Rec. Res. Sci. Tech.* 4: 01-05.

Kumar, V. and Narula, N.1999. Solubilization of inorganic phosphates and growth emergence of wheat as affected by *Azotobacter chroococcum* mutants. *Biol. Fert. Soils*, 28: 301–5.

Kundu, B.S., Nehra, K., Yadav, R. and Tomar, M.2009. Biodiversity of phosphate solubilizing bacteria in rhizosphere of chickpea, mustard and wheat grown in different region of Haryana. *Indian J. Microbiol.* 49: 120–127.

Laheurite, F. and Berthelin, J. 1988. Effect of phosphate-solubilizing bacteria on maize growth and root exudation over four levels of labile phosphorus. *Plant Soil.*105:11–17.

Lcbuhn, M., Heulin, T. and Hartmann, A. 1997. Production of auxin and other indolic and phenolic compounds by *Paenibacillus polymyxa* strains isolated from different proximity to plant rook. *FEMS Microbiol. Ecol.* 22: 325-334.

Leinhos, V. and Vacek, O. 1994. Biosynthesis of auxins by phosphate solubilizing rhizobacteria from wheat (*Triticum aestivum*) and rye (*Secale cereale*). *Microbiol. Res.* 149: 31-35.

Lemanceau, P. 1992. Effets benefiques de rhizobacteries sur les plantes: exemple des *Pseudomonas* spp. fluorescent. *Agron.*12: 413–437.

Lifshtiz, R., Kloepper, J.W.E., Kozlowski, M., Simonson, C., Carlson, J., Tipping, E.M. and Zaleska, I. 1987. Growth promotion of canola (rapeseed) seedlings by a strain of Pseudomonas putida under gnotobiotic conditions. *Can. J. Microbiol.* 33: 309-395.

Linu, M.S., Stephen, J. and Jisha, M.S. 2009. Phosphate solubilizing *Gluconacetobacter* sp., *Burkholderia* sp. and their potential interaction with cowpea (*Vigna unguiculata* (L.) Walp.). *Int. J. Agric. Res.* 4: 79-87.

Lipping, Y., Jiatao, X., Daohong, J., Yanping, F., Guoqing. L. and Fangcan, L. 2008. Antifungal substances produced by *Penicillium oxalicum* strain PY-1-potential antibiotics against plant pathogenic fungi. *World J. Microbiol. Biotechnol.* 24:909-915.

Loper, J.E and Buyer, J.S.1991. Siderophores in microbial interactions on plant surfaces. Moleculare *Plant-Microbe Interactions*.4:5-13.

Lucy, M., Reed, E. and Glick, B.R. 2004. Application of free living plant growth promoting rhizobacteria. Antonie van leeuwenhoek. 86(1):1-25.

Ludwig, W., Strunk, O., Klugbauer, S., Klugbauer, N., Weizenegger, M., Neumaier, J., Bachleitner, M. and Schleifer, K.H. 1998. Bacterial phylogeny based on comparative sequence analysis. *Electrophoresis*. 19: 554-568.

Lynch, J.M. 1985. Origin, nature and biological activity of aliphatic substances and growth hormones found in soil. In: Soil Organic Matter and Biol. Activity. Eds. Vaughan D and Malcom RE. MartinusNijhoff/Dr. W. Junk Publishers. Dordrecht, Boston, Lan Lancaster pp.151-174.

Lynch, J.M.1990. Introduction: some consequences of microbial rhizosphere competence for plant and soil. In: Lynch, J.M. (ed.), *The Rhizosphere*. John Wiley and Sons, New York, pp.1-10.

MacFaddin, J.F. 2000. Biochemical tests for identification of medical bacteria (3rd ed.), Lippincott Williams and Wilkins, U.S.A. 912pp.

Mahidi, S.S., Hassan, G.I., Hussain, A. and Rasoo, I. F. 2011. Phosphorus Availability Issue- Its Fixation and Role of Phosphate Solubilizing Bacteria in Phosphate Solubilization. *Res. J. Agric. Sci.* 2:174-179.

Maheswar, N.U and Sathiyavani, G.2012. Solubilization of phosphate by *Bacillus* sp., from groundnut rhizosphere (*Arachis hypogaea* L). *J. Chem. Pharm. Res.* 4(8):4007-4011.

Mallesh, S.B., Narendrappa, T., and Kumari.2008. Management of root rot of sage (*Salvinia officinalis*) caused by *Fusarium solani* and *Rhizoctonia solani. Int. J. Plant Prot.*2(2):261-264.

Manivannan, M., Ganesh, P., Suresh Kumar, R., Tharmaraj, K. and Shiney R.B. 2012. Isolation, Screening, Characterization and Antagonism Assay of PGPR Isolates from Rhizosphere of Rice Plants in Cuddalore District. *Int. J. Pharma. Biol. Arch.* 3(1):179-185.

Manjaiah, K.M., Channal, H.T., and Satyanarayana, T. 1996. Influence of rock phosphate on nutrient dynamics in the presence of organic amendment and P solubilizer at different stages of growth of markers associated with biological control activities in *Bacillus subtilis. Biol. Control.* 96:145–154.

Manjunath, M., Prasanna, R., Sharma, P., Nain, L., and Singh, R. 2011. Developing PGPR consortia using novel genera *Providencia* and *Alcaligenes* along with cyanobacteria for wheat. *Arch. Agron. Soil Sci.* 57(8):873–887.

Marschner, H. 1995. *Mineral nutrition of higher plants* (2nd Ed.). Academic press, San Diego, 889pp.

Mathew, M.M. and Hameed, S.M.S.2002. Influence of microbial inoculants and phosphorus levels on root characters, growth and yield of vegetable cowpea (*Vigna unguiculata* sub sp. *sesquipedalis* (L) verdcourt). *J. Trop. Agric.* 40: 71-73.

McVicker, M. H., Bridger, G. L. and Nelson, L. B. 1963. *Advances in phosphate fertilization.* Fertilizer Technology and Usage, Soil Science Society of America, Madison II, Wisconsin, 155-187 pp.

Mikanova, O. and Kubat, J. 1994. Phosphorus solubilization from hardly soluble phosphates by soil microflora. Rostlina Vyroba 40: 833–840

Mishra, R. K., Prakash, O., Alam, M. and Dikshit, A. 2010. Influence of plant growth promoting rhizobacteria (PGPR) on the productivity of *Pelargonium graveolens* L. herit. *Rec. Res. Sci. Tech.* 2(5): 53-57.

Mittal, V., Singh, O., Nayyar, H., Kaur, J. and Tewari, R. 2008. Stimulatory effect of phosphate solubilizing fungal strains (*Aspergillus awamori* and *Penicillium citrinum*) on the yield of chickpea (*Cicer arietinum* L. cv. GPF2). *Soil Biol. Biochem.* 40:718-727.

Moat, A. D. and Foster, J.W. 1988. *Microbial Physiology.* John Wiley & Sons, Inc. USA., pp. 175-182.

Mohammadi, K., Ghalavand, A., Aghaalikhani, M., Heidari, G. and Sohrabi, Y. 2011. Introducing a sustainable soil fertility system for chickpea (*Cicer arietinum* L.). *Afr. J. Biotechnol.* 10(32):6011-6020.

Moretti, M., Gilardi, G., Gullino, M.L., and Garibaldi, A. 2008. Biological control potential of *Achromobacter xylosoxydans* for suppressing Fusarium wilt of tomato. *Int. J. Bot.* 4:369–375.

Moyer, C. L., Dobbs, F.C. and Karl, D.M .1994. Estimation of diversity and community structure through restriction fragment length polymorphism distribution analysis of bacterial 16S rRNA genes from microbial mat at an active, hydrothermal vent system, Loihi Seamount, Hawaii. *Appl. Environ. Microbiol.* 60: 871–879.

Mundra, S.. Arora, R.. Stobdan, T.2011. Solubilization of insoluble inorganic phosphates by a novel temperature, pH and salt tolerant yeast, *Rhodotorula* sp. PS4, isolated from seabuckthorn rhizosphere, growing in cold desert of Ladakh, India. *World. J. Micobiol.Technol.*27:2387-2396.

Muralidharan, A. and George, C.M. 1971. Effect of phosphorous and molybdenum on the growth and nodulation on ground nut. *Kerala Agricultural University J. Res.* 9:48:53.

Murray, R. G. E., Brenner, D. J., Colwell, R. R., De Vos, P., Goodfellow, M., Grimont, P. A. D., Pfenning, N., Stackebrandt, E. and Zavarzin, G. A. 1990. Report of the ad hoc committee on approaches to taxonomy within the Proteobacteria. *Int. J. Sys.t Bacteriol.* 40: 213–215.

Murthy, K.N., Uzma, F. and Srinivas, C. 2013. Induction of systemic resistance by *Trichoderma asperellum* against bacterial wilt of tomato caused by *Ralstonia solanacearum. Int. J. Adv. Res.*1 (10):181-194.

Nahas, E., 1996. Factors determining rock phosphate solubilization by micro–ganisms isolated from soil. *World J. Microbiol. Biotech.*, 12: 567–72.

Nair, C.B. and Anith, K.N. 2009. Efficacy of acibenzolar-S-methyl and rhizobacteria for the management of foliar blight disease of amaranth. *J. Trop. Agric.* 47 (1-2): 43-47.

Nair, S. K. and Subba Rao, N.S. 1977. Distribution and activity of phosphate solubilizing microorganisms in the rhizosphere of coconut and cocoa under mixed cropping. *J. Plantn. Crops.* 5: 67-70.

Najjar, G., Godlinski, F., Vassilev, N., Eichler-Löbermann, B. 2012. Dual inoculation with *Pseudomonas fluorescens* and arbuscular mycorrhizal fungi increases phosphorus uptake of maize and faba bean from rock phosphate. *Agric. For. Res.* 62:77-82.

Nath, A. K. and Borah, D. K. 1983. A study on the release on native and applied fixed phosphate as affected by pH and moisture regime. *Indian. J. Agric. Chem.* 16:247–251.

Nath, R., Sharma, G.D. and Barooah, M.2012. Efficiency of Tricalcium Phosphate Solubilization by Two Different Endophytic *Penicillium* sp.

Isolated from Tea (*Camellia sinensis* L.). *Eur. J. Exp. Biol.* 2 (4):1354-1358.

Nautiyal, C.S., Bhadauria, S., Kumar, P., Lal, H., and Verma, M.D. 2000. Stress induced phosphate solubilization in bacteria isolated from alkaline soils. *FEMS Microbiol. Lett.* 182:291–296.

Nautiyal, S.C. 1999. An efficient microbiological growth medium for screening phosphate solubilizing micro - organisms. *Microbiol. Lett.* 170: 265-270.

Neilands, J.B. and Nakamura, K. 1997. Detection determination isolation characterization and regulation of microbial iron chelates. In: Winkelmann G (Ed) CRC Handbook of Microbial Iron Chelates. CRC Press, Boca Raton. 1-14pp.

Neilands, J. B. 1981.Microbial iron compounds. *Annu. Rev. Biochem.* 50: 715-731.

Ng, L. C., Sariah, M., Sariam, O., Radziah, O. and Zainal, A. M.A. 2012. Rice seed bacterization for promoting germination and seedling growth under aerobic cultivation system. *Austr. J. Crop Sci.* 6(1):170-175.

Ngamau, C.N., Matiru, V.N., Tani, A. and Muthuri, C.W. 2012. Isolation and identification of endophytic bacteria of bananas (*Musa* spp.) in Kenya and their potential as biofertilizers for sustainable banana production. *Afri. J. Microbiol. Res.* 6(34): 6414-6422.

Ngoma, L., Esau, B., and Babalola, O.O. 2013. Isolation and characterization of beneficial indigenous endophytic bacteria for plant growth promoting activity in Molelwane Farm, Mafikeng, South Africa. *Afr. J. Biotechnol.* 12:4105-4114.

Ngoma, L., Mogatlanyane, K., and Babalola, O.O.2014. Screening of Endophytic Bacteria towards the Development of Cottage Industry: An *in Vitro* Study. *J. Hum. Ecol.* 47(1): 45-63.

Nguyen, M.T. and Ranamukhaarachchi, S.L. 2010. Soil-borne antagonists for biological control of bacterial wilt disease caused by *Ralstonia solanacearum* in tomato and pepper. *J. Plant .Pathol.* 92:395-406.

Nguyen, C., Yan, W., Le Tacon, F. and Lapayne, F.1992. Genetic variability of phosphate solubilizing activity by monocaryotic and dicaryotic mycelia of the ectomycorrhizal fungus *Laccaria bicolor* (Maire) P.D. Orton. *Plant and Soil.*143: 193-199.

Noel, T.C., Sheng, C., Yost, C., Pharis, R., and Hynes, M. 1996. *Rhizobium leguminosarum* as a plant growth promoting rhizobacterium: direct growth promotion of canola and lettuce. *Can. J. Microbiol.* 42: 279-283.

Nuraini, Y., Abadi, A.L., Soemarno and Ardyati, T. 2011. Potensial of legume and maize to stimulate population of nitrogen-fixing bacteria, phosphate solubilizing bacteria and indole acetic acid production. *J. Agric. Food Technol.* 1(12): 218-226.

Ogut, M., Er, F., and Kandemir, N. 2010. Phosphate solubilization potentials of soil *Acinetobacter* strains. *Biol. Fertil. Soils.* 46:707–715.

Okon, Y. and Kapulnik, Y. 1986. Development and function of *Azospirillum* inoculated roots. *Plant Soil.* 90: 3–16.

Olsen, S.R., Cole, C.V., Watanabe, F.S. and Dean, L.A.1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate, *USDA Circular No. 939*, US Govt. Print. Office, Washington, DC. pp 1-19

Omar, S. A. 1998. The role of rock-phosphate-solubilizing fungi and vesicular–arbuscular mycorrhiza (VAM) in growth of wheat plants fertilized with rock phosphate. *World J. Microbiol. Biotechnol.* 14:211-218.

Oosterhuis, D.M. and Jernstedt, J. 1999. Morphology and anatomy of the cotton plant. Chapt 2.1, pp. 175-206. In: W. Smith and J.S. Cothren (eds.). Cotton: Origin, History, Technology and Production. John Wiley & Sons, Inc., New York.

Oosterhuis, D. M., Okuba, M.A., and Mozaffari, M. 2008. Effect of soil-applied nitrogen fertilizer on the nitrogen content of cotton flowers. pp. 43-44. In: N.A. Slaton (ed.) Arkansas Soil Fertility Studies 2007. Ark. Agr. Exp. Sta., University of Arkansas, Fayetteville Arkansas. Research Series 558.

Osorio, N. W. and Habte, M. 2001. Synergistic influence of an arbuscular mycorrhizal fungus and a P solubilizing fungus on growth and P uptake of *Leucaena leucocephala. Arid Land Res. Manag.*15:263-274.

Pal, S.S. 1998. Interactions of an acid tolerant strain of phosphate solubilizing bacteria with a few acid tolerant crops. *Plant Soil.* 198:169-177.

Pandey, P., Kang, S.C., and Maheswari, D.K. 2005. Isolation of endophytic plant growth-promoting *Burkholderia* spp. MSSP from root nodules of *Mimosa pudica. Curr. Sci.* 89:177-180.

Pandey, A., Das, N., Kumar, B., Rinu, K. and Trivedi, P. 2008. Phosphate solubilization by *Penicillium* spp. isolated from soil samples of Indian Himalayan region. *W. J. Microbiol. Biotechnol.* 24: 97-102.

Pandey, A., Trivedi, P., Kumar, B., and Palni, L.M.S. 2006. Characterization of a phosphate solubilizing and antagonistic strain of *Pseudomonas putida* (B0) isolated from a sub-alpine location in the Indian central Himalaya. *Curr. Microbiol.* 53:102-107.

Panhwar,Q.A Naher, U. A., Jusop, S., Othman,R., Latif, M A.,and Ismail M.R.2014. Biochemical and Molecular Characterization of Potential Phosphate-Solubilizing Bacteria in Acid Sulfate Soils and Their Beneficial Effects on Rice Growth. 9(10): 97-241.

Paramageetham, C.H. and Babu, G.P. 2012. Antagonistic activity of Fluorescent Pseudomonads against a Polyphagous soil born plant pathogen- *Sclerotium rolfsii*. 1:436. Doi: 10.4172/ scientific reports.

Parani, K., and Saha, B.K. 2012. Prospects of Using Phosphate Solubilizing *Pseudomonas* as Bio Fertilizer. *Eur. J. Biol. Sci.* 4 (2): 40-44.

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 phospahte by a novel enivironmental stress-tolerant *Burkholderia vietnamiensis* M6 isolated from ginseng rhizospheric soil. *Appl. Microbiol. Biotechnol.* 86: 947-955.

Patten, C.L., and Glick, B.R. 2002. Role of *Pseudomonas putida* Indoleacetic Acid in Development of the Host Plant Root System. *Appl. Environ. Microbiol.* 68(8): 3795–3801.

Paul, D., Anandraj, M., Kumar, A. and Sharma, Y. R. 2005. Antagonatic mechanisms of florescent pseudomonad, against *Phytophthora capsicii* in black pepper (*Piper nigrum* L.). *J. Spices, Aromatic Crops.* 14 (2): 122-129.

Pavlica, D.A., Hora, T.I., Bradshaw, J.J., Skogerboe, R.K., and Baker, R. 1978. Volatiles from soil influencing activities of soil fungi. *Phytopathol.* 68: 758–765.

Perix, A., Rivas, R., Santa-Regina, I., Mateos, P., Martinez-Molina, E., Rodriguez-Barrueco, C. and Velazquez, E. 2004. *Pseudomonas lutea* sp. a novel phosphate-solubilizing bacterium isolated from the rhizosphere of grasses. *Internatl. J. Syst. Evol. Microbiol.* 54:847-850.

Perix, A., Rivas, R., Mateos; P.F., Martinez-Molina, E., Rodriguez , B.C. and Velazquez , E. 2003. *Pseudomonas rhizosphaerae* sp. nov., a novel

species that actively solubilizes phosphate *in vitro*. *Internatl. J. Syst. Evol. Microbiol.* 53:2067-2072.

Perneel, M., Heyrman, J., Adiobo, A., De Maeyer, K., Raaijmakers, J.M., De Vos, P., and Hofte, M. 2008. Characterisation of CMR5c and CMR12a, novel fluorescent *Pseudomonas* strains from the cocoyamrhizosphere with biocontrol activiy. *J. Appl. Microbiol.*103:1007-1020.

Pierre, W.H. 1938. *Phosphorus deficiency and soil fertility*. Soil and Men, Year book of Agric, 106pp.

Pieterse, C.M.J., Pelt, J.A.V., Wees, S.C.M.V., Ton, J.,Kloostererziel, K.M.L., Keurentjes , J.J.B., Verhagen, B.W.M., Knoester, M., Sluis, I.V.D., Bakker, P.A.H.M., and Loon, L.C.V.2001. Rhizobacteria mediated induced systemic resistance: Triggering, signaling and expression. *Eur. J. Plant Pathol.*107:51-61.

Pikovskaya, R.I.1948. Mobilization of phosphorus in soil in connection with the vital activity of some microbial species. *Microbiologiya.* 17:362–70.

Pitt, J.I. 1979. The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*. London: Academic Press Inc. 634 p.

Pleban, S., Ingel, F., and Chet, I.1995. Control of *Rhizoctonia solani* and *Sclerotium rolfsii* in the green house using endophytic *Bacillus* spp. *Euro. J. Pl. Pathol.* 101:665-672.

Ponmurugan, P. and Gopi, C.2006. *In vitro* production of growth regulators and phosphatase activity by phosphate solubilizing bacteria. *Afri. J. Biotechnol.* 5 (4): 348-350.

Poonguzhali, S., Madhaiyan, M., and Sa, T.M. 2008. Isolation and identification of phosphate solubilizing bacteria from Chinese cabbage and their effect on growth and P-utilization of plants. J. Microbiol. Biotechnol. 18:773-777.

Powell, A.A. and Matthews. 2005. Towards the validation of the controlled detoriation vigor test for small seeded vegetables. Seed Testing Int. *ISTA News Bull.*129: 21-24.

Pradhan, N. and Sukla, L.B. 2005. Solubilization of inorganic phosphate by fungi isolated from agriculture soil. *Afr. J. Biotechnol.* 5: 850-854.

Prakash, O., Verma, M., Sharma, P., Kumar, M., Kumari, K., Singh, A., Kumari H., Jit, S., Gupta, S.K., Khanna, M. and Lal, R. 2007. Polyphasic approach of bacterial classification – an overview of recent advances. *Indian. J. Microbiol.* 47:98–108.

Prasad, K., Aggarwal, A., Yadav, K., and Tanwar, A. 2012. Impact of different levels of superphosphate using arbuscular mycorrhizal fungi and *Pseudomonas fluorescens* on *Chrysanthemum indicum* L. *J. Soil. Sci. Plant. Nutr.* 12 (3): 451–462.

Radhakrishnan, Ramalingam, Kang-Bo, S., Byeong-Won, L., Chung-Dong, H., Suk-Bok, P., Chang-Hwan, P., Sung-Up, K., Choon-Ki, L., and In-Youl, B.2013. IAA-Producing *Penicillium* sp. NICS01 Triggers Plant Growth and Suppresses *Fusarium* sp.-Induced Oxidative Stress in Sesame (*Sesamum indicum* L.). *J. Microbiol. Biotechnol.* 23(6):856–863.

Raghothama, K.G .1999. Phosphate acquisition. *Annu. Rev. Plant Physiol.* 50: 665–693.

Rahi, P., Vyas, P., Sharma, S., Gulati, A., and Gulati, A. 2009. Plant growth promoting potential of the fungus *Discosia* sp. FIHB 571 from tea rhizosphere tested on chickpea, maize and pea. *Indian Microbiol.* 49 (2) :128–133.

Raaijmakers, J. M., Vlami, M., and de Souza, J. T. 2002. Antibiotic production by bacterial biocontrol agents. *Antonie van Leeuwenhoek.* Kluwer Academic Publishers.**81**: 537–547.

Raj, J., Bagyaraj, D. J. and Manjunath, A. 1981. Influence of soil inoculation with vesicular arbuscular mycorrhizae and a phosphate-dissolving bacterium on plant growth and P^{32} uptake. *Soil Biol. Biochem.*13: 105-108.

Rajan, P.P., Sarma, Y.R., Anandaraj, M. 2002.Management of foot rot disease of black pepper with *Trichoderma* sp. *Indian Phytopath.*55(1):17-21.

Rajasekharan, P., Nair, K. M., Rajasree, G., Sureshkumar, P., and Narayanan Kutty, M. C. 2013. *Soil Fertility Assessment and Information Management for Enhancing Crop Productivity in Kerala.* Kerala State Planning Board, Thiruvananthapuram, 1–514pp.

Rakh, R. R .2011.Biological Control of *Sclerotium rolfsii*, Causing Stem Rot of Groundnut By *Pseudomonas* Cf *monteilii* 9. *Recent Res. Sci. Technol.* 3(3): 26-34.

Ramachandran, K., Srinivasan, V., Hamza, S., and Anadaraj, M. 2007. Phosphate solubilizing bacteria isolated from the rhizosphere soil and its growth promotion on black pepper (*Piper nigrum* L.) cutting. *Plant Soil Sci.* 102: 325-331.

Ramani, V. and Patel, H.H. 2011. Phosphate solubilization by *Bacillus sphaericus* and *Burkholderia cepacia* in presence of pesticides. *J. Agric. Technol.* 7(5): 1331-1337.

Ramesh, R., Joshi, A. A. and Ghanekar, M.P. 2009. Pseudomonads: major antagonistic endophytic bacteria to suppress bacterial wilt pathogen, *Ralstonia solanacearum* in the eggplant (*Solanum melongena* L.). *World J. Microbiol. Biotechnol.* 25:47- 55.

Ramette, A., Irapolli, M., Defago, G. and Moenne-Loccoz, Y. 2003. Phylogeny of HCN synthase encoding hcnbc genes in biocontrol fluorescent pseudomonads and its relationship with host plant species and HCN synthesis ability. *Mol. Biol. Plant Microb. Interact.* 16 : 525-535.

Ramkumar, V. and Kannapiran, E. 2011. Isolation of total heterotrophic bacteria and phosphate solubilizing bacteria and in vitro study of phosphatase activity and production of phytohormones by PSB. *Arch. Appl. Sci. Res.* 3:581-586.

Ramzan, N., Noreen, N., and Shahzad, S. 2014. Inhibition of *in vitro* growth of soil-borne pathogens by compost-inhabiting indigenous bacteria and fungi. *Pak. J. Bot.* 46(3): 1093-1099.

Rana, A., Saharan, B., Kabi, S.R., Prasanna, R., and Nain, L. 2011. *Providencia,* a PGPR with biocontrol potential elicits defense enzymes in wheat. *Ann. Plant Prot. Sci.* 19 (1): 138-141.

Rane, M.R., Sarode, P.D., Chaudhari, B.L. and Chincholkar, S.B. 2008. Exploring antagonistic metabolites of established biocontrol agent of marine origin. *Appl. Biochem. Biotechnol.*151:665-675.

Rani, M.S., Lakshmi, V.K., Suvarnalatha, D.P., Madhuri, R.J., Aruna, S., Jyothi, K., Arasimha, G., and Venkateswarlu, K. 2008. Isolation and characterization of a chlorpyrifos degrading bacterium from agricultural soil and its growth response. *Afr. J .Microbiol. Res.* 2:026–031.

Ravikumar, S., Shanthy, S., Kalaiarasi, A. and Sumaya, S.2010. Bioinoculation of halophilic phosphobacteria for raising vigorous seedlings of *Rhizophora mucronata. Arch. Appl. Sci. Res.* 2 (6):261-266.

Reddy, M.S. and Singh, H. 2011. Field evaluation of P solubilizing bacteria on the growth and yield of wheat and maize grown in rock phosphate amended soils. Proceedings of the 2nd Asian PGPR Conference, Beijing, P.R. China, pp 92.

Reddy, M.S., Kumar, S., Babita, K., Reddy,M.S. 2002.Biosolubilization of poorly soluble rock phosphates by *Aspergillus tubingensis* & *Aspergillus niger.Bioresource Technol.*84, 187- 189.

Reetha, S., Bhuvaneswari, G., Thamizhiniyan, P., and Mycin, T.R. 2014. Isolation of indole acetic acid (IAA) producing rhizobacteria of *Pseudomonas fluorescens* and *Bacillus subtilis* and enhance growth of onion (*Allim cepa*.L). *Int. J. Curr. Microbiol. App. Sci.* 3(2): 568-574.

Ren, Y.X., Zhu, X.L., Fan, D.D., Ma, P., and Liang, L.H .2013. Inoculation of phosphate solubilizing bacteria for the improvement of lead accumulation by *Brassica juncea. Environ. Technol.* 34: 436-469.

Richa, G., Khosla, B., and Sudhakara Reddy, M .2007. Improvement of maize plant growth by phosphate solubilizing fungi in rock phosphate amended soils. *World J. Agric.Sci.*, 3(4): 481-484 .

Richardson, A. E. 2004. *Soil microorganisms and phosphorus availability.* Management in Sustainable Farming Systems Melbourne, Australia: CSIRO, pp50-62.

Richardson, A.E. 2001. Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. *Aust. J. Plant. Physiol.* 28: 897–906.

Rini, C.R. and Sulochana, K.K. 2007. Usefulness of *Trichoderma* and *Pseudomonas* against *Rhizoctonia solani* and *Fusarium oxysporum* infecting tomato. *J. Trop. Agric.* 45 (1-2): 21–28.

Rivas, R., Trujillo, M.E., Sanchez, M., Mateos, P.F., Martinez-Molina, E. and Velasquez, E. 2004. *Microbacterium ulmi* sp. Nov. a xilanolytic, phosphate-solubilizing bacterium isolated from sawdust of *Ulmus nigra. Int. J. Syst. Evol. Microbiol.*54:513-517.

Rodriguez, H. and Fraga, R. 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnol. Adv.*17: 319-339.

Ryder, M. H., Stephens, P. M., and Bowen, G. D. 1994. *Improving Plant Productivity with Rhizosphere Bacteria.* Commonwealth Scientific and Industrial Research Organization, Adelaide, Australia. 278p

Sabannavar, S.J. and Lakshman. H.C.2009. Effect of rock phosphate solubilization using mycorrhizal fungi and phosphobacteria on two high yielding varieties of *Sesamum indicum* L. *World J. Agric. Sci.* 5(4):470–479.

Sahin, F., Cakmakci, R. and Kantar, F .2004. Sugar beet and barely yields in relation to inoculation with N2 fixing and phosphate solubilizing bacteria. *Plant Soil.* 265:123-129.

SailajaKumari, M.S. and Ushakumari, K.2002. Effect of vermicompost enriched with rock phosphate on the yield and uptake of nutrients in cowpea (*vigna unguiculata* l. walp). *J. Tro. Agric.* 40: 27-30.

Sajnanath, K. 2000. Soil health management practices. ICAR–KVK– Alappuzha, CPCRI. 23p

Sakthivel, N. and Gnanamanikam, S. S.1987. Evaluation of *Pseudomonas fluorescens* for suppression of sheath rot disease and enhancement of grain yields in rice (*Oryza sativa* L.). *Appl. Environ. Microbiol.* 2056-2059.

Salisbury, F.B. 1994. The Role of Plant Hormones. In: Plant-Environment Interactions, Wilkinson, R.E. (Ed.). Marcel Dekker, New York, USA. pp: 39-81.

Sambrook, J., Fritsch, E.F and Maniatis, T.1989.Molecular cloning: A Laboratory Manual. 2nd edition. Cold Spring Harbor Laboratory Press. New York, USA, 1322p.

Samuel, S. and Muthukkaruppan, S. M. 2011. Characterization of plant growth promoting rhizobacteria and fungi associated with rice, mangrove and effluent contaminated soil. *Curr. Bot.* 2(3): 22-25.

Sanjotha, P., Mahantesh, P., and Patil, C.S.2011. Isolation and Screening of efficiency of Phosphate Solubilizing Microbes. *Int. J. Microbiol. Res.* 31:56-58.

Saraf, M., Thakker, A. and Patel, B.V. 2008. Biocontrol activity of different species of *Pseudomonas* against phytopathogenic fungi *in vivo* and *in vitro* conditions. *Inter. J. Biotechnol. Biochem.* 4: 223-232.

Saxena, A., Sharma, A., Goel, R. and Johri, B. N. 1996. Functional characterization of a growth promoting fluorescent pseudomonad from rajanigandha rhizosphere[Abstract]. *37th Annual. Conference of Association of Microbiologists*. India, December 4-6, IIT, Chennai, 135p.

Sayyed, R. Z., Badgujar, M. D., Sonawane, H. M., Mhaske, M. M., and Chincholkar, S. B. 2005. Production of microbial iron chelators (sideropores) by fluorescent pseudomonads. *Indian. J. Biotechnol.* 4: 484-549.

Schaeffer, A.B. and Fulton, M. 1993. A simplified method of staining endospores. *Sci.* 77-194.

Selvakumar, G., Piyush, J., Sehar, N., Mishra, P.K., Bisht, J.K., and Gupta, H.S. 2009. Phosphate solubilization and growth promotion by *pseudomonas fragi* cs11rh1 (mtcc 8984) a psychrotolerant bacterium isolated from a high altitude himalayan rhizosphere. B*biologia.* 64: 239-245.

Selvakumar, G., Joshi, P., Suyal, P., Mishra, P.K., Joshi, G.K., Venugopalan, R., Bisht, J.K., Bhatt, J.C., and Gupta, H.S. 2013.Rock phosphate solubilization by psychrotolerant *Pseudomonas* spp. and their effect on lentil growth and nutrient uptake under polyhouse conditions. *Annal Microbiol.* 63(4):1353–1362.

Sendukumaran, S., Natarajan, S. and Thamburaj, S. 1998. Effect of organic and inorganic fertilizers on growth, yield and quality of tomato. *S. Indian Hortic.* 46: 203-205.

Shabani, G., Ardakani, M.R., Chaichi, M.R., Friedel, J.K., Khavazi, K. and Eshghizadeh, H.R. 2011. Effect of different fertilizing systems of seed

yield and phosphorus uptake in annual medics under dryland farming conditions. *Not. Bot .Horti .Agrobo.* 39:191-197.

Shah, S., Nasreen, S., and Sheikh, P.A. 2012. Cultural and Morphological Characterization of *Trichoderma* spp. Associated with Green Mold Disease of *Pleurotus* spp. in Kashmir. *Res. J. Microbiol.* 7: 139-144.

Shahab, S., Ahmed, N. and Khan, N.S. 2009. Indole acetic acid production and enhanced plant growth promotion by indigenous PSBs. *Afr. J. Agric. Res.* 4:1312-1316.

Sharma, B.C., Subba, R. and Saha, A. 2012. *In-vitro* solubilization of tricalcium phosphate and production of IAA by phosphate solubilizing bacteria isolated from tea rhizosphere of Darjeeling Himalaya. *Plant Sci. Feed.* 2 (6):96-99.

Sharma, P. 2011. Complexity of Trichoderma –Fusarium interaction and manifestation of biological control. *Aust. J. Crop. Sci.* 5(8):1027-1038.

Sharma, K., Dak, G., Agrawal,A., Bhatnagar, M., and Sharma, R. 2007. Effect of phosphate solubilizing bacteria on the germination of *Cicer arietinum* seeds and Seedling growth *J. of Herbal Med and Toxicol* 1(1) 61-63.

Sharma, K.2005. Manual of Microbiology.In: Isolation, purification and identification of bacteria. Ane Books Pub. New Delhi, p. 41.

Shehana, R.S. and Abraham, A. 2001. Efficiency of Phosphorous solubilising organisms in acidic laterite soil. *J. Trop. Agr.* 39 (2001) : 57-59

Sherene, T. 2012. Jhabua rock phosphate as a phosphorus source in maximizing yield potential of cotton. *J. Cotton Res. Dev.* 26 (1) 90-91.

Shovitri, M., Kuswytasari, N.D., and Zulaika, E. 2014. P-solubilizing Microorganisms from The Poteran Island, Madura [abstract]. In: *The 8th Korean-Asean Joint Symposium on Biomass Utilization and Renewable Energy*; 18-22, August, 2014, Seoul, Korea.p.7

Singal, R., Gupta, R.; and Saxena, .R.K .1994. Rock phosphate solubilization under alkaline conditions by *Aspergillus japonicus* and *A. foetidus*. *Folia Microbiol.* 39:33–36.

Singh, H., Reddy .M.S .2011. Effect of inoculation with phosphate solubilizing fungus on growth and nutrient uptake of wheat and maize plants fertilized with rock phosphate in alkaline soils. *Eur. J. Soil. Biol.* 47:30-34.

Singh, S., Singh, R.D., and Girish, B.H.2013. Effect of Different Form of Phosphorous Nutrition on Growth and Yield of Cauliflower (*Brassica Olaracea* Var. *Botrytis* L.) .*Int. J. Agric. Environ. Biotechnol.* 6(2): 283-286.

Sivaprasad, P and Meenakumari, K.S. 2005. National workshop on Microbial inoculants for crop Nutrition and Health. College of Agriculture, Vellayani, Thiruvananthapuram, Kerala, India. 116-118 p.

Sivasakthi, S., Kanchana, D., Usharani, G., and Saranraj, P. 2013. Production of plant growth promoting substance by *Pseudomonas fluorescens* and *Bacillus subtilis* isolated from paddy rhizosphere soil of Cuddalore district, Tamil Nadu, India. *Inter. J. Microbiol. Res.* 4(3): 227-233.

Skrary, F.A. and Cameron, D.C .1998. Purification and characterization of a *Bacillus licheniformis* phosphatase specific for d-alphaglycerphosphate. *Arch. Biochem. Biophys.* 349:27-35.

Sneath, P.H.A. 2005. The preparation of the Approved Lists of Bacterial Names. *Int. J. Syst. Evol. Microbiol.* 55: 2047–2048.

Son, T. T. N., Diep, C.N., and Giang, T.T.M. 2006. Effect of bradyrhizobia and phosphate solubilizing bacteria application on Soybean in rotational system in the Mekong delta. *Omonrice.* 14:48-57.

Song, O.R., Lee, S.J., Lee, S.C., Kim, K.K., and Choi, Y.L. 2008. Solubilization of insoluble inorganic phosphate by *Burkholderia cepacia* DA23 isolated from cultivated soil. *Braz. J. Microbiol.* 39: 151-156.

Sowmya , M., Rejula , M.P., Rejith , P.G., Mohan , M., Karuppiah, M. and Hatha; A.A.M. 2013. Heavy metal tolerant halophilic bacteria from Vembanad Lake as possible source for bioremediation of lead and cadmium. *J. Enviro. Biol.* 35: 655-660.

Sparks, D.L. 1999. Kinetics and mechanisms of chemical reactions at the soil mineral/water interface. In: D.L. Sparks (ed.), *Soil physical chemistry* (2^{nd} Ed.). CRC Press, Boca Raton, FL, pp. 135-191.

Sperber, J. I. 1958. The incidence of apatite solubilizing organisms in the rhizosphere and soil. *Aust. J. Agric. Res.* 9, 778–781

Sreenivasa, M.N., Nagaraj, N. and Bhat, S. N. 2009. Beneficial traits of microbial isolates of organic liquid manures, Proc. First Asian PGPR Congress for Sust. Agric., 21-24, June, 2009, ANGRAU, Hyderabad.

Sridevi, M.. Mallaiah, K.V.; Yadav, N.C.S. 2007. Phosphate solubilization by *Rhizobium* isolates from *Crotalaria* species. *J. Plant Sci.* 2: 635-639.

Srinivasan, R. 2002. Studies on phosphate solubilizing microorganisms from salt affected soils. M. Sc.(Agri.) Thesis, Univ. Agril. Sci., Dharwad. 140pp.

Srivastav, S., Yadav, K. S., and Kundu, B. S. 2004. Prospects of using phosphate solubilizing *Pseudomonas* as biofungicide. *Indian J. Microbiol.* 44:91–94.

Stackebrandt, E. and Goebel, B.M. 1994. Taxonomic Note: A Place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int. J .Syst. Bact.* 44: 846-849.

Stephen, J. and Jisha, M.S.2011. Short communication Gluconic acid production as the principal mechanism of mineral phosphate solubilization by *Burkholderia* sp. (MTCC 8369). *J. Trop. Agric.* 49 (1-2): 99-103.

Stevenson, F.J. 1986. *Cycles of soil carbon, nitrogen, phosphorus, sulphur micronutrients*. Wiley, New York, 231-284p.

Sturz, A. V., Christie, B. R , Matheson, B. G. and Nowak, J. 1997. Biodversity of endophytic bacteria which colonize red clover nodules, roots, stems and foliage and their influence on host growth. *Biol. Fertil. Soils*.25:13-19.

Subbarao, N. S. 1988. Phosphate solubilizing micro-organism. In: Biofertilizer in agriculture and forestry. Regional Biofert. Dev. Centre, Hissar, India. pp. 133-142.

Sultana, S. M., Maya, C., Peter , V. and Farooq, O. 2010. Biochemical characterisation of phosphobacterium isolated from rhizosphere of *Costus* sp. with special reference to *Bacillus* species. *Int. J. Pharma. Sci.Res.* 1:116-119.

Sundara Rao, W.V.B., Mann, H.S., Pal, N.B. and Mathur, R.S.1963. Bacterial inoculation experiments with special reference to Azotobacter. *I. J. Agric. Sci.* 33: 279-290.

Sundara, B., Natarajan, V. and Hari, K. 2002. Influence of phosphorus solubilizing bacteria on the changes in soil available phosphorus and sugarcane yields. *Field Crops Res.* 77:43-49.

Sundari, A. and Sureshkumar, S. M. 2004. Influence of DAP and phosphobacteria on growth and yield of blackgram under rice fallow condition. *J. Ecobiol.*16 (1): 75-77.

Supanjani, Han, H.S., Jung, S.J. and Lee, K.D. 2006. Rock phosphate potassium and rock solubilizing bacteria as alternative sustainable fertilizers. *Agron. Sustain. Dev.* 26: 233-240.

Surapat, W., Pukahuta, C., Rattanachaikunsopon, P., Aimi, T. and Boonlue, S. 2013. Characteristics of Phosphate Solubilization by Phosphate-Solubilizing Bacteria Isolated from Agricultural Chili Soil and Their

Efficiency on the Growth of Chili (*Capsicum frutescens* L. cv. Hua Rua). *Chiang Mai .J. Sci.* 40(1): 11-25.

Suslov, T.V. 1982. Role of root-colonizing bacteria in plant growth,.InMount MS, Lacy GH (ed), Phytopathogenic prokaryotes. Academic Press, London, United Kingdom. p 187–223.

Suslov, T. V., Schroth, M. N., and Isaka, M. 1982. Application of a rapid method for gram differentiation of plant pathogenic and saprophytic bacteria without staining. *Phytopathol.*72: 917-918.

Swaby, R. J., and Sperber, J. 1958. Phosphate dissolving microorganisms in the rhizosphere of legumes. Nutrition of the legumes. Proc. Univ. Nottingham 5th Easter *Sch. Agric. Sci.*, 289–294.

Swamy, C.A.K., Raghunandan, B.L., Chandrashekhar, M., and Brahmaprakash, G.P. 2010. Bio-activation of rock phosphate vis- a- vis seed treatment with phosphorus solubilizing microbes (PSM) in enhancing P nutrition in cowpea and ragi. *Indian J. Sci. Technol.* 3:4.

Sylvia, D.M., Fuhrmann, J.J., Hartel . P.G., Zuberer, D.A.1999. Principles and Applications of Soil Microbiology. Prentice Hall Inc. Upper saddle River. NJ. 672 pp.

Tambong, J.T., Hofte, M. 2001. Phenazines are involved in biocontrol of *Pythium myriotylum* on cocoyam by *Pseudomonas aeruginosa* PNA1. *Eur. J. Plant Pathol.* 107: 511-521.

Thaha, S.M., Mahmoud, S.A.Z., Halim, El-Damaty, A. and Abd. El-Hafez, A.M. 1969. Activity of phosphate dissolving bacteria in Egyptian soils. *Plant and Soil.*31:149-160.

Thakuria, D., Talukdar, N.C., Goswami, C., Hazarika, S., Boro, R.C. and Khan, M.R. 2004. Characterization and screening of bacteria from the rhizo-

sphere of rice grown in acidic soils of Assam. *Current Science* 86: 978-985.

Thaller, M.C., Berlutti, F., Schippa, S., Iori, P., Passariello, C. and Rossolini, G.M. 1995. Heterogeneous patterns of acid phosphatases containing low-molecular-mass Polipeptides in members of the family Enterobacteriaceae. *Int. J. Syst. bacteriol.* 4: 255–261.

Thomas, G.J., Sweetingham, M.W., Yang, H.A. and Speijers, J. 2008. Effect of temperature on growth of *Colletotrichum lupini* and on anthracnose infection and resistance in lupins. *Aust. Plant Pathol.* 37:35-39.

Thomas, R.1996. Effect of biofertlizers on N and P economy in palmarosa (*Cymbopogon* martinii). MSc(Ag) thesis, Kerala Agricultural University, Thrissur, 136p.

Tilak, K.V.B.R. 1991. Bacterial fertilizers. New Delhi: Publications and Information Division I.C.A.R. p 66.

Tilak, K. V. B. R., Saxena, A. K., and Datt, N.1995. Dynamics of microbes in agricultural soil with different management practices. *J. Soil Biol. Ecol.*15:117–126.

Tiwari, V.N., Lehri, L.K. and Pathak, A.N.1989. Effect of inoculationg crops with phospho-microbes. *Expl. Agric.*25:47-50.

Tjamos, E.C., Papavizas, G.C., Cook, R.J. 1992. *Biological control of plant diseases. Progress and challenges for the future.* Plenum Press, New York, 462p.

Tomar, N.K., Gupta, A.P., and Khanna, S.S. 1984.Evaluation of rock phosphate super phosphate mixtures by incubation in organic matter for efficient use in wheat. *Fertil. News.*29:37-38.

Tomar, R.K.S.1998. Effect of phosphate solubilizing bacteria and farmyard manure on the yield of blackgram (*Phaseolus mungo*). *Indian J. Agric. Sci.*, 68: 81-83.

Tripti, Kumar, V. and Anshumali. 2012. Phosphate Solubizing Activity of Some Bacterial Strains Isolated from Chemical Pesticide Exposed Agricultre soil. *Int. J. Eng. Res. Dev.* 39:01-06.

Trivedi, P. and Pandey, A. 2008. Plant growth promotion abilities and formulation of *Bacillus megaterium* strain B 388 (MTCC6521) Isolated from a Temperate Himalayan Location. *Indian J. Microbiol.* 48(3): 342-347.

Uchida, R. 2000. *Essential nutrients for plant growth: Nutrient functions and deficiency*. USDA, Washington, D.C., 377-396p.

Van Peer, R., Niemann, G.J. and Schippers, B.1991. Induced resistance and phytoalexin accumulation in biological control of *Fusarium* wilt of carnation by *Pseudomonas* sp. *Phytopathol.* 81:728–734.

Van Schie, B.J., Van Dijken, J.P. and Kuenen, J.G. 1984. Non-coordinated synthesis of glucose dehydrogenase and its prosthetic group PQQ in *Acinetobacter* and *Pseudomonas* species. *FEMS Microbiol. Lett.* 24 (1): 133–138.

Vandamme, P., Pot, B., Gillis, M., De Vos, P., Kersters, K. and Swings, J. 1996. Polyphasic taxonomy, a consensus approach to bacterial systematics. *Microbiol. Rev.* 60: 407–438.

Van Hamme, J.D., Singh, A., and Ward, O.P. 2003. Recent Advances in Petroleum Microbiology. *Microbiol. Mol. Biol. Rev.* 67(4): 503–549.

Vassilev, N. and Vassileva, M.2003. Biotechnological solubilization of rock phosphate on media containing agro-industrial wastes. *Appl. Microbiol. Biotechnol.* 61(5-6):435-40.

Vazquez, P., Holguin, G., Puente, M.E., Lopez-Cortes, A. and Bashan, Y. 2000. Phosphate-solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. *Biol. Fertil. Soils*.30:460–468.

Venkateswaran, K. and Natarajan, R. 1985. Seasonal distribution of inorganic phosphate solubilizing bacteria and phosphatase producing bacteria in Porto Novo waters. *Indian J. Mar. Sci.* 12: 213-217.

Venkateswarlu , B., Rao, A.V., Raina, P., and Ahmad, N.1984.Evaluation of phosphorous solubilization by microorganisms isolated from aridisols. *J. Indian Soc. Soil Sci.* 32:273-277.

Vessey, J. K. 2003. Plant growth promoting rhizobacteria as biofertilizer. *Plant soil.* 255: 571- 586.

Victoria, D. E., Reyes L. L. and Benitez, A. D. L. C. 2009. Use of 16S ribosomal gene for characterization of phosphate solubilizing bacteria associated with corn. *Rev. Fitotec. Mex.* 32(1): 31-37.

Vikram, A., Hamzehzarghani, H., Alagawadi, A.R.,Krishnaraj, P.U., and Chandrashekar, B. S. 2007. Production of plant growth promoting substances by phosphate solubilizing bacteria isolated from vertisols. *J. Plant Sci.* 2: 326-333.

Vincent, J.M. 1927. Distortion of fungal hyphae in the presence of certain inhibitors. *Nat.* 59:850.

Vyas, P. and Gulati, A. 2009. Organic acid production *in vitro* and plant growth promotion in maize under controlled environment by phosphate-solubilizing fluorescent *Pseudomonas*. *BMC Microbiol.* 9(174):1-15.

Walpola, B.C and Yoon, M. 2013. Isolation and characterization of phosphate solubilizing bacteria and their co-inoculation efficiency on tomato plant growth and phosphorous uptake. *Afr. J. Microbiol. Res.* 7(3):266-275.

Walpola, B.C., Arunakumara, K.K.I.U., Yoon, M.H.2014. Isolation and characterisation of phospahte solubilizing bacteria (*Klebsiella oxytoca*) with enhanced tolerant to environmental stress. *African J. Micobiol Res.* Vol8(31).2970-2978.

Walsh, U.F, Morrissey, J.P. and Gara, F. 2001. *Pseudomonas* for biocontrol of phytopathogens: from functional genomics to commercial exploitation. *Curr. Opin. Biotechnol.* 12:289 – 295.

Wani, P.A., Khan, M.S. and Zaidi, A. 2007. Co inoculation of nitrogen fixing and phosphate solubilizing bacteria to promote growth, yield and nutrient uptake in chickpea. *Acta. Agron. Hung.* 55:315-323.

Wasule, D. L., Wadyalkar, S. R. and Buldeo, A. N. 2007. Effect of Phosphate Solubilizing Bacteria on Role of *Rhizobium* on Nodulation by Soybean. In: "First International Meeting on Microbial Phosphate Solubilization", (Eds.): Velazquez, E. and Rodriguez- Barrueco, C. PP.139-142.

Watanabe, F.S. and Olsen, S.R. 1965. Test of an ascorbic acid method for determining phosphorus in water and $NaHCO_3$ extracts from soil. *Soil Sci. Soc. Am. Proc.* 29:677-8.

Wayne, L. G., Brenner, D. J. and Colwell, R. R. 1987. International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int. J. Syst. Bacteriol.* 37: 463–464.

Wei , Z., Yanga, X., Yinb, S., Shena, Q., Rana, W., Xua,Y. Efficacy of Bacillus-fortified organic fertiliser in controlling bacterial wilt of tomato in the field. *Applied Soil Ecology* 48. 152–159.

Wei ,Z., Yanga,X., Yin, S., Shen, Q., Rana, W., Xu, Y.2011. Efficacy of Bacillus-fortified organic fertiliser in controlling bacterial wilt of tomato in the field. *Applied Soil Ecology* 48: 152–159.

Weller, D.M.1988. Biological control of soil borne plant pathogens in the Rhizosphere with Bacteria. *Ann.Rev.Phytopathol.* 26:379-407.

Whitelaw, M.A., Harden, R.J., and Helyar, K.R. 1999. Phosphate solubilisation in culture by the soil fungus *Penicillium radicum.* 31:655-665.

Whitelaw, M.A. 2000. Growth promotion of plants inoculated with phosphate solubilizing fungi. *Adv. Agron.* 69:99-151.

Woyessa, D. and Assefa, F. 2011. Diversity and Plant Growth Promoting Properties of Rhizobacteria Isolated from tef (*Eragrostis tef*). *Ethiop. J. Educ. Sci.* 1-14.

Xiao, C., Chi, R., Huang, X., Zhang, W., Qiu, G., and Wang, D. 2008. Optimization for rock phosphate solubilizing fungi isolated from phosphate mines. *Ecol. Eng.* 33: 187-193.

Xiao ,C., Chi , R., He , H., Zhang ,W.2009. Characterization of tricalcium phosphate solubilization by *Stenotrophomonas maltophilia* YC isolated from phosphate mines. *J. Central South University of Technol.* V(16). pp 581-587

Yadav, S.K., Dave, A., Sarkar, A., Singh, H.B. and Sharma, B.K. 2013. Coinoculated biopriming with *Trichoderma, Pseudomonas* and *Rhizobium* improves crop growth in *Cicer arietinum* and *Phaseolus vulgaris. Int. J. Agric. Biol.* 6(2):255-259.

Yadav, B. K. and Tarafdar, J.C. 2011. *Penicillium purpurogenum,* unique P mobilizers in arid agro-ecosystems. *Arid Land Res. Manag.* 25(1): 87-99.

Yadav, J., Verma J.P., and Tiwari, K.N. 2011. Plant Growth Promoting Activities of Fungi and their Effect on Chickpea Plant Growth. *Asian J. Biol. Sci.* 4: 291-299.

Yahya, A.I and Al-Azawi, S.K. 1989. Occurrence of Phosphate –solubilizing Bacteria in some Iraqi soils. *Plant Soil.* 117:135-141.

Yu, X., Ai, C., Xin, L. and Zhou, G. 2011. The siderophore-producing bacterium, *Bacillus subtilis* CAS15, has a biocontrol effect on *Fusarium* wilt and promotes the growth of pepper. *Eur. J. Soil. Biol.* 47:138–145.

Yuen, G.Y. and Schroth, M.N. 1986. Inhibition of *Fusarium oxysporum* f. sp. *dianthi* by iron competition with an *Alcaligenes* sp. *Phytopathol.* 76: 171–176.

Zaidi, A., Khan, M.S. and Aamil, M .2004.Bio-associative effect of rhizospheric microorganisms on growth, yield and nutrient uptake of greengram. *J. Plant Nutr.*27:599-610.

Zaidi, A., Khan, M.S., Ahemad ,M., Oves, M., and Wani, P.A. 2009. Recent Advances in Plant Growth Promotion by Phosphate-Solubilizing Microbes. In: Khan MS et al (Eds) Microbial Strategies for Crop Improvement. Springer-Verlag, Berlin Heidelberg. Pp. 23 50.

Zain, M.E., Razak, A.A., El-Sheikh, H.H., Soliman, H.G. and Khalil, A.M. 2009. Influence of growth medium on diagnostic characters of *Aspergillus* and *Penicillium* species. *Afr. J. Microbiol. Res.* 3:280–286.

Zarrin, F., Saleemi, M., Zia, M., Sultan, T., Aslam, M., Rehman, R.U., and Chaudhary, F. M. 2009. Antifungal activity of plant growth-promoting rhizobacteria isolates against *Rhizoctonia solani* in wheat. *Afr. J. Biotechnol.* 8(2):219–225.

Zambare, V.P., Padul, M.V., Yadav, A.A. and Shete, T.B. 2008. Vermiwash: Biochemical and microbiological approach as ecofriendly soil conditioner. *J. Agric. Biol. Sci.* 3(4): 1-5.

Zeid, A. M., Altalhi, A. D. and Abd El-Fattah, R.I. 2008. Fungal Control of Pathogenic Fungi Isolated From Some Wild Plants in Taif Governorate, Saudi Arabia. *Mal. J. Microbiol.* 4(1): 30-39.

Zhao, Q., Shen, Q., Ran, W., Xiao, T., Xu, D. and Xu, Y. 2011. Inoculation of Soil by *Bacillus subtilis* Y-IVI Improves Plant Growth and Colonization of the Rhizosphere and Interior Tissues of Muskmelon *(Cucumis melo* L.). *Biol. Fertil. Soils.* 47:507-514.

Zhou, J., Bruins, M.A., and Tiedje, J.M. 1995. DNA recovery from soils of diverse composition. *Appl. Environ. Microbiol.* 62:316 – 322.

Zhou, T., Chen, D., Li, C., Sun, Q., Li, L., Liu, F., Shen, Q. and Shen, B.2012. Isolation and characterization of *Pseudomonas brassicacearum* J12 as an antagonist against *Ralstonia solanacearum* and identification of its antimicrobial components. *Microbiol. Res.* 167(7):388–394.

Zhu, F., Qu, L., Hong, X. and Sun, X. 2011. Isolation and characterization of a phosphate-solubilizing halophilic bacterium *Kushneria* sp. YCWA18 from Daqiao Saltern on the coast of Yellow Sea of China. *Complement. Altern.. Med.*2011. 6p.

Zourob, M., Elwary, S. and Turner, A. 2008. Principles of Bacterial Detection: Biosensors, Recognition Receptors, and Microsystems. Springer, New York, NY, USA.45pp.

Appendices

APPENDIX- I

A. Composition of media used in the Study

1. Nutrient agar

Beef extract	-3.0g
Peptone	-5.0g
NaCl	-5.0g
Agar	-20.0g
Distilled water	-1000 ml

2. Phenol red medium

Peptone	– 10.0g
Carbohydrate	– 5.0g
NaCl	- 5.0g
Phenol red	- 0.018 g
Distilled water	- 1000 ml
p^H	-7.0

3. Pikovskaya' Agar medium

Glucose	-10.0g
$Ca_3(PO_4)_2$	- 5.0g
$(NH_4)_2SO_4$	-0.5g
KCl	-0.2g
$MgSO_4.7H_2O$	- 0.1g
$MnSO_4.\ 7H_2O$	-trace
$FeSO_4$	-trace
Yeat extract	-0.5g
Distilled water	- 1000 ml
p^H	-7.2

4. King's B medium

Peptone	- 20.0g
Glycerol	- 10.0g
K_2HPO_4	- 1.5g
$MgSO_4.\ 7H_2O$	- 0.5g
Agar	- 20 .0g
Distilled water	- 1000 ml
p^H	- 7.2-7.4

5. Czapek Dox broth

$FeSO_4.7H_2O$	- 0.01g
$MgSO_4$	- 0.5g
KCl	- 0.5g
K_2HPO_4	- 1.0g
Sodium nitrate	- 3.0g
Sucrose	- 3.0g
Distilled water	- 1000 ml
p^H	- 5.8

6. Potato Dextrose Agar

Potato (peeled)	-200.0g
Dextrose	-20.0g
Agar	-15.0g
Distilled water	-1000ml
p^H	-5.6

7. Chrome Azurol S blue Agar

CAS	-60.5 mg
Distilled water	-50 ml
Iron solution	-10 ml
Hexadecyl trimethyl ammonium bromide	-72.9 mg
KB broth	-100 ml
PIPES	-30.2 g
Difco agar	-18 g
Double distilled water	-750 ml
p^H	-6.8

APPENDIX-II

I. Reagents used for Gram staining

1. Crystal violet/ Gram stain

Solution A

Crystal violet (90 % dye content)	-2.0 g
Ethyl alcohol (95%)	-20.0 ml

Solution B

Ammonium oxalate	-0.8 g
Distilled water	-80.0 ml

Mix solution A and B

2. Gram's iodine

Iodine	-1.0g
Potassium iodide	-2.0g
Distilled water	-300.0 ml
3. Ethyl alcohol (100 %)	-95.0 ml
Distilled water	-5.0 ml

4. Safranine

Safranine O	-0.25 ml
Ethyl alcohol	-10.0 ml
Distilled water	-100.0 ml

3. Lactophenol cotton blue (stain)

Lactic acid	-20.0 ml
Phenol crystals	-20.0 ml
Glycerol	-40.0 ml
Distilled water	-20.0 ml
Cotton blue (1% aqueous)	-2.0 ml

3. Endospore stain

A. Malachite green (5%)

Malachite green	-5.0 g
Distilled water	-100.0 ml

B. Safranin

Safranine O (2.5 %) solution in 95 % ethyl alcohol	-10.0 ml
Distilled water	-100.0 ml

4. Kovac's reagent

P-dimethylaminobenzaldehyde	-5.0 **g**
Amyl alcohol	-75.0 ml
Concentrated hydrochloric acid	-25.0 ml

5. Nessler's reagent

Potassium iodide	-50.0 g
Distilled water (ammonia free)	-25.0 ml
Mercuric chloride (saturated)	-35.0 ml
Potassium hydroxide (50 %aqueous)	-400.0 ml

APPENDIX-III

Weather data during crop growth (June-August 2013)

Weather parameters	June	July	August
Mean maximum ^{0}C	28.5	28.4	29.9
Mean minimum ^{0}C	22.7	22.7	22.9
High maximum ^{0}C	31.6	30.7	33.0
Lowest minimum ^{0}C	21.0	21.6	21.8
Mean R^H morning %	97	97	96
Mean R^H evening%	83	84	72
Mean R^H %	90	91	84
Rainfall (mm)	1031.8	932.3	305.9
Rainy days	28	30	16
Sunshine (hrs)	29.4	23.8	134.3
Mean sunshine (hrs)	1	0.8	4.3

EXPLORATION OF NATIVE MINERAL PHOSPHATE SOLUBILIZING MICROORGANISMS AS BIOFERTILIZER FOR THE ACIDIC SOILS OF KERALA

By

SARANYA, K. S.

(2011-11-134)

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the requirement for the degree of

Master of Science in Agriculture

Faculty of Agriculture

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P.G. Thesis Seminar

Topic: Exploration of native mineral phosphate solubilizing microorganisms as biofertilizer for the acidic soils of Kerala

Name of the student : Saranya K.S.

Admission No. : 2011-11-134

Date : 28-01-2014

Venue: Library Hall

Time: 2.15pm

ABSTRACT

A study was undertaken on 'Exploration of native mineral phosphate solubilizing microorganisms as biofertilizer for the acidic soils of Kerala'. The main objective was to exploit native microorganisms with mineral phosphate solubilization and plant growth promoting activities for the acidic soils of Kerala.

Two hundred microbial isolates (173 bacteria and 27 fungi) from the repository maintained at Dept. of Agril. Microbiology were screened on Pikovskaya's agar medium. Phosphate Solubilization Efficiency (PSE) ranged from 106.6% to 555.5%. PSE was higher in bacteria but fungi were faster in solubilization of P. Twenty most efficient isolates were selected for quantitative estimation of P-solubilization , using Mo-blue method. Two fungal isolates namely PSF-183 and PSF-182 solubilized maximum amount of P (103.5µg/ml and 102.5µg/ml respectively) in 14 days. A corresponding drop in pH was recorded in all the isolates, which indicated acid production.

The 20 selected isolates were also characterized using cultural, biochemical, physiological and molecular tests. All the isolates tested positive for catalase and oxidase production. PSB-14 ranked first with respect to tolerance to acidity, followed by PSB-22. Bacterial isolates were identified as *Acinetobacter* sp., *Providencia* sp., *Achromobacter* sp., *Providencia alcalifaciens*, *Bacillus cereus*, *Stenotrophomonas maltophilia*, *Ochrobactrum* sp., *Pseudomonas* sp., and *Burkholderia* sp. using 16S rDNA sequencing. Fungal isolates were identified as *Aspergillus* sp., *Penicillium* sp., *Trichoderma* sp. and yeast. Six isolates of *Aspergillus niger* and *A. flavus* were discarded and fourteen isolates were further tested for plant growth promoting (PGPR) and antagonistic activities.

IAA production was noticed in four isolates: *Providencia* sp., *Pseudomonas* sp., *Penicillium* sp. and *Trichoderma* sp. Significant enhancement of seedling vigour in cowpea was observed with *Pseudomonas* sp. followed by *Providencia* sp. (seedling vigour index of 3058.66 and 2954.33 respectively). The fourteen isolates were further tested *in vitro* for antagonistic activity against major soil borne pathogens (*Rhizoctonia solani, Fusarium oxysporum, Sclerotium rolfsii, Pythium aphanidermatum* and *Ralstonia solanacearum*). Most of the isolates inhibited *R. solani* whereas none was effective against the bacterial pathogen *R. solanacearum.*

Based on the efficiency of P-solubilization and plant growth promoting activities, three native isolates *Providencia* sp., *Pseudomonas* sp. and *Trichoderma* sp. were selected for evaluation in pot culture experiment, with cowpea (variety Bhagyalakshmi) as the test crop. KAU commercial formulation of P-solubilizing microorganisms was used as reference culture. Three treatments of P were included as no P, rock phosphate and factamphos.

Pseudomonas sp. (PSB149) recorded maximum shoot weight, root weight, no. of nodules/ plant, pod length, no. of grains per pod and pod yield per plant, in combination with rock phosphate. Chemical analysis of plants after harvest revealed that P content in roots and grains was highest in KAU reference bio-inoculant, whereas highest P in shoot was observed in *Pseudomonas* sp. There was an increase in available P content of soil when *Pseudomonas* sp. (PSB149) was applied along with rock phosphate (17.67mg/kg). Final population of P-solubilizing microorganisms in soil was highest in *Pseudomonas* sp. (33X104cfu/g). This is indicative of the rhizosphere colonization efficiency of the bioinoculant *Pseudomonas* sp.

The *in vitro* and pot culture experiments revealed the efficiency of *Pseudomonas* sp. as a P-solubilizing microorganism. When tested in pot culture, this isolate in combination with rock phosphate gave the same pod yield as that of factamphos, indicating its suitability as a biofertilizer for Kerala soils. Evaluation under field conditions will be required for validation of the results, before the bacterium can be recommended as a biofertilizer.