ISOLATION, CHARACTERIZATION AND EFFICACY OF PHOSPHATE SOLUBILISING BACTERIA

By

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(J-13-MBS-06)

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Needless to say, all omissions and errors are mine.

Aijaz Ahmad Dar

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<td>Arbuscular micorrhizal fungi</td>
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<tr>
<td>bp</td>
<td>Base pairs</td>
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<tr>
<td>Bv</td>
<td>Biovar</td>
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<tr>
<td>cfu</td>
<td>colony forming unit</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<td>Exopolysaccharides</td>
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<td>MIC</td>
<td>Minimum inhibition concentration</td>
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<tr>
<td>NBRIIP</td>
<td>National botanical research institute phosphate</td>
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<tr>
<td>NFW</td>
<td>Nuclease free water</td>
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<tr>
<td>PGPB</td>
<td>Plant growth promoting bacteria</td>
</tr>
<tr>
<td>PGPTs</td>
<td>Plant growth promoting traits</td>
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<tr>
<td>PSM</td>
<td>Phosphate solubilizing microorganisms</td>
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<tr>
<td>PVK</td>
<td>Pikovskaya’s medium</td>
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<tr>
<td>TCP</td>
<td>Tricalcium phosphate</td>
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<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
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<td>PSB</td>
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<tr>
<td>PSM</td>
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<tr>
<td>PGPR</td>
<td>Plant growth promoting rhizobacteria</td>
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<tr>
<td>rRNA</td>
<td>Ribosomal RNA</td>
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<tr>
<td>rpm</td>
<td>Revolutions per minute</td>
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<tr>
<td>Taq</td>
<td><em>Thermophilus aquaticus</em></td>
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Title of thesis : Isolation, Characterization and Efficacy of Phosphate Solubilising Bacteria
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ABSTRACT

The present investigation was carried out with the objectives to explore rhizospheres for isolation of phosphate solubilizing bacteria vis a vis evaluation of the efficient isolated phosphate solubilizers in maize (Zea mays). Eight locations viz. R.S. Pura, Burj Tanda, Chakrohi, Kaili mandi, Chatha farm-I, Chatha farm-II, Gurha slathian and Rakhi Dhiansar were surveyed for rhizosphere soil sample collection. Soil varied in pH, E.C, O.C, available N and available P between 6.68 – 7.12, 0.10 – 0.19 E.C µS cm⁻¹, 0.41-.53%, 112-166 kg ha⁻¹ and 10-14.2 kg ha⁻¹, respectively. The different isolated PSB showed the clearing zones from 0.95 mm to 7.79 mm whereas the phosphorus solubilizing efficiency determined was between 38.56 % and 78.52 %. The total culturable bacteria from the locations showed the peak values on the 6th day of incubation varying between 6.13×10⁶ to 8.93×10⁶ cfu g⁻¹ soil, whereas the PSB population from the locations showed the peak values on 7th day of incubation and varied between 2.50×10⁸ - 8.86×10⁸ cfu g⁻¹ soil. PSB induced the pH changes in the Pikovskaya broth and showed a drop of pH from 6.47 (Control) to 3.63 (PSB). The optimum temperature for PSB survival has been found to vary between 25-35⁰C. Maximum phosphatase activity in 24 hrs varied between 12.9-36.8 µg PNPP ml⁻¹h⁻¹ whereas the maximum phytase activity was observed in 72 hrs and varied between 4.2-10.2 µg P ml⁻¹ h⁻¹. The three phosphorus solubilizing efficient bacterial cultures were subjected to the 16S rRNA sequence analysis, two cultures were identified as Bacillus pumilus (PSB1 and PSB2), and one as Bacillus atrophaeus (PSB3). These isolates were blended in a sterile carrier maintaining the population from 10⁹ to 10¹⁰ were evaluated for their efficiency in promoting the dry matter yield of Maize (Zea mays) variety CM-135 in the pot experiment and showed a significant increase in dry matter yield of the Zea mays as compared to the control. A present investigation revealed diverse PSB population in the rhizospheric soil samples of different areas of Jammu that showed their potential role as biofertilizers for growth promotion of crop plants. Endeavours needed to be directed to identify, screen and characterize PSB from more niches in Jammu region for their ultimate application under field conditions. So that, the successful implementation in use of PSB to exploit soil fixed P that can be an alternative sustainable strategy for management of soil to optimize P bioavailability.

Key words: Phosphate solubilising bacteria, solubilization, Rhizosphere, Bacillus pumilus, Bacillus atrophaeus. Maize, 16s rRNA, Phosphorous
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INTRODUCTION
CHAPTER-I

INTRODUCTION

Phosphorus (P) is second important plant nutrient most ordinarily limiting the growth and development of the crops after nitrogen. It is usually present in nature as organic or inorganic forms. The availability of P in soil is generally low as it gets fixed in different types of soils and becomes unavailable (Tanwar and Shaktawat, 2003). Phosphorous makes up around 0.2% of plant dry weight and is a crucial plant supplement. It plays an important role in plant metabolism involving cell division, development, photosynthesis, breakdown of sugar, nutrient uptake, mineral transport inside of the cell, regulation of metabolic pathways and resistance against diseases.

Phosphate-solubilizing bacteria (PSB) assume a critical part in minimizing the phosphorus deficiency in soil by solubilizing the insoluble forms of phosphate to soluble forms and renders P available for plants (Yadav and Dadarwal, 1997). In order to improve the P solubilization, the seeds or soil is inoculated with the PSB to get the better crop production (Abd-Alla, 1994; Jones and Darrah, 1994; Yahya and Al-Azawi, 1998).

Phosphate solubilizing microorganisms includes the diverse groups of microorganisms including the bacteria, fungi, actinomycetes, and arbuscular mycorrhizal fungi. Phosphate solubilization by these microbes occurs by various mechanisms, such as acidification, chelation, ion exchange reactions and polymeric substance formation (Delvasto et al., 2006). The important genera of fungi are Aspergillus and Penicillium species and among the bacterial genera are Pseudomonas, Azospirillum, Bacillus, Rhizobium, Burkholderia, Arthrobacter, Alcaligenes, Serratia, Enterobacter, Acinetobacter, Flavobacterium, and Erwinia are considered as the best P solubilizers (Whitelaw, 2000).

Evidence of normally occurring rhizospheric phosphorus solubilizing microorganisms (PSM) goes back to 1903 (Khan et al., 2007). Bacteria are more efficient in phosphorus solubilization than fungi (Alam et al., 2002). PSB constitutes 1 to 50%, while phosphorus-solubilizing fungi (PSF) are just 0.1 to 0.5% among the entire microbial population in the soil (Chen et al., 2006). Majority of the phosphorus fertilizers are added in the soil but these become unavailable to plants by converting into insoluble forms such as iron phosphates, aluminum phosphates and calcium...
phosphates (Altomare et al., 1999). The issue of inaccessibility of phosphorous can be overcome by utilizing advantageous microorganisms that guarantees P accessibility to plants by synthesis of different types of organic acids (Poonamgautam et al., 2003; Deubel and Merbach, 2005).

PSB are well adapted to different soil conditions including variable pH, temperarture and organic carbon because of their genetic potential for solubilising inorganic P (Johri et al., 1999) and have a capacity to change over the insoluble phosphatic mixes into solvent structures (Kang et al., 2002; Pradhan and Sukla, 2005) in soil and make them accessible to the crops Bacillus megaterium, B. circulans, B.subtilis, B. polymyx, B. sircalmous, Pseudomonas striata, and Enterobacter could are considered as the most important strains (Kucey et al., 1989). Pseudomonas aeruginosa is known to enhance the plant growth and suppress many infectious diseases by prompted systemic resistance (Audenaert et al., 2002) besides some strains like Pseudomonas putida and Pseudomonas fluorescens help in mobilizing P in canola, lettuce, tomato and increased yields of potato, radishes, rice, sugar beet, tomato, lettuce, apple, citrus, beans, ornamental plants and wheat (Kloepper, 1994; Glick, et al., 1997). The utilization of these inoculants by distinctive techniques can be sure to improve the productivity of usually and artificially created P resources and thus, augment the chemical fertilizer application for the crop production (Salimpour et al., 2010).

The diversity of PSB relies upon diverse soil properties (physical and chemical properties, organic matter, and P content) and common exercises (Kim et al., 1998). Soils of Jammu are unexplored for the microbial diversity; no specific work has been done for the isolation of phosphate solubilizing bacteria in soils of Jammu except quantitative analysis. Keeping in view of the need to explore soils of Jammu for their potential to harbour phosphate-solubilizing bacteria, a present investigation was carried out with the following objectives:

1. Exploration of rhizospheres for isolation of phosphate solubilizing species.
2. Evaluation and efficacy of isolated phosphate solubilizers in maize (Zea mays).
REVIEW OF LITERATURE
CHAPTER-II

REVIEW OF LITERATURE

The objectives pertaining to the of the research work is reviewed as under

2.1. Phosphorus solubilizing Bacteria (PSB)

2.2. Mechanisms of Phosphorus Solubilization

2.3. Diversity of P solubilizers

2.4. Factors effecting PSB diversity

2.5. Techniques adapted in Isolation of phosphate solubilizing bacteria

2.6. Isolation of PSB from different niches

2.7. Morphological and biochemical based Identification of Phosphate Solubilizing Bacteria

2.8. 16S rRNA sequence based Identification of Phosphate Solubilizing Bacteria

2.9. Production of PSB bio fertilizers

2.1. Phosphorus solubilizing Bacteria (PSB)

Phosphorous (P) is an essential macro nutrient applied to soil in the form of phosphate fertilizers and is important for the plant growth and development which constitutes about 0.2 % plant dry weight (Harrison, et al.,2002). Phosphorous is mostly insoluble or weakly soluble in soils and the average P availability of soils is nearly 0.05% (W/W) and 0.01% of the total P exists in accessible form for the plants (Illmer and Schimmer, 1995). In order to attain the larger crop production, P fertilizers are applied to the soils and most of these applied fertilizers gets fixed into insoluble forms and becomes unavailable to plants. Phosphate solubilizing bacteria (PSB) are the beneficial bacteria having the ability to solubilize fixed forms of phosphorous into soluble forms. PSB are used as inoculums for releasing the fixed P for the plant development and yield. Currently, different strains of the bacteria has been identified for using in biofertilizer, among them Pantoea agglomerans (P5), Microbacterium laevaniformans (P7) and Pseudomonas putida (P13) strains are efficient insoluble phosphate solubilizers (Malboobi et al., 2009). The use of PSB as inoculants simultaneously increases P uptake by the plants and crop yield. Strains
from the genera *Pseudomonas, Bacillus* and *Rhizobium* are among the most powerful phosphate solubilizers and acid phosphatases play a major role in the mineralization of organic phosphorous in soil (Whitelaw, 2000).

### 2.2. Mechanisms of Phosphorus Solubilization

The ability of PSB to release metabolites for P solubilization, for example, organic acids, which through their hydroxyl and carboxyl groups chelate the cation bound to phosphate and the latter being changed over to soluble forms (Sagoe et al., 1998). Certain bacterial species have the potential for mineralization and solubilization for natural and inorganic phosphorus (Hilda and Fraga, 2000; Khiari and Parent, 2005). Phosphate solubilization is carried out by different processes like natural acid secretion and proton discharge carried out by the microbes (Surange et al., 1995; Dutton and Evans, 1996; Nahas, 1996). Phosphorus solubilization is completed by a variety of bacteria and fungi by acting upon the sparingly soluble soil phosphate chelates (Whitelaw, 2000). The P solubilization is carried out by PSB through generation of low molecular weight organic acids particularly gluconic and keto gluconic acids bringing down the pH of rhizosphere (Goldstein, 1995; Deubel et al., 2000). The pH of rhizosphere is also brought down by the proton/bicarbonate discharge (anion/cation balance) and gaseous (\(O_2/CO_2\)) interactions. Phosphorus solubilization capacity of PSB has a direct connection with pH of the medium. The maintenance of the pH within the cells of the plant roots can be changed by the strong or weak organic acids via carboxylation/decarboxylation mechanisms (Davies, 1986). The secreted organic acids help in the solubilization of phosphorus by chelating metal ions that are responsible for immobilization of soluble phosphorus by either chelating with metal ions or forming complexes with phosphorus or both (Kirk, 1999; Neumann et al., 2000). These pH variations occur in the rhizosphere soil in response to adverse nutritional conditions like P deficiency (Haynes, 1990).

Entrance of root exudates, for example, natural ligands can likewise change the concentration of P in the soil solutions (Hinsinger, 2001). Hydrochloric acid (HCL) (inorganic acids) can also solubilise the P, but they are not so effective in lowering down the pH as compared to Organic acids (Kim et al., 1997). PSB secretes the organic acids and solubilize the insoluble phosphates by bringing down the pH and in specific cases; phosphate solubilization is induced by phosphate starvation (Gyaneshwar et al., 1999). A number of reporters has seen mono-, di-, and tri-
carboxylic acids from the supernatants of growing mineral phosphate solubilizing (MPS) bacteria. It has been estimated that the acids produced are approximately more than 5% of the carbohydrate consumed by the bacteria (Banik and Dey, 1983). Some researchers have observed a direct relation of pH decrease with the availability of phosphorus in the culture media (Sperber, 1958; Agnihotri, 1970; Liu et al., 1992). While few others could not find any positive correlation between degree of solubilization and decline in pH (Mehta and Bhide, 1970; Wani et al., 1979; Goldstein, 1986). A number of strains of PSBs have been found associated with organic acids such as lactic acid, citric acid and tartaric acid. Strains of Bacillus were found to produce mixtures of lactic, isovaleric, isobutyric and acetic acids (Illmer and Schiner, 1992; Banik and Dey, 1982). Other organic acids, such as glycolic, oxalic, malonic, and succinic acid, have also been identified among phosphate solubilizers (Illmer and Schiner, 1992; Banik and Dey, 1982). In addition, other mechanism of phosphate solubilization involve chelating substances and inorganic acids such as sulphideric (Rudolfs, 1922), nitric, and carbonic acid (Hopkins and Whiting, 1916). But, they are not as effective in the phosphate solubilization as organic acid production (Rudolfs, 1922; Vázquez, 1996).

The PSB cause acidification of soil which is responsible for release of P from apatite by proton substitution/discharge of \( H^+ \) or release of \( Ca^{2+} \) (Goldstein 1994; Illmer and Schinner, 1995) while the reverse happens when uptake of anions surpasses that of cations, with discharge of \( OH^-/HCO_3^- \) surpassing that of \( H^+ \) (Tang and Rengel, 2003). The secretion of Carboxylic anions by PSB contribute more to phosphate solubilization than cationic secretion of \( H^+ \) due to the high affinity of carboxylic anions to calcium and solubilize more P than acidification alone (Staunton and Leprince, 1996). But, Cationic complexing is commanding component in P solubilization if the natural acidic structure favours complexation (Fox et al., 1990) and is controlled by nourishing, physiological, and development states of the microbial society (Reyes et al., 2007), in any case, it is for the most part because of the lowering down of pH alone by natural acids (Moghimi and Tate, 1978) or generation of microbial metabolites (Abd Alla, 1994). Natural anions and related protons are compelling in solubilizing precipitated types of soil P (e.g., Fe - and Al-P in acid soils, Ca-P in alkaline soils), chelating metal particles that may be connected with complex types of P or may encourage the arrival of adsorbed P through exchange
reactions of ligand (Jones, 1998). Separation of calcium phosphate (Ca-P) results from the consolidated impacts of carboxylic acids combination and consequent pH decline including normal system, yet proton discharge is the result of a few systems (Deubel et al., 2000). Other mechanisms which have been suggested are production of H$_2$S, which interacts with ferric phosphate to produce ferrous sulphate releasing phosphate simultaneously (Swaby and Sperber, 1958). It has been suggested that the MPS process occurs because of microbial sulphur oxidation (Rudolph, 1922), nitrate production and CO$_2$ formation and thus results in the formation of inorganic acids such as sulphuric acids.

H$^+$ excretion originating from NH$_4^+$ assimilation could be alternative mechanism for P solubilization (Parks et al., 1990). HPLC analysis of the culture solution of the Pseudomonas sp., did not notice any organic acid but solubilization occurred (Illmer and Schinner, 1995). They concluded that the best reason for solubilization without production of acid is the excretion of protons associated respiration or NH$_4^+$ assimilation.

Extracellular oxidation via direct oxidation pathway may play a crucial part in soils where calcium phosphates deliver a sufficient amount of unavailable mineral phosphorous (Goldstein, 1995). This mechanism was confirmed by biochemical analysis of lowering of pH in insoluble P solubilization by Burkholderia cepacia DA23 (Song, et al., 2008). Every organism can react in one or more than one way to bring about the phosphorous solubilization. Therefore, it is difficult to select a single mechanism, which is responsible for the P solubilization.

**Soil phosphorous**

Soil generally contains a satisfactory amount of organic and inorganic phosphorus. The measure of organic phosphorus is generally low and its recovery does not surpass 20%. Phosphate accessibility in soil and the utilization of phosphate by plants are increased with the assistance of phosphate solubilizers. A high percentage of PSBs are usually found in rhizospheres in comparison with the non-rhizosphere soil (Kundu et al., 2009). Many physiological factors are responsible for p-solubilizing capacity of PSBs. Henceforth different bacterial species solubilize phosphorus at a diverse extent. With an increase in phosphorus uptake by plants, PSBs can play an important role in plant nutrition (Rodriguez et al., 2006). The
mineralization of phosphorus compound is done by the action of a few phosphates (additionally called phosphor hydrolase), present in various soil microorganism and assume a crucial part in assimilation of phosphate from organic compounds by plants and microorganisms (Sharma et al., 2011).

Despite the fact that majority of available phosphorus in the soil is organic but largest available source of soluble reactive phosphorus comes from inorganic phosphorus (Boto and Wellington, 1988). The dominant orthophosphate ions contributing to the pH of the soil are $\text{H}_2\text{PO}_4^-$ and $\text{HPO}_4^{2-}$ (Lindsay, 1979).

The nucleic acids, phospholipids and ATP are essentially having Phosphorus as key element for continuous synthesis (Marschner, 1995). The plants mainly obtain phosphorus in inorganic form based on methodology viz. extractable, exchangeable, and labile and bioavailability (Salcedo and Medeiros, 1995). The growth of plants is highly influenced by the availability of the inorganic phosphorus in the soil (Smith et al., 2003) which in turn depends on the cationic-anionic balance in the rhizosphere. Major factors contributing to it involve (1) extrusion of $\text{H}^+$ or $\text{OH}^-$and (2) accumulation and degradation of organic acids (Haynes, 1990). The plant roots depending on the unequal absorption of the nutritive cations or anions influence the pH of the rhizospheric soil. The uptake of the anionic Phosphate ion ($\text{PO}_4^{3-}$, $\text{H}_2\text{PO}_4^-$, $\text{HPO}_4^{2-}$) causes change in the electronegativity of the soil which is balanced by the active excretion of $\text{OH}^-$ ions, similarly the uptake of a cation (e.g. $\text{NH}_4^+$, $\text{Ca}^{2+}$, $\text{Mg}^{2+}$, $\text{K}^+$, $\text{Na}^+$) is balanced by $\text{H}^+$ excretion (Haynes, 1990).

### 2.2.1. Role of Siderophores in P solubilization

Siderophores are the agents having high affinity for iron and are usually secreted by almost all the microorganisms in response to the iron deficiency. Therefore, under iron limitations, the siderophores serves as the solubilizing agents for iron from mineral or organic complexes. Nearly 500 known siderophores are being exclusively employed by the microbial species and the strains which produce them. The excretion of the siderophores has also been found in the P solubilizing microorganisms (Vassilev et al., 2006; Caballero-Mellado et al., 2007; Hamdali et al., 2008). Despite the production of siderophores by the PSM, these agents are not regarded as responsible for the P solubilization apparently.
2.2.2. Role of EPS in P-solubilization

Exopolysaccharides (EPS) are the compounds comprising mainly of carbohydrates secreted by some bacteria and fungi outside their cell walls. The bacterial strains (Enterobacter sp. (EnHy-401), Arthrobacter sp. (ArHy-505), Azotobacter sp. (AzHy-510) and Enterobacter sp. (EnHy-402)) having the ability to solubilize TCP (tri calcium phosphate), were used to evaluate the role of EPS in P-solubilization (Yi et al., 2008). The tested isolates produced a significant amount of EPS and presented a strong ability for P-solubilization. However, more research is needed to understand the association between EPS production and P-solubilization.

2.2.3. Enzymes responsible for P-solubilization

A group of three enzymes are mainly responsible for release of phosphorus from organic compounds: (1) Phosphatases (phosphohydrolase), which accomplish dephosphorylation of phospho-ester or phosphoanhydride bonds in organic matter, (2) Phytases, which specifically cause P release from phytic acid, and (3) Phosphonatases and C-P lyases, enzymes that complete C-P cleavage in organophosphonates. The core action speciously corresponds to the effort of acid phosphatases and phytases because of their predominant occurrence in soil.

**Phosphatases (phosphohydrolase)**

Phosphatases are the group of enzymes which help in releasing inorganic phosphorus from nucleotides and sugar phosphates and thus can be regarded as organic phospho-ester scavengers. These enzymes have been used in a number of biotechnological applications over the recent past (Rodriguez et al., 2006). The phosphatases from PSB have been divided into two categories based on the pH optima viz. Alkaline (pH>7) or Acidic (pH<6) phosphatases. A number of phosphate systems including primary, secondary, cyclic and sugar alcohols as well as phenols and amines can be hydrolyzed by these enzymes leading to release of phosphorus. Acidic phosphatases from certain PSB have been reported to work very efficiently as far as mobilization of phosphorus is considered (Rossolini et al., 1998). One of the example is of acpA gene isolated from Francisella tularensis expresses an acid phosphatase with optimum action at pH 6, with an extensive range of substrate specificity (Reilly et al., 1996). Also, genes encoding nonspecific acid phosphatases class A (PhoC) and class B (NapA) isolated from Morganella morganii are very
satisfactory in phosphate solubilization. Among rhizobacteria, a gene from *Burkholderia cepacia* that aids phosphatase activity was isolated (Rodríguez et al., 2000). This gene codes for an outer membrane protein that boosts production in the absence of soluble phosphates in the medium. In order to greatly improve organic phosphate mineralization in plant growth promoting bacteria (PGPB) workers are looking for heterologous expression of genes encoding different types of phosphates in agriculturally important bacterial strains. In an attempt to achieve so, a group of workers attempted to transfer the napA phosphatase gene from the soil bacterium *Morganella morganii* to *Burkholderia cepacia* IS-16, a strain used as a biofertilizer, using the broad-host range vector pRK293 (Fraga et al., 2001). This transfer of foreign gene from another bacterial strain in a biofertilizer strain proved beneficial in terms of stability and ecological safety. Therefore, biotechnological application for NSAPs gene transfer and expression in plant PGPB is at forefront research, which involves latest recombinant DNA technology.

Another group of phosphates (alkaline phosphatase) which work efficiently at high pH are a homo dimeric enzyme with a molecular weight of 56 kD. It is a metallo enzyme, binding two zinc atoms and one magnesium ion per monomer. This enzyme catalyzes the hydrolysis of a wide variety of phosphomono esters and catalyzes trans phosphorylation reaction by transferring phosphoryl group to alcohol in the presence of certain phosphate acceptors (Coleman, 1992). Alkaline phosphatase serves as a valuable reagent for the removal of terminal mono esterified phosphate from both ribo- and deoxyribo-oligonucleotides in *Escherichia coli*. Alkaline phosphatase gene has been cloned and expressed from various species. Several studies have been conducted in order to clone sequence and express the alkaline phosphatase encoding gene. These studies have helped in production of large amounts of alkaline phosphatase in vectored bacteria. For example, the alkaline phosphatase gene from *Sphingomonas* sp. (Nilgiriwala et al., 2008) and some thermophilic bacteria (Li et al., 2007) has been cloned and expressed in *E. coli* and their biochemical behaviors have been investigated (Zhang and Shan, 2008).
Phytases

Majority of the phytases (myo-inositol hexakisphosphate phosphohydrolases) are included in the high molecular weight acid phosphatases. Phytate is the primary source of inositol and the main stored form of phosphate in plant seeds and pollen. Utmost genetic engineering work have concentrated on the exploration for phytases that are optimal for enhancing the animal nutrition. Additional important application of these enzymes that is not currently exploited is solubilization of soil organic phosphorus through phytate degradation. Phytate serves as the important component of organic forms of P in soil (Rodríguez et al., 2006). The capability of plants to attain phosphorus straight from phytate is very inadequate. However, Arabidopsis plants supplied with phytate has the significant impact on growth and phosphorus nutrition when they were genetically altered with the phytase gene (phyA) from Aspergillus niger (Richardson, 2001). Thermally stable phytase genes (phy) from Bacillus subtilis VTT E- 68013 and from Bacillus sp. DS11 (Kim et al., 1998). (Kerovuo et al., 1998) has been replicated. Acid phosphatase/phytase genes from E. coli (appA and appA2 genes) have also been isolated and characterized (Golovan et al., 2000). The multi functionality of phytate enzymes makes them striking for solubilization of organic P in soil. In addition, neutral phytases have great potential for genetic expansion of PGPB. The genes from neutral phytase have been recently cloned from B. subtilis and Bacillus licheniformis (Tye et al., 2002)

2.3. Diversity of P solubilizers

A significant number of microbial species display P solubilization ability; these incorporate bacteria, fungi, actinomycetes, and even algae. Apart from Pseudomonas and Bacillus, other microorganisms reported as P-solubilizers incorporate Rhodococcus, Arthrobacter, Serratia, Chryseobacterium, Gordonia, Phyllobacterium, Delftia sp. (Wani et al., 2005; Chen et al., 2006), Azotobacter (Kumar et al., 2001), Xanthomonas (De Freitas et al., 1997), Enterobacter, Pantoea, and Klebsiella (Chung et al., 2005), Vibrio proteolyticus, Xanthobacter agilis (Vazquez et al., 2000). Moreover, beneficial nitrogenous rhizobia, which alter environmental nitrogen into ammonia and disseminate the fixed nitrogen to the host plants, have additionally demonstrated phosphate solubilization action (Zaidi et al., 2009), for instance, Rhizobium leguminosarum bv. Trifolii (Abril et al., 2007), and Rhizobium species nodulating Crotalaria species (Sridevi et al., 2007) enhanced plant P-solubilization by
assembling inorganic and natural phosphorous. These microorganisms are abundant but vary in density and mineral phosphate solubilizing (MPS) ability from soil to soil or from one production system to another. Certain phosphate solubilizing bacteria have additionally been isolated from stressed environments like the halophilic microbes Kushneria sinocarni separated from the deposit of Daqiao saltern on the eastern coast of China (Zhu et al., 2011). P solubilizing bacteria constitute 1-50% and fungi 0.1-0.5% of the total respective population in the soil (Kucey, 1983). In addition, P-solubilizing fungi do not lose the P dissolving ability in vitro upon repeated sub culturing as is common with the P solubilizing bacteria (Sperber, 1958, Kucey, 1983). P-solubilizing fungi create a larger number of acids than bacteria and thus show more prominent P-solubilizing action (Venkateswarlu et al., 1984). The genera Aspergillus and Penicillium (Fenice et al.,2000; Khan and Khan 2002; Reyes et al., 1999, 2002) are the most illustrative among the filamentous fungi that solubilize phosphate, in spite of the fact that strains of Trichoderma (Altomare et al., 1999) and Rhizoctonia solani (Jacobs et al., 2002) have additionally been accounted for as P solubilizers. A nemato fungus Arthrobotrys oligospora likewise can solubilize phosphate in vivo and also in vitro (Duponnois et al., 2006). Only a couple of studies have been led to evaluate the capacity of Yeasts to solubilize phosphate, e.g. Yarrowia lipolytica (Vassilev et al., 2001), Schizosaccharomyces pombe and Pichia fermentans. Most of the of the recognized filamentous fungi, are numerous found in agrarian soils, for example, Penicillium sp., Mucor sp. and, Aspergillus sp. which has been indicated to enhance plant development by 5-20% after inoculation (Gunes et al., 2009). Nearly 20% of actinomycetes can solubilize P, including those in the basic genera Streptomyces and Micromonospora (Hamdali et al., 2008).

Moreover, cyanobacteria and mycorrhiza have also been accounted for P solubilization activity. The P solubilizing ability of the arbuscular micorhizal fungi (AMF) and rhizobacteria were evaluated on the growth and nutrient uptake of Sorghum bicolor in acid and poor availability phosphate soils. The microbial inocula consisted of the AMFs Glomus manihotis and Entrophosphora colombiana, Pseudomonas sp., and the results demonstrated that the cooperation of AMF and the effective rhizobacteria could possibly be formulated as bio fertilizers in acid soil. The effect of dual inoculation with AMF and rhizobacteria should be further assessed on
diverse crops and agro climatic environments, especially in the field (Widada et al., 2007).

Therefore, the results have shown that diversity of P solubilizing microorganisms varied in different ecological niches and there is abundant possibility to identify novel isolates from variety of environments in near future.

2.4. Factors effecting PSB diversity

Due to the soil P fertility concerns, farmers often apply excess phosphates than required by the plants (Goldstein, 1986) which gets deposited progressively and results in soil pollution and pollution of other water resources such as streams, groundwater, and lakes. (Del Campillo et al., 1999; Reddy et al., 2002). Contrarily, the degree of replacement and accessibility of P in soil is governed largely by many environmental factors, like, soil pH, temperature, humic substances, soil P concentration, its fixation by soil, microbial composition including PSM (Hameeda et al., 2008; Henri et al., 2008; Srividya et al., 2009) and their functions, and several plant exudates (Hoffland et al., 1989; Ae et al., 1990; Gillespie and Pope 1990). Also, the complex phenomenon of P solubilization process depends on many other factors such as the nutritional fertility of soils and growth dynamics and physiological functions of the organisms required in mineralization or solubilization (Reyes et al., 1999; Chen et al., 2006). The capacity of PSM has also been found to be severely affected by several agents and flora (Gupta et al., 2007; Yadav et al., 2010). The soil microflora should be provided with the healthy and nutritive environment for the proper growth, establishment, and normal functioning of PSM in soils (Vassileva et al., 1999). Such microbes have been found to be effective in P uptake and in turn enhanced the crop yield in stressed environments when applied on seeds or inoculated in the soils (Zaidi et al., 2003; Hamdali et al., 2012) despite contradictory reports on efficiency of PSM in fluctuating environments (Kern et al., 2012). The effect of various environmental factors on the organizational and efficient diversity of phosphate solubilizing microbes is reviewed as follows.
2.4.1. Factors Affecting Inorganic P Solubilization

2.4.1.1 Hydrogen Ion Concentration (pH)

The pH among the various environmental factors affects the growth and metabolic activities of microbial populations including PSM (Reyes et al., 2002; Khan et al., 2007). Solubilization of inorganic P by bacteria at optimum pH has been found to be neutral or slightly acidic (Bajpai and Sundara, 1971). The decrease in pH of the medium by P solubilizing microbes is associated with the medium in which they are growing. The significant negative correlation between pH and solubilization of inorganic phosphate has been found alongside, for example, calcium phosphate (Wani et al., 2008). Nahas (1996) tested 42 bacterial isolates for their capacity to solubilize rock phosphate (RP) and calcium phosphate (Ca-P) in culture medium and he has reported a significant correlation between final pH value and titratable acidity and between titratable acidity and soluble.

2.4.1.2. Temperature

The bacteria react differently to varying temperatures and are capable of growing at the higher temperature (thermophiles, thermo tolerant) and also at extremely low temperatures such as at or below 0, 15, and 20°C (psychrophiles, psychrotolerant) by producing a group of heat and cold shock proteins, respectively. The P-solubilizing abilities of the PSB are greatly affected by the temperature variations, the biological activities of the PSB remain low at low temperatures which increase with the increase in temperature towards the optimum range after that the organisms show variable responses. Generally, the PSB identified so far belongs to the group mesophiles (Khan et al., 2007, 2010), suggesting that they could be only efficient in the mesophilic environment. However, some thermotolerant (Chang and Yang 2009; Rao et al., 2009; Panda et al., 2013) and psychrophilic PSB have also been identified showing P solubilization ability activity (Negi et al., 2009; Pallavi and Gupta 2013).

The bacterial cultures particularly B. subtilis and B. circulans (Gaind and Gaur, 1991) showed consistent P solubilization even at 45°C which was found to be due to the ability of their enzyme systems to stand higher temperatures. Such a situation of higher soil temperature is generally found during summer in tropics where the temperature may go up to 50°C. Thus, there is a need to isolate such PSM that can
withstand high temperatures of tropics. *P. fluorescence* showed enhanced P solubilization at 35°C (Panda *et al.*, 2013), while *P. corrugata* isolated from a temperate region in Sikkim (Himalaya) exhibited solubilization of TCP both at psychrophilic and mesophilic temperature ranges (Pandey *et al.*, 2002, 2006). Likewise, the P solubilization at lower temperatures was effectively shown by cold-tolerant species of *Pantoea dispersa* and *Exiguobacterium acetylicum* (Selvakumar *et al.*, 2010).

### 2.4.1.3. Carbon and energy source

The PS activity is also being found to be effected by the availability of various carbon sources, like, glucose, galactose, fructose, starch, and mannitol present in the rhizospheres, discharged by many plants as photosynthates (Derrien *et al.*, 2004; McRae and Monreal, 2011) which are utilized as C and energy source by many soil microbes including PSM (Yadav *et al.*, 2010; Khan *et al.*, 2013). Such carbon sources have been noted to affect the production of enzymes responsible for the dissolution of organic P (Qureshi *et al.*, 2010) and solubilization of inorganic P by *A. niger*. In another report, sucrose was found as the best C source for *P. rugulosum* for solubilization of hydroxylapatite and FeSO₄ (Reyes *et al.*, 1999). According to Nautiyal (1999) the microorganisms produced higher amounts of organic acids which cause more insoluble P solubilization when glucose was used as C source. Different sugars such as glucose, sucrose, or maltose were used to determine the influence of C on the solubilization of insoluble P in order to assess the P solubilization activity of *Burkholderia cepacia* (DA23) (Song *et al.*, 2008). Extensive solubilization of calcium P was found when glucose was utilized as sugar and was found to be much lesser in a medium containing sucrose (Panda *et al.*, 2013).

### 2.4.1.4. Nitrogen source

Nitrogen (N), like many other elements, determine the growth and functionality of PSM. PSB take N as nitrite, nitrate, or amino form, subject to the enzyme and greatly influencing P solubilization activity (Bar-Yosef *et al.*, 1999; Habte and Osorio 2012).

Ammonium in most of the studies has been found as a better N source than nitrate (Wenzel *et al.*, 1994; Asea *et al.*, 1988), and *P. fluorescence*, for example,
utilized $(\text{NH}_4)_2\text{SO}_4$ most efficiently and significantly minimized the pH during P solubilization in the medium.

2.4.1.5. Effect of CaCO$_3$ and Aeration

CaCO$_3$ when added to the medium markedly decreases P solubilization by bacteria and fungi in liquid media. For example, the efficient P solubilizer *Enterobacter intermedium*, isolated from grass rhizosphere, was found to be having the decreased P solubilization 200-250 mg L$^{-1}$, when inoculated in the medium treated with 1 % CaCO$_3$ compared to medium without CaCO$_3$ (1,000 mg/L) (Hwangbo et al., 2003). Similarly, the P solubilization by *Rhizobium* and *Bradyrhizobium* from RP was observed to be reduced calcium added as CaCl$_2$, CaCO$_3$, and Ca(OH)$_2$ to the medium because CaCO$_3$ increases the pH of the medium towards alkalinity, which inhibits the growth of bacteria, resulting in reduced solubilization (Halder et al., 1991).

Aeration is another important factor contributing hugely to P solubilization by microbes. The P solubilization was found to be improved when the strains of *P. striata* and *A. awamori* were allowed to grow in shake cultures as compared to stationary cultures. The enhanced aeration of shaking cultures attributed to the increased P solubilization. The P concentration has been observed to increase aeration from 349 ppm to 1,675 ppm, as compared to non-aerated environment from 242 ppm to 1,164 ppm (Jung et al., 2002).

2.4.1.6. Humic substances and organic compounds

Humic substances play some important roles in soil conditioning and plant growth (Benedetti et al., 1996), improving nutrient uptake, especially P, S, N, and Zn, removing toxins, stimulating soil biological activity, solubilizing minerals, improving soil structure, protecting soil from degradation; and enhancing water-holding capacity (WHC) for better drought resistance and reduction in water usage. The microbial metabolism is stimulated by the humic substances (containing humic acid (HA), a naturally occurring polymeric organic compound) by releasing the PO$_4$ from hardly soluble rock minerals because of high total acidity and its ability to complex and chelate the resulting solutions (Schnitzer and Khan, 1972; Sposito, 1989).

Moreover, HA has the capacity to form complexes that can convert soil constituents into forms that are suitable for uptake by the plant (Vaughan and
McDonald, 1976). As HA contains 51-57 % organic C, 4-6 % N, and 0.2-1 % P, it increases crop yields by supplying N and P to the plants together with the improvement in the physicochemical and biological features of the soils (Hajra and Debnath, 1987). The use of humic compounds in the presence of PSB enhances the available P and pH and reduces the exchangeable ions. P solubilization by *B. megaterium* var. phosphaticum from insoluble TCP and the amounts of P solubilized was found to improve by the presence of sodium humate and fulvic acid (Khan *et al*., 2009).

The physical, chemical, and biological properties of soil were found to be increased by the application of organic matter, which in turn provide a better environment for the growth, and activity of the indigenous/introduced PSM.

### 2.4.1.7. Effect of Salt Concentrations

The variable response is shown by the PSB when they are grown in salt-affected environments (Srinivasan *et al*., 2012). *Pseudomonas aeruginosa*, *P. putida*, *P. cepacia*, and *P. fluorescens* were allowed to grow in the presence of varying concentrations of salts (NaCl) and showed optimum P solubilization at 0-1.25 % NaCl, but at higher concentrations of NaCl the P solubilization process was found to be delayed (Deshwal and Kumar, 2013). In a similar study, the effect of salt concentrations (0 %, 2 %, 4 %, 6 %, and 8 %) on the PS ability of *Bacillus* was assayed (Cherif-Silini *et al*., 2013). The bacterial cultures in NBRIP medium containing varying rates of salts had variable solubilization activity which declined with the gradual rise in salinity.

### 2.4.2. Factors Affecting Organic P Mineralization

The release of available P by the breakdown of organic matter is governed by the microbial activity and the availability of P depends upon the physicochemical characteristics of soils like soil conditions and weathering process which influence microbial activity; soil pH, temperature, and warm humid conditions and nutrient levels of soils. Temperature above 30°C has the maximum positive effect on mineralization of organic P and the optimum temperature enabling P solubilization at 35°C. The optimum moisture range for mineralization of organic P is 50-75 % of total WHC even though it may also occur in flooded conditions. Alternate wetting and drying favor mineralization of P as it splits water stable soil aggregates. The
cultivation practices also affect organic P mineralization (Hedley et al., 1982; Miguel and Wright, 2008).

2.5. Techniques adapted in Isolation of phosphate solubilizing bacteria

PSB are generally isolated from rhizosphere and non-rhizosphere soils, rhizoplane, phyllosphere, and P fixed soil and even from stressed soils using serial plate dilution method or by enrichment culture technique (Zaidi et al., 2009). With a report from Pikovskaya, (1948) that microbes could solubilize non-readily available forms of soil P and play a role in P release to plants, different methods and media, such as Pikovskaya (Pikovskaya, 1948), bromophenol blue dye method (Gupta et al., 1994) and National Botanical Research Institute Phosphate (NBRIP) medium (Nautiyal, 1999) have been proposed. Plate screening method is usually used for the isolation of the phosphate solubilizing bacteria by the visual detection of clear halos formed around the microbial colonies in a media containing insoluble mineral phosphates (tri-calcium phosphate or hydroxyapatite) as P source (Pikovskaya, 1948; Gupta et al., 1994). The method is considered generally suitable for isolation and preliminary characterization of phosphate solubilizing microorganisms (PSMs) (Katznelson et al., 1962; Bardiya and Gaur, 1974; Goldstein and Liu, 1987; Illmer and Schinner, 1995). A modified method developed by Gupta et al., (1994) using a Pikovskaya medium containing bromophenol blue. By releasing the organic acids by PSBs, yellow colored halos are formed around the colonies in response to the pH drop in this medium. In certain cases, there have been contradictory results between plate halo detection and P solubilization in liquid culture brought about by the PSB. It was observed that some of the bacteria which do not show any clearing zones around the colonies in the agar plates but could solubilize insoluble inorganic phosphates in broth medium (Das, 1963; Louw and Webley, 1959) due to the difference in diffusion rates of various organic acids discharged by an organism (Johnson, 1959). NBRIP liquid broth media with bromophenol blue as a pH indicator has been created for the screening of phosphate solubilizing microorganism (Nautiyal 1999).

The isolation of PSB on artificial culture media is critically dependent on the source of insoluble phosphate in the culture media. The different components of the media have been manipulated for getting a good result. The use of TCP usually yields many (up to several thousand per study) isolates of “supposed” PSM. However, the bacterial isolates obtained from such media containing TCP are not exclusively PSB.
Therefore, a number of other compounds have been also tried like iron/aluminium phosphate and several calcium phosphates but majority of them are even less soluble than TCP in water. Since, there is great variation in the pH and chemical properties of soil, therefore it is very hard to have some metal-Phosphate compound that can serve as the universal selection factor for PSM. Although, knowledge of type of soil can guide the selection of the metal-Phosphate for potential PSM cultivation like alkaline, acidic, or organic-rich soil. A study made by Bashan et al., (2013) suggested adding calcium phosphate compounds (including rock phosphates) for alkaline soils, iron/aluminium phosphate compounds for acidic soils, and phytates for soils rich in organic matter greatly improves availability of the phosphorus to the plants.

The bacterial and fungal strains showing P solubilizing activity are distinguished by the formation of clear halo (a sign of solubilization) around their colonies. However, formation of only halo zone around a bacterial/fungal colony on a solid agar medium should not be considered the sole test for P solubilization. The colony showing halo after repeated culturing essentially needs to be tested in liquid media to assay P dissolution. The isolates, which are found positive in this test, are further processed for detection of production of organic acids. The isolates found positive for organic acid production are finally tested on a plant as the ultimate test for potential P solubilization (Bashan et al., 2013). The cultures showing P-solubilizing activity are termed as microphos (Zaidi et al., 2009). These phosphate solubilizing cultures are tested in field for test trial to check their effect on growth of plants and finally produced in bulk for ultimate transmission as a biofertilizer. Furthermore, a potential PSB isolate must be further tested for direct contribution to P plant nutrition and not necessarily to general growth promotion, as commonly done because promotion of growth, even by PSB, can be the outcome of other mechanisms (Bashan et al., 2013) and ability to solubilise P is not necessarily correlated with the ability to promote plant growth (Collavino et al., 2010).

2.6. Isolation of PSB from different niches

Vazquez et al., (2000) isolated Phosphate Solubilizing Bacterial strains from the rhizosphere of mangroves utilizing culture media containing tri basic calcium phosphate and they analyzed the bacterial culture for the generation of organic acids.
Fankem et al., (2006) collected seven rhizosphere soil samples from oil palm tree of Cameroon. The collected soil samples were air desiccated, crushed to pass through 2 mm sieves to isolate the Phosphate Solubilizing Bacteria. Reyas fundamental medium having Calcium, Aluminum, and Iron phosphates was used for the calculation of phosphate solubilization. At the termination of incubation time, it appeared that phosphate solubilization resulted from a combined impact of pH lowering of the media and organic acids production. Furthermore, each of the standard isolates was capable of exhibiting at least one of essential organic acids such as Citrate, Malate, and Tartrate. Among the ten isolates tested, three were recognized as Pseudomonas fluorescens and would be considered as influential bio fertilizers.

Thirty-six strains of Phosphate Solubilizing Bacteria (PSB) from Central Taiwan were isolated, screened and characterized. Mineral Phosphate Solubilizing (MPS) activities of all strains were verified on tricalcium phosphate medium by analyzing the soluble phosphate content after 72 hours of incubation at 30 °C (Chen et al., 2006).

The phosphate solubilizing fungi Aspergillus spp., Penicillium spp. and Fusarium spp. and bacteria Bacillus subtilis and B.megatherium were collected from saline affected area of Amravati district. The strains were used to diminish the salinity of soil by employing their organic acid metabolic activity (Rajankar et al., 2007).

Vassilev et al., (2007) exploited dry olive waste, as a substrate for phytase production and Rock Phosphate solubilization by Citric Acid producing Aspergillus niger. For phosphate solubilization corn steep liquor, Yeast Extract and Ammonium Nitrate were used as the nitrogen source. Both enzyme production and phosphate solubilization depended on water medium content, type of nitrogen source, inoculum size, and the present, and initial concentration of Phosphate in the medium.

The strain Paecilomyces marquandii from phosphate deficient soil on Pikovskaya’s medium was isolated. The medium was altered and tricalcium phosphate was substituted with other phosphate sources and glucose was replaced with 9 individual carbon compounds viz. Fructose, Galactose, Glycerol, Lactose, Maltose, Mannose, Sorbitol, Starch and Sucrose to examine the effect of different carbon sources on phosphate solubilization. The nitrogen sources were assessed similarly by switching ammonium sulphate with six different nitrogen sources viz. ammonium chloride, asparagine, calcium nitrate, potassium nitrate, sodium nitrate
and urea and their effect on phosphate solubilization were reported by Ahuja et al., (2007).

The phosphate solubilization effectiveness of ten soil bacteria for various parameters like carbon sources such as glucose, fructose, sucrose and lactose, the variable concentration of sodium chloride and glucose were examined by Shahab and Ahmed, (2008). Glucose was found to be the most positive carbon source for solubilization while lactose was found to be the least favorable carbon source. *Acinetobacter lwoffii*, *Pseudomonas aeruginosa*, and *Bacillus thuringiensis* were detected to be the most favorable isolates.

Hamdali et al., (2008) isolated the phosphate solubilizing strains from Moroccan phosphate mine and showed that the isolated strains produce siderophores but not organic acids.

Chang and Yang, (2009) separated the thermo tolerant phosphate solubilizing microbes including bacteria, actinomycetes, and fungi from diverse compost plants and biofertilizers to prepare the multi-useful biofertilizer. Most of the isolates possessed amylase, carboxy methyl cellulase, chitinase, pectinase, protease, lipase, and nitrogenase activities. All isolates could solubilize calcium phosphate and Israel Rock Phosphate. Adding these microbes can shorten the period of maturity, improve the quality, increase the soluble phosphorus content, and enhance the populations of phosphate solubilizing and proteolytic microbes in biofertilizers.

Two possible mechanisms viz. proton excretion by ammonium assimilation and organic acid production were used to inspect the ability of *Pseudomonas fluorescens* to solubilize insoluble phosphate. Phosphate solubilization was significantly promoted with glucose compared to fructose and there were no clear differences in pH and phosphate solubilization between Glucose-ammonium and Glucose-nitrate media. *Pseudomonas fluorescens* solubilized little insoluble phosphate with fructose, regardless of nitrogen sources used. *P. fluorescens* produced mainly gluconic and tartaric acids with small amounts of 2-ketogluconic, formic and acetic acids showed by High-performance liquid chromatography analysis. The pH was reduced with an increase in gluconic acid concentration and was inversely correlated with soluble phosphate concentration during the process (Park et al., 2009).
Khan et al., (2009) studied the occurrence, mechanisms and character of phosphorus solubilizing bacteria in crop production. Plants attain phosphorus from soil solution as phosphate anion and are the least mobile element in plant and soil contrary to other macronutrients. It precipitates in the soil as orthophosphate or is absorbed by ferric and aluminum oxides through ligand exchange.

Srividya et al., (2009) isolated and categorized the fungal strains from cultivated soil, having potential to solubilize insoluble inorganic phosphates on Pikovskya’s medium with tricalcium phosphate. Aspergillus niger and Penicillium sp. displayed high phosphate solubilization effectiveness on Pikovskya’s medium with tricalcium phosphate in liquid broth in 5 days of growth. A. niger exhibited maximum phosphate solubilization efficiency on Pikovskya’s agar solid and liquid medium in 5 days of growth. Aspergillus sp. displayed varied levels of phosphate solubilization activity in both solid and liquid broth culture in presence of various carbon and nitrogen sources and diverse media. PSMs alter insoluble phosphates into soluble forms generally through the process of acidification, chelation and exchange reactions.

The influence of phosphate solubilizing rhizobacteria to advance plant health of tomato was studied by Hariprasad and Niranjana, (2009) and discovered that phosphate solubilizing rhizobacteria endorsed plant growth significantly in 30-day-old-seedlings. One out of total 16 isolates of phosphate solubilizing rhizobacteria, PSRB19 showed significant increase in shoot length (14.0 cm), root length (18.0 cm), fresh mass (0.762 g/seedling) and dry mass (0.110 g/seedling) followed by isolate PSRB8 as compared to control.

Joseph and Jisha, (2009) separated PSB having the capacity to solubilize insoluble inorganic phosphates from rhizosphere soil. Eighty-one potential PSBs isolated were quantitatively examined for phosphate solubilization. Of these, four microorganisms, for example, Acetobacter liquefaciens, Acetobacter sp., Pseudomonas gladioli and one unknown strain observed to be effective phosphate solubilizers. They were chosen for further assessment and found that they solubilize tricalcium phosphate in buffered and additionally non-buffered media. The proficiency of phosphate solubilization was diminished in buffered media contrasted with non-buffered media. The buffering limit of the medium decreased the viability PSB in discharging phosphate from tricalcium phosphates.
PSB from distinct fertility gradient with respect to N, P and K condition of soil through their insoluble mineral phosphate-source utilization patterns were isolated, purified and categorized by Saha and Biswas, (2009). Purulia Rock Phosphate, Mussourie Rock Phosphate, crystalline iron and Aluminum Phosphate (insoluble phosphate sources) were charged in fundamental Pikovskaya solid medium. Growth pattern of the separates on those phosphate sources was recorded and was verified that different communities utilized distinct Phosphate sources in distinct magnitudes.

Chakraborty et al., (2010) collected four hundred isolates from soil samples from a forest, river basin, agricultural fields and rhizosphere of plantation crops of North Bengal and the isolates were selected for phosphate solubilizing activity on Pikovskaya’s agar medium. Ninety showed phosphate solubilizing activity among the screened isolates. Out of these, ten isolates belonging to Aspergillus niger, A. melleus and A. clavatus were selected for further in vitro evaluation of phosphate solubilization using Tricalcium Phosphate and Rock Phosphate. The study discovered that the isolates could solubilize tricalcium phosphate better than Rock Phosphate.

Samiran et al., (2010) isolated the two stress tolerant (Arthrobacter sp. and Bacillus sp.) phosphate solubilizing bacteria on the basis of their phosphate solubilization from tomato rhizosphere and strains with high phosphate solubilizing ability were then tested against a wide range of temperature, pH, and salt stresses. Their capacity to solubilize other insoluble phosphates, such as ferric phosphate and aluminum phosphate was also calculated. The strains also demonstrated various plant growth promoting and biocontrol actions including indole acetic acid production.

Six Phosphate Solubilizing Bacteria were isolated from paddy fields of Eastern Uttar Pradesh, India and identified as members of Enterobacter and Exiguobacterium genera. Out of the six isolates, Enterobacter sp. exhibited a high level of phosphate solubilization in the liquid medium. Exiguobacterium sp. exhibited increased phosphate solubilization efficiency under alkaline pH (Kumar et al., 2010).

Crespo et al., (2011) examined the capacity to solubilize insoluble inorganic phosphate compounds by Gluconacetobacter diazotrophicus using different culture methods. Qualitative plate assays using tricalcium phosphate as the sole phosphate source showed that G. diazotrophicus had solubilization only when aldoses were used as the carbon source. In batch cultures with hydroxyapatite as the phosphate source
and Glucose as the carbon source, more than 98% of insoluble phosphate was solubilized. Continuous cultures of *G. diazotrophicus* exhibited significant activities under carbon or phosphate limitation. It was proposed that *G. diazotrophicus* is an excellent candidate to be employed as biofertilizer because in addition to the already defined plant growth promoting abilities of this organism, it shows a substantial mineral phosphate solubilization ability.

Babana *et al.*, (2013) isolated the six strains of bacteria and two phosphorus solubilizing strains of fungi from wheat rhizosphere in Mali. Two bacterial strains (*Pseudomonas* sp.BR2 and non-identified bacteria B3), which did not form halo zone around colonies, were able to release phosphorus from Tilemsi rock phosphate in the liquid medium. *Vibrio splendidus* B27 was the most efficient rock phosphate solubilizer on NBRIP agar plates (SI = 3.60) based on solubilization index (SI) (298.9 mg l⁻¹) for fungi. Citric acid showed a high significant positive correlation with P solubilized among nine different organic acids produced by the tested microorganisms, no bacterium was able to produce cyanhydrique acid, *Pseudomonas* sp. BR2, *Agrobacterium tumefaciens* BR10, and non-identified bacteria B3 produced siderophores during their growth.

Asuming-Brempong and Aferi, (2014) isolated the phosphate solubilizing microorganisms from the Typic Paleudalf and the Pellustert. Pikovskaya cultural medium was used to determine the ability of the isolates to solubilize calcium phosphate, iron phosphate, and rock phosphate. Most of the isolates were able to solubilize Fe-P, and then the Ca-P medium but little rock phosphate and it was noticed that there were no differences in solubility of inorganic phosphates between the isolates from the different soils types. The colonies of the isolates produced yellow halo zones were produced by the colonies of the isolates on agar plates indicating the production of weak organic acid by the addition of bromothymol blue.

Mujahid *et al.*, (2014) determined the potential of indigenous bacterial isolates from rhizosphere soil and marine environment to promote plant growth. Eight bacterial isolates were characterized for phosphorous solubilization activity isolated from the soil and marine environments. Quantitative and qualitative estimation for phosphorus solubilization was done and minimum inhibition concentration (MIC) of
antibiotic and heavy metals were tested for the isolated strains. The strains exhibited the diverse pattern for antibiotic and heavy metal resistance.

Delgado et al., (2014) isolated the 20 phosphate solubilizing bacterial strains and were screened for their phosphorus solubilizing ability. Among the total 20 bacterial isolates, only four were selected based on phosphorus solubilizing efficiency for molecular characterization and were identified as Pseudomonas sp. and Enterobacter sp.

Saxena et al., (2014) studied the synergistic effect of an arbuscular mycorrhizal fungus (AMF), Glomus etunicatum and an indigenous PSB strain, Burkholderia cepacia BAM-6 on wheat plants growing in pots containing soil with low phosphorous to analyze their potential to be used as bio-inoculants in semi-arid regions. The study showed that the dual inoculation enhanced the growth and yield parameters in comparison to the individual inoculations with AMF and PSB. Therefore, showed that the dual inoculation of the bacteria and fungus can be used as a biofertilizer for the wheat crop grown in arid and semi-arid regions.

Henri et al., (2014) screened phosphate solubilizing bacteria from two agro-ecological zones of Cameroon for their phosphate solubilizing activity on solid and liquid media cultures supplemented with Mallan, Moroccan or Mexican rock phosphates and were subsequently experimented on maize plants grown in pots containing the unsterile soil altered with Mallan rock phosphate for their ability to promote maize growth. The Mallan (402.5 µg P/g) rock phosphates appeared to be the favorable phosphates to be solubilized by the variety of strains, while the Mexican rock phosphate (345.3 µg P/g) was least solubilized in broth. This study showed that all the strains in single and in consortia significantly enhanced the number of leaves, stem base diameter, dry mass, shoot and root dry mass in comparison to the non-inoculated control. The result of inoculation with single strain varied between 27.5 and 59.3 % growth increase, while the result of inoculation with consortis varied between 54.1 and 19.3 % as compared to the non-inoculated control. Thus, the results of the study show the benefit of rock phosphate and PSB that would increase maize productivity.

Kaur and Reddy, (2015) tested the beneficial effects of two phosphate solubilizing bacteria, Pantoea cypripedii (PSB-3) and Pseudomonas plecoglossicida
(PSB-5) on maize and wheat plants inoculated together or singly with rock phosphate (RP). They found that the crop growth increased in terms of shoot and root dry biomass, shoot height, grain yield, and P uptake when PSB were inoculated together with rock phosphate in both maize and wheat crops compared to other treatments. The combined inoculation of PSB and RP also enhanced the enzyme activities and PSB population in both the crops compared to DAP treatment. They suggested that the inoculation of PSB along with RP would be the best alternative for chemical fertilizer use in sustainable agriculture systems.

Maitra et al., (2015) conducted a study in floodplain wetlands to correlate PSB abundance, a decline in sediment pH, and natural mobilization of sediment Ca-P. They found PSBs were abundant in floodplain wetland waters, sediments, and in river and ponds, showing a low to moderate Ca-P solubilization activity. The PSB activity was found to be more in Churni River and Bhomra wetland sediments than other environments and were effective in enhancing available P concentration in interstitial water indicating their P release potential. P fractionation of incubated sediments exhibited only a short-term decline in Ca-P by PSB, indicating that Ca-P might not be their only or preferred metabolic target. Even with low to moderate activity in the culture medium, high population density, and efficacy in P release in sediment propose a significant role of PSB in P cycling in freshwater environments.

Kumar et al., (2015) isolated One hundred and six P-solubilizing bacteria (PSB) from rhizosphere soil and root endosphere of Hippophae rhamnoides L.(Sea-buckthorn) from two sites viz. Lahaul and Spiti of Himachal Pradesh using culture-dependent procedures and were screened for various plant growth-promoting traits (PGPTs). Indole acetic acid produced was detected in 76.41 % isolates, siderophore synthesis in 43.39 % isolates and hydrogen cyanide in 19.4 % isolates. The percent growth inhibition against Alternaria sp. and Fusarium oxysporum was detected in 47.2 % and 25.5 isolates respectively. In General, 23.56 % of PSB isolates from rhizosphere soil and 16.85 % from root endosphere exhibited none of the PGPTs tested. 36.58 % PSB showing PGPTs had a single trait and 43.08 % had multiple traits showing two (29.68 %) and three (13.82 %) types of PGPTs. According to Shannon-Weaver diversity index the proportion of PSB isolates possessing PGPTs was higher for rhizosphere soil than for the root endosphere. Besides, the five best performing PSBs under in vitro conditions were selected for pot experiment to evaluate their
efficiency for growth promotion of tomato seedlings under greenhouse conditions. Among the five isolates, *Bacillus subtilis* CKS1 showed a significant increase in shoot length (13.82 %), root length (25.07 %), shoot dry weight (29.47 %) and root dry weight (33.33 %).

2.7. Morphological and biochemical based Identification of Phosphate Solubilizing Bacteria

Yasmin and Bano, (2011) identified the phosphate solubilizing bacteria (1P, 2P, 3P, 4P and 5P) isolated from rhizosphere soil of various plant species on the basis of colony and cell morphology. The morphology of the bacteria formed on the PVK agar plates was observed after 24 hours. Also the cell motility and shape of the colonies were studied by taking the single colony from each isolate from the agar plates on to the glass slide containing a drop of sterile water and observed under light microscope. They also performed the various biochemical tests including gram staining, oxidase tests, catalase tests. They utilized microbial identification kits QTS-24 to perform the physiological and biochemical tests for the final identification of PSBs.

Sharma, (2011) observed the morphological features (shape, size, elevation, surface form, margins and surface texture, color) of the isolated PSBs for their characterization. The Isolates having the morphological characters like colorless non-pigmented colonies and gram negative, rod shaped, based on the biochemical reactions was identified as *Pseudomonas* fluorescens. The isolate showing slimy, white colonies with irregular margins, having gram positive character and on the basis of biochemical reactions the isolate was identified as *Bacillus megaterium*.

Bhoosreddy, (2014) collected the soil samples for screening of PSB and phosphate solubilizing fungi (PSF) from different rhizospheric and root regions. The colonies showing clear zones around the colonies on PVK agar were selected as PSMs. The isolates A1, A3, A5, A7, A9 upon microscopic observation were confirmed to be Gram positive motile bacilli, whereas isolates A2, A4, A6, A8, A10 were identified to be Gram negative, motile bacilli. The isolates A1, A3, A5, A7, and A9 exhibited large, luxuriant colonies on Bacillus Differentiating Agar medium and A2, A4, A6, A7, A8, and A10 displayed green colored colonies on *Pseudomonas* Isolation Agar medium. Based on characters observed it was established that A1 to
A9 had identical morphological, biochemical and cultural characteristics as that of Bacillus subtilis, and A2 to A10 as Pseudomonas aeruginosa.

Bagalkar, (2013) isolated the different PSBs from various soil samples and were identified on the basis of morphological and biochemical characters which included Grams, IMViC reaction, catalase test, starch hydrolysis test oxidation fermentation test, Phenyl alanine deamination test, Nitrate reduction, Gelatin hydrolysis test, Urea hydrolysis test, Dehydrogenase test, Casein hydrolysis test, Citrate utilization test, Indol production test, Triple sugar iron (TSI) test, Carbohydrate fermentation test (Glucose, Fructose, Sucrose, Arabinose, Mannitol, Lactose, Trehalase, Galactose, Raffinose), Motility test, Endospore staining and capsule staining. The results interpreted were compared with the ‘Bergey’s Manual’ and five phosphate solubilizing bacteria were identified as Pseudomonas spp., Acinetobacter, Enterobacter spp. and Micrococcus spp.

Karpagam and Nagalakshmi, (2014) isolated 37 Phosphate solubilizing microbial colonies from the agricultural soil on the Pikovskaya’s agar medium, containing insoluble tricalcium phosphate (TCP). The colonies showing clear halo zones around the microbial growth were considered as phosphate solubilization. The eight isolates out of 37 microbial isolates showed highest phosphate solubilization index (PSI) ranged from 1.13 - 3.0, were selected for further study as qualitative as well as quantitative actions. Out of these 8 effective isolates, 3 strains showed maximum PSI of psm1, psm2 and psm6 in agar plates along with high soluble phosphate production of 0.37 mg L⁻¹, 0.30 mg L⁻¹ and 0.28 mg L⁻¹ in broth culture. A drop of lactophenol cotton blue was placed on the glass slide and observed under microscope for identification of fungi.

On the basis of morphological and biochemical characters (gram staining, shapes, IMViC test and motility including catalase, oxidase test, sucrose, lactose fermentation, starch hydrolysis, Gelatin hydrolysis and Nitrate reduction) of the bacteria it was concluded that psm1, psm2 and psm6 belongs to genus Pseudomonas, Bacillus, and Rhizobium respectively.

2.8. 16S rRNA sequence based Identification of Phosphate Solubilizing Bacteria

Chen et al., (2006) carried out a study for isolation, screening and characterization of 36 strains of phosphate solubilizing bacteria (PSB) from Central
Taiwan. Tricalcium phosphate medium were used to test the mineral phosphate solubilizing (MPS) activities of all isolates by examining the soluble-P content after 72 hours of incubation at 30 °C. 16S rDNA sequencing of 36 isolates was carried out for Identification and phylogenetic analysis and found ten isolates belonged to genus Bacillus, nine to genus Rhodococcus, seven to genus Arthrobacter, six to genus Serratia and one each to genera Chryseobacterium, Delftia, Gordonia and Phyllobacterium. The p-solubilizing activity of the isolated strains was associated with the release of organic acids and a fall in the pH of the medium. From the cultures of these isolates, eight different kinds of organic acids, viz. citric acid, gluconic acid, lactic acid, succinic acid, propionic acid and three unknown organic acids were detected by HPLC analysis.

Islam et al., (2007) isolated 30 bacteria from the rhizoplane of rice cv. BR29 in Mymensingh, Bangladesh and from the seedlings from the surface sterilized seeds of the same crop. Six isolates showed the varying levels of phosphate solubilization upon screening in both broth and agar plate assays using National Botanical Research Institute’s phosphate medium(NBRIP). The bacterial isolates were identified as Acinetobacter sp. BR-12, Klebsiella sp. BR-15, Acinetobacter sp. BR-25, Enterobacter sp. BR-26, Microbacterium sp. BRS-1 and Pseudomonas sp. BRS-2 on the basis of their phenotypic and 16S rRNA gene sequencing data and was found that BR-25 showed the highest phosphate solubilizing activity followed by BR -15.

A survey was conducted by Perez et al., (2007) for Phosphate-solubilizing bacteria naturally colonizing a limonitic crust in the southeast region of Venezuela. A total of 130 heterotrophic bacterial isolates showing dissimilar degrees of mineral tri calcium Phosphate (Ca₃ (PO₄)₂ solubilizing activities were separated and their phosphate solubilizing ability were identified with different phosphate source. The 10 superlative Ca₃ (PO₄)₂ solubilizers were characterized by partial sequencing analysis of their particular 16S rRNA genes.

Singh and Prakash, (2011) studied the possible role of phosphate solubilizing bacteria (PSB) available in farming soils and isolated 31 bacterial isolates showing solubilization activities from sandy loam soils under chickpea cropping of Patiala, Punjab on Pikovskaya agar plates. The isolated strains were characterized on the basis of partial sequencing analysis of their 16S rDNA gene and were found to belong the
genera *Pseudomonas* and Serratia. For tracking the survival of introduced populations of the PSB ERIC-PCR based finger printing was done during mass inoculation of the strains under chickpea plots. The results showed a positive correlation (*r*2 = 0.853) among soil phosphatase activity and phosphate solubilizers population which was also positively correlated (*r*2= 0.730) to available phosphorus.

Sultan *et al.*, (2012) isolated and identified the bacteria from farming soil, unrelated and treated with four organophosphates (Malathion, Chlorpyrifos, Phorate, and Dimethoate) and found the effect of these organophosphates on them. Bacterial identification was carried out by 16S rDNA sequence analysis and were identified as *Sphingomonas* sp.SZL-1, *Pseudomonas mendocina* strain PC19 and *Brevundimonas* sp. XJ-412. *Sphingomonas* was affected the most followed by *Pseudomonas* by all the four organophosphates considered for study and was absent in most of the treated soil plates up to 21st day whereas *Brevundimonas* was least affected, present in almost all the treated plates as compared with controls. These bacteria were found to be more significant in bioremediation and maintaining soil fertility.

The degree to which soil phosphorus level influenced the incidence of soil phosphate-solubilising bacteria (PSB) and their taxonomic abundance and diversity was studied at three long term fertilizer trials (Whatawhata, Winchmore, and Ballantrae) in New Zealand. Bacteria were isolated from rhizosphere (ryegrass and clover) and non-rhizosphere soils differing in P status. The P-solubilizing phenotype was determined on agar supplemented with sparingly soluble mineral phosphates (Ca₃HOH(PO₄)₃ and CaHPO₄). The extent of P-solubilization in the bacterial population was significantly greater (P<0.001) in soils of low P status, demonstrating a choice pressure for this trait based on soil P availability. P-solubilising bacteria from high P level soils and soils which had not received P fertilizer (nill P soils), were identified based on 16S rRNA-gene sequence analysis. Across the samples, the P-solubilising community was very rich with 39 genera of PSB found, covering 24 families and 4 phyla. At Ballantrae and Winchmore, the PSB composition differed (P<0.05) across soil P status, which was associated with a variation in abundance of *Actinobacteria, Pseudomonadaceae, and Moraxellaceae*. The phylogenetic composition of PSB varied significantly (P<0.05) between sites, however nearly half the families were common across all sites, constituting a ‘core community’ of P-solubilising bacteria for these New Zealand pasture soils (Mander *et al.*, 2012).
Zhao et al., (2013) isolated twelve bacterial strains from maize rhizosphere presenting different degrees of phosphate solubilizing activity. Over 300 µg mL\(^{-1}\) phosphate were solubilized by four strains from insoluble Ca\(_3\)(PO\(_4\))\(_2\), with isolate SCAUK0330 solubilizing over 450 µg mL\(^{-1}\). The strain SCAUK0330 was identified as *Burkholderia cepacia* based on the 16S rRNA gene sequence analysis. SCAUK0330 showed growth at 10-40\(^\circ\)C and pH 4-10 and tolerated up to 5% NaCl and also showed the antagonistic effect against the nine pathogenic fungi. This strain also enhanced the growth of both healthy and *Helminthosporium maydis* infected maize plants showing that the isolate was a good candidate to be used as a biofertilizer and biocontrol agent under a variety of environmental conditions.

Walpola and Yoon, (2013) isolated two phosphate-solubilizing bacteria viz *Pantoea agglomerans* and *Burkholderia anthina*. They conducted a pot experiment under greenhouse conditions to study their effect on growth and phosphorous uptake in tomato plants and the pots were arranged in a randomized block design having three replications for every treatment. The experiment was designed on eight treatments that is: (i) Soil with bacterial inoculation control and without tricalcium phosphate (TCP), (ii) soil + TCP, (iii) Soil + *P. agglomerans*, (iv) Soil + *P. agglomerans* + TCP, (v) Soil + *B. anthina*, (vi) Soil + *B. anthina* + TCP (vii) Soil + *P. agglomerans* + *B. anthina* and (viii) Soil + *P. agglomerans* + *B. anthina* + TCP. Both the strains showed the positive results for all the tested plant growth-promoting traits. The production of indole acetic acid (IAA) was 10 and 7.5µg/ml respectively for *P. agglomerans* and *B. anthina* and were considered as effective siderophore producers as they produced >80% siderophore. Both the strains increased the plant height, root length, shoot and root dry weight, phosphorous uptake and available phosphorous content of the soil as compared to the control. They also noted that the effects were more pronounced in co-inoculation of PSB strains with tricalcium phosphate.

Kang et al., (2014) conducted an experiment to discover the effectiveness of phosphate solubilizing bacteria *Bacillus megaterium* MJ1212 for increasing the growth of mustard plants. The bacterium was isolated from the soil and was identified by the phylogenetic analysis of its 16S rRNA gene and its phosphorus solubilization by the formation of a clear zone on National Botanical Research Institute’s Phosphate medium (NBRIP). An increase in shoot and root length and fresh weight of plants determined the beneficial effect of *Bacillus megaterium* MJ1212. The biochemical
analysis revealed the *Bacillus megaterium* MJ 1212 treated plants had higher chlorophyll, sugars, and amino acids than control.

Panhwar *et al.*, (2014) conducted a study to examine the total microbial populations, the occurrence of growth promoting bacteria and their beneficial effects in acid soils. They selected the four sites and collected the soil, and rice root samples randomly in Kelantan, Malaysia. The microbial population was found to be more than fungal populations. They isolated the 21 phosphate solubilizing bacteria including 19 N₂-fixing strains from the acid surface soil. Three potential strains based on their beneficial effects were identified as *Burkholderia thailandensis*, *Sphingomonas pituitosa*, and *Burkholderia seminalis* and were able of producing indole acetic acid (IAA) and organic acids. Upon inoculation of these strains to the rice seedling variety MR 219 with different concentrations of Al (0, 50, and 100 µM) and pH 4 increased the pH with associated reduction in Al concentration. The beneficial effect of these strains showed their ability to be used as a biofertilizer for rice cultivation on acid sulfate soils.

Rahman *et al.*, (2014) isolated, screened and characterized thirty-four bacteria isolated from about ten plant and soil samples collected from six different sites from an industrially polluted location of Bhaluka of district Mymensingh and evaluated their phosphorus solubilization ability on the NBRIP media.

Midekssa *et al.*, (2015) isolated and characterized 41 phosphate-solubilizing bacteria from rhizospheres of lentil growing areas of Ethiopia and evaluated their beneficial effects on the growth of lentil crop under greenhouse conditions. The isolated bacteria were identified as *Acinetobacter*, *Agrobacterium*, *Bacillus*, *Burkholderia*, *Chryseomonas*, *Enterobacter*, *Pseudomonas*, *Ralstonia* and *Sphingomonas* genera using biochemical tests and 16S rDNA sequence analysis. Inoculation of lentil crop with these strains significantly enhanced the lentil growth as compared to that of the uninoculated control plants. They concluded that the lentil rhizosphere soil from Ethiopia contains the diverse phosphate solubilization bacterial population with plant growth promotion ability and phosphate solubilization potential.

Mehta *et al.*, (2015) isolated the phosphate solubilizing bacteria from the apple rhizosphere soil from Himachal Pradesh, India and was recognized as *Bacillus circulans* on the basis of phenotypic characteristics, biochemical tests, fatty acid
methyl esters analysis and 16S rRNA gene sequence. The isolate displays the plant
growth promoting traits of siderophore, nitrogenase, phosphorous solubilization,
auxin, 1-aminocyclopropane-1-carboxylate deaminase activity and antagonistic effect
against Dematophora necatrix. They explore under the in vitro studies that the P-
solubilization and other plant growth-promoting traits were dependent on the presence
of glucose in PVK medium and removal of yeast extract had no significant effect on
plant growth-promoting traits. P-solubilization activity was combined with the release
of organic acids and a drop in the pH of the Pikovskaya’s medium. HPLC analysis
detected gluconic and citric acid as major organic acids in the course of P-
solubilization. Remarkable increase was observed in seed germination (22.32%),
shoot length (15.91%), root length (25.10%), shoot dry weight (52.92%) and root dry
weight (31.4%), nitrogen (18.75%), potassium (57.69%) and phosphorus (22.22%)
content of shoot biomass over control. These results exhibit that isolate CB7 has the
promising PGPR. Their results reveal that the isolate CB7 is a promising candidate to
be improved as a biofertilizer to increase the plant growth and enhance the soil
fertility.

Mishra et al., (2015) isolated sixteen phosphate solubilizing bacteria from
fennel soil samples in Rajasthan on Pikovskaya medium and were further screened
using the NBRIP broth for acid production and for quantitative assay of phosphate
solubilization rhizospheric soils of Rajasthan.

Baliah and Begum, (2015) isolated, identified and characterized phosphate-
solubilizing bacteria. The selected strains were identified as Bacillus and
Pseudomonas spp. and were characterized under in vitro conditions. This study
showed that population level of PSB was higher in cluster bean rhizosphere soils.
They also examined that all the strains differ in P solubilization, pH change,
enzymatic activity, organic acid production, and utilization of different carbon,
nitrogen, amino acid and vitamin sources.

Messaoud et al., (2015) collected rhizosphere soil samples of different legumes
from sixteen sites in Errachidia province to isolate the potential phosphate bacteria.
They isolated the 62 strains and out of these 19.4% were selected as PSBs and were
identified by 16S rDNA gene analysis. They concluded that the isolated PSB strains
could be a better choice for the promotion of agricultural production without harming the environmental balance.

2.9. Production of PSB biofertilizers

In order to minimize the problems relating to land degradation, declining soil fertility and hastily declining the production levels that occur in large parts of the world needing the basic principles of good farming practice. The three major solid components of the soil including minerals, organic components and microorganisms. These components greatly affect the physical, chemical, and biological properties and processes of global systems. Biofertilizer are the formulations containing cells of various types of beneficial microorganisms and are regarded as important components of integrated nutrients management. The potential biofertilizers plays a key role in productivity and sustainability of soils and farms. Therefore, the introduction of PSB as biofertilizer would not only counterbalance the huge cost of chemical phosphate fertilizers but would also solubilize the insoluble P in the fertilizers and soils to which they are applied (Chang and Yang, 2009; Banerjee et al., 2010). Strains from bacterial genera *Pseudomonas*, *Bacillus*, *Rhizobium* and *Enterobacter* along with *Penicillium* and *Aspergillus* fungi are the most powerful P solubilizers (Whitelaw, 2000). The PSB strains show the P-solubilization of the inorganic phosphate abilities extending between 25-42µg P mL⁻¹ and organic P mineralizing capacities between 818 µg P m⁻¹ (Tao et al., 2008). The PSB in aggregation with rock phosphate and particular super phosphate decreases the P dosage by 50 and 25%, respectively (Sundara et al., 2002).
MATERIALS AND METHODS
CHAPTER-III

MATERIALS AND METHODS

The present study was undertaken in the Division of Microbiology, Faculty of Basic Sciences, SKUAST-J, Chatha and is been given in this chapter under the following sub-heads.

3.1. Survey and Collection of Soil samples
3.2. Physico Chemical properties of soil
3.3. Enumeration of total rhizospheric bacterial population
3.4. Isolation of Phosphate Solubilizing Bacteria (PSB)
3.5. Determination of phosphate solubilizing activity
3.6. Effect of Temperature and pH
3.7. Morphological and Biochemical Characteristics of the isolates
3.8. Genotypic Confirmation
3.9. Determination of acid phosphatase activity
3.10. Determination of phytase enzyme activity
3.11. Determination of the Antagonistic effect of the PSB strains
3.12. Mass production of efficient cultures
3.13. Seed germination
3.14. Statistical analysis

3.1. Survey and Collection of Soil samples

District Jammu and district Samba were surveyed for the collection of rhizospheric soil samples. Jammu is located at 32.73°N 74.87°E with a humid subtropical climate. The average yearly precipitation of Jammu is about 42 inches (1,100 mm) with the bulk of the rainfall in the months from June to September. Jammu city is at an elevation of 1030 feet above the sea level. The area forming north
of Jammu-Chamb road and Jammu-Pathankot road which is known as Kandi area and is comparatively under developed and is mostly rainfed.

R.S. Pura, Burj Tanda, Chakrohi, Kaili Mandi, Gurhaslathia, Chatha farm-I, Chatha farm-II and Rakh Dhiansar (Map 1 and Table 3). Rhizospheric soil samples from Rice, Radish, Brinjal, Cauliflower and Wheat were collected from each location. A distance of about 500 m interval was used from one sampling site to another within the sub location. Collected samples were transferred to plastic bags, brought to laboratory and prepared for the analyses.

The soil samples were then air dried in the laboratory for a period of 4-5 days and were stored in plastic bags at 4±1°C prior to biochemical analysis.

3.2. Physico Chemical properties of soil

The collected rhizospheric soil samples were analysed for their physical and chemical properties. The samples were analyzed for their pH, electric conductivity (E.C), Organic carbon (O.C), available nitrogen (N), available phosphorous (P) and texture.

3.2.1. Laboratory procedures

The soil analysis was carried out on air-dried soil portions (<2mm).

3.2.1.1. pH (1:2.5)

The soil pH was determined potentiometrically in 1:2.5 (W/V) suspension of water (Jackson, 1973).

3.2.1.2. Electric conductivity

Electric conductivity was measured in 1:2.5 soil water suspension with Electrical Conductivity meter (Jackson, 1973).

3.2.1.3. Organic carbon

Organic carbon was determined by the chromic acid titration method (Walkley and Black, 1934).

3.2.1.4. Available nitrogen

The available nitrogen was determined by the Kjeldahl method.
3.2.1.5. Available phosphorous

Available phosphorous was determined following the method of Murphy and Riley. (1962).

3.2.1.6. Particle size analysis

Hydrometer method using bouyoucous hydrometer as outlined by Piper. (1966) was used for mechanical analysis of soil and following textural diagram texture of the soil was computed (Bouyoucos, 1962).

3.3. Enumeration of total rhizospheric bacterial population

A 10-fold serial dilution was prepared for the determination of rhizospheric population by spread plate count method. Total bacterial population was determined from the soil rhizosphere using Nutrient agar (HiMedia Laboratories Pvt Ltd. Mumbai, India). 0.1 mL aliquots were taken from $10^{-7}$, $10^{-8}$ and $10^{-9}$ and were spreaded on Nutrient agar plates with sterile L-shaped loop. The plates were maintained in triplicates and incubated at $30\pm1^\circ C$. The total microbial population was enumerated at 2, 4, 6, 10 and 15 days of incubation period.

3.3.1. Enumeration of indigenous Phosphate Solubilizing Bacterial (PSBs) strains

The soil samples collected from various areas were diluted to a 10-fold serial dilution and 0.1 mL aliquots were taken from $10^{-7}$ and spreaded on Pikovskaya media (HiMedia Laboratories Pvt Ltd., Mumbai, India) plates (Pikovskaya, 1948) with sterile L-shaped loop. The plates were incubated at $29\pm1^\circ C$ for 15 days and periodic observation for the halo formation was recorded at 2, 4, 7, 12 and 15 days.

3.4. Isolation of Phosphate Solubilizing Bacteria (PSB)

3.4.1. Isolation and Purification of PSBs

The strains were isolated from the rhizospheric soils of rice, brinjal, radish wheat and cauliflower grown at different locations. The isolation was made by using serial dilution method.

One gram of fine powdered soil from each sample was dispersed in 10 ml of sterilized water blanks and was thoroughly shaken. From the first dilution 1 ml was
Map 1. Satellite imagery of the sampling area
transferred to 9 ml of sterile water blank to form 10^{-1} dilution. Similarly, 10^{-2}, 10^{-3}, 10^{-4} to 10^{-10} dilutions were made for each soil sample. 0.1 ml from 10^{-5}, 10^{-6} and 10^{-7} dilutions were taken by sterile pipette and were spreaded on (maintained in triplicates) Pikovskaya agar medium containing calcium phosphate. The plates were incubated at 30±1°C for 4-5 days. Colonies showing halo zones with a large relative diameter were picked and purified further using same medium. Isolated colonies were further sub cultured by using the Pikovskaya (PVK) media preserved under refrigerated conditions for further study.

3.5. Determination of phosphate solubilizing activity

The qualitative as well as quantitative analysis for phosphate solubilization of the selected isolates were conducted by plate screening method and broth culture method.

3.5.1. Qualitative method

The phosphate solubilizing activities of each isolate was determined by spotting 10 µl of pure cultures of phosphate solubilizing bacteria on the Pikovskaya’s agar media plates in three replicates. The potential isolates which solubilize the insoluble phosphate on the Pikovskaya medium by forming the halos. The growth and solubilization diameter was determined after incubation at 30 ±1°C for 15 days. The zone of phosphate solubilization (mm) formed around colonies was recorded after a period of 4, 10 and 15 days. The colonies forming more than 3.0 mm zone of solubilization were stocked. The solubilization efficiency and the solubilization index on the basis of diameter of clearing halo zones were measured according to Nguyen et al. (1992).

\[
\text{Solubilizing Efficiency (} \% \ \text{S.E)} = \frac{\text{Solubilization Diameter}}{\text{Growth Diameter}} \times 100
\]

\[
\text{Solubilization Index (S.I)} = \frac{\text{Colony Diameter + Halozone Diameter}}{\text{Colony Diameter}}
\]
3.5.2. Quantitative method

In order to determine the P solubilization quantitatively 100 ml of the Pikovskaya’s broth (PB) medium (HiMedia Laboratories Pvt Ltd. Mumbai) with Tricalcium phosphate (0.3g 100 ml⁻¹) was prepared and sterilized. Each isolated pure bacterial colony was inoculated into the broth medium and the inoculated broth cultures were incubated at 32 ±1°C for 4-5 days on rotatory shaker. After incubation, the broth cultures were centrifuged at 10,000 rpm for 30min. Uninoculated sterilized broth served as the control. The available Phosphorous was determined by colorimetrically at 420 nm with standard KH₂PO₄ (Murphy and Riley, 1962).

3.6. Effect of Temperature and pH

3.6.1. Temperature

To determine the phosphate solubilization activity under different temperatures, 72 hour (hr) old pure bacterial colonies were inoculated in a 250 ml Erlenmeyer flasks containing 100 ml sterilized PB medium (HiMedia Laboratories Pvt Ltd. Mumbai) and were incubated at 5°C, 15°C, 25°C, 35°C, 40°C and 50°C under shaking conditions for 36 hours (hrs). Uninoculated sterile media was used as a control.

3.6.2. pH

The variation in pH was studied by inoculating the 72 h old pure bacterial colonies in 250 ml Erlenmeyer flasks containing 100 ml sterilized PB medium incubated at 30±1°C under shaking conditions for 3 days. Sterile uninoculated medium served as control. pH fluctuations were observed through 3 days period at 6, 12, 24, 36 and 72 hrs.

3.7. Morphological and Biochemical Characteristics of the isolates

Identification of pure cultures was made by using morphological and biochemical characteristics (Sneath-Peter et al., 1994). The commercially accessible biochemical test kits have an advantage of fewer requirements for setting up a range of test media and reagents and the time required for interpretation of results. HiMedia Biochemical Identification kits, involving single step procedure of inoculation, which leads to final identification of test organism being studied. Each Biochemical
Identification test kit is a standardized colorimetric recognizable proof framework using traditional biochemical tests and carbohydrate utilization tests. The tests depend on the principle of pH change and substrate utilization. Organisms undergo metabolic changes under incubation, which are designated by a color change either in the media that is deciphered visually or after expansion of a reagent.

The probably positive phosphate solubilizing bacteria were subjected to various biochemical tests using commercially available kits KB002 HiAssorted™ Biochemical Test Kit and KB004 HiStaph™ Identification Kit (HiMedia Laboratories Pvt Ltd., Mumbai).

3.7.1 Preparation of inoculums

For biochemical characterisation, bacterial inoculums were prepared by inoculating individual colonies in sterilized test tubes containing 5 ml of Brain Heart Infusion broth and incubated at 37ºC for 6 hrs.

3.7.2. Inoculation of bacteria in KB002 HiAssorted™ Biochemical Test Kit and KB004 HiStaph™ Identification Kit (HiMedia Laboratories Pvt Ltd., Mumbai)

The kits were opened aseptically and each of the well in the kits was inoculated with 50 µl of inoculum by surface inoculation and was incubated at 37±1ºC for 24 hrs. At the end of the incubation period, a series of reagents were added in designated wells as per manufacturer’s specifications to carry out different biochemical tests. The results were interpreted as per the guidelines of manufacturer’s instructions.

The biochemical characterization conducted on commercially available Kits tested the following biochemical tests as proposed in Bergey’s Manual of Systematic Bacteriology.

3.7.2.1. Citrate utilization

Citrate utilization identifies the capacity of the organisms to use citrate as the sole source of carbon.

3.7.2.2. Ornithine utilization

The test determines the ability of the microorganisms to use the amino acid
ornithine as a source of carbon and energy for growth. Use of ornithine is accomplished by the enzyme ornithine decarboxylase.

3.7.2.3. H₂S Production

This test determines whether the bacteria reduce sulfur-containing compounds to sulfides during the process of metabolism. The test recognizes the hydrogen sulfide production.

3.7.2.4. Voges Proskauer's Test

The Voges-Proskauer's test determines the capability of bacteria to produce non acidic or neutral end products, such as acetyl methyl carbinol from the organic acids that result from glucose metabolism. It identifies the acetoin creation.

3.7.2.5. O-Nitrophenyl-β-D-galactopyranoside (ONPG) test

ONGP test is used to check β-Galactosidase enzyme activity.

3.7.2.6. Urease activity

The urease test identifies those bacteria that are capable of hydrolyzing urea to produce ammonia and carbon dioxide and is used to check the urease activity of the microorganisms.

3.7.2.7. Arginine utilization

The purpose of this test is to distinguish the bacteria that can use the amino acid arginine as a source of carbon and energy for growth. Use of arginine is accomplished by the enzyme arginine dihydrolase.

3.7.2.8. Carbohydrate fermentation

Carbohydrate fermentation tests (mannitol, sucrose, lactose, arabinose, raffinose, trehalose and maltose). For the ultimate characterization of isolated strains, the following biochemical tests were performed that are being discussed as below:

3.7.2.9. Oxidase test

Pure colonies of each isolate were streaked with the help of sterilized inoculation loop on the commercially available Oxidase discs DD018 (HiMedia). The change in color was observed with 5- 10 seconds. If the color changed from white to
purple, then the result was considered to be positive and if there was no color change then the result was considered to be negative.

3.7.2.10. Catalase test

Catalase is an enzyme, which is produced by microorganisms that live in oxygenated environments to neutralize toxic forms of oxygen metabolites. To find out if a particular bacterial isolate is able to produce catalase enzyme, small inoculums of bacterial isolate was mixed into hydrogen peroxide solution (3%) and the rapid evolution of oxygen bubbles were interpreted as a positive result for the tested isolates.

3.7.3. Colonies and Cell Morphology

The pure isolates were further cultured on new plates for colony morphology. The colony morphology was studied on the basis of their form, color, pigmentation, configuration, luster, topography and optical characteristics. To study cell shape/size and structure, the bacterial strains were distinguished based on Gram staining.

3.7.3.1. Gram Staining

Slides of pure bacterial strains were prepared for Gram staining (Vincent, 1970). A thin smear of each bacterial isolate was separately made on a clean glass slides and heat fixed. Then the smear was stained by crystal violet for one minute and washed with water followed by flooding with Gram’s iodine. After one minute the slides were washed with tap water and decolorized with alcohol. After decolorization, the smears were counter stained with saffranin for one minute. The slides were washed, air-dried and were observed under microscope using oil immersion for studying the Gram nature of isolates. Pink colored bacteria were Gram -ve while purple colored were Gram +ve. After Gram staining the slides were studied for cell morphology under microscope.

3.7.4. Utilization of the Carbon sources

The utilization of the different carbon sources was done on using commercially available kit (HiMedia Laboratories Pvt Ltd. Mumbai,). The carbohydrates tested were glucose, mannitol, sucrose, lactose, arabinose, raffinose, trehalose and maltose.
3.8. Genotypic Confirmation

3.8.1. DNA extraction

The DNA from all of the isolates was extracted by Snap chilling method as reported earlier by Yousr et al. (2007). The method involved heating two to three well-isolated colonies of bacterial cultures in nuclease-free water at 100°C for 15 min in a water bath. The bacterial suspension was immediately subjected to rapid chilling to release DNA by cold shock. The lysate obtained was centrifuged at 13,000 rpm for 5 min, and the supernatant was used directly as a template for PCR.

3.8.2. 16S rDNA genus specific PCR Using Universal Primers:

The Universal primers were used to amplify the whole region of 16S rDNA as per the method of Lane. (1991). PCR amplification was carried out 0.2 ml PCR tubes with 25 µl of reaction volume (Table 1).

Table 1. Reaction mixture for PCR based detection of PSB using Universal 16S rDNA gene Primers

<table>
<thead>
<tr>
<th>PCR Ingredients</th>
<th>Volume in µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR Assay buffer (10 X) (without MgCl₂)</td>
<td>3.0 µl</td>
</tr>
<tr>
<td>dNTP mix (2.5mM each)</td>
<td>2.0 µl</td>
</tr>
<tr>
<td>MgCl₂ (25mM)</td>
<td>3.5 µl</td>
</tr>
<tr>
<td>Forward Primer (25 pm/µl)</td>
<td>1.0 µl</td>
</tr>
<tr>
<td>Reverse primer (25 pm/µl)</td>
<td>1.0 µl</td>
</tr>
<tr>
<td>Taq polymerase (3 units/µl)</td>
<td>0.33 µl</td>
</tr>
<tr>
<td>DNA template</td>
<td>3.0 µl</td>
</tr>
<tr>
<td>Nuclease free water</td>
<td>11.17 µl</td>
</tr>
<tr>
<td>Total</td>
<td>25.00 µl</td>
</tr>
</tbody>
</table>

The reaction conditions in PCR for all the three were 1 cycle at 94°C for 5 min, followed by 30 cycles of 95°C for 0.5 min, 55°C for 0.5 min, 72°C for 1.5 min and a final extension step at 72°C for 10 min. The amplified products were analyzed by electrophoresis on 1.5% (w/v) agarose gel and visualized in UV trans illuminator. Detail of the primer sequences used in this study is given in Table 2.
Table 2. List of Primers used in the study

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Prime Name</th>
<th>Primer Sequence (5' →3')</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>UNI 27F</td>
<td>AGAGTTTGATCMTGGGCTCAG</td>
<td>1500</td>
</tr>
<tr>
<td></td>
<td>UNI 1525R</td>
<td>AAGGAGGTTGWTCCARCC</td>
<td></td>
</tr>
</tbody>
</table>

3.8.3. Agarose gel electrophoresis:

Agarose gel electrophoresis was carried out in a horizontal, submerged electrophoresis unit to check the integrity of DNA. Each DNA sample was mixed with one-fifth volume of the gel loading buffer. For electrophoresis, 1.5 percent agarose gel in TBE buffer containing ethidium bromide (0.5 μg⁻¹) was used. After the addition of tracking dye (bromophenol blue), each sample was loaded in the well of gel and electrophoresis was carried out at 100 V at room temperature for about 1-2 hr depending upon the length of the gel or till the dye migrated more than half of the length of the gel. At the end of the electrophoresis, the gel was visualized under UV trans-illuminator.

3.8.4. Gene Sequencing of samples

All the three isolates were sequenced in order to determine the identity of the isolated cultures by comparing the sequences with the database. For this, the PCR products were sequenced commercially from Chromous Biotech (Bengaluru) using dye terminator chemistry. The sequences obtained were subjected to nucleotide BLAST (Basic Local Alignment Search tool) (http://blast.ncbi.nlm.nih.gov/Blast.cgi) at the server of the National Centre for Biotechnology information (NCBI) to determine the similarity with the already submitted gene sequences and identity was affirmed by highest percentage of similarity match.

3.9. Determination of acid phosphatase activity

The acid phosphatase activity of the phosphate solubilizing bacteria was evaluated as per Tabatabai and Bremner. (1969) method. The method involved following steps:
I. A 3 ml of an aliquot (48 h PSB culture), 1 ml modified universal buffer (MUB) and 1 ml 0.115 M p-NPP were pipetted into a 20 ml reagent vial.

II. The mixture was incubated at 37±1°C for 1 hr.

III. A Phosphatase reaction was stopped by the addition of 20 ml of 0.5 N NaOH. The mixture was then transferred to a 50 ml volumetric flask and the absorbance was read with a spectrophotometer at 410 nm along with standards.

3.10. Determination of phytase enzyme activity

Phytase activity was measured by the method as described below:

After 48 h of incubation, exact 150 µl of PSB culture was added with 600 µl of substrate (3 mM Na-phytate in 0.2 M Na-acetate, pH 4.0) and incubated at 45°C temperature. The released inorganic phosphate was measured by an ascorbic acid method.

3.11. Determination of the Antagonistic effect of the PSB strains

For this study Rhizoctona solani was received from MTCC wide accession no. 4633. Antagonistic effect of PSB strains against the rice pathogen R. solani was determined by using the dual culture technique (Sariah, 1994). An exact 5 mm diameter agar plug with mycelium was placed on the centre of PDA media plates. The PSB isolates were spotted (20 µl) approximately 3 cm distances from the pathogen on the same plates. The plates were incubated under room temperature and checked zones of inhibition of mycellium growth after 7 days when the fungal mycelium had reached the edge of the plates. The measurement of growth inhibition was done by using following formula:

\[ \% \text{ inhibition in radial growth} = 100 \times \frac{r1-r2}{r1} \]

Where, \( r1 \) is the radial mycelia growth in control and \( r2 \) is the radial mycelia growth in treatment.

3.12. Mass production of efficient cultures

The mass production of the efficient PSB strains was done by carrier mixed cell cultures. Three days old bacterial colonies grown on PVK agar media plates were
selected and inoculated in a 500 ml sterilized Erlenmeyer flasks containing PVK broth medium at 30 ±1°C for 72 hrs under shaking at 120 -140 rpm. This has served as the mother culture. 1 % of the mother culture was transferred to the 500 ml flask after getting a desirable growth (10^7-10^8 cfu mL⁻¹). The broth was mixed with the carrier (compost and soil) by maintaining the moisture content 70% and pH near neutral (7), this was used as inoculum (Gaur, 1990).

3.13. Seed germination

The maize grains (*Zea mays* var.CM-135) were selected for germination under laboratory conditions in the seed germinator for 72 hrs at 28°C.

The grains were surface sterilized by using 70% ethanol for 10 minutes and 100% ethanol for five minutes respectively. After each step, the grains were washed by distilled water for five times.

3.13.1. Seedling treatment

The germinated healthy seedlings were selected for PSB treatment. The treatment was done by preparing a suspension of 1 Kg of biofertilizer in 10 litre of water. Seedling was dipped into suspension for 20-30 minutes and transplanted treated seedling as the method given by Motsara *et al.* (1995).

3.13.2. Bioassay

A pot experiment was set up by transferring the inoculated maize seedlings into the sterilized polythene bags containing sterilized soil (1 Kg) under greenhouse conditions (Plate 13).

Two treatments were set up for carrying out a pot experiment. The inoculated seedlings and the uninoculated seedlings which served as the control. Both the treatments were replicated in 5 replications.
Plate 13. Inoculation of PSB strains on maize grains
3.13.4. Enumeration of viable cell population

The survival of the PSB in the rhizosphere of Zea mays Var. CM-135 in polythene bags was monitored through 30 days interval by serial dilution technique by taking 1 ml of the rhizospheric soil and aseptically transferring it to 9 ml sterile water blank and shaking it vigoursly, so as to form homogeneous suspension. The enumeration was carried out using spread plate technique using PVK agar medium. These plates were incubated at 30±1°C for 48 hrs. Plates containing 30-300 colonies were recorded and the counts were expressed as log cfu per ml of carrier material.

3.13.5. Dry matter yield

To evaluate the impact of inoculated PSB strains on the dry matter yield of maize, plants were uprooted after 30 days. Fresh and dry weight of root and shoot were documented. Dry weight was determined by placing the root and shoot samples separately into small, pre weighted brown paper bags and drying them in an oven at 80°C for 48 hrs. Data concerning to dry matter yield, plant biomass and P changes was verified through 30 days period and was subjected to statistical analysis.

3.14. Statistical analysis

Experimental data obtained from the investigation was subjected to statistical analysis by using Analysis of Variance (ANOVA) as per Snedecor and Cochran, (1980) in triplicate along with appropriate controls. The SPSS software version 2.0 were used for analysing the data statistically.
RESULTS
CHAPTER-IV

RESULTS

The present study involved isolation of phosphate solubilising bacteria from rhizospheric soil samples taken from the predominant crops growing in the areas of R.S. Pura, Burj Tanda, Chakrohi, Kaili mandi, Chatha farm-I, Chatha farm-II, Gurha slathian and Rakh Dhiansar.

4.1. Physico-chemical properties of the rhizospheric soil samples

The collected rhizospheric soil samples were air dried, thoroughly mixed, sieved and analyzed for various physico-chemical characteristics. The observed physical and chemical properties of the concerned soil samples are presented below and shown in Table 3.

pH (1:2.5)

The pH of the soil samples varied from 6.72-7.2. The pH of Kaili mandi was observed least (6.72) among all the soil samples whereas the highest pH was noticed in samples from Chakrohi.

Electric Conductance

The E.C of the soil samples ranged from 0.1-0.19 µS cm⁻¹ with highest E.C observed from Chatha farm I and II regions (0.19 µS cm⁻¹) and least from Burj Tanda and Gurha Slathian.

Organic carbon

The O.C recorded had a variation from 0.41-.53%. The maximum organic carbon was noticed from Chatha farm I and II (0.53%). A low O.C was found from the soil samples of Burj Tanda and Gurha slathian (0.41%).

Available Nitrogen

The available N estimated during the study was found to vary from 112-166 kg ha⁻¹ with least available N found in the soil samples of Gurha slathian (112 kg ha⁻¹) and maximum was observed in the soil samples of Chatha farm II (166 kg ha⁻¹).
Available phosphorous

The available P recorded during the study was found to vary from 10-14.2 kg ha\(^{-1}\). The lowest available P was observed in the soil samples of Gurha slathian (10 kg ha\(^{-1}\)) while maximum available P was recorded from Chatha farm I (14.2 kg ha\(^{-1}\)).

Texture

The texture of all the soil samples from various regions was found to have sandy clay loam texture.
Table 3. Physico-chemical properties of soil samples collected from different areas

<table>
<thead>
<tr>
<th>Location</th>
<th>Rhizosphere</th>
<th>pH (1:2.5)</th>
<th>E.C µS cm(^{-1})</th>
<th>Organic Carbon (%)</th>
<th>Available N (kg ha(^{-1}))</th>
<th>Available P (kg ha(^{-1}))</th>
<th>Texture</th>
</tr>
</thead>
<tbody>
<tr>
<td>R.S. Pura (L1)</td>
<td>Rice</td>
<td>6.8</td>
<td>0.16</td>
<td>0.5</td>
<td>134</td>
<td>12.8</td>
<td>Sandy clay loam</td>
</tr>
<tr>
<td>Burj Tanda (L2)</td>
<td>Radish</td>
<td>6.78</td>
<td>0.10</td>
<td>0.41</td>
<td>132</td>
<td>12.4</td>
<td>Sandy clay loam</td>
</tr>
<tr>
<td>Chakrohi (L3)</td>
<td>Rice</td>
<td>7.2</td>
<td>0.15</td>
<td>0.6</td>
<td>171</td>
<td>14.0</td>
<td>Sandy clay loam</td>
</tr>
<tr>
<td>Kaili Mandi (L4)</td>
<td>Brinjal</td>
<td>6.72</td>
<td>0.13</td>
<td>0.47</td>
<td>138</td>
<td>12.56</td>
<td>Sandy clay loam</td>
</tr>
<tr>
<td>Gurhaslathia (L5)</td>
<td>Cauliflower</td>
<td>6.75</td>
<td>0.10</td>
<td>0.41</td>
<td>112</td>
<td>10</td>
<td>Sandy clay loam</td>
</tr>
<tr>
<td>Chatha farm-I (L6)</td>
<td>Rice</td>
<td>6.68</td>
<td>0.19</td>
<td>0.53</td>
<td>164</td>
<td>14.2</td>
<td>Sandy clay loam</td>
</tr>
<tr>
<td>Chatha farm-II (L7)</td>
<td>Wheat</td>
<td>6.68</td>
<td>0.19</td>
<td>0.53</td>
<td>166</td>
<td>14.0</td>
<td>Sandy clay loam</td>
</tr>
<tr>
<td>Rakh dhiansar (L8)</td>
<td>Rice</td>
<td>7.12</td>
<td>0.17</td>
<td>0.48</td>
<td>114</td>
<td>10.5</td>
<td>Sandy clay loam</td>
</tr>
</tbody>
</table>
4.2. Enumeration of total rhizospheric bacterial population from soil samples:

The total bacterial population was determined by spread plate count method from different locations using Nutrient agar medium (Table 4). Highest population was obtained on 6 days of incubation period from locations L1, L4, L7 and L8 and showed the density of total culturable bacteria from $6.13 \times 10^6$ to $8.93 \times 10^6$ cfu g$^{-1}$ soils. On 15$^{th}$ day the bacterial density from all the locations shows downward trend (Fig. 1). The total bacterial population obtained from various rhizospheric samples at $10^7$ dilutions is presented as log value of the total bacterial population in the Fig. 1.

Table 4. Total culturable bacteria from the rhizosphere of different locations

<table>
<thead>
<tr>
<th>Location</th>
<th>2days</th>
<th>4days</th>
<th>6 days</th>
<th>10 days</th>
<th>15 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>$1.7 \times 10^6$</td>
<td>$4.9 \times 10^6$</td>
<td>$7.5 \times 10^6$</td>
<td>$6.5 \times 10^6$</td>
<td>$5.8 \times 10^6$</td>
</tr>
<tr>
<td>L2</td>
<td>$1.6 \times 10^6$</td>
<td>$5.4 \times 10^6$</td>
<td>$7.4 \times 10^6$</td>
<td>$6.56 \times 10^6$</td>
<td>$4.5 \times 10^6$</td>
</tr>
<tr>
<td>L3</td>
<td>$1.2 \times 10^6$</td>
<td>$3.8 \times 10^6$</td>
<td>$6.13 \times 10^6$</td>
<td>$5.71 \times 10^6$</td>
<td>$3.33 \times 10^6$</td>
</tr>
<tr>
<td>L4</td>
<td>$2.67 \times 10^6$</td>
<td>$4.98 \times 10^6$</td>
<td>$8.93 \times 10^6$</td>
<td>$6.31 \times 10^6$</td>
<td>$5.56 \times 10^6$</td>
</tr>
<tr>
<td>L5</td>
<td>$1.76 \times 10^6$</td>
<td>$4.36 \times 10^6$</td>
<td>$7.53 \times 10^6$</td>
<td>$6.35 \times 10^6$</td>
<td>$4.76 \times 10^6$</td>
</tr>
<tr>
<td>L6</td>
<td>$1 \times 10^6$</td>
<td>$4.59 \times 10^6$</td>
<td>$6.31 \times 10^6$</td>
<td>$5.55 \times 10^6$</td>
<td>$4.6 \times 10^6$</td>
</tr>
<tr>
<td>L7</td>
<td>$2.7 \times 10^6$</td>
<td>$5 \times 10^6$</td>
<td>$8.11 \times 10^6$</td>
<td>$6.12 \times 10^6$</td>
<td>$4.15 \times 10^6$</td>
</tr>
<tr>
<td>L8</td>
<td>$1.72 \times 10^6$</td>
<td>$3.97 \times 10^6$</td>
<td>$7.52 \times 10^6$</td>
<td>$5.95 \times 10^6$</td>
<td>$4.17 \times 10^6$</td>
</tr>
</tbody>
</table>
4.2.1. Enumeration of indigenous Phosphate Solubilizing Bacterial (PSBs) strains

The total PSB count was obtained by spread plate method using Pikovskaya agar plates. Out of the eight locations viz R.S. Pura (L1), Burj Tanda (L2), Chakrohi (L3), Kaili mandi (L4), Chatha farm-I (L5), Chatha farm-II (L6), Gurha slathian (L7) and Rakh Dhiansar (L8), the highest population of PSB was obtained in the locations L1, L4, L7, and L2 followed by the isolates collected from L8, L6 and L5 respectively (Table 5). Conversion log values of PSB density in the locations showed a curvilinear graph (Fig. 2).
Table 5. PSB population from various locations

<table>
<thead>
<tr>
<th>Location</th>
<th>2days</th>
<th>4days</th>
<th>7 days</th>
<th>12 days</th>
<th>15 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>2.67×10^8</td>
<td>4.13×10^8</td>
<td>8.86×10^8</td>
<td>7.92×10^8</td>
<td>6.87×10^8</td>
</tr>
<tr>
<td>L2</td>
<td>1×10^8</td>
<td>3.90×10^8</td>
<td>5.33×10^8</td>
<td>4.71×10^8</td>
<td>3.98×10^8</td>
</tr>
<tr>
<td>L3</td>
<td>0.7×10^8</td>
<td>1.68×10^8</td>
<td>2.50×10^8</td>
<td>2.16×10^8</td>
<td>2.07×10^8</td>
</tr>
<tr>
<td>L4</td>
<td>1.83×10^8</td>
<td>4.33×10^8</td>
<td>7.33×10^8</td>
<td>5.78×10^8</td>
<td>4.90×10^8</td>
</tr>
<tr>
<td>L5</td>
<td>0.67×10^8</td>
<td>2.89×10^8</td>
<td>3.43×10^8</td>
<td>3.01×10^8</td>
<td>2.90×10^8</td>
</tr>
<tr>
<td>L6</td>
<td>1.53×10^8</td>
<td>2.5×10^8</td>
<td>3.68×10^8</td>
<td>2.95×10^8</td>
<td>2.18×10^8</td>
</tr>
<tr>
<td>L7</td>
<td>1.67×10^8</td>
<td>2.33×10^8</td>
<td>6.68×10^8</td>
<td>5.98×10^8</td>
<td>4.87×10^8</td>
</tr>
<tr>
<td>L8</td>
<td>1.67×10^8</td>
<td>2×10^8</td>
<td>4×10^8</td>
<td>3.45×10^8</td>
<td>2.98×10^8</td>
</tr>
</tbody>
</table>

Fig.2. Log values of PSB population from various samples
4.3. Isolation and Purification of PSBs

The phosphate solubilizing bacteria were screened from the Pikovskaya media on the basis of production of halo zone as shown in the plate. One isolate from each rhizospheric soil sample was isolated and total eight PSB strains were isolated based upon the solubilization zone produced in PVK agar plates and the isolates were purified by repeated sub culturing on the same medium (Plate 1). Halozones exhibited by different isolates from the different locations (Table 6) showed the minimum of 0.95 mm and maximum 7.79 mm. The corresponding diameter was recorded minimum of 2.1 and maximum 9.92 mm for the isolates from the location L6 and L7 respectively.

4.4. Phosphorous solubilization activity

All the isolates were tested for their phosphate solubilizing activity qualitatively and quantitatively using the PVK agar media and PVK broth. The highest phosphate solubilizing activity was found in PSB7 (78.52%) followed by PSB4 (76.50%) while the lowest activity (35.76%) was found in PSB3 (Table 7). The overall range of P solubilising efficiency (PSE) by the different strains varied between 30.76 and 78.52% and was significant as compared to control at LSD 0.05. Strains have differed significantly with respect to the incubation time in solubilization of phosphorous.

The isolated strains were further assayed qualitatively by inoculated in the Pikovskaya broth medium to check their P solubilization efficiency. All the strains solubilized P efficiently during the initial stages of incubation but subsequently a reduction in the P solubilisation was observed (Fig.3). Reasonably, the three isolates (PSB1, PSB4 and PSB7) showed the consistent results in solubilizing the phosphate. The maximum P solubilization was observed at different periods of incubation for all the tested isolates. PSB1, PSB4 and PSB7 showed a peak value of P solubilisation after 36 hrs and varied between 0.9-1.3 µg ml⁻¹, whereas other PSB isolates showed a decrease in P solubilisation after 24 hrs of time varying between 0.3-0.5 µg ml⁻¹ (Fig.3). Maximum P solubilization was observed in PSB1, PSB4 and PSB7 as 0.9, 1.3 and 1.0 µg ml⁻¹ (Table 7).
Plate 1. Isolated PSB strains on Pikovskaya agar media showing halozones.
Table 6. Phosphate Solubilization Efficiency and Index of various cultures

<table>
<thead>
<tr>
<th>Location</th>
<th>PSB Strain</th>
<th>Colony Diameter (mm)</th>
<th>Halozone Diameter (mm)</th>
<th>% Efficiency</th>
<th>PSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>PSB₁</td>
<td>4.9</td>
<td>3.1</td>
<td>63.26</td>
<td>1.63</td>
</tr>
<tr>
<td>L2</td>
<td>PSB₂</td>
<td>2.11</td>
<td>1.01</td>
<td>47.86</td>
<td>1.47</td>
</tr>
<tr>
<td>L3</td>
<td>PSB₃</td>
<td>4.8</td>
<td>1.87</td>
<td>38.96</td>
<td>1.38</td>
</tr>
<tr>
<td>L4</td>
<td>PSB₄</td>
<td>7.9</td>
<td>6.01</td>
<td>76.05</td>
<td>1.76</td>
</tr>
<tr>
<td>L5</td>
<td>PSB₅</td>
<td>4.67</td>
<td>1.67</td>
<td>35.76</td>
<td>1.35</td>
</tr>
<tr>
<td>L6</td>
<td>PSB₆</td>
<td>2.1</td>
<td>0.95</td>
<td>45.23</td>
<td>1.45</td>
</tr>
<tr>
<td>L7</td>
<td>PSB₇</td>
<td>9.92</td>
<td>7.79</td>
<td>78.52</td>
<td>1.78</td>
</tr>
<tr>
<td>L8</td>
<td>PSB₈</td>
<td>4.33</td>
<td>1.67</td>
<td>38.56</td>
<td>1.38</td>
</tr>
</tbody>
</table>

Table 7. Phosphate solubilization induced by PSB strains in PVK broth (µg ml⁻¹)

<table>
<thead>
<tr>
<th>Strain</th>
<th>6</th>
<th>12</th>
<th>24</th>
<th>36</th>
<th>72</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.3±0.01</td>
<td>0.4±0.08</td>
<td>0.5±0.01</td>
<td>0.5±0.02</td>
<td>0.5±0.02</td>
</tr>
<tr>
<td>PSB₁</td>
<td>0.6±0.01</td>
<td>0.7±0.01</td>
<td>0.4±0.01</td>
<td>0.6±0.0</td>
<td>0.9±0.03</td>
</tr>
<tr>
<td>PSB₂</td>
<td>0.3±0.04</td>
<td>0.3±0.03</td>
<td>0.5±0.01</td>
<td>0.6±0.11</td>
<td>0.5±0.01</td>
</tr>
<tr>
<td>PSB₃</td>
<td>0.3±0.01</td>
<td>0.3±0.3</td>
<td>0.5±0.01</td>
<td>0.5±0.05</td>
<td>0.5±0.02</td>
</tr>
<tr>
<td>PSB₄</td>
<td>0.5±0.01</td>
<td>0.5±0.03</td>
<td>0.7±0.01</td>
<td>0.7±0.1</td>
<td>1.3±0.12</td>
</tr>
<tr>
<td>PSB₅</td>
<td>1±0.02</td>
<td>0.3±0.03</td>
<td>0.6±0.02</td>
<td>0.6±0.1</td>
<td>0.3±0.02</td>
</tr>
<tr>
<td>PSB₆</td>
<td>1±0.02</td>
<td>0.3±0.03</td>
<td>0.6±0.02</td>
<td>0.6±0.1</td>
<td>0.3±0.02</td>
</tr>
<tr>
<td>PSB₇</td>
<td>0.4±0.03</td>
<td>0.6±0.01</td>
<td>0.8±0.06</td>
<td>0.9±0.01</td>
<td>1.0±0.04</td>
</tr>
<tr>
<td>PSB₈</td>
<td>0.3±0.01</td>
<td>0.3±0.04</td>
<td>0.5±0.02</td>
<td>0.5±0.02</td>
<td>0.5±0</td>
</tr>
<tr>
<td>L.S.D(P=0.05)</td>
<td>0.1</td>
<td>0.2</td>
<td>0.1</td>
<td>0.2</td>
<td>0.1</td>
</tr>
</tbody>
</table>
Fig. 3. P solubilization induced by PSB strains in PVK broth

4.4.1. Effect of pH and Temperature

All the isolated PSB strains lowered the pH of the Pikovskaya broth significantly in comparison to control. Fluctuations in pH changes were noted. A drop of pH from 6.47 (Control) to 3.63 (PSB₁) was observed on studying the pH. The first reading was taken after 6 hrs. Subsequently the further reading was taken after 12, 24, 36 and 72 hrs. The pH variation is significantly different in PSB₁, PSB₄ and PSB₇ in comparison to control. Even they are significantly different in pH alterations from the strains PSB₃, PSB₅ and PSB₈.

Strain PSB₁ showed the pH changes from 3.63 to 6.53 which is significant as compared to control. Strains PSB₂ and PSB₃ showed the pH drop from 4.23 to 6.7 and 4.2 to 6.07 respectively significantly compared to control. Strains PSB₄ and PSB₅ displays the pH changes from 4 to 6.5 and 3.73 to 6.73 respectively which is also significant as compared to control. The alterations in pH brought about by the strains PSB₆, PSB₇ and PSB₈ were 3.57 to 6.5, 4.23 to 6.17, and 4.33 to 6.47 which are also significant as compared to control (Table 8 and Fig. 4). However, post 36 hrs pH changes are buffered automatically.
Table 8. Alterations of pH in PVK broth exhibited by different PSB strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Time (Hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Control</td>
<td>6.5±0.1</td>
</tr>
<tr>
<td>PSB1</td>
<td>5.5±0.1</td>
</tr>
<tr>
<td>PSB2</td>
<td>6.1±0.1</td>
</tr>
<tr>
<td>PSB3</td>
<td>5.5±0.1</td>
</tr>
<tr>
<td>PSB4</td>
<td>6.5±0.1</td>
</tr>
<tr>
<td>PSB5</td>
<td>5.7±0.1</td>
</tr>
<tr>
<td>PSB6</td>
<td>5±0.1</td>
</tr>
<tr>
<td>PSB7</td>
<td>6.2±0.6</td>
</tr>
<tr>
<td>PSB8</td>
<td>6.5±0.1</td>
</tr>
<tr>
<td>L.S.D(P=0.05)</td>
<td>0.34</td>
</tr>
</tbody>
</table>

Fig.4. Alterations of pH in PVK broth exhibited by different PSB strains
In order to evaluate the capacity of the efficient strains to survive under different temperatures viz. 50°C, 150°C, 250°C, 350°C, 400°C and 500°C. The preferred temperature for the isolated efficient strains was found to be ranging from 20°C to 35°C. The isolated strains showed poor to moderate growth above or below these temperatures. The selected PSBs grows well at temperature 27°C to 35°C (Table 9).

Table 9. Temperature tolerance of efficient PSB strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>5°C</th>
<th>15°C</th>
<th>25°C</th>
<th>35°C</th>
<th>40°C</th>
<th>50°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSB₁</td>
<td>*</td>
<td>*</td>
<td>***</td>
<td>***</td>
<td>**</td>
<td>-</td>
</tr>
<tr>
<td>PSB₄</td>
<td>*</td>
<td>*</td>
<td>***</td>
<td>***</td>
<td>**</td>
<td>-</td>
</tr>
<tr>
<td>PSB₇</td>
<td>*</td>
<td>*</td>
<td>***</td>
<td>***</td>
<td>**</td>
<td>-</td>
</tr>
</tbody>
</table>

- → No growth  
* → Poor growth  
** → Moderate growth  
*** → Best growth

4.5. Morphological and Biochemical Characteristics of the isolates

PSB₁, PSB₄ and PSB₇ were isolated from the rhizospheric soil samples taken from the R.S. Pura, Kaili Mandi and chatha farms respectively. They were Gram +ve long rods arranged in chains and were having the yellowish colony morphology (Plate 2) except the PSB₂ which were observed in the form of groups of 2 and 4 (Plate 2) with creamy white morphology. The colony form of the PSB₁ and PSB₄ was irregular whereas the PSB₇ was observed as circular. All the three isolates were medium in size and were pigmented with none of the culture being motile. The configuration of the PSB₁ and PSB₇ was heave whereas the configuration of the PSB₄ was comprehensive. The isolates had also the glossy lusture with sleek topography. The optical characteristics of the PSB₁ were opaque whereas the optical characteristics of the PSB₄ and PSB₇ were translucent (Table 10).
Plate 2. Gram positive rods of the three efficient PSB cultures
<table>
<thead>
<tr>
<th>Strain</th>
<th>PSB₁</th>
<th>PSB₄</th>
<th>PSB₇</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram nature</td>
<td>Gram positive long rods</td>
<td>Gram positive long rods</td>
<td>Gram positive long rods</td>
</tr>
<tr>
<td>Cell arrangement</td>
<td>Chains</td>
<td>Groups of 2 and 4</td>
<td>Chains</td>
</tr>
<tr>
<td>Color of the colony</td>
<td>Yellow</td>
<td>Creamy White</td>
<td>Yellow</td>
</tr>
<tr>
<td>Size</td>
<td>Medium</td>
<td>Medium</td>
<td>Medium</td>
</tr>
<tr>
<td>Form</td>
<td>Irregular</td>
<td>Irregular</td>
<td>Circular</td>
</tr>
<tr>
<td>Pigmentation</td>
<td>Pigmented</td>
<td>Pigmented</td>
<td>Pigmented</td>
</tr>
<tr>
<td>Motility</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>Configuration</td>
<td>Heave</td>
<td>Comprehensive</td>
<td>Heave</td>
</tr>
<tr>
<td>Luster</td>
<td>Glossy</td>
<td>Glossy</td>
<td>Glossy</td>
</tr>
<tr>
<td>Topography</td>
<td>Sleek</td>
<td>Sleek</td>
<td>Sleek</td>
</tr>
<tr>
<td>Optical characteristic</td>
<td>Opaque</td>
<td>Translucent</td>
<td>Translucent</td>
</tr>
</tbody>
</table>

### 4.6. Identification of PSB on the basis of biochemical testing

The three cultures showing efficient phosphate solubilizing activity were subjected to a range of biochemical tests for identification on the basis of an interpretation of biochemical tests from ABIS online website (www.abisonline.com). The detailed results of all the biochemical tests are presented in Table 11 and shown in the Plates 3 and 4. Biochemical results showed citrate utilization was negative for PSB₇ and while other two isolates (PSB₁ and PSB₄) were positive for it. Ornithine, Hydrogen sulphide production, and Arginine utilization tests were negative for all the three isolates while ONPG test was found positive for each of the tested isolates. PSB₁ and PSB₇ showed showed positive results for Voges Proskauer’s test whereas the strain PSB₄ was found to be negative to the Voges Proskauer’s test.
Plate 3. Biochemical tests on KB004 HiStaph™ Identification of PSB isolates

Plate 4. Biochemical tests on KB002 HiStaph™ Identification of PSB isolates
Plate 5. PSB Strains showing oxidase positive

Plate 6. PSB Strains showing catalase positive reactions
Urease activity was observed positive for PSB₁ and PSB₇ while PSB₄ showed the negative reaction. Among sugar fermentation test; glucose, mannitol, and lactose were exclusively fermented by all the three tested cultures while none of the cultures was able to ferment sucrose and maltose. PSB₁ and PSB₇ were found positive for fermentation of arabinose and trehalose. Raffinose sugar was fermented only by PSB₇. All the cultures were found positive for catalase and oxidase activity (Plates.5,6).

**Table 11. Biochemical tests of the efficient bacterial strains**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Biochemical test</th>
<th>PSB₁</th>
<th>PSB₄</th>
<th>PSB₇</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Citrate Utilization</td>
<td>+</td>
<td>+</td>
<td>_</td>
</tr>
<tr>
<td>2.</td>
<td>Ornithine</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>3.</td>
<td>Hydrogen sulphide</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>4.</td>
<td>Voges Proskauer’s</td>
<td>+</td>
<td>_</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>ONPG</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>Urease</td>
<td>+</td>
<td>_</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td>Arginine Utilization</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>8.</td>
<td>Glucose</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9.</td>
<td>Mannitol</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10.</td>
<td>Sucrose</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>11.</td>
<td>Lactose</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>12.</td>
<td>Arabinose</td>
<td>+</td>
<td>_</td>
<td>+</td>
</tr>
<tr>
<td>13.</td>
<td>Raffinose</td>
<td>_</td>
<td>_</td>
<td>+</td>
</tr>
<tr>
<td>14.</td>
<td>Trehalose</td>
<td>+</td>
<td>_</td>
<td>+</td>
</tr>
<tr>
<td>15.</td>
<td>Maltose</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>16.</td>
<td>Catalase</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>17.</td>
<td>Oxidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

The results of biochemical tests for PSB₁ when subjected to ABIS software showed 79% of similarity matches with *Bacillus pumilus*. For PSB₄ culture, results of biochemical tests showed similarity match upto 84% with *Lysinibacillus sphaericus*. Similarly, in case of PSB₇, a similarity of 76% was found with *Bacillus subtilis*. 
4.6.1. Utilization of the Carbon sources

All the three isolates were tested for the utilization of different carbohydrates as their carbon sources and it was observed that all the three isolates utilized glucose, Mannitol and lactose as their carbon sources while none of the isolate could ferment sucrose and maltose. Arabinose and trehalose was utilized by PSB$_1$ and PSB$_7$. In addition, Raffinose was fermented only by PSB$_7$ (Table 11).

4.7. Antagonistic effect of PSB strains

The isolated PSB from the different niches of Jammu were tried to restrict the mycelium growth of *Rhizoctonia solani* *in vitro* studies using dual culture technique on PDA plates. Culture plates showed that isolates PSB could not inhibit the fungal growth (Plate 7). The incubation study was taken through one week to observe the growth pattern, when no inhibition effect was displayed by the PSB isolates and experiment was stopped. In order to minimise error experiment was repeated for another week but the result remained unaltered.

4.8. Genotypic Confirmation

For confirmation of the biochemical tests at 4.5, 4.6 and 4.6.1 the Universal primers were used to amplify the whole region of *16S rDNA* and the primers successfully amplified the region in all the three isolates which is shown in the Plate 8 the amplified gene product was around 1500 bp in size and was gel eluted for nucleotide sequencing. The sequences of *16S rDNA* of all the three tested cultures are given in fasta format (Plate 9) of sequencing and data analysis.

The sequences of *16S rDNA* from PSB$_1$, PSB$_4$ and PSB$_7$ culture were subjected to BLAST at http://blast.ncbi.nlm.nih.gov/Blast.cgi, the results showed 99% similarity match of sequence with *Bacillus pumilis* for culture PSB$_1$ and PSB$_4$ and *Bacillus atrophaeus* for culture PSB$_7$. The phylogenetic tree showing relationship of PSB isolates with closely resembling bacteria is shown in the Plates 10, 11 and 12.
Plate 7. Effect of PSB strains on the inhibition of mycellium growth of the *R. solani*
Plate 8. PCR amplified 16S rRNA gene product using Universal 16S rRNA Primers

PSB₁
ccatcgacaccactgtcaacctgtcccccgaagggaaagccctctctcggtggattgctagaggtgtcagacctggttaaggcttcgcgtttgcttcgaatttaaaacacatgcctccaccgctcttgggcccgcctcaatttctggtttcagtctctgcagacgtactcaccagggagagttcttaat

PSB₂
gacaaactcgacaccactgtcaacctgtccccgaagggaaagccctctctcggtggattgctagaggtgtcagacctggttaaggcttcgcgtttgcttcgaatttaaaacacatgcctccaccgctcttgggcccgcctcaatttctggtttcagtctctgcagacgtactcaccagggagagttcttaat

PSB₃
aggtcgacaccactgtcaacctgtccccgaagggaaagccctctctcggtggattgctagaggtgtcagacctggttaaggcttcgcgtttgcttcgaatttaaaacacatgcctccaccgctcttgggcccgcctcaatttctggtttcagtctctgcagacgtactcaccagggagagttcttaat

Plate 9. 16S rRNA gene sequence of three PSB isolates
Plate 10. Dendrogram by neighbor joining method of 16S rRNA sequence of PSB₁ with known bacterial cultures from database in NCBI
Plate 11. Dendrogram by neighbor joining method of 16S rRNA sequence of PSB\textsubscript{4} with known bacterial cultures from database in NCBI
Plate 12. Dendrogram by neighbor joining method of 16S rRNA sequence of PSB7 with known bacterial cultures from database in NCBI
colorimetric method by measuring the absorbance at 420 nm (Tabatabai and Bremner, 1969). All the isolates except PSB2 and PSB8 displayed an increased phosphatase activity as compared to control. Maximum phosphatase activity was shown by the strains PSB1, PSB7 and PSB4 respectively while the least phosphatase activity was shown by PSB2 (Table 12 and Fig. 5).

The phosphate enzyme activity was significantly higher in PSB1, PSB4 and PSB7 and also varied significantly with in the different isolates from the different niche areas, Standard deviation Table (12) indicates that replications of each isolate showed almost similar pattern and quantum of enzyme activity. P solubilization activity also showed significant impact on releasing the enzymes at different period of time.

**Table 12. Phosphatase activity of the isolated PSB strains (µg PNPP ml\(^{-1}\)h\(^{-1}\))**

<table>
<thead>
<tr>
<th>Strain</th>
<th>6</th>
<th>12</th>
<th>24</th>
<th>36</th>
<th>72</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14.7±0.2</td>
<td>16.1±0.4</td>
<td>18.4±0.3</td>
<td>18.2±0.8</td>
<td>15.8±0.0</td>
</tr>
<tr>
<td>PSB1</td>
<td>30.5±0.6</td>
<td>32.4±0.3</td>
<td>34.2±0.3</td>
<td>36.1±0.1</td>
<td>24.6±0.5</td>
</tr>
<tr>
<td>PSB2</td>
<td>12.3±0.3</td>
<td>12.2±0.3</td>
<td>12.9±0.3</td>
<td>13±0.2</td>
<td>10.3±0.2</td>
</tr>
<tr>
<td>PSB3</td>
<td>13.2±0.0</td>
<td>15.4±0.0</td>
<td>18±0.2</td>
<td>19.3±0.1</td>
<td>15.4±0.4</td>
</tr>
<tr>
<td>PSB4</td>
<td>31.5±0.5</td>
<td>32.5±0.0</td>
<td>33±0.1</td>
<td>28.5±0.1</td>
<td>26.4±0.1</td>
</tr>
<tr>
<td>PSB5</td>
<td>12.6±0.1</td>
<td>15±0.8</td>
<td>18±0.2</td>
<td>21.2±2</td>
<td>18.9±1.9</td>
</tr>
<tr>
<td>PSB6</td>
<td>20.3±0.1</td>
<td>20.8±0.0</td>
<td>20.6±0.0</td>
<td>18.8±0.1</td>
<td>16.8±0.1</td>
</tr>
<tr>
<td>PSB7</td>
<td>36.2±0.0</td>
<td>37±0.2</td>
<td>36.8±0.2</td>
<td>24.6±0.1</td>
<td>22.4±0.1</td>
</tr>
<tr>
<td>PSB8</td>
<td>14.6±0.0</td>
<td>15.5±0.1</td>
<td>17.5±0.6</td>
<td>19.4±0.9</td>
<td>12.4±0.1</td>
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<tr>
<td>L.S.D(P=0.05)</td>
<td>1.5</td>
<td>0.6</td>
<td>1.4</td>
<td>1.3</td>
<td>1.4</td>
</tr>
</tbody>
</table>
Fig. 5. Phosphatase activity of the isolated PSB strains (µg PNPP ml⁻¹ h⁻¹)

4.10. Determination of phytase enzyme activity

The phytase activity of the various isolates was determined by using a modification of the method of Fiske and Subbarow. (1925). The phytase enzyme activity shown by the PSB strains was significantly more than the control (Uninoculated sterilized PVK broth). Most of the strains displayed phytase activity during the initial stages of incubation, but on subsequent incubation a reduction in Phytase activity was observed. Maximum Phytase activity was shown in PSB₁, PSB₄ and PSB₇ and the least activity was observed in PSB₂ (Table 13 and Fig. 6). Though the phytase activity showed by the different strains as shown in Table 14 and Fig.6 showed no significant effect as compared to control at LSD 0.05 level.
Table 13. Phytase activity of the isolated PSB strains (µg P ml⁻¹ h⁻¹)

<table>
<thead>
<tr>
<th>Strain</th>
<th>6</th>
<th>12</th>
<th>24</th>
<th>36</th>
<th>72</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
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<tr>
<td>PSB₁</td>
<td>10.6±0.3</td>
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<td>10.2±0.4</td>
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<tr>
<td>PSB₂</td>
<td>3.4±0.5</td>
<td>3.4±0.7</td>
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<tr>
<td>PSB₃</td>
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<td>PSB₄</td>
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<td>9.4±0.6</td>
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<td>PSB₅</td>
<td>4.8±0.2</td>
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<td>6.4±0.5</td>
<td>6.8±0.2</td>
<td>6.9±0.2</td>
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<tr>
<td>PSB₆</td>
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<td>6.4±0.3</td>
<td>7.8±0.9</td>
<td>8.4±0.5</td>
<td>8.6±0.5</td>
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<tr>
<td>PSB₇</td>
<td>9.2±0.4</td>
<td>9.2±0.5</td>
<td>8.2±0.5</td>
<td>8.2±0.5</td>
<td>8.4±0.5</td>
</tr>
<tr>
<td>PSB₈</td>
<td>4.6±0.4</td>
<td>3.7±0.4</td>
<td>3.8±0.2</td>
<td>4.5±0.4</td>
<td>4.6±0.3</td>
</tr>
<tr>
<td>L.S.D(P=0.05)</td>
<td>N.S</td>
<td>N.S</td>
<td>N.S</td>
<td>N.S</td>
<td>N.S</td>
</tr>
</tbody>
</table>

Fig. 6. Phytase activity of the isolated PSB strains

4.11. Bioassay

Pot experiment

Pot experiment was carried out by evaluating three strains of PSBs. Maize variety CM-135 was grown in pots for the period of 30 days. Treatment consisted of 3 strains of PSB isolated from the rhizosphere and total replications were maintained
five. Control was also maintained by growing uninoculated maize grain. Moisture of the soil was maintained throughout the growing season by compensating pots with the sterile water. Total dry matter yield and Phosphorous content were determined after 30 days. The total dry matter yield and available phosphorous were significantly improved by all the three strains as compared to control (Table 14, Fig. 7 and Plate 14, 15).

Strain PSB₁ increased the dry matter yield as compared to control to the tune of 17.41 % whereas strain PSB₄ and PSB₇ increased the dry matter yield up to 17.03 % and 8.97 % respectively as compared to control.

The results indicate that efficient PSB isolated from different niches in lab has shown the effective results in the pot experiment trials.

**Table 14. Dry matter yield (g plant⁻¹) and available P content (%)**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Dry matter Yield (g plant⁻¹)</th>
<th>P content %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.168</td>
<td>0.3</td>
</tr>
<tr>
<td>PSB₁</td>
<td>4.894</td>
<td>0.8</td>
</tr>
<tr>
<td>PSB₄</td>
<td>4.878</td>
<td>0.62</td>
</tr>
<tr>
<td>PSB₇</td>
<td>4.542</td>
<td>0.4</td>
</tr>
<tr>
<td>L.S.D (P=0.05)</td>
<td>0.518</td>
<td>0.191</td>
</tr>
</tbody>
</table>
Fig. 7. Dry matter yield (g plant⁻¹) and P uptake (%) obtained by the inoculation of isolated PSB strains in maize

Phosphorous uptake was measured by using diacid method and quantitative analysis was done spectrophotometrically. P uptake using different effective inoculants have shown the availability of solubilized P in the rhizosphere and its availability to plants.

Inoculants of PSB strains in the maize (CM-135) has mobilized P in the rhizosphere, facilitates its availability to plants. Uptake of P indicated by the P content of plants compared to control was 62.5 %, 51.61 % and 25 %.
Plate 14. Effect of inoculated PSB strains on dry matter yield of *Zea mays*

Plate 15. Dry matter yield of *Zea mays* (CM-135)
DISCUSSION
CHAPTER-V

DISCUSSION

The soils of Jammu are unexplored for the microbial diversity; no specific work has been done for the isolation of phosphate solubilizing bacteria in soils of Jammu except quantitative analysis. Keeping in view, the need to explore soils of Jammu for their potential to harbor phosphate-solubilizing bacteria, an investigation was carried out with the objectives to explore rhizospheres for isolation of phosphate solubilizing bacteria and evaluate the efficient isolated phosphate solubilizers in maize (Zea mays).

5.1. Enumeration of total rhizospheric bacterial and PSB population

Density of total culturable bacteria assessed from the different rhizospheres inferred that varied from $6.13 \times 10^6$ to $8.93 \times 10^6$ cfu g$^{-1}$ soil in different locations: L1, L4, L7 and L8, whereas the population of PSB was higher in rhizosphere soil samples obtained from the locations L1, L4, L7, and L2. The total culturable bacteria using serial dilution technique varied between $1 \times 10^6$ to $8.93 \times 10^6$ cfu g$^{-1}$ from location L6 and L4 respectively (Table 4) and the total PSB population level varied between $0.67 \times 10^8$ to $8.86 \times 10^8$ cfu g$^{-1}$ from the locations L5 and L1 respectively (Table 5). The probable reason for higher counts of PSBs from location L1 could be the nature of the soil and higher fixation of fertilizer P in the area. The total culturable bacteria seems not to have taken care of PSB since the isolation of these bacteria needs enrichment technique using a culture specific media.

5.2. Phosphorous solubilization and enzyme activity

The isolated phosphate solubilizing bacterial strains showed the clearing zones from 0.95 mm to 7.79 mm whereas the phosphorus solubilizing efficiency determined was between 38.56 % - 78.52 % (Table 6). The results were nearly similar to that of Panhwar et al., (2014) who found the range of PSB solubilization efficiency in the range of 23.23% to 78.52%. In vitro phosphate solubilization efficiency was maximum for the Bacillus pumilus (PSB$_1$) isolate which was reflected in the pot experiment of the culture with the maize plants.

The comparison of the phosphate solubilizing efficiency of the three efficient PSB isolates with the acid phosphate activity were found to have positive correlation
(r= 0.886) to each other as highest PSB isolate had highest acid phosphatase activity. The PSE was also correlated with Phytase enzyme and the relation was found positive (r=0.749).

Similar findings have been made by Kaur and Reddy, (2013); De Freitas et al., (1997) who also found the high efficiency in phosphate solubilization due to the acid phosphate activity. Regression equation was also worked out amongst the enzymes phosphatase and phytase P solubilization. Linear equation worked out as \( y = 0.411x + 0.002 \) (\( r^2=0.786 \)) and \( y= 0.057x+2.577 \) (\( r^2 = 0.103 \)). Though the phytase activity showed by the different strains has no significant effect as compared to control at LSD 0.05 which is contradicted to Panhwar et al., 2012 and Kaur and Reddy, (2013).

The P-solubilizing activity is determined by the microbial biochemical ability to produce and release organic acids, which through their carboxylic groups chelate the cations (mainly Ca) bound to phosphate converting them into the soluble forms (Kpomblekou and Tabatabai, 1994). From the results of this study, it is being reaffirmed that the phosphate solubilization by different PSBs is involved with the production of organic acids (Halder et al., 1990; Goldstein, 1995; Kim et al., 1998; Rashid et al., 2004). The inverse relationship observed between the pH and Soluble-P concentration indicates that organic acid production by these PSB strains plays a significant role in the acidification of the medium facilitating the P solubilization. Regression equation was worked out between the changes in pH and the phosphate solubilization which deduced as \( y=0.268x -0.975 \) (\( r^2=0.049 \)). The present results are also in agreement with other researchers (Yu et al., 2011; Walpola and Yoon, 2013), who reported similar negative relationships. Upon reaching the maximum solubilization, two cases were noticed; the first phenomenon was the reduction of the concentration of the solubilized phosphorus in the medium which could be explained by the fact that these strains used this solubilized phosphorus as a nutrient for their growth. For the second case, a stabilization of the maximum concentration of the solubilized phosphorus was observed; in this case we cannot conclude if these strains used or not used the solubilized phosphorus since we had stopped the study at the fourth day (Table 7).

It was noticed that the concentration of the solubilized phosphorus differed from one strain to another (Table 7) and this result was in accordance to those of
Andrade et al., (2014) and Rfaki et al., (2014) and no correlation was demonstrated between the index of solubilization and the concentration. Hence, it needs further studies to understand the characteristics and mechanisms of phosphate solubilization by PSB.

In this study the isolated PSB strains were able to produce the phosphatase and phytase enzymes which are considered to have a significant impact on the P solubilization and in addition to plant growth (Glick, 2005). But in present study phytase showed no significant effect (Table 13). The production of these two enzymes were also reported by Ponmurugan and Gopi, (2006); Sharma et al., (2011).

From this present study, the solubilization is only based characteristic feature of PSB such P solubilization zone production in the solid medium, pH change of the liquid medium and phosphatase activity, organic acids production and ability to release phosphorous.

5.3. Utilization of the Carbon sources

The PSB strains utilized different types of carbohydrates compounds as carbon source. The utilization of different types of carbons sources varied from strain to strain. All the three PSB strains preferred glucose, mannitol and lactose as carbon source and arabinose and trehalose was utilized by two PSB strains and one strain was only able to utilize raffinose. None of the PSB strains could utilize sucrose and maltose (Table 11). These variations are mainly due to the nature of strains and differences in the species of respective genus as has been reported by Baliah and Begum, (2015). Susilowati and Syekhfani, (2014).

5.4. Phenotypic confirmation

The efficient phosphate solubilizing bacteria were subjected to the morphological, identification and biochemical analysis on the basis of an interpretation of biochemical tests from ABIS online website.

The results of biochemical tests when subjected to ABIS software showed 79%, 84% and 76% similarity match with Bacillus pumilus, Lysinibacillus sphaericus and Bacillus subtilis for PSB₁, PSB₄ and PSB₇ respectively. This finding is also supported by the earlier reports that say that the most efficient and frequently
encountered PSBs belong to the genus *Bacillus* (Elliott *et al*., 1987; Venkateswaran and Natarajan, 1983).

5.5. Genotypic Confirmation

Among the eight phosphate solubilizing isolates, three efficient PSB were selected for further studies on the basis of formation of clear zones around the colonies on PVK agar plates. According to 16S rDNA sequence analysis (Plate 9), two strains were identified as *Bacillus pumilus* (PSB1 and PSB3) and one as *Bacillus atrophaeus* (PSB7) (Plate 10, 11, 12). *Bacillus pumilus* is a Gram-positive, aerobic spore forming bacillus commonly found in soil. The bacterium has been reported as an active ingredient in agricultural fungicides. Several reports have described the growth promoting effect of *Bacillus* species including *B. pumilus* either due to enhance in the production of phytohormones or increased phosphate solubilisation activity (Lee *et al*., 2102; Wang *et al*., 2014. *Bacillus atrophaeus* is a Gram-positive rod shaped bacterium commonly isolated from soil. It has been reported by several authors to have Phosphate solubilisation activity (Khan *et al*., 2007; Dhankar *et al*., 2013).

Thus, the study shows the potential of the isolated PSBs in growth promotion of plant crops which can serve as a revolutionary cheap biofertiliser aiding in the growth promotion and increase the yields of the various crops.

5.6. Pot experiment

Pot experiment was carried out by evaluating three strains of PSBs. Maize variety CM-135 was grown in pots for the period of 30 days. The total dry matter yield and available phosphorous were significantly improved by all the three strains as compared to control (Fig 7, Plate 15).

The results of the pot experiment showed an efficient increase in the dry matter yield compared to the control. The results obtained could be associated with cell elongation and multiplication induced by greater absorption of nutrients, particularly phosphorous. In addition, there are some similar reports on enhanced dry matter content of maize due to inoculation of PSB. The results were in agreement to Hameeda *et al*., (2006) and Pandey *et al*., (2006) who demonstrated the increase in the plant biomass and grain yield in maize plants using different PSB.
SUMMARY AND CONCLUSIONS
Phosphorus (P) is biologically important and an integral component of every organism, from bacteria and plankton to organisms of higher trophic levels. Phosphorus is the second most essential macronutrient after nitrogen needed by the plants. The availability of P in soil is generally low as it is generally fixed to inorganic phosphates as iron, aluminum and calcium. Phosphate-solubilizing bacteria (PSB) assume a critical part in minimizing the phosphorus deficiency in soil by fixing the insoluble forms of phosphate to soluble forms and therefore make P available for plants.

Sampling was done from R.S. Pura, Burj Tanda, Chakrohi, Kaili Mandi, Gurhaslathia, Chatha farm-I, Chatha farm-II and Rakh Dhiansar as per the map. Physico chemical properties of the rhizosphere soil samples were analysed and was observed that the pH of various soils samples varied between 6.68 and 7.75. The organic carbon, available nitrogen and available phosphorous were 0.41- .53 %, 112- 171 Kg ha\(^{-1}\) and 12-14 Kg ha\(^{-1}\) respectively. Soils are low to medium in P. Keeping in view the inorganic phosphorous added to these soils, there seems to be fixation of the phosphorous.

Primarily total bacterial counts were obtained by spread plate method for the period of 15 days at 30±1\(^{0}\)C. The log cfu varied between 1.7×10\(^6\) - 5.8×10\(^6\) cfu g\(^{-1}\) in the locations R.S. Pura (L1), Burj Tanda (L2), Chakrohi (L3), Kaili mandi (L4) and between 1.76×10\(^6\) - 4.76×10\(^6\) cfu g\(^{-1}\) from the locations Chatha farm-I (L5), Chatha farm-II (L6), Gurha slathian (L7) and Rakh Dhiansar (L8) whereas the peak values of bacterial population was observed on 6\(^{th}\) day in most of the isolates to assess the presence of bacterial loads in different sites indicating low bacterial presence in these areas.

The total PSB count obtained by spread plate method was found to be between 2.67×10\(^8\) - 6.87×10\(^8\) cfu g\(^{-1}\) from the rhizosphere soil samples collected from L1 whereas the total PSB count obtained from locations L2 - L8 varied between 0.67×10\(^8\)- 4.90×10\(^8\) cfu g\(^{-1}\). The total PSB population from all the soil samples was found to be more on the 7\(^{th}\) day indicating the presence of indigenous strains of PSB in local
niche which can be utilized for improving the yields as has been improved in the present study

Isolated phosphate solubilisers depicted the clearing zones that varied from 0.95 mm to 7.79 mm with the concomitant the phosphorus solubilizing efficiency between 38.56 % - 78.52 %.

All the PSB strains showed a significant decrease in the pH as compared to the control during the initial stages of incubation and on further incubation pH changes were buffered.

Preferred temperature for the isolated PSB strains were found to be between 20°C to 35°C. A decrease in bacterial growth was observed below or above this temperature.

Three efficient phosphate solubilizing bacteria among the eight isolated PSB strains were further confirmed by biochemical testing and morphological observations confirmed the presence of effective bacterial species in solubilizing phosphorous as *Bacillus pumilus, Lysinibacillus sphaericus*, and *Bacillus subtilis*.

The nomenclature of the bacterial species was ascertained at the DNA level using the Universal primers to amplify the whole region of *16S rDNA*. The sequences obtained confirmed the presence of *Bacillus pumilus, and Bacillus atrophaeus*.

All the isolated and confirmed PSB strains are able to display phosphatase and phytatase activity and which were correlated with the P solubilizing activity confirmed by linear regression equation.

These identified isolates blended in a sterile carrier maintaining the population from $10^{-9}$ to $10^{10}$ were evaluated for their efficiency in promoting dry matter yield of a Maize (*Zea mays*) variety CM-135 in the pot experiment.

All the three tested strains tend to enhance the growth of *Zea mays*. The strains also improved the uptake of phosphorous by maize plants and the available phosphorous content in the soil compared to the control.

Future endeavours should be directed to identify, screen and characterize PSB from more niches in Jammu region for their ultimate application under field conditions. So that, the successful implementation in use of PSB to mobilise soil fixed
P that can be an alternative sustainable strategy for management of soil to optimize P bioavailability.
REFERENCES
REFERENCES


Audenaert, K., Pattery, T., Cornelis, P. and Höfte, M. 2002. Induction of systemic


APPENDIX
# APPENDIX

## BACTERIOLOGICAL MEDIA

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VITA
VITA

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EDUCATIONAL QUALIFICATIONS

Bachelor’s degree : B.Sc.
Master’s degree : M.Sc Microbiology
University and year of award : Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu-180009, 2016
OGPA : 7.56
Title of Master’s Thesis : Isolation, Characterization and Efficacy of Phosphate Solubilising Bacteria