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Isolation and Characterization of Phosphate Solubilizing Bacteria from Tomato (*Solanum l.*) Rhizosphere and Their Effect on Growth and Phosphorus uptake of the Host Plant under Green House Experiment.

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Summary / abstract of thesis: A total of eleven phosphate solubilizing bacteria were isolated from rhizosphere soil of tomato (*Solanum lycopersicum*) plants. Isolates designated as PSB1, PSB2, PSB4, PSB5 and PSB7 were selected out of eleven isolates based on their clear zone diameters. They were characterized to generic level and found to belong to Pseudomonas spp. Tricalcium phosphate (TCP), rock phosphate (RP) and bone phosphate (BP) solubilizing efficiency of these isolates was studied in the laboratory at 5, 10, and 15 days of incubation. All bacterial isolates solubilized significantly ($P< 0.05$) higher amounts of TCP over uninoculated control. The highest P was release upon 5 days of incubation was recorded from the isolate PSB1 with 7.64 mg/50ml in the medium and the least P release was recorded by isolates PSB4 and PSB5 with a P content of 4.79 mg /. At 10^{th} day of incubation, the highest P release was recorded by isolates PSB2 (8.19 mg/50ml), PSB1 (8.10mg/ 50ml). At 20^{th} days of incubation, the highest P release was recorded by isolate PSB7 (11.77 mg/ 50ml) and PSB2 (11.33 mg/ 50ml) and the least P released was recorded by isolate PSB5 (5.44 mg/ 50ml)). Under RP solubilization, the p released from all isolates upon 5 days of incubation was not significant as compared to the control. At 10^{th} day of incubation, the highest P release was recorded by isolates PSB5 (5.56 mg/50ml), PSB7 (5.02 mg/ 50ml) and the least P release was observed by isolate PSB4 (3.46mg/g). At 20^{th} days of incubation, the highest P release was recorded by isolate PSB7 (7.928 mg/ 50ml) and the least P release was recorded by isolate PSB4 (4.025mg/ 50ml). Regarding BP, The p released from all isolates except isolate PSB7 (3.020mg /50ml) upon 5 days of incubation was not significant as compared to the control. At 10^{th} day of incubation, the highest P release was recorded by isolates PSB5 (7.37 mg/50ml), PSB7 (7.025 mg/ 50ml) and the least P released was observed by isolate PSB2 (5.47 mg/50ml). At 20^{th} days of incubation, the highest P release was recorded by isolate PSB7 (11.09 mg/ 50ml) and the least P release was recorded by isolate PSB4 (9.06 mg/ 50ml). Under greenhouse experiment, root length and shoot length of inoculated plant did not show variation as compared to positive control. In shoot dry weight all inoculated treatments except PSB4+TCP were effective as equal as that of positive control. Upon determination of phosphorus uptake efficiency of the plant, treatments PSB2 (1.17 mg/g), PSB4 (1.09 mg /g) and PSB7 (1.08 mg /g) showed maximum p uptake while isolate PSB1 (0.94 mg/g) showed the least p uptake as compared to the positive control.

Key words: Biofertilizers, clear zone, Phosphorus, solubilization efficiency

INTRODUCTION

Phosphorus is second only to nitrogen in mineral nutrients most commonly limiting the growth of crop. It is an essential element for plant development and growth making up about 0.2% of plant dry weight. It plays an indispensable biochemical role in photosynthesis, respiration, energy storage and transfer, cell division, cell enlargement and several other processes in the living plant. An adequate supply of phosphorus in the early stages of plant growth promotes physiological functions including early root formation, and is important for laying down the primordia for reproductive parts of plants. It is also known to improve quality of many fruits, vegetables and grain crops.

It exists in nature in variety of organic and inorganic forms. P availability is low in soils because of its fixation as insoluble phosphates of iron, aluminum and calcium. Since deficiency of P is the most important chemical factor restricting plant growth, chemical phosphatic fertilizers are widely used to achieve optimum yields. Soluble forms of P fertilizer used are easily precipitated as insoluble forms, this leads to excessive and repeated application of P fertilizer to crop land.

Currently, the main purpose in managing soil phosphorus is to optimize crop production and minimize P loss from soils. Plant growth promoting bacteria (PGPB) are soil and rhizosphere bacteria that can benefit plant growth by different mechanisms (Glick, 1995), and P-solubilization ability of the microorganisms is considered to be one of the most important traits associated with plant P nutrition. Given the negative environmental impacts of chemical fertilizers and their increasing costs, the use of PGPB is advantageous in the sustainable agricultural practices. Recently, phosphate solubilizing microorganisms have attracted the attention of agriculturists as soil inoculums to improve the plant growth and yield (Young, 1994; Young et al., 1998; Goldstein et al., 1999; Fasim et al., 2002).

The main sources for phosphate fertilizers are rock phosphates (Van Kauwenbergh, 1997). They are much less expensive than soluble phosphorus fertilizers. Phosphate rocks generally are apatitic, containing varying percentages of P_2O_5 in a calcium matrix. Mineral forms of phosphorus constitute the biggest reservoirs of phosphorus, represented primarily by rocks and deposits formed during geological age. The principal characteristic of these primary minerals (oxyapatite, hydroxyapatite, and apatite) is their insolubility. A large portion of soluble inorganic phosphate applied to agricultural soil as chemical fertilizer is rapidly immobilized soon after application and becomes unavailable to plants (Dadarwal *et al*., 1997). Less phosphorus is available immediately upon application, but in acid soils with adequate rainfall the dissolution of rock phosphate can maintain recommended levels of P over time (Khasawneh et al., 1980, Bolan et al., 1990).

The production of chemical phosphatic fertilizers is a highly energy-intensive process requiring energy worth US $ 4 billion per annum in order to meet the global need (Goldstein et al., 1993). The situation is further compounded by the fact that almost 75–90% of added phosphatic fertilizer is precipitated by metal cation complexes present in the soils (Stevenson, 1986). Thus, the dependence of fertilizer production on a fossil energy source and the prospects of the diminishing availability of costly input of fertilizer production in years to come have obviously brought the subject of mineral phosphate solubilization to the forefront.

The mechanism of mineral phosphate solubilization by PSB strains is associated with the release of low molecular weight organic acids (Goldstein, 1995; Kim et al., 1997a), which through their hydroxyl and carboxyl groups chelate the cations bound to phosphate, thereby converting it into soluble forms (Kpomblekou and Tabatabai, 1994). However, P-solubilization is a complex phenomenon, which depends on many factors such as nutritional, physiological and growth conditions of the culture (Reyes et al., 1999). Phosphate solubilizing bacteria (PSB), phosphate solubilizing fungi (PSF) and actinomycetes are found to be active in conversion of insoluble phosphate to soluble primary and secondary orthophosphate ions (Chabot *et al.,* 1993; Pal, 1998).

The microbial system can bring appreciable amounts of nutrients from the natural reservoir and enrich the soil with the important but scarce nutrients. The crop microbial ecosystem can thus be energized in sustainable agriculture with considerable ecological stability and environmental quality. The organisms especially with phosphate solubilizing potential increase the availability of soluble phosphate andcan enhance plant growth by increasing the efficiency of biological nitrogen fixation or enhancing the availability of other trace elements such as iron, zinc, etc., and by production of plant growth promoting regulators(Sattar and Gaur, 1987; Kucey et al., 1989; Ponmurugan and Gopi, 2006). Under phosphorus-deficient conditions this association could result either in improved uptake of the available phosphates or rendering unavailable phosphorus sources accessible to the plant.it has been found that *Pseudomonas* spp. enhanced the number of nodules, dry weight of nodules, yield components, grain yield, nutrient availability and uptake in soybean crop (Son *et al.*, 2006).

Phosphate solubilizing bacteria enhanced the seedling length of *Cicerarietinum* (Sharma *et al*., 2007), while co-inoculation of PSM and PGPR reduced P application by 50 % without affecting corn yield (Yazdani *et al*., 2009). Inoculation with PSB increased sugarcane yield by 12.6 percent (Sundara *et al*., 2002).

The essential elements especially the major nutrients (NPK) are considered the most important among nutrients and factors limiting growth and yield of tomato*(Solanum lycopersicum)* plants. Since tomato plant growth is negatively affected by their deficiency, optimum levels of fertilizers are used. However, Efficient and economic use of nutrients would help in decreasing the costs of application and the risk due to the application of high amount of fertilizers (Venkatasalam and Krishnasamy, 2005).

Stimulation of different crops by PGPR has been demonstrated in both laboratory and field trials.It has been found that Strains of *Pseudomonas putida* and *Pseudomonas fluorescen* have increased root and shoot elongation in canola, lettuce, and tomato (Hall, 1996) as well as crop yields in potato, radishes, rice, sugar beet, tomato, lettuce, apple, citrus, beans, ornamental plants, and wheat (Suslov,1982; Lemanceau,1992; Kloepper,1994).

Tomato*(Solanum lycopersicum)* is among the most important vegetable crops in Ethiopia. The total production of this crop in the country has shown a marked increase (Lemma et al., 1992) since it became the most profitable crop providing a higher income to small scale farmers compared to other vegetable crops. However, tomato*(Solanum lycopersicum)* production is highly constrained by several factors especially in developing nations like Ethiopia (FAO Production Year Book, 2004). The national average of tomato*(Solanum lycopersicum)* fruit

yield in Ethiopia is often low (125 q/ha) compared even to the neighboring African countries like Kenya (164 q/ha) (FAO Production Year Book, 2004).

In Ethiopia, farmers get lower yield mainly due to diseases and pests as well as due to sub-optimal fertilization. Mehla et al., (2000) and Pandey et al., (1996) reported that fruit yield in tomato*(Solanum lycopersicum)* is highly influenced by the NP fertilizers rates applied. Similarly, Sherma et al., (1999) also reported average fruit weight of tomato*(Solanum lycopersicum)* has been influenced by the amount of NP fertilizers rates applied. Thus, tomato*(Solanum lycopersicum)* plant should receive optimum amount of NP fertilizers to produce higher fruit yield. As reported by de Groot *et al.*, (2002), the relative growth rate of tomato*(Solanum lycopersicum)* increases sharply with increasing plant P concentration. Results from recent research have indicated that foliar application of phosphorus in greenhouse tomato*(Solanum lycopersicum)* enhances the concentrations of chlorophyll, K, P, Mg and Fe in the leaves, accelerates fruit maturity and increases marketable yield and quality (Chapagain and Wiesman 2004). According to de Groot *et al.* (2001), at mild P limitation the assimilate supply is not the limiting factor for reduced growth rates, but at severe P limitation the rate of photosynthesis is depressed, as indicated by the decrease in starch accumulation. Under conditions of severe P deficiency, the leaf N concentration is also suppressed, due to a decrease in leaf cytokinin levels (de Groot *et al.* 2002).

Although, tomato*(Solanum lycopersicum)* is the second major crop of the world after potato, there is lack of research, particularly under field conditions, to show the effect of phosphate solubilizing bacteria on tomato *(Solanum lycopersicum)* growth. Consequently, this study mainly emphasizes on isolation of phosphate solubilizing bacteria from rhizosphere of tomato*(Solanum lycopersicum)* plant and observing their effect on their growth.

OBJECTIVES OF THE STUDY

2.1. General objective

- To isolate and characterize phosphate solubilizing bacteria from the rhizosphere soils of tomato*(Solanum lycopersicum)* plants and evaluate their efficiency on the growth of tomato *(Solanum lycopersicum)* plants in greenhouse experiment

2.2. Specific objectives

- solation of phosphate solubilizing bacteria from tomato rhizosphere
- Characterization and *in vitro* screening of isolates for their ability to solubilize phosphate
- To assess the influence of selected efficient phosphate solublizing bacteria on growth of tomato*(Solanum lycopersicum)* plants

REVIEW OF LITERATURE

3.1. Phosphorus in soil

Phosphorus (P) is one of the major essential macronutrients for plants and is applied to soil in the form of phosphatic fertilizers. However, a large portion of Soluble inorganic phosphate applied to the soil a schemical fertilizer is immobilized rapidly and becomes unavailable to plants (Goldstein, 1986).

Microorganisms are involved in a range of processes that affect the transformation of soil P and are thus an integral part of the soil P cycle. In particular, soil microorganisms are effective in releasing P from inorganic and organic pools of total soil P through solubilization and mineralization (Hilda and Fraga, 1999). Recently, phosphate solubilizing microorganisms have attracted the attention of agriculturists as soil inoculums to improve the plant growth and yield (Young, 1994; Young et al., 1998; Goldstein et al., 1999; Fasim et al., 2002).

Most agricultural soils contain large reserves of phosphorus, a considerable part of which has accumulated as a consequence of regular applications of P fertilizers (Richardson, 1994). However, a large portion of soluble inorganic phosphate applied to soil as chemical fertilizer is rapidly immobilized soon after application and becomes unavailable to plants (Dey, 1986).

The phenomena of fixation and precipitation of P in soil is generally highly dependent on pH and soil type. Thus, in acid soils, phosphorus is fixed by free oxides and hydroxides of aluminum and iron, while in alkaline soils it is fixed by calcium, causing a low efficiency of soluble P fertilizers, such as super calcium (Goldstein, 1986; Goldstein, 1994 and Jone, 1991). According to Lindsay (1979), superphosphate contains a sufficient amount of calcium to precipitate half of its own P, in the form of dicalcium phosphate or dicalcium phosphate dihydrated.

3.2. Interaction between Microorganisms and soil Rhizosphere

Soil P dynamics is characterized by physicochemical (sorption-desorption) and biological (immobilization-mineralization) processes. Large amount of P applied as fertilizer enters in to the immobile pools through precipitation reaction with highly reactive Al^{3+} and Fe^{3+} in acidic, and Ca^{2+} in calcareous or normal soils (Gyaneshwar *et al.*, 2002; Hao *et al.*, 2002).

Efficiency of P fertilizer throughout the world is around 10 - 25 % (Isherword, 1998), and concentration of bioavailable P in soil is very low reaching the level of 1.0 mg kg–1 soil (Goldstein, 1994). Soil microorganisms play a key role in soil P dynamics and subsequent availability of phosphate to plants (Richardson, 2001). Inorganic forms of P are solubilized by a group of heterotrophic microorganisms excreting organic acids that dissolve phosphatic minerals and/or chelate cationic partners of the P ions i.e. PO_4^{3-} directly, releasing P into solution (He *et al.*, 2002).

Microbial community influences soil fertility through soil processes like decomposition, mineralization, and storage / release of nutrients. Microorganisms enhance the P availability to plants by mineralizing organic P in soil and by solubilizing precipitated phosphates (Chen *et al.*, 2006; Kang *et al.*, 2002; Pradhan and Sukla, 2005). These bacteria in the presence of labile carbon serve as a sink for P by rapidly immobilizing it even in low P soils (Bünemann *et al.*, 2004). Subsequently, PSB become a source of P to plants upon its release from their cells. The PSB and plant growth promoting rhizobacteria (PGPR) together could reduce P fertilizer application by 50 % without any significant reduction of crop yield (Jilani *et al.*, 2007; Yazdani

et al., 2009). It infers that PSB inoculants / biofertilizers hold great prospects for sustaining crop production with optimized P fertilization.

The complexity of the soil system is determined by the numerous and diverse interactions among its physical, chemical, and biological components, as modulated by the prevalent environmental conditions (Buscot, 2005). In particular, the varied genetic and functional activities of the extensive microbial populations have a critical impact on soil functions, based on the fact that microorganisms are driving forces for fundamental metabolic processes involving specific enzyme activities (Nannipieri et al., 2003). Many microbial interactions, which are regulated by specific molecules/signals (Pace, 1997), are responsible for key environmental processes, such as the biogeochemical cycling of nutrients and matter and the maintenance of plant health and soil quality (Barea et al., 2004).

The different physical, chemical, and biological properties of the root-associated soil, compared with those of the root-free bulk soil, are responsible for changes in microbial diversity and for increased numbers and activity of micro-organisms in the rhizosphere micro-environment (Kennedy, 1998). Carbon fluxes are crucial determinants of rhizosphere function (Toal et al., 2000). The release of root exudates and decaying plant material provide sources of carbon compounds for the heterotrophic soil biota as growth substrates, structural material or signals for the root associated micro biota (Werner, 1998).

Factors underlying the differential capacities of plant genotypes to access soil nutrients include differences in the surface area of contact between roots and soil (e.g. Sadana *et al*., 2002) and in the composition and amount of root exudates (Rengel, 2002; Jones *et al*., 2004) and rhizosphere micro flora (Marschner *et al*., 2005b), resulting in differences in the chemistry and biology of the rhizosphere. The availability of nutrients in the rhizosphere is controlled by the combined effects of soil properties, plant characteristics, and the interaction of roots with microorganisms (Jones et *al*., 2004). The concentration of nutrients and their availability to plants differ between the rhizosphere and the bulk soil (e.g. Marschner *et al*. 2003).

Many studies have demonstrated that soil-borne microbes interact with plant roots and soil constituents at the root–soil interface (Lynch, 1990; Linderman, 1992; Glick, 1995; Kennedy, 1998; Bowen and Rovira, 1999; Barea et al., 2002b). The great array of root–microbe interactions results in the development of a dynamic environment known as the rhizosphere where microbial communities also interact. Microbial activity in the rhizosphere affects rooting patterns and the supply of available nutrients to plants, thereby modifying the quality and quantity of root exudates (Bowen and Rovira, 1999; Grayndler, 2000; Barea, 2000).

3.3. Biofertilizers

Phosphate solubilizing bacteria (PSB) are being used as biofertilizer since 1950s (Kudashev, 1956; Krasilinikov, 1957). Release of P by PSB from insoluble and fixed forms is an import aspect regarding P availability in soils. Biofertilizers are products containing living cells of different types of microorganisms which when applied to seed, plant surface or soil, colonize the rhizosphere or the interior of the plant and promotes growth by converting nutritionally important elements (nitrogen, phosphorus) from unavailable to available form through biological process such as nitrogen fixation and solubilization of rock phosphates (Rokhza et al., 1995).

Beneficial microorganisms in biofertilizers accelerate and improve plant growth and protect plants from pests and diseases (El-yazied et al., 2007). Use of PSMs can increase crop yields up to 70 percent (Verma, 1993). Integration of half dose of NP fertilizer with biofertilizer gives crop yield as with full rate of fertilizer; and through reduced use of fertilizers the production cost is minimized and the net return maximized (Jilani *et al*., 2007). Combined inoculation of arbuscular mycorrhiza and PSB give better uptake of both native P from the soil and P coming from the phosphatic rock (Goenadi *et al.*, 2000; Cabello *et al.*, 2005).

Organisms that are commonly used as biofertilizer component are nitrogen fixers, potassium solubilizers and phosphorus solubilizers, or with the combination of molds and fungi (Lee and Pankhurst, 1992; Wani et al., 1995). Studies have shown that these microorganisms are present in the soil in different numbers (Katznelson et al. 1962; Khan and Bhatnagar 1917; Louw and Webley, 1959), and that a large proportion of the bacterial phosphate-solubilizing (PS) population is found in the rhizosphere of plants (Sperber 1958a). However, the PS bacteria, when viewed as a percentage of the total soil microbial population, were not found to constitute a significantly larger proportion of the rhizosphere microbial population (Sperber 1958a; Katznelson and Bose, 1959).

Strains of *Pseudomonas putida* and *Pseudomonas fluorescens* was found to increase root and shoot elongation in canola, lettuce, and tomato (Hall , 1996, and Glick,1997) as well as crop yields in potato, radishes, rice, sugar beet, tomato, lettuce, apple, citrus, beans, ornamental plants, and wheat **(**Suslov**,** 1982, Lemanceau**,** 1992, Kloepper **,** 1994**)** . According to Kloepper (1989) Wheat yield increased up to 30% with *Azotobacter* inoculation and up to 43% with *Bacillus* inoculants, and a 10–20% yield increase in the same crop was reported in field trials using a combination of *Bacillus megaterium* and *Azotobacter chroococcum* (Brown, 1974). *Azospirillum* spp. have increased yield in maize, sorghum, and wheat (Kapulnik ,1981) and *Bacillus* spp. has increased yield in peanut, potato, sorghum, and wheat (Broadbent,1977, Burr ,1978 and Capper ,1985).

Kobus (1962) reported that the numbers of PS bacteria in a soil were influenced more by soil type and the manner of its cultivation than by the physical composition or content of humus, N or P in the soil. Among the soil bacteria communities, *Pseudomonas strata* S., *Bacillus sircalmous and* intrubacters could be referred to as the most important strains (Hall, 1996). In particular, *Pseudomonas flourcents*is considered as an important member of rhizosphere organism community. The positive effect of *Pseudomonas* inoculation on plant growth has been reported in many researches. It has been found that mycorrhiza along with *Pseudomonas putida* increased leaf chlorophyll content in barley (Mehrvarz *et al.*, 2008).
Seed yield of green gram was enhanced by 24 % following triple inoculation of *Bradyrhizobium+ Glomusfasciculatum+ Bacillus subtilis* (Zaidi and Khan, 2006). Growth and phosphorus content in two alpine Carex species was also increased by inoculation with *Pseudomonas sfortinii* (Bartholdy et *al.*, 2001).

3.4. Phosphate solubilization

3.4.1. Mineral phosphate solubilization

Several reports have examined the ability of different bacterial species to solubilize insoluble inorganic phosphate compounds, such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite, and rock phosphate (Goldstein, 1986). There are considerable populations of

phosphate-solubilizing bacteria in soil and in plant rhizosphere (Sperberg et al; 1958). These include both aerobic and anaerobic strains, with a prevalence of aerobic strains in submerged soils (Ragh, 1966). A considerably higher concentration of phosphate solubilizing bacteria is commonly found in the rhizosphere in comparison with non rhizosphere soil (Raghu, 1966; Alexander, 1977). Among the bacterial genera with this capacity are *Pseudomonas, Bacillus, Rhizobium, Burkholderia, Achromobacter, Agrobacterium, Micrococcus, Aereobacter, Flavobacterium* and *Erwinia.*

3.4.2. Organic phosphate solubilization

Soil contains a wide range of organic substrates, which can be a source of P for plant growth. To make this form of P available for plant nutrition, it must be hydrolyzed to inorganic P. Mineralization of most organic phosphorous compounds is carried out by means of phosphatase enzymes. The presence of a significant amount of phosphatase activity in soil has been reported (Lynch et al., 1990). Important levels of microbial phosphatase activity have been detected in different types of soils (Kirchner, 1993; and Kurchaski, 1996). In fact, the major source of phosphatase activity in soil is considered to be of microbial origin (Garcia, 1992; and Xu, 1995). In particular, phosphatase activity is substantially increased in the rhizosphere (Tartdar, 1987). The presence of organic phosphate-mineralizing bacteria in soil has been surveyed by Greaves and Webley (Greaves, 1965) for the rhizosphere of pasture grasses, by Raghu and MacRae (1966) for rice plants, as well as by Bishop et al. (1994) and Abd-Alla (1994). The pH of most soils ranges from acidic to neutral values. Thus, acid phosphatases should play the major role in this process.

3.5. Occurrence of Phosphate Solubilizing Bacteria (PSB)

Evidence of naturally occurring rhizospheric phosphorus solubilizing microorganism (PSM) dates back to 1903 (Khan *et al.,* 2007). Bacteria are more effective in phosphorus solubilization than fungi (Alam *et al.,* 2002). Among the whole microbial population in soil, PSB constitute 1 to 50 %, while phosphorus solubilizing fungi (PSF) are only 0.1 to 0.5 % in P solubilization potential (Chen *et al.,* 2006)). Microorganisms involved in phosphorus acquisition include mycorrhizal fungi and PSMs (Fankem*et al.,* 2006). Among the soil bacterial communities, ectorhizospheric strains from *Pseudomonas* and *Bacilli*, and endosymbiotic rhizobia have been described as effective phosphate solubilizers (Igual *et al.,* 2001). Strains from bacterial genera *Pseudomonas, Bacillus, Rhizobium* and *Enterobacter* along with *Penicillium* and *Aspergillus* fungi are the most powerful P soluiblizers (Whitelaw, 2000). *Bacillus megaterium*, *B. circulans*, *B.subtilis*, *B. polymyxa*, *B. sircalmous, Pseudomonas striata*, and *Enterobacter* could be referred as the most important strains (SubbaRao, 1988; Kucey *et al.,* 1989). A nematode fungus *Arthrobotrysoligospora* also has the ability to solubilize the phosphate rocks (Duponnois *et al.,* 2006).

High proportion of PSM is concentrated in the rhizosphere, and they are metabolically more active than from other sources (Vazquez *et al.,* 2000). Bacilli are common in soil, whereas spirilli are very rare in natural environments (Baudoin*et al.* 2002). The PSB are ubiquitous with variation in forms and population in different soils. Population of PSB depends on different soil properties (physical and chemical properties, organic matter, and P content) and cultural activities (Kim *et al.,* 1998). Larger populations of PSB are found in agricultural and range land soils (Yahya and Azawi, 1998).

3.6. Mechanisms of Phosphate Solubilization

Many microorganisms in the soil are able to solubilize "unavailable" forms of calcium-bound P by excreting organic acids which either directly dissolves rock phosphate or chelate calcium ions to bring the P into solution (Katznelson and Bose 1959; Sperber 1958b). Some bacterial species have mineralization and solubilization potential for organic and inorganic phosphorus, respectively (Hilda and Fraga, 2000; Khiari and Parent, 2005). Phosphate solubilization takes place through various microbial processes / mechanisms including organic acid production and proton extrusion (Surange, 1995; Dutton and Evans, 1996).

Phosphorus solubilizing activity is determined by the ability of microbes to release metabolites such as organic acids, which through their hydroxyl and carboxyl groups chelate the cation bound to phosphate, the latter being converted to soluble forms (Sagoe *et al.*, 1998).
Phosphorus solubilization is carried out by a large number of saprophytic bacteria and fungi acting on sparingly soluble soil phosphates, mainly by chelation-mediated mechanisms (Whitelaw, 2000). Inorganic P is solubilized by the action of organic and inorganic acids secreted by PSB in which hydroxyl and carboxyl groups of acids chelate cations (Al, Fe, and Ca) and decrease the pH in basic soils (Kpomblekou and Tabatabai, 1994).

3.7. Effect of Phosphate Solubilizing Bacteria on Crop Production

Solubilization of fixed soil P by PSB resulted in higher crop yields (Zaidi, 1999). Microorganisms with phosphate solubilizing potential increase the availability of soluble phosphate and enhance the plant growth by improving biological nitrogen fixation (Kucey *et al.*, 1989; Ponmurugan and Gopi, 2006). *Pseudomonas* spp. enhanced the number of nodules, dry weight of nodules, yield components, grain yield, nutrient availability and uptake in soybean crop (Son *et al.*, 2006). Phosphate solubilizing bacteria enhanced the seedling length of *Cicerarietinum* (Sharma *et al.*, 2007), while co-inoculation of PSM and PGPR reduced P application by 50 % without affecting corn yield (Yazdani *et al.*, 2009). Inoculation with PSB increased sugarcane yield by 12.6 percent (Sundara *et al*., 2002). Sole application of bacteria increased the biological yield, while the application of the same bacteria along with mycorrhiza achieved the maximum grain weight (Mehrvarz *et al.*, 2008). Single and dual inoculation along with P fertilizer was 30-40 % better than P fertilizer alone for improving grain yield of wheat, and dual inoculation without P fertilizer improved grain yield up to 20 % against sole P fertilization (Afzal and Bano, 2008).

3.8. Tomato plant

Tomato belongs to the *Solanaceae* family along with other well-known species, such as potato, tobacco, peppers and eggplant. Tomato has its origin in the South American Andes. The cultivated tomato was brought to Europe by the Spanish conquistadors in the sixteenth century and later introduced from Europe to southern and eastern Asia, Africa and the Middle East (Upendra et al., 2003). Tomato (*Lycopersicon esculentum*) is the most widely grown and most consumed vegetable in the world being recognized as a rich source of vitamins and minerals. Tomatoes are now eaten freely throughout the world, and their consumption is believed to benefit the heart among other things. Recent studies suggest that tomatoes contain the antioxidant lycopene, the most common form of carotenoid, which markedly reduces the risk of prostate cancer (Kucuk, 2001). It is tasty and easily digestible and its bright color stimulates appetite. As a result, it is grown in the backyard of most people’s home. It is consumed as salad

with other leafy vegetables, in sandwiches, and as stewed, fried, and baked singly or in combination with other vegetables. It is an essential ingredient in pizza, pasta, hamburger, hot dogs, and other foods. It is also rich in nutrients and calories. It is a good source of Fe and vitamin A, B, and C (USDA, 1963).

Since the mineral composition of tomato depends on the amount and type of nutrients taken from the growth medium, such as soil, it is necessary that adequate amount of nutrients should be available for the production and nutrient content of tomatoes. Water soluble P fertilizers, such as nitro-phosphate or triple super phosphate, are desirable to tomato for its rapid availability (von Uexkull, 1979).

Phosphorus helps to initiate root growth of tomato and therefore aids in early establishment of the plant immediately after transplanting or seeding. Starter solution containing high concentration of P is normally applied to tomato plants within few days after transplanting for early root development and establishment in the soil. The vigorous root growth stimulated by P helps in better utilization of water and other nutrients in the soil and promotes a sturdy growth of stem and healthy foliage (Nelson, 1978).

Several reports revealed that use of PSB along with phosphate source increased shoot length, root length, shoot dry weight and phosphorus uptake of tomato plants than without use of phosphate solubilizing bacteria. Shahram (2012) reported that *Azotobacter* + *Azosprillum* and *Pseudomonas* + *Azotobacter* + *Azosprillum* increased shoot fresh, root fresh shoot dry weight weight of tomato plant. Viswanathan (2011) also reported that coinoculation of *Azospirillum* and AM recorded significantly increased shoot height and dry weight of tomato plants than either *Azospirillum* or AM fungi singly inoculated ones.

Inoculation of phosphate solubilizing bacteria was also found to increase phosphorus uptake of many crop plants including tomato plants. Subba Rao *et al.* (1985a) reported that seed inoculation with *A. brasilense* in conjunction with soil inoculation with *Gigaspora margarita* or *Glomus fasciculatum* produced significantly higher dry matter content of shoots, root biomass, P content and phosphorus uptake by pearl millet than the soil inoculation with VAM alone.
Subba Rao *et al.* (1985b) studied the dual inoculation effects of *Azospirillum brasilense* and various AM fungi in barley, among the different AM fungi, soil inoculation with *Glomus mosseae* and *Glomus fasciculatum* produced significantly higher dry matter production and grain yield than their corresponding controls in pot culture condition. Konde *et al.* (1988) also reported that simultaneous inoculation of either *Glomus* or *Gigaspora* and *Azospirillum* resulted in significant increase in fresh and dry weights and N and P uptake by shoots of bulbs of onion (*Allium cepa*) over their corresponding controls in a pot culture experiment.

MATERIAL AND METHODS

4.1. Isolation of Phosphate Solubilizing Bacteria

4.1.1 Collection of soil samples

Soil samples at a depth of 0-30 cm were collected from tomato rhizosphere On October 2004 E.C from Debrezeit, located 47km south of Addis Ababa. It is found at an altitude of about 1920 meters above sea level with Absolute Location of 8044’40’’N latitude and 38059’9’’E longitude.The samples were randomly picked, and collected in alcohol sterilized plastic bags and preserved at 4^0C for further analysis.

4.1.2. Isolation and purification of phosphate soluiblizers

Phosphate solubilizing bacteria were isolated from collected soil samples by serial dilution plate count method using Pikovskaya medium (pikovskaya, 1948) which is a selective medium for isolation of phosphate soluiblizers. The ingredients of pikovyskaya are:

Glucose----------10g
Ca3 (po4)2----------5g
(NH4)2so4----------0.5g
Yeast extract----------0.5g
$Mgso_4$----------0.2g
$Mnso_4$----------trace
$FeSO_4$----------trace
Agar----------15g
Distilled water----------—1000ml
PH----------–7.0

Ten grams of soil from each sample was suspended in 90 ml of sterilized water in 250ml flask to make 1:10 dilution. These were agitated on a shaker for 30 minutes to break clogs. Then series of dilutions 10^{-1}, 10^{-2}, 10^{-3}, 10^{-5} and 10^{-6} were made. From dilutions of 10^{-4}, 10^{-5} and 10^{-6}, 0.1 ml of suspension was transferred on petridish containing Pikovskaya's medium. The suspension was spread uniformly on petridish using glass rod spreader and incubated at 30±1°C for 7 days and the colonies exhibiting clear zones were selected, purified by streak plate method. The diameter of zone of solubilization was measured and expressed in centimeter and the selected isolates were preserved on agar slants for further use.

4.2. Identification and Characterization of the Bacterial Isolates

All the selected isolates were examined for the colony morphology, cell shape, colony type .

4.2.1. Gram reaction; twenty four hour young cultures of PSB isolates were gram stained and the results were recorded.

Procedure for gram stain: The twenty four hour culture of PSB isolates were covered with crystal violet for 20 seconds.

- The stain was gently rinsed off with water and shaked off and Covered with gram's iodine for one minute
- The gram's iodine was poured off with excess water
- 95% ethyl alcohol was run down the slide until the solvent runs clear (about 10-20 seconds) and rinsed with water to stop the action of the alcohol.
- The culture was covered with safranin for 20 seconds (counter stain) and gently rinsed with water.

4.2.2. Spore staining; forty eight hours old cultures of PSB isolates were spore stained as described by Aneja (1993).

A smear is prepared and fixed with 20 passes in a flame .A generous amount of saturated aqueous malachite green is applied to the slide and allowed to steam of for 10 -20 minutes, dye should be added so not dry up .After cooling it was rinsed with tap water to remove excess stain,

and it was counter stained with 0.5% safranin, blotted and dried .Spores stain light green while the rest of the cells stains pink and finally they were observed under oil emersion microscope.

4.2.1. Biochemical Characterization

The PSB isolates were further characterized using the following biochemical tests.

4.2.1.1. Fluorescent Pigment Production

King's B medium was used for this experiment (korobko, 1988). The ingredients for Kings Medium are:

Peptone---------- 20g
$K2HSO_4$----------1.5g
$MgSO_4.7H_2O$----------1.5g
Glycerin----------10ml
Agar----------17g
Distilled water----------–1000ml
PH----------7.2

Each isolate was incubated in the King's B medium and allowed to grow for 5 days .The diffusing pigment of each growth was observed using uv - light at wave length of 230nm .

4.2.1.2. Starch hydrolysis

The ability of the isolates to hydrolyse starch was examined by the procedure of Aneja (2003). Petriplates containing starch agar were inoculated with test cultures and incubated at 30°C for three days. After incubation the plates were flooded with Lugol's iodine solution and allowed to stand for 15-20 minutes. The clear zone around the colony was considered as positive for the test.

Ingredients of starch agar:

Soluble starch----------–20g
Peptone----------5g
Beef extract----------5g
Agar----------–15
Distilled water----------1000ml
PH ----------7.0

4.2.1.3. Catalase test

Nutrient agar slants were inoculated with test organisms and were incubated at 30°C for 24 hours. After incubation the tubes were flooded with one ml of three per cent hydrogen peroxide and observed for production of gas bubbles. The occurrence of gas bubbles was scored positive for catalase activity (Blazevic and Ederer, 1975).

4.2.1.4. Gelatin liquefaction

To the pre sterilized nutrient gelatin deep tubes, the test cultures were inoculated and tubes were incubated at 28±2°C for 24 hours. Following this, the tubes were kept in a refrigerator at 4° for 30 minutes. The tubes with cultures that remained liquefied were taken as positive and those that solidified on refrigeration were taken as negative for the test (Aneja, 2003).

Ingredients for nutrient gelatin deep tubes:(g/l)

Peptone----------–5
Beef extract----------3
Gelatin ----------120
Distilled water---------- 1000
PH----------7.0

4.2.1.**5. Oxidase test**

A small piece of whatman filter paper was soaked with 1% aqueos solution of tetra methyl-p-phenyldiaminedihydrochloride and a loop full of 24 hours old culture was scrapped and rubbed on the filter paper. The appearance of a blue color in 10-20 seconds was considered as a positive test for oxidase (Collins and Lyne, 1976).

4.2.1.6. Methyl red and Voger – Proskauer test

Ingredients of MRVP medium (g/l)

Peptone……7
Glucose……5
K_2SO_4…… 5
Distilled water…….1000
pH…….7

Ingredient s of methyl red indicator

Methyl red…….. ..0.1g
Ethyl alcohol……..300ml
Distilled water……100ml

Reagents

Vp1: 0.6g of ά –naphtol was dissolved in 100ml of 96% ethanol
Vp 2: 40 g of KOH was dissolved in 100ml of distilled water

5ml of MRVP broth was distributed in test tubes and autoclaved at 121^0C with a pressure of 15 psi for 15 min .Two MRVP tubes were inoculated with 24 hr old culture and incubated at 28^0C for 2-3 days .Then five drops of methyl red indicator was added to one of the tubes inoculated with each culture .The development of red color indicated a positive test. With regard to VP test 12 drops of VP 1 reagent and 2-3 drops of VP 2 reagent were added to the other test tubes. The presence of pink color was the characteristics of acetone production.

4.2.1.8. Utilization of different carbon sources

The isolates were examined for their ability to utilize different carbon sources *viz*, Lactose, maltose, and dextrose. The test was carried out according to Amarger *et al.*, 1997, the composition of the media were (g/l)

K_2HPO_4 1
KH_2PO_4……1
$FeCl_3$.$6H_2O$……0.01
$MgSO_4.2H_2O$ ……0.2
$CaCl_2$ ……..0.1
$(NH4)_2SO4$……..1
Agar ……20
Carbon source …… ……...1

The carbon sources were autoclaved together with the medium at 121^0c for 15 minutes and a pressure of 15psi, the growth was observed and recorded.

4.3. Screening of Isolates for Phosphate Solubilization

4.3.1. Quantitative Estimation of P Released From Insoluble P Source

The test PSB isolates used for this experiment were PSB1, PSB2, PSB4, PSB5 and PSB7. Fifty ml of pikovskaya's broth (without phosphorus source) was dispensed in 250 ml Erlenmeyer flasks and to this 250mg of tricalcium phosphate, 333.33mgof rock phosphate, and 200mg of old bone meal were separately added to each flask.

The above quantities gave equivalent amount of phosphate ions. The flasks were sterilized at 121^0c and pressure 15psi for 15 minutes. This autoclaved and cooled medium was inoculated with 0.1 ml of 24 hrs active culture of each isolates. Three replicate flasks were used for each PSB isolates and the amount of Pi released in the broth was estimated at 5, 10 and15 days after inoculation Keneni et al., (2000).

The available Pi content in the flask broth was estimated by phosphor molybdic blue color method (Jackson, 1973) as follows.

4.3.2 Reagents used

Chloromolybdic acid

Chloromolybdic acid reagent was prepared by dissolving 7.5 g of ammonium molybdate in 150 ml distilled water to which 162 ml of concentrated HCl was added. The volume was made up to one liter with distilled water.

Chlorostannous acid

Chlorostannous acid reagent was prepared by dissolving 25 g of $SnCl_2.2H_2O$ in 100 ml concentrated HCl and making the volume to one liter with distilled water. Both reagents were stored in amber colored bottles in a refrigerator.

One ml of the culture/supernatant was taken in a 50 ml volumetric flask to which 10 ml of chloromolybdic acid was added and mixed thoroughly. The volume was made up to approximately three fourth with distilled water and then 0.25 ml. Chlorostannous acid was added to it and mixed. Immediately the volume was made up to 50 ml with distilled water and the contents were mixed thoroughly. Within 15 minutes, the blue color developed was read on a spectrophotometer at 610 nm using a reagent blank. Simultaneously, a standard curve was prepared using various concentrations of standard 2 ppm KH_2PO4 solution. The amount of phosphorus solubilized was calculated from the standard curve.

4.3.3. Preparation of standard curve

Potassium dihydrogen phosphate was dried at 40^0C and 0.2195 g of it was dissolved in 400 ml distilled water. Twenty five ml of 7 N H_2SO_4 was added to it and the volume was made up to one litre with distilled water and mixed thoroughly. Twenty ml of this was diluted further to 500 ml with distilled water to obtain two ppm solution and used further.

4.5. Green House Evaluation of Efficient Phosphate Solubilizing Bacteria (PSB) On Growth of Tomato Plants

Clay soil was collected from agricultural fields of Holta ,50km from Addis Ababa was used to evaluate the effects of inoculation of selected strains of p- solubilizing bacteria on yield and

uptake of nutrients by tomato .The surface soil collected from the top 15 cm layer was dried and ,grounded to pass through a 2mm sieve and filled with alcohol sterile plastic pots of 3kg capacity. tricalcium phosphate at a rate of 200g/hectare(17.2mg P_2O_5/kg of soil) was weighed separately added in to the soil as basal dose before sowing (Haile, 1999). Soil pot culture experiment was conducted using five efficient phosphate solubilizing bacteria to study their performance in enhancing the growth, and p uptake of tomato plants.
The treatments fixed for soil pot culture experiment are presented below. Seven treatments, each with three replications were designed.

Details of the treatments used for pot culture experiment

1. Control (No inoculation, no phosphate (p) source
2. Positive control (soluble KH_2Po_4)
3. AAUPSB1 +TCP
4. AAUPSB2+TCP
5. AAUPSB4+TCP
6. AAUPSB5+TCP
7. AAUPSB7+TCP

Total number of treatments: 7
Number of replication: 3

4.5.2. Soil characteristics

Some of the properties of the soil are presented as follow.

Table 1:- **Soil analysis was done in jije laboratory PLC, Addis Ababa, Ethiopia**

Parameter	p^H	CEC/kg soil	Available phosphorus(mg/kg)	texture			
				%sand	%silt	%clay	Soil class
Value	6.72	28.53	4.04	13	29	58	clay
Methods for analysis	pH meter (Jackson, 1967)	Ammonia distillation method	Olsen et al.	Hydrometer method			

4.5.6 Seed sterilization, inoculation and sowing

Tomato seeds were obtained from Agricultural house of Mercato, Addis Ababa, Ethiopia. Tomato seeds were surface sterilized with sodium hypochlorite (4%) for 25 minutes and then thoroughly rinsed twice with sterile water. The seeds were then placed in dry flask and air dried overnight. All isolates were propagated in nutrient broth medium and incubated on a rotary shaker (150 rpm) for 5 days (Reyes *et al*., 2006). Starter cultures of the selected test isolates (PSB1, PSB2, PSB4 PSB5 and PSB7) were grown in test tubes containing 10ml PVK broth on orbital shaker at 150 rev/min at room temperature(average 25$_o$C) for 3 days. One ml PVK broth culture of each test strain (10^{9}cells) was transferred in to 100ml sterilized PVK broth in 250ml Erlenmeyer flask and placed on orbital shaker at 150 rev/min at room temperature for 4 days. One ml of each undiluted 4 days old PVK broth culture (about10^{9}cells,) was inoculated by pipetting on to the base of the seedlings of Tomato plant when they emerged according to Vincent (1970).

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The inoculated seeds were sown in pots at 4 seeds per pot in three replications. After growth, thinning was done to retain only one plant in each pot. The pots were watered regularly to maintain optimum moisture and other routine care was taken to protect the plants from pests and diseases.

4.5.9 Plant growth parameters

4.5.9.1 shoot length

The plant height was measured at 60 days after sowing from the base of the plant to the base of fully opened top leaf and expressed in centimeters (cm).

4.5.9.2 Root growth

Root length was recorded at 60 days after sowing by uprooting the plants and measuring the root length from tip of the longest root to the neck region and expressed in cm.

4.6. Analysis of phosphorous contents of plant samples

The oven dried plant samples were ground to fine powder and used for estimation of phosphate content.

4.6.1. Digestion of plant samples

The phosphorus content of plant samples was determined using metavandate method (NSL, 1994).the plant samples were calcinated in the furnance at 450^{o}c and digested with strong acid to liberate the organic phosphorus. The phosphorus in the solution was determined calorimetrically by using molybdate and metavandate for color development.

Reagent preparation

A) Solution 1; 20 ammonium molybdate was dissolved in 25 ml distilled water.
B) Solution 2; 1.25g of ammonium metevandate was dissolved in 300ml distilled water by heating. Then 425 ml of concentrated perchoric acid was added. Then solution 2 was added to solution one and the volume was adjusted to a litre with distilled water.

Procedure:

5ml of sample from digests was pippeted in to 50 ml volumetric flasks. The ten ml of the molybdate and vandate solutions was added to the samples and the standards. The volume was brought up to 50ml with distilled water. After 10 minutes the color developed was read on spectrophotometer at 460nm wavelength.

Standard phosphorus preparation

A) 500ppm p solution: 3g of KH_2PO_4 was oven dried at 105^{o}c for two hours. After cooling 2.197g KH_2PO_4 was measured and dissolved in 1lt of distilled water.
B) 100ppm p solution: 50 ml of the 500ppm p solution was taken in 250 ml volumetric flask and diluted to 250 ml with distilled water.
C) 10ppm p solution: 50 ml of the 100ppm p solution was taken in 500ml volumetric flask and diluted to 500ml with distilled water.
D) Phosphorus working solution standards of 0, 1, 2, 3, 4, and 5 ppm p; 0, 5, 10, 15, 20, and 25 ml of the 10 ppm p solution were pippeted in 50ml volumetric flasks.

Standard curve was prepared with absorbance on x- axis and concentrations on y-axis, from which p-values in the samples were calculated.

Calculations:

PPM = C.V1.V2 / S.A

Where C = p concentration in the sample digest

V1- volume of the digest

V2- volume of the digestion.

S-Weight of the material digested in the gram

A- Aliquots (5ml)

Ppm - parts per million (conc. × 1000)

4.7. Statistical analysis of the data

The data obtained from the experiments were subjected to statistical analysis of variance (ANOVAS). Analysis was performed for all data with triplicates for each. The data groups were analyzed using the statistical Soft Ware JMP (John's Macintosh Project) IN version 5.0.1 with stastical significant difference at (p<_0.05).

RESULTS

In this experiment a total of 11 phosphate solubilizing bacteria (PSB) isolates were isolated from tomato rhizosphere. The isolates were examined for their ability to solubilize insoluble tricalcium phosphates. The selected isolates were characterized and tentatively identified up to genus level based on morphological and biochemical properties. The efficient p soluiblizers were further subjected for their ability to release p from different insoluble phosphate sources. Highly efficient p solubilizing strains were also tested for their influence on growth and nutrient uptake of tomato plant under pot culture conditions.

5.1. Isolation of Phosphate Solubilizing Bacteria (PSB) from Rhizosphere Soils of Tomato Plant

The rhizosphere soil samples of tomato plant were collected and used for the isolation of PSB. Out of the total 11 PSBisolates isolated, five were selected for further experiment based on their solubilizing efficiency. These isolates were purified, identified and maintained for further use.

5.2 Identification of PSB Isolates

All the selected isolates of PSB were identified up to genus level based on their morphological and biochemical characters and the results are presented below.

Tests	Isolates				
	PSB_1	PSB_2	PSB_4	PSB_5	PSB_7
Shape	rod	rod	rod	rod	Rod
Gram reaction	negative	negative	negative	negative	negative
Catalase test	+	+	+	+	+
Oxidase test	+	+	+	-	-
Gelatin liquefaction test	+	+	+	+	+
Starch	+	+	+	+	+

hydrolysis						
Methyl red test		-	+	+	-	-
Vp test		-	-	+	-	-
Pigment production test		+ve yellowish	+ve yellowish	+ve greenish blue	+ve yellowish	+ve greenish blue
Endo spore test		-	-	-	-	-
Carbohydrate Utilization test	lactose	+	+	+	+	+
	Maltose	+	+	+	+	+
	dextrose	+	+	+	+	+

Table 1. Some morphological and biochemical tests of PSBs.

"+" : stands for positive result "- ": stands for negative result

By comparing the cultural, morphological and biochemical characteristics on the characteristics described in the Berge's manual of systematic Bacteriology (Krieg and Holt, 1984), attempts were made to classify them in to their respective genera. Accordingly the isolates were found to belong to the genera pseudomonas.

5.3 Phosphate Solubilization by the Isolates

Qualitative analysis of the isolates for p solubilization is presented in Table 2.

All the isolates were examined for their ability to solubilize phosphate sources on agar media supplemented with tricalcium phosphate.

	Colony diameter (cm)	Halo zone Diameter(cm)	Solubilization index
PSB1	1.3	2.1	2.61
PSB2	1.4	2.0	2.42
PSB4	0.9	1.1	1.80
PSB5	1.05	1.6	2.52
PSB7	1.0	2.5	3.50

Table2. All values are means of triplicates.

These isolates formed a clear zone diameter of between 1 cm and 3cm and the largest clear zone diameter of 2.5mm was recorded in PSB7 followed by PSB1 (2.1) and PSB5(2.52) and the least clear zone was obtained in PSB4 (Table 2). Solubilization index can be calculated using the formula;

$$SI = \frac{\text{colony diameter + clearing zone}}{\text{Colony diameter}}$$ (Ed Premono et al, 1996)

The diameter of zone of solubilization formed by the isolates ranged from 1.80 to 3.50cm at 72 hours after incubation. Among the isolates pSB4, recorded maximum solubilization zone (3.50 cm diameter) followed by PSB1 (2.61cm) and PSB5 (2.52 cm). However, the isolate PSB7 showed the least solubilization zone of 1.80 cm diameter.

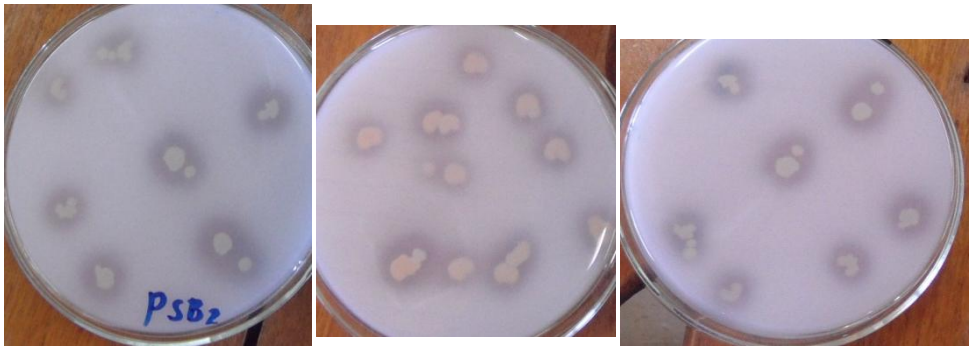


Fig. phosphate solubilizing bacteria isolates

5.4 Quantitative Estimation of P Solubilizing Activity of the Isolates from Different Phosphate Sources

The amount of P released from tricalcium phosphate, rock phosphate and bone phosphate in a broth by the isolates was studied at 5, 10, 15 days after incubation (DAI).

5.4.1. Tricalcium phosphate solubilization efficiency of isolates

Isolates	5th day		10th day		15th day	
	P^H	Pmg/50ml	P^H	Pmg/50ml	P^H	Pmg/50ml
PSB1	6.82	7.642 ± 0.347^{a}	6.25	8.103 ± 0.660^{a}	5.89	10.396 ± 0.802^{b}
PSB2	7.60	6.428 ± 0.440^{b}	6.67	8.195 ± 1.019^{a}	5.46	11.337 ± 1.042^{a}
PSB4	7.60	4.792 ± 0.345^{c}	6.49	5.974 ± 0.223^{b}	6.28	6.694 ± 0.270^{c}
PSB5	7.57	4.98 ± 0.415^{c}	7.10	5.266 ± 0.485^{b}	6.13	5.440 ± 0.253^{d}
PSB7	7.29	6.471 ± 0.875^{b}	6.70	7.558 ± 0.425^{a}	5.22	11.772 ± 0.175^{a}
Control	6.64	2.201 ± 0.100^{d}	6.61	2.558 ± 0.152^{c}	6.58	2.94 ± 0.427^{e}

Table 3. All values are means of triplicates ± SD. Levels not connected by same letter under the same column are significantly different ($p\leq0.05$) using JMP method of analysis.

The results are (Table 3) indicated that the amount of P released from mineral P by all strains increased with increase in incubation time and it was greatest at 15 DAI. The P released from TCP by the strain at 15 DAI ranged from5.44mg/50ml to 12.33mg/50ml. there was also a decrease in p^H as incubation time was increased from 5 days to 15 days. Maximum release of P was observed by PSB7 followed by PSB2 and the least P was released by PSB5 at 15 DAI.

5.4.2. **Rock phosphate solubilizing efficiency of isolates**

Isolates	5th day		10th day		15th day	
	P^H	Pmg/50ml	P^H	Pmg/50ml	P^H	Pmg/50ml
PSB1	5.74	3.694 ± 1.489^{a}	4.37	4.383 ± 1.11^{abc}	3.63	4.714 ± 1.273^{cd}

PSB2	6.13	3.701±0.264[a]	4.59	4.077±0.55[bc]	3.61	5.363±0.739[c]
PSB4	5.68	3.324±1.048[ab]	4.37	3.467±0.88[cd]	3.80	4.025±0.725[d]
PSB5	6.04	3.240±0.341[ab]	4.40	5.564±0.645[a]	3.54	6.883±0.232[a]
PSB7	6.22	3.660±0.225[a]	6.16	5.025±0.87[ab]	3.16	7.928±0.440[b]
Control	6.82	2.105±0.360[b]	6.78	2.158±0.370[d]	6.62	2.34±0.173[e]

Table 4. **All values are means of triplicates ± SD. Levels not connected by same letter under the same column are significantly different (p≤0.05)) using JMP method of analysis.**

The results are (Table 4.) indicated that the amount of P released from RP by all strains increased with increase in incubation time and it was greatest at 15 DAI. The P released from RP by the strain at 15 DAI ranged from 4.025 mg/50ml to 7.928mg/50ml. there was also a decrease in p^H as incubation time was increased from 5 days to 15 days. Maximum release of P was observed by PSB7 followed by PSB5 and the least p was released by PSB4 at 15 DAI.

5.4.3. Bone phosphate solubilizing efficiency of the isolates

Isolates	5th day		10th day		15th day	
	P^H	Pmg/50ml	P^H	Pmg/50ml	P^H	Pmg/50ml
PSB1	5.83	4.500±0.278[a]	4.39	6.058±0.530[bc]	3.78	9.120±0.361[c]
PSB2	5.25	4.290±0.486[a]	4.33	5.47±0.430[c]	3.58	10.461±0.373[b]
PSB4	5.60	4.318±0.453[a]	4.36	6.316±0.531[b]	4.17	9.064±0.382[c]
PSB5	6.13	3.935±0.199[a]	4.35	7.370±0.330[a]	3.79	10.974±0.373[b]
PSB7	5.57	3.020±0.193[b]	4.38	7.025±0.252[a]	3.67	11.093±0.491[a]
Control	6.45	0.480±0.223[c]	6.33	0.62±0.127[d]	6.17	0.66±0.126[d]

Table 5. All values are means of triplicates ± SD. Levels not connected by same letter under the same column are significantly different (p≤0.05) using JMP method of analysis.

Among the isolates PSB7 released maximum amount of P from BP11.093mg/50mlfollowed by PSB5 10.974mg/50ml, both were significantly superior over all other isolates. Out of 5 isolates examined 3 isolates showed more than10mg/50ml of phosphate solubilization from bone phosphate.

The results of tricalcium phosphate solubilization by the selected isolates and the associated pH changes in the medium are shown in (Table 3). The result showed that PSB7was found to be more efficient in solubilizing tricalcium phosphate than the rest of the isolates. All the bacterial isolates solubilized significantly higher amounts of tricalcium phosphate over uninoculated control. The highest amount of solubilization was recorded for the bacterial isolate PSB7 followed by PSB2 (34 mg), PSB1 (27 mg), PSB4 (20.4 mg), and PSB5 at 15 days of incubation time. An inverse relation between the amount of soluble P and the reduction of pH in the inoculated medium was found when compared with the uninoculated medium.

There was significant solubilization of rock phosphate over the uninoculated control by the six isolates. The amount of soluble phosphorus and corresponding pH change of the medium is presented in Table 4. The solubilization increased steadily up to 15 days of incubation where the maximum amount of soluble phosphorus was released. The highest amount of soluble phosphorus was obtained with PSB7, (31 mg) followed by PSB5, (25 mg), PSB2, (22.8 mg), PSB4, (22.5 mg), and PSB1, (20.5 mg)). There was a drastic drop in the pH of the medium inoculated with all PSB isolates; whereas the pH changes in the uninoculated flask throughout

the 15 days was not significant. The results for the phosphorus solubilization efficiency of PSB isolates on bone phosphate are shown in (Table 5).
The amount of phosphorus released by different isolates of PSB on the bone phosphate as a function of time was not significant unlike the tricalcium phosphate and rock phosphate.

5.5. Effect of Inoculation of Selected Phosphate Solubilizing Bacteria on Growth and P Uptake of Tomato Plant

5.5.1. Root length

A significant difference in the root length of tomato plants was observed at 60 days of plant growth due to various inoculation treatments.

treatments	Root length in average In cm	Shoot length in average In cm	Shoot dry weight in gram
Positive control(KH_2PO_4)	8.33 ± 2.02^{ab}	$19.33\pm0.1.52^{a}$	0.829 ± 0.385^{b}
Absolute control	6.66 ± 0.28^{b}	12.00 ± 0.173^{b}	0.442 ± 0.155^{b}
PSB1	8.16 ± 0.22^{ab}	17.16 ± 0.292^{ab}	0.536 ± 0.356^{b}
PSB2	10.5 ± 0.327^{a}	19.33 ± 0.550^{a}	0.674 ± 0.471^{ab}
PSB4	7.66 ± 0104^{ab}	$16.83\pm0.175a^{b}$	0.743 ± 0.329^{ab}
PSB5	11.16 ± 0.152^{a}	21.16 ± 0.152^{a}	1.127 ± 0.068^{a}
PSB7	10.16 ± 0.325^{ab}	20.5 ± 0.433^{a}	1.108 ± 0.087^{a}

Table 6. All values are means of triplicates ± SD. Levels not connected by same letter under the same column are significantly different ($p\leq0.05$) using JMP method of analysis.

A significant difference in the root, shoot length and shoot dry weight of tomato plants was observed at 60 days of plant growth due to various inoculation treatments. (Table 6).The treatment receiving inoculation of PSB5 recorded maximum root length (11.16 cm) and was significantly superior over all other inoculation treatments followed by PSB7 (10.16) and PC (8.33). All treatments showed significant increase in root growth over absolute control (AC).

5.5.2. Shoot Length

All the treatment receiving inoculation of bacteria increased the shoot length of tomato plants significantly over absolute control, but not significant with PC.
Among the inoculated treatments the PSB5 strain showed maximum shoot length of tomato at 60 days of growth (21.16. cm) followed by isolates PSB7(20.5) and positive control (19.33) which were significantly superior over the all other inoculated treatments and the absolute control (table 6). The simulative effect of PSB on growth and consequently on dry weight may be due to the activity of P solubilization caused by the used isolate(Kim et al.,1997) and increase further mineral availability uptake and plant growth(Han et al., 2006).

Figure.1.tomato plants in green house treatments

5.6. Phosphorus Analysis of Tomato Plants

5.6.1. Phosphorus uptake of tomato plants

The application of PSB with TCP significantly increased soluble P and plant P uptake in tomato plant seedlings (Table 7).

treatment	Absolute control	Positive control (soil+KH_2PO_4)	PSB1+ TCP	PSB2+ TCP	PSB4+ TCP	PSB5+ TCP	PSB7+ TCP
P_{av}. in mg/g	0.959 ± 0.04^{b}	0.993 ± 0.02^{b}	0.94 ± 0.03^{b}	1.17 ± 0.02^{a}	1.09 ± 0.03^{a}	1.06 ± 0.02^{ab}	1.08 ± 0.03^{b}

- **Analysis was done in jije laboratory PLC, Addis Ababa, Ethiopia**.

Table 7. All values are means of triplicates ± SD. Levels not connected by same letter under the same column are significantly different ($p\leq0.05$) using JMP method of analysis.

Phosphorus uptake of tomato plants on each treatment was evaluated as described above. Data in Table (7) show that significant increases in available P were observed in inoculated tomato treatments compared to uninoculated ones. All inoculations except PSB1+TCP showed significant p uptake as compared to the un inoculated control. The maximum uptake was observed with TCP + PSB2 followed by TCP+PSB4 (table 7). Similar results were reported by Mehasen *et al*. (2002) who found that available-P content was increased when the plants were inoculated with P-solubilizer with rock phosphate.

DISCUSSION

6.1. Phosphate Solubilization of the Isolates

In the present study five phosphate solubilizing bacteria designated as PSB1, PSB2 PSB4, PSB5, and PSB7 were isolated from soil samples collected from Debrezeit district. They were all found to belong to the genus pseudomonas.

This finding is supported by an earlier report that says that most efficient and frequently encountered phosphate solubilizing bacteria belonging to the genus Pseudomonas or the genus Bacillus (Sundram, 1994). Haile (1999) and Keneni et al., (2000) also isolated phosphate solubilizing bacteria all of which are characterized as belonging to the genus Pseudomonas.

When grown in culture media supplemented with Tricalcium phosphate, all the isolates produced halo zone around the colonies, indicating the solubilization of phosphate source used. Phosphate solubilizing microbes are detected by the formation of clear halos around their colonies. The halo is produced due to solubilization of insoluble phosphates, which in turn is mediated via the production of organic acid in the surrounding medium (Gaur, 1990).

The isolate PSB7 showed maximum solubilization index, followed by PSB1 (Table 2). All isolates were confirmed for their P solubilization ability by phospho- molybdate test. Phospho-molybdate test for quantitative determination of available phosphorous indicated that the isolates PSB7 and PSB5 solubilized significantly higher phosphate than all other bacterial strains. An inverse relationship was observed between pH value of culture medium and concentration of phosphate solubilized, indicating the organic acid secretion (Table 3, 4, 5). Difference in the P solubilization was reflected by the change in the pH of the culture medium.

In the present study an inverse relationship between pH and soluble phosphate concentration was observed among all the isolates. This result is in agreement with the report of Rashid et al., (2004). In this study, a drastic drop in pH was observed in all treatments in opposite to the phosphorus amount released (table 3, 4, 5). This was due to the fact that 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 -concentrations indicates that organic acid production by these PSB strains plays a significant role in the acidification of the medium facilitating the P solubilization. Similar inverse relationship between pH and soluble phosphate was reported earlier by Illmer and Schinner (1995) and Hwangbo et al. (2003).
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).

6.2. Tricalcium phosphate (TCP), bone phosphate (BP) and rock phosphate (RP) solubilization of PSB isolates

All PSB isolates solubilized significantly greater amount of TCP, RP, and BP over un inoculated control (table 3, 4, 5). The presence of small amount of soluble phosphorus in the un inoculated control flask is assumed to be due to the release of phosphate ions during autoclaving. This is in line to the idea proposed by Agnihotri (19790) cited in Haile (1999).

In the present study there was a progressive increase in the solubilization of TCP by all PSB isolates. This result is in agreement with Keneni et al., (2000) and Kumlachew (2011) that the solubilization of TCP was progressive and maximum at 20 of days of incubation.
Chen et al., (2005) also reported that the maximum P solubilization was recorded at the last day of incubation by Arthrobacter sp. followed by S. marcescens with a maximum drop in the pH to 4.9.

The solubilization of rock phosphate with the isolates continued up to 15 days without drop with maximum solubilization by PSB7 (7.92mg/50ml) followed by PSB5 (6.88mg/50ml) (table. 4). Similarly Asfaw (1988) and Haile (1999) reported that the maximum solubilization RP was recorded at 20 days of incubation. Kumlachew (2011) also reported that the maximum solubilization was observed at the fifth day of inoculation. Hamdali (2011) reported that actinobacteria strains showed different abilities to release soluble phosphate from RP. According to him strain AT12 was the most efficient strain releasing 25.87 µg/ml soluble P in the growth medium.

Similarly the trend of bone phosphate solubilization by the isolates was progressive up to 15 days of incubation. As in the case of TCP and RP, PSB7 released the maximum p in BP. This result was in contrary with the report of Haile (1999) who reported that Jim41was the most efficient solubilization in TCP and RP but least in solubilizing bone phosphate. This variation may be due to the efficiency of the isolate PSB7.
In all cases of phosphorus solubilization, there was a perfect inverse relationship between release of soluble phosphorus and reduction in the pH of the medium for all substrates. In this investigation the highest soluble phosphorus was observed in tricalcium phosphate inoculated with PSB7 (11.77mg/50ml) and the corresponding pH decrease during 15 days of incubation was 5.22 (table 3). In agreement with this result, Paul and SunduraRao (1971) revealed that there was a perfect inverse correlation with the amounts of tricalcium solubilized and the pH of the medium inoculated with PSB cultures. Among twelve PSBs tested, the highest amount of tricalcium phosphate solubilization was achieved by Bacillus brevis with the lowest pH of 4.4. Acid production and reduction of the pH of the medium is one of the mechanisms by which soluble phosphorus is released by PSB (Gaur, 1988).

The drop in pH of the inoculated medium containing rock phosphate was more drastic than tricalcium phosphate and bone phosphate. Among the different phosphorus sources tested, the lowest pH value was observed in rock phosphate inoculated with PSB7 isolate. This is supported by Haile (1999) and Keneni et al., (2010) this could be due to the high insolubility of rock phosphate compared to tricalcium phosphate. Thus, some PSB isolates when inoculated in a medium with rock phosphate released more acid than they did with tricalcium phosphate.

Unlike isolate PSB7, which showed maximum solubilization for all phosphate sources, the other isolates showed variations of solubilization in each sources. This suggests that the mechanism employed by PSB isolates in solubilizing insoluble phosphates is variable. This variation is probably attributed to the fact that certain isolates could produce enzymes such as phosphophatases. This reason can be reinforced by the report which suggested that the solubilization of calcium phytases and lecithin by microorganisms was due to the production of enzymes such as phytases and lecithinnases (Gaur 1972).

6.3. The effect of inoculation of phosphate solubilizing bacteria (PSB) on shoot, root length and shoot dry matter of tomato plant

Inoculation of PSB isolates together with TCP was found to increase shoot length, root length and dry matter yield of tomato plant as compared to uninoculated control. There have been a number of reports on plant growth promotion by bacteria that have the ability to solubilize inorganic and/or organic P from soil after their inoculation in soil or plant seeds (Gaur et al., 1972).

In the present study, all the isolates used as inoculants exhibited significant increase in shoot and root length of tomato over control, the maximum record was shown by isolate PSB5 (21.16) followed by PSB7 (20.5) (Table 6). All isolates except isolate PSB1 showed significant difference in shoot dry weight compared to un inoculated control. Positive growth promotion by inoculation with P-solubilizing bacteria has been is attributed to the ability of these bacteria to solubilize P and produce siderophores and hormones (Khan et al., 2009).

Similarly, Hariprasad and Niranjana (2009) studied the effect of phosphate solubilizing rhizobacteria to improve plant health of tomato and revealed that phosphate solubilizing rhizobacteria promoted plant growth significantly in 30 day-old-seedlings. Of the 16 isolates of phosphate solubilizing rhizobacteria, PSRB19 showed significant increase in shoot length (14.0 cm), root length (18.0 cm), followed by isolate PSRB8. In the control, shoot length, root length, and dry mass was found to be 9.0 cm, 12.5 cm, 0.225 and 0.041 g/seedling respectively. Likewise Alagawadi and Gaur (1994) conducted a field experiment in medium black soil under rain fed conditions to evaluate the effect of combined inoculation of *Azospirillum brasilense* and *Pseudomonas striata* or *Bacillus polymyxa* (with and without fertilizer nitrogen and phosphate) on the yield and nutrient uptake of sorghum. The results showed a significant increase in grain and dry matter yields and N and P uptake of sorghum from the combined inoculation over single inoculation of individual organisms.

Bacteria isolated from rhizosphere soils are known to produce growth regulating substances and some of them are capable of dissolving phosphate. Some of the PSB are also able to produce vitamins towards the dissolution of bicalcium phosphate and all the strains of phosphate bacteria were able to solubilize inorganic phosphate (Ponmurugan and Gopi, 2006). Increasing the bioavailability of P with the inoculation of PGPR or with a combination of inoculation and rock materials has been also reported by Han and Lee (2005).

The application of PSB significantly increased soluble P and plant P uptake in tomato plant seedling (Table 6 and 7).The amount of P uptake in plant tissue differed significantly between bacterial strains. Highest P uptake was recorded by PSB2 (1.174mg/g) and PSB4 (1.174mg/g) followed by PSB7 (1.081mg/g) and PSB5 (1.067mg/g) (table 7). Results of this study indicate the highly beneficial effect of PSB inoculation on P release from tricalcium phosphate and the consequent significant increase in P uptake in tomato plants compared to non-inoculated treatments. This result concides with (Turan *et al.,* 2007) who studied the effects of PSB (*Bacillus* FS- 3) application on phosphorous content of tomato (*Lycopersicon esculentum*) under green house conditions with five different fertilizer treatments. A greater increase was noticed in plant phosphorous uptake in treatments with PSB application than without PSB in all of treatments. Tantawy *et al*. (2009) also observed the effects of inoculation with phosphate solubilizing bacteria on tomato rhizosphere, colonization process plant growth and yield. The inoculation with *Pseudomonas sp.* had a positive significant effect on tomato leaves phosphorous content.

The present result also reinforces the reports of Gull et al. (2004) who reported that PSB have potential to solubilize fixed P resulting higher p uptake and crop yields. The importance of PSB was also shown in other crops. The application of bradyrhizobia (Bradyrhizobium japonicum) and PSB (Pseudomonas spp.) enhanced the number of nodules, dry weight of nodules, yield components, grain yield, soil nutrient availability and p uptake of soybean crop. In wheat, inoculation of PSB strains along with P fertilizer were 30 - 40% more efficient than P fertilizer alone in improving grain yields, and dual inoculation of the microorganisms without P fertilizer improved yields up to 20 % against sole P fertilization (Afzal and Bano, 2008).

CONCLUSION AND RECOMMENDATION

7.1. Conclusion

In the present study five phosphate solubilizing bacteria coded as PSB1, PSB2, PSB4, PSB5 and PSB7 were isolated and characterized under genus *pseudomonas.*

All isolates were found to be efficient in solubilization of tricalcium phosphate, rock phosphate, and bone phosphate. However, except PSB7 which solubilized maximum in all phosphate sources, and there was variation of solubilization efficiency among other isolates. This fact may be due to the mechanisms employed to solubilize, species difference and other environmental factors. The maximum amount of soluble p was released by PSB isolates from tricalcium phosphate, followed by bone phosphate and rock phosphate.

Inoculation of PSB along with application of tricalcium phosphate increased the shoot dry matter yield and phosphorus uptake of tomato plant significantly over the control.

7.2. Recommendation

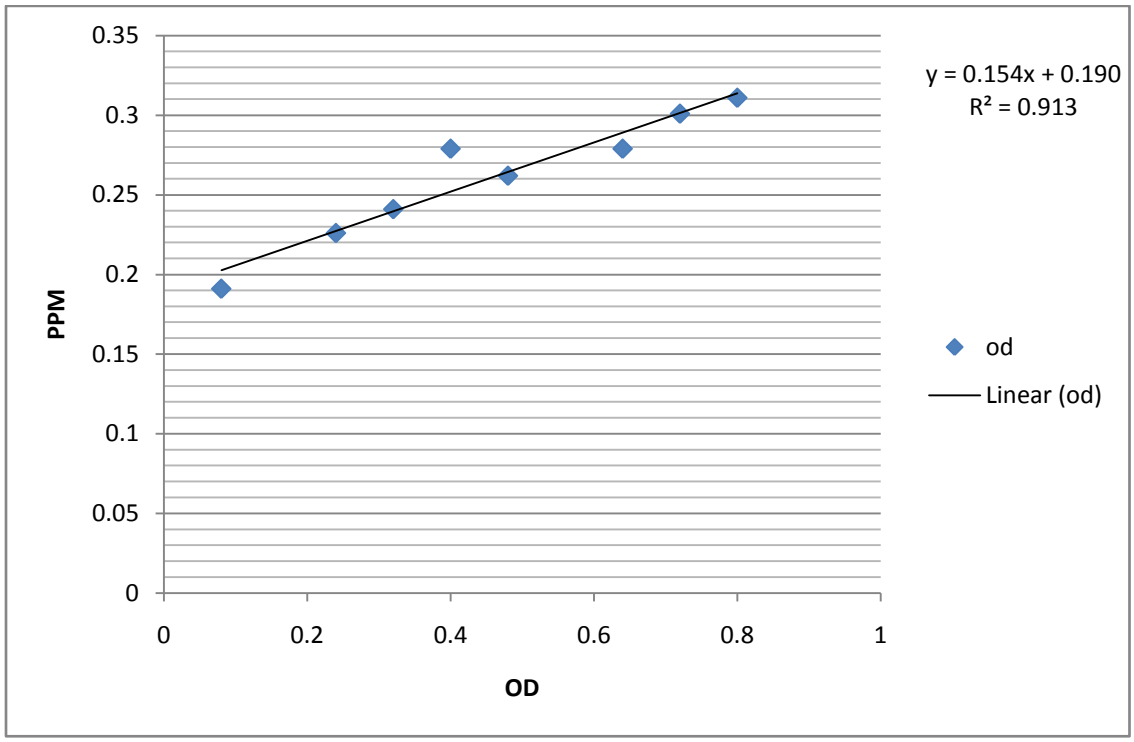
Based upon the results of the study the following points can be recommended:

- Further isolation and characterizations of PSB isolates has to be made so as to get more efficient PSB isolates.
- Further inoculation experiments in field should be undertaken especially with non-leguminous plants so that the importance of these PSMs in growth promotion of plants can be clear cut.
- The various environmental factors that directly or indirectly affect the activities of phosphate solubilizing microorganisms should be investigated.
- Good PSB isolates must be produced in large scale and commercialized and get accessed to farming communities.

Appendices . 1.

Halo zones formed by PSB isolates

Appendice 2. Standard curve of soluble phosphorus from which amount of phosphorus released from insoluble phosphate sources was calculated.



References

Abd-alla, M. H. (1994). Phosphatases and the utilization of organic P by *Rhizobium leguminosarum*biovar viceae. Lett. Appl. Microbiol. 18:294-296.

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

Alam S., Khalil S., Ayub N. and Rashid M. (2002). In *vitro* solubilization of inorganic phosphate by phosphate solubilizing microorganism (PSM) from maizerhizosphere. Intl. J. Agric. Biol. **4**: 454-458.

Alexander, M. (1977). *Introduction to Soil Microbiology.* Wiley and Sons, New York.

Amarger, N., Macheret, V. & Laguerre, G. (1997). *Rhizobium gallicum* sp. nov. and *Rhizobium giardinii* sp. nov., from *Phaseolus vulgaris* nodules. *Int J Syst Bacteriol* **47**, 996±1006.

Anderson, G. (1980). Assessing organic phosphorus in soils. **In:** *The Role of Phosphorus in Agriculture*, pp. 411–32, (Khasawneh, F.E., Sample , E.C. and Kamprath, E.J., eds). Amer Soc Agronomy, Madison, Wis.

Anje,K.R. (2003). Experiments in microbiology, plant pathology and biotechnology. 4th ed., New age international publishers, New Delhi, pp.74-128

Baldani, V.L.D., Baldani, J.I. and Döbereiner, J. (1987). Inoculation on field-grown wheat (*Triticum aestivum*) with *Azospirillum* spp. in Brazil. *Biol Fert Soils* **4**:37–40.

Bardiya, M.C. and Gaur, A.C. (1974). Isolation and screening of microorganisms dissolving low grade rock phosphate. *Folia Microbiol* **19**:386–9.

Barea, J.M., Azco´n, R., Azco´n-Aguilar, C. (1983). Interactions between phosphate solubilizing bacteria and VA mycorrhiza to improve plant utilization of rock phosphate in non-acidic soils. 3rd international congress on phosphorus compounds, Brussels, pp127–144.

Barton, C. J. (1948). Photometric analysis on phosphate rock. *Ind. Anal. Eng. Chem.* **20**:1068–1073.

Bartholdy B. A., M. Berreck and K. Haselwandter.(2001). Hydroxamate siderophore synthesis by *Phialocephala fortinii*,. Bio. Metals **14**:33-42.

Bishop, M..L, Chang A. C, Lee R.W.K..(1994). Enzymatic mineralization of organic phosphorusin a volcanic soil in Chile. Soil Sci; **157:238**–43

Bowen, G.D. and Rovira, A.D. (1999). The rhizosphere and its management To improve plant growth. *Advances in Agronomy* **66**:1–102.

Broadbent P, Baker KF, Franks N, Holland J. (1977). Effect of *Bacillus* spp. on incrased growth of seedlings in steamed and in nontreated soil. Phytopathology;67:1027–34.

Brown, M.E. (1974). Seed and root bacterization. *Annu Rev Phytopatol* **12**:181 - 97.

Bünemann, E. K., D. A. Bossio, P. C. Smithson, E. Frossard and A. Oberson. 2004.Microbial community composition and substrate use in a highly weathered soil as affected by crop rotation and P fertilization. Soil Biol. Biochem. **36**:889-901

Burr, T.J., Schroth, M.N. and Suslow, T. (1978). Increased potato yields by treatment of seedpieces with specific strains of *Pseudomonas fluorescens* an *Pseudomonas putida.* Phytopathology **68**:1377–83.

Buscot, F. (2005). What are soils? In: Buscot F, Varma S, eds. Microorganisms

Cabello, M., G. Irrazabal, A. M. Bucsinszky, M. Saparrat and S. Schalamuck. (2005). Effect of G. mosseae and a rock- phosphate-solubilizing fungus, J. the Basic Microbiol. **45**:182-189.

Capper, A.L. and Campbell, R. (1986). The effect of artificially inoculated antagonistic bacteria on the prevalence of take-all disease of wheat in field experiment. *J Appl Bacteriol* **60**:155–60.

Chabot, R., Antoun, H., Kloepper, J.W. and Beauchamp, C.J. (1996a). Root colonization of maize and lettuce by bioluminescent Rhizobium leguminosarumbiovar. *phaseoli. Appl Environ Microbiol* **62**:2767–72.

Chabot, R., Hani, A. and Cescas, P.M. (1996b). Growth promotion of maize and lettuce by phosphate-solubilizing Rhizobium leguminosarumbiovar. phaseoli. *Plant Soil* **184**:311–21.

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

Chapagain BP, Wiesman Z (2004) Effect of potassium magnesium chloride in the fertigation solution as partial source of potassium on growth, yield and quality of greenhouse tomato. *Scientia Horticulturae* **99**, 279-288

Chen, Y. P., Rekha, P. D., Arunshen, A. B., Lai, W. A. and Young, C. C. (2006). Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. *Appl. Soil Ecol.* **34**: 33-41.

Chen,Y.P., Rkha,P.D., Arun,A.B. and Shen, F.T. (2005). Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities.

Dadarwal , Yadav K.S and , K.R.(1997). Phosphate solubilization and mobilization through soil microorganisms. **In**: biotechnological approaches in soil microorganisms for sustainable crop production (Dadarwal, K.R., Ed.) pp. 293-308. Scientific Publishers, Jodhpur.

Dalal, R.C. (1977). Soil organic phosphorus. *Adv. Agron*. **29**: 83–117.

Daniel, P., Robert, J. and Reid, S. (1998). Phosphorus uptake by plants: from soil to cell. *plant physiol*. **11**: 447-453.

Das, A.C. (1963). Utilization of insoluble phosphates by soil fungi. *J. Indian Soc. Soil Sci*. **11**:203–207.

Datta, M., Banish, S. and Dupta, R.K. (1982).Studies on the efficacy of a phytohormone producing phosphate solubilizing Bacillus firmus in augmenting paddy yield in acid soils of Nagaland. *Plant Soil* **69**:365–73.

de Groot, C.C., Marcelis, L.F.M., van den Boogaard, R. and Lambers, H. (2001). Growth and dry-mass partitioning in tomato as affected by phosphorus nutrition and light. *Plant, Cell and Environment* **24**:1309-1317

de Groot, C.C., Marcelis, L.F.M., van den Boogaard, R., and Lambers, H. (2002). Interactive effects of nitrogen and irradiance on growth and partitioning of dry mass and nitrogen in young tomato plants. *Functional Plant Biology* **29**:1319-1328

Dey, K.B. (1986). Phosphate solubilizing organisms in improving fertility status. **In:** *Biofertilizers: Potentialities and Problems*, pp. 237–48, (Sen, S. P and Palit, P., eds). Plant Physiology Forum, Naya Prokash, Calcutta.

Donahue, R.L., Milller, R.W. and Shickluna, J.C. (1990). Soils: An Introduction to Soils and Plant Growth. Prentice Hall of India private Limited, New Delhi, pp: 222–4.

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

Dutton V.M. and Evans C.S. (1996). Oxalate production by fungi: its role in pathogenicity and ecology in the soil environment. Can J. Microbiol. 42: 881-895.

Eckford, M.D. (1927). Thermophillic bacteria in milk. *American J. Hyg.*, **7:**200-201.

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

Ehrlich, H.L. (1990). Mikrobiologische und biochemische Verfahrenstechnik. **In:** *Geomicrobiology* (Einsele, A., Finn, R.K. and Samhaber ,W., eds). VCH Verlagsgesellschaft, Weinheim.

El-Yazeid A.A., Abou-Aly H.A., Mady M.A. and Moussa S.A.M. 2007. Enhancing growth, productivity and quality of squash plants using phosphate dissolving microorganisms (bio phosphor) combined with boron foliar spray. Res. J. Agric. Biol. Sci. 3**(4):** 274-286.

Ethiopian Economic Association (EEA**)** (2002). Second annual report on the Ethiopian Economy, Vol II 2000/2001. Addis Ababa: EEA.

Ezawa, T., Smith, S. E. and Smith, F. A. (2002). P metabolism and transport in AM fungi. *Plant Soil* **244**: 221-230.

FAO. (2004). Production year book.

Fankem H., Nwaga D., Deubel A., Dieng L., Merbach W. and Etoa F.S. (2006). Occurrence and functioning of phosphate solubilizing microorganisms from oil palm tree rhizosphere in Cameroon. Afr J. Biotech. 5: 2450-2460.

Fasim, F., Ahmed, N., Parson, R. and Gadd, G.M. (2002). Solubilization of zinc salts by bacterium isolated from air environment of a tannery. *FEMS Microbiol.Lett.* **213**:1–6.

Feoli1, E., Gallizia, L.V., and Woldu, Z. (2002). Processes of Environmental Degradation and Opportunities for Rehabilitation in Adwa, Northern Ethiopia. Landscape Ecology **17**: 315–325.

Garcia C, Fernandez T, Costa F, Cerranti B, Masciandaro G1992. Kinetics of phosphatase activity in organic wastes. Soil Biol Biochem; **25** :361–5.

Gaur, A.C. and Ostwal, K.P. (1972). Influence of phosphate dissolving Bacilli on yield and phosphate uptake of wheat crop. *Indian J ExpBiol* **10**:393–4.

Gaur, A. C. (1972). Role of Phosphate Solubilizing Microorganisms and Organic Matter in Soil Productivity. Proc.Of Symposium on Soil Fertility, National Academy of Science, India. PP. 259- 268.

Gaur, A. C. (1988). Phosphorus Solubilizing Biofertilizer. Division of Microbiology, Indian Agricultural Research Institute, New Delhi. PP. 45-46.

Gaur, A.C. (1990). Phosphate solubilizing microorganisms as biofertilizers. Omega Scientific Publishers, New Delhi, India, **57**(61), pp114.

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

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

Glick, B.R., Changping, L., Sibdas, G. and Dumbroff, E.B. (1997). Early development of canola seedlings in the presence of the plant growth-promoting rhizobacterium *Pseudomonas putida* GR12-2. *Soil Biol Biochem* **29**:1233–9.

Glick, B.R. (1995). The enhancement of plant growth by free-living bacteria. *Can. J. Microbiol*. **41**: 109–117.

Glick, B.R.(1995). The enhancement of plant growth by free-living bacteria. *Can. J. Microbiol.* **41**:109–117.

Goldstein, A. H. (1994). Involvement of the quinoprotein glucose dehydrogenises in the solubilization of exogenous phosphates by gram-negative bacteria. **In:** *Phosphate in Microorganisms: Cellular and Molecular Biology*, pp. 197-203, (Gorini, A. T., Yagil, E. and Silver,S., eds). ASM Press, Washington, D. C.

Goldstein, A.H., R. D. Rogers and G. Mead.(1993). Mining by microbe, Bio.Technol. 11, Univ. 1250–1254.

Goldstein, A.H. (1986). Bacterial solubilization of mineral phosphates: historical perspectives and future prospects. *Am. J. Altern. Agricult*. **1:** 57–65.

Goldstein, A.H. (1986). Bacterial solubilization of mineral phosphates: historical perspective and future prospects. *Am. J. Altern. Agr*i.**1**: 51–57.

Goldstein, A.H. (1986). Bacterial solubilization of mineral phosphates: historical perspective and future prospects. *Am. J. Altern. Agri*. **1**:51–57.

Goldstein, A.H. (1994). Involvement of the quinoprotein glucose dehydrogenase in the solubilization of exogenous phosphates by gram-negative bacteria. **In:** *Phosphate in Microorganisms:Cellular and Molecular Biology*, pp.197–203, (Torriani-Gorini A, Yagil, E. and Silver, S. eds). ASM Press,Washington, DC.

Goldstein, A.H. (1995). Recent progress in understanding the molecular genetics and biochemistry of calcium phosphate solubilization by Gram negative bacteria. *Biol. Agric. Hort.* **12**:185–193.

Goldstein, A.H. and Liu, S.T. (1987). Molecular cloning and regulation of a mineral phosphate solubilizing gene from *Erwinia herbicola. Bio/Technology* **5**:72-4.

Goldstein, A.H., Braverman, K. and Osorio, N. (1999). Evidence for mutualism between a plant growing in a phosphate-limited desert environment and a mineral phosphate solubilizing (MPS) bacterium. *FEMS Microbiol. Ecol.* **3**: 295–300.

Goldstein, A.H., Braverman, K. and Osorio, N. (1999). Evidence for mutualism between a plant growing in a phosphate-limited desert environment and a mineral phosphate solubilizing (MPS) bacterium. *FEMS Microbiol. Ecol.* **3**:295–300.

Greaves MP, Webley DM. 1965. A study of the breakdown of organic phosphates by microorganisms from the root region of certain pasture grasses. J Appl Bact.**28**:454–65.

Gruhn, P., Goletti, F. and Yudelman, M. (2000). Integrated Nutrient management, soil fertility and sustainable agriculture: Current issues and future challenges. International Food Policy Research Institute, Washington, D.C.

Gulati, A., P. Rahi and Vyas, P. (2007). Characterization of phosphate solubilizing florescent Pseudomonas from the rhizosphere of sea buckthorn growing in the cold desert of Himalayas. *Curr. Microbiol.* **56:**73-79.

Gull, M., Hafeez, F.Y., Saleem, M. and Malik, K. A. (2004). Phosphorus uptake and growth promotion of chickpea by co-inoculation of mineral phosphate solubilizing bacteria and a mixed rhizobial culture. *Aus. J. Exp. Agric*. **44**:623-628.

Gupta, R., Singal, R., Sankar, A., Chander, R.M. and Kumar, R.S. (1994). A modified plate assay for screening phosphate solubilizing microorganisms. *J. Gen. Appl Microbiol* **40**:255–60.

Gyaneshwar, P., Kumar, G. N., Parekh, L. J. and Poole. P. S. (2002). Role of soil microorganisms in improving P nutrition of plants. *Plant Soil* **245**: 83-93.

Haile, W., Fassil, A. and Asfaw, H. (1999). Studies on Phosphate Solubilizing Ability of Bacteria Isolated from Some Ethiopia Soils.

Halder, A.K., Mishra, A.K., Bhattacharya, P. and Chakrabarthy, P.K. (1990). Solubilization of rock phosphate by Rhizobium and Bradyrhizobium. *J. Gen. Appl. Microbiol.* **36:**81–92.

Hall, J.A., Pierson, D., Ghosh, S. and Glick, B.R. (1996). Root elongation in various agronomic crops by the plant growth promoting rhizobacterium *Pseudomonas putida* GR12-2. *Isr J Plant Sci* **44**:37–42.

Hamdali, H., Moursalou, K., Tchangebedji, G., Ouhdoouch,Y.and Hafidi, M. (2011). Isolation and Characterization of Rock Phosphate Solubilizing Actinobacteria from a TopolesePhosphate Mine.African j.Biotech.Volume11 (2).pp.32-320.

Han, H. S. and Lee, K. D. (2005). Phosphate and Potassium Solubilizing Bacteria Effect on Mineral Uptake, Soil Availability and Growth of Eggplant. *Res .J. Agric. Biol. Sci.* **1**(2): 176-180.

Han, H., Supanjani, S. and Lee, K.D. (2006). Effect of co-inoculation with phosphate and potassium solubilizing bacteria on mineral uptake and growth of pepper and cucumber. *Plant Soil Environ*. **52** (3): 130-136.

Hao, X., Cho, C. M., Racz, G. J. and Chang, C. (2002). Chemical retardation of phosphate diffusion in an acid soil as affected by liming. *Nutr. Cycl. Agroecosys.* **64**: 213-224.

Hariprasad, P., Niranjana, S.R.(2009). Isolation and characterization of phosphate solubilizing rhizobacteria to improve plant health of tomato. Plant Soil 316, 13–24.

Harley, J.L. and Smith, S.E. (1983). *Mycorrhizal symbiosis*. Academic Press, London.

Havlin, J., D. Beaton, J., L.Tisdale, S. and L.Nelson, W. (1999). Soil Fertility and Fertilizers: An Introduction to Nutrient management. 6th Ed. Pearson Edition (Singapore) Pte. Ltd., Indian Branch, India

He, Z. L., Bian, W. and Zhu, J. (2002). Screening and identification of microorganisms capable of utilizing phosphate adsorbed by goethite. *Comm. Soil Sci. Plant Anal.* **33**: 647-663.

Hilda, R. and Fraga, R. (1999). Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnol. Adv.* **17**: 319–359.

Hilda, R. and Fraga, R. (1999). Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnol. Adv.* **17**:319–359.

Hwangbo, H., Park, R.D., Kim, Y.W., Rim, Y.S., Park, K.H., Kim,T.H., Suh, J.S., Kim, K.Y., (2003). 2-Ketogluconic production and phosphate solubilization by Enterobacter intermedium. *Curr.Microbiol.* **47**:87–92.

Illmer, P. and Schinner, F. (1995).Solubilization of inorganic calcium phosphates-solubilization mechanisms. Soil Biol. Biochem. 27, 257–263. in soils: roles in genesis and functions. Heidelberg, Germany: Springer-Verlag, 3–18.

Isherword, K. F. (1998). Fertilizer use and environment. **In:** Symp. *Plant Nutrition Management for Sustainable Agricultural Growth*, pp. 57-76, (Ahmed, N. and A. Hamid, A., eds), Proc NFDC, Islamabad.

Jackson, M. L., 1973, *Soil Chemical Analysis*, Prentice Hall of India Pvt. Ltd., New Delhi.

Jilani, G., Akram, A., Ali, R. M., Hafeez, F. Y., Shamsi, I. H., Chaudhry, A. N. and Chaudhry, A. G. (2007). Enhancing crop growth, nutrients availability, economics and beneficial rhizosphere microflora through organic and biofertilizers. *Ann. Microbiol.* **57**: 177-183.

Jones DL, Hodge A, Kuzyakov Y. (2004). Plant and mycorrhizal regulation of rhizodeposition. *New Phytologist* 163: 459–480.

Jones, D.A., Smith, B.F.L., Wilson, M.J. and Goodman, B.A. (1991). Solubilizator fungi of phosphate in rise soil. *Mycol. Res.* **95**:1090– 1093.

Kang, S. C., Hat, C. G. Lee, T. G. and Maheshwari, D. K. (2002). Solubilization of insoluble inorganic phosphates by a soil-inhabiting fungus *Fomitopsis sp.* PS 102. *Curr. Sci.* **82**: 439-442.

Kapulnik J, Gafny R, Okon Y. (1985). Effect of *Azopirillum* spp. inoculation on root development and NO-3 uptake in wheat (*Titicum aestivum* cv. Miriam) in hydroponic systems. Journal. Botany; **63**:627–31.

Katznelson , H., Peterson, E.A. and Rovatt, J.W. (1962). Phosphate dissolving microoganisms on seed and in the root zone of plants. *Can. J. Bot.* **40**:1181–1186.

Kennedy, A.C. (1998). The rhizosphere and spermosphere. In: Sylvia DM, Fuhrmann JJ, Hartel PG, Zuberer DA, eds. Principles and applications of soil microbiology. Upper Saddle River, New Jersey: Prentice Hall, 389–407.

Khiari L. and Parent L.E. 2005. Phosphorus transformations in acid light-textured soils treated with dry swine manure. Can J. Soil Sci. 85: 75-87

Khan, A., G. Jilani, M.S. Akhtar, S.M.S. Naqvi and M. Rasheed. 2009. Phosphorus Solubilizing Bacteria: Occurrence, Mechanisms and their role in crop production. Journal of Agriclture and Biological Sciences, 1(1): 48-58.

Khan, K. S. and Joergensen, R. G. (2009). Changes in microbial biomass and P fractions in biogenic household waste compost amended with inorganic P fertilizers. *Bioresour. Technol.***100**: 303-309.

Kim, K.Y., Jordan, D. and Krishnan, H.B. (1997a). Rahnellaaqualitis, a bacterim isolated from soybean rhizosphere, can solubilize hydroxyapatite. *FEMS Microbiol.Lett.* **153**: 273–277.

Kim, K.Y., Jordon, D. and McDonald, G.A. (1997). Effect of Phosphate- solubilizing bacteria and vesicular – arbuscular mycorrhizae on tomatogrowth and soil microbial activity. *Biology and Fertility of Soils*, **265**(2): 7987.

King, E.O., M.K. Ward and D.E. Raney, 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab Clin. Med., **44**: 301-307.

Kirchner MJ, Wollum AG, King LD. (1993). Soil microbial populations and activities in reduced chemical input agroecosystems. Soil Sci Soc Amer J; **57**:1289–95.

Kloepper, J.W., Lifshitz, K. and Schroth, M.N. (1988). *Pseudomonas* inoculants to benefit plant production. ISI Atlas Sci Anim Plant Sci. pp. 60–4.

Kloepper, J.W., Lifshitz, K. and Zablotowicz, R.M. (1989). Free-living bacterial inocula for enhancing crop productivity. *Trends Biotechnol* **7**:39–43.

Kloepper, J.W. (1994). Plant growth promoting bacteria (other systems). In: Okon J, editor. Azospirillum/Plant Association. Boca Raton, FL: CRC Press, pp. 137–54.

Konde, B. K., Tamble, A. D. and Ruikar, S. K. (1988). Yield of nitrogen and phosphorusuptake by onion as influenced by inoculation of VAM fungi and *Azospirillum brasilense*. **In** : *Mycorrhiza for Green Asia, Proceedings of the First Asian Conference on Mycorrhiza*, University of Madras, Chennai, India, 29-1 January 1989 (Eds. Mahadevan A., Raman, N. and Natarajan, K.), pp. 222-224.

Kpomblekou, K. and Tabatabai, M.A. (1994). Effect of organic acids on release of phosphorus from phosphate rocks.*Soil Sci.* **158**: 442– 453.

Krasilinikov, N. A. (1957). On the role of soil micro-organism in plant nutrition. *Microbiologiya* **26**: 659-672.

Krieg, N.R. and Holt, J.G. (1989). Bergey's manual of systematic bacteriology. Williams, Baltimore and London, Pages: 964

Kucuk, O. (2001). Phase II randomized clinical trial of lycopene supplementation before radical prostatectomy. *Cancer Epidem. Biom. Prev.* **10:** 861-868.

Kucey, R.M.N., H.H. Jenzen and M. Leggett(1989). Microbially mediated increases in plant available phosphorus. Adv. Agron., **42**, 199-228.

Kudashev, I. S. (1956). The effect of phosphobacterin on the yield and protein content in grains of Autumm wheat, maize and soybean. *Doki. Akad. Skh. Nauk.* **8**: 20-23.

Kucharski J, Ciecko Z, Niewolak T, Niklewska-Larska T. (1996). Activity of microrganisms in soils of different agricultural usefulness complexes fertilized with mineral nitrogen. Acta Acad Agric Technicae-Olstenensis; **62**:25–35.

Lee K.E. and Pankhurst C.E. 1992. Soil organisms andsustainable productivity. Australian J. Soil Res. **30**: 855-92.

Lemanceau, P. (1992). Effects benefiques de rhizobacteries sur les plantes: exemple des *Pseudomonas* spp. fluorescent. *Agronomie* **12**:413–37.

Lemma, D., Yayeh, Z. and Herath, E. (1992). Agronomic Studies in Tomato and Capsicum. *In*: Herath and Lemma (eds.). Horticulture Research and Development in Ethiopia: Proceedings of the Second National Horticultural Workshops of Ethiopia. 1-3 December. Addis Ababa, Ethiopia. pp 153-163.

Lifshitz, R., Kloepper, J.W., Kozlowski, M., Simonson, C., Carlson, J., Tipping, E.M. and Zalesca, I. (1987). Growth promotion of canola (rapeseed) seedlings by a strain of Psedomonasputida under gnotobiotic conditions. *Can J Microbiol* **33**:390–5.

Lifshitz, R., Kloepper, J.W., Kozlowski, M., Simonson, C., Carlson, J., Tipping, E.M. and Zalesca, I. (1987). Growth promotion of canola (rapeseed) seedlings by a strain of Psedomonasputida under gnotobiotic conditions. *Can J Microbiol* **33**:390–5.

Linderman, R.G. (1992). Vesicular–arbuscular mycorrhizae and soil microbial interactions. **In**: Bethlenfalvay GJ, Linderman RG, eds. Mycorrhizae in sustainable agriculture. Madison, Wisconsin: ASA Special Publication, 45–70.

Lindsay, W.L. (1979). *Chemical Equilibrial in Soil.* John Wiley and Sons, New York.

Louw, H.A. and Webley, D.M. (1956). A study of soil bacteria dissolving certain phosphate fertilizers and related compounds. *J.Appl.Bacteriol*. **22**: 227–33.

Lynch, J.M. (1990). The rhizosphere. New York: John Wiley.

Marschner, P., Joergensen, R.G., Piepho, H.P. and Buerkert, A. (2004b). Legume rotation effects on early growth and rhizosphere microbiology of sorghum in West African soils. *Plant and Soil* **264**: 325–334.

Marzouk, E.R.E., (2002). Effect of organic residues on phosphorus availability in some soils of North Sinai. M.Sc. Thesis. Fac. Environ. Agric. Sci., El- Arish, Suez Canal Univ.,

Egypton phosphorus availability in some soils of North Sinai. M.Sc. Thesis. Fac. Environ. Agric. Sci., El-Arish, Suez Canal Univ., Egypt.

Mehasen, S.A.S., Zaghloul, R.A. and El-Ghozoli, M.A. (2002). Effectiveness of dual inoculation with *Bradyrhizobium* and endomycorrhizae in presence of different phosphatic fertilizer sources on growth and yield of soybean. *Annals Agric. Sci., Ain Shams Univ., Cairo*, **47**(2): 477-500.

Mehrvarz, S., M. R. Chaichi and H. A. Alikhani. 2008. Effects of phosphate solubilizing microorganisms and phosphorus chemical fertilizer on yield and yield components of Barely (*Hordeum vulgare* L.). Am-Euras. J. Agric. & Environ. Sci. 3:822-828.

Mehla, C.P., Srivastava, V.K., Jage, S., Mangat, R., Singh, J. and Ram, M. 2000. Response of tomato varities to N and P fertilization and spacing. Indian Jornal of Agricultural Research. **34** (3): 182-184.

Nannipieri, P., Ascher, J., Ceccherini, M.T., Landi, L., Pietramellara, G. and Renella, G. (2003). Microbial diversity and soil functions. European Journal of Soil Science 54, 655–670.

Nautiyala, C.S., P. Bhadauria, Kumar, H. Lal and R. Mondal. (1999). Stress induced phosphate solubilization in bacteria isolated from alkaline soils. *FEMS Microbiology Letters* **182**: 291-296.

Nelson, W.L. (1978). Phosphorus and moisture. In Phosphorus for Agriculture. Phosphate and Potash Institute, Atlanta, GA. pp.160.

Ochs, M., (1996). Influence of humidified and nonsolubilizingbacteria and vesicular mycorrhizal fungi humidified natural organic compounds on mineral with and without rock phosphate on four forest tree dissolution. *Chemical Geol.* **132**: 119-124.

Olsen, S. Cole, R., Watanable, F.S. and Dean, L.A. (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. *Circ. U.S. Dep. Agric.* pp939.

Ostwal, K.P. and Bhide, V.P. (1972). Solubilization of tricalcicum phosphate by soil *Pseudomonas. Indian J Exp Biol* **10**:153–4.

Pace, N.R. (1997). A molecular view of microbial diversity in the biosphere. *Science* **276**:734–740.

Pal, S.S. (1998). Interaction of an acid tolerant strain of phosphate solubilizing bacteria with a few acid tolerant crops. *Plant Soil*, **198**: 167–77.

Pandey, R.P, Solanki, P.N, Saraf R.K and Parihar, M.S. 1996. Effect of Nitrogen and Phosphorus on growth and yield of tomato varieties. Punjab Vegetable Grower. 31: 1-5.

Paul, E.A. and Clark, F.E. (1988). Soil Microbiology and Biochemistry. Academic Press, San Diego, CA.

Paul, N. B. and SunduraRao, W. B. (1971). Phosphate Dissolving Bacteria in Rhizosphere of Some Cultivated Legumes. *Plant Soil* **35**: 127-132

P i k o v s k a y a, R. I. (1 9 4 8). Mobilization of phosphorus in soil in connection with vital activity of some microbial species, Microbiology **17**: 362 70.

Ponmurugan, P. and Gopi, C. (2006). Distribution Pattern and Screening of Phosphate Solubilizing Bacteria Isolated from Different Food and Forage Crops. *J.Agron.* **5:**600-604.

Pradhan, N. and Sukla, L. B. (2005). Solubilization of inorganic phosphate by fungi isolated from agriculture soil. *African J. Biotechnol*. **5**: 850-854.

Raghu, K. and MacRae, I.C. (1966). Occurrence of phosphate-dissolving microorganisms in the rhizosphere of rice plants and in submerged soils. *J. Appl. Bacteriol*. **29**: 582–586.

Rashid, M., S. Khalili, N. Ayub, S. Alam and F. Latif. 2004. Organic acid production and phosphate solubilization by phosphate solubilizing microorganisms (PSM) under in vitro conditions. *Pakistan Journal of Biological Science* **7**:187 196.

Reddy, T.Y. and Reddi, G.H.S. (2002). Principles of agronomy.Kalyani Publishers, 3rd Edn. India, pp: 526.

Rengel Z. (2002). Genetic control of root exudation. *Plant and Soil* **245**: 59–70.

Reyes, I., A. Valery and Z. Valduz, (2006). Phosphate-solubilizing microorganisms isolated from rhizospheric and bulk soils of colonizer plants at an abandoned rock phosphate mine. *Plant and Soil* **287**(1-2): 69-75.

Reyes, I., Bernier, L., Simard, R. and Antoun, H. (1999). Effect of nitrogen source on solubilization of different inorganic phosphates by an isolate of Pencilliumrugulosum and two UV-induced mutants.FEMS Microbiol. Ecol. **28**:281–290.

Richardson, A.E. (1994). Soil microorganisms and phosphorus availability. In: *Soil Biota, Management in Sustainable Farming Systems*, pp. 50–62, (Pankhurst, C.E., Doube , B.M., Grupta, V.V.S.R. and Grace, P.R., eds). Melbourne, Australia: CSIRO.

Richardson, A. E. (2001). Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. *Aust. J. Plant Physiol*. **28**: 897-906.

Rodríguez, H. and Fraga, R. (1999). Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnol. Adv.* **17**:319-339.

Rokhzadi A., Asgharzadeh A., Darvish F., Nourmohammadi G. and Majidi E. 2008. Influence of plant growth-promoting rhizobacteria on dry matter accumulation and yield of chickpea (*Cicer arietinum* L.) under field condition. Am-Euras. J. Agric. Environ. Sci. 3(**2):** 253-257.

Sadana, U.S., Kusum L. and Claassen, N. (2002). Manganese efficiency of wheat cultivars as related to root growth and internal manganese requirement. *Journal of Plant Nutrition* **25**: 2677–2688.

Sagoe C., Ando T., Kouno K. and Nagaoka T. (1998). Relative importance of protons and solution calcium concentration in phosphate rock dissolution by organic acids. Soil Sci. Plant Nutr. **44**: 617-625.

Sarig, S., Okon, Y., and Blum, A. (1990). Promotion of leaf area development and field in *Sorghum bicolor* inoculated with *Azospirillum brasilense. Symbiosis* **9**:235–45.

Shahram, s. (2012). Effect of PGPR on growth and nutrient uptake of tomato. International journal of advances in engineering and technology, **27**(2):pp1-31.

Sharma K, Dak G, Agrawal A, Bhatnagar M, Sharma R (2007). Effect of phosphate solubilizing bacteria on the germination of *Cicer arietinum* seeds and seedling growth. J. Herb. Med. and Toxicol. **1**:61-63.

Sherma, K.C., Singh, A.K. and Sharma, S.K. 1999. Studies on Nitrogen and Phosphorus requirement of tomato hybrids. Annals of Agricultural Research. **20 (4):** 339-402.

Sperberg, J.I. (1958). The incidence of apatite-solubilizing organisms in the rhizosphere and soil. *Aust. J. Agric. Res*. **9**:778.

Stewart, J.W.B., Hedley, M. J. and Chauhan, B.S. (1980). The immobilization, mineralization, and redistribution of phosphorus in soils. Pages 276-306 in Western Canadian Phosphate Symposium Proceedings. Alta. Soil Sci. Workshop, Calgary, Alta.

Son TTN, Diep NC, Giang TTM. (2006). Effect of Bradyrhizobia and phosphate solubilizing bacteria application on soybean in rotational system in the mekong delta, Omonrice. **14**:48-57.

Subba Rao, N. S., Tilak, K. V. B. R. and Singh, C. S. (1985a). Effect of combined inoculation of *Azospirillum brasilense* and vesicular-arbuscular mycorrhiza on pearl millet (*Pennisetum americanum*). *Plant and Soil*, **84** : 283-286.

Subba Rao, N. S., Tilak, K. V. B. R. and Singh, C. S. (1985b). Synergistic effect of vesicular-arbuscular mycorrhizas and *Azospirillum brasilense* on the growth of barley in pots. *Soil Biology and Biochemistry* **17:** 119-121.

SubbaRao, N.S. (1982). Advances in agricultural microbiology. In: SubbaRao NS, editor. Studies in the Agricultura and Food Sciences. London: Butterworth Scientific pp. 295–303.

SubbaRao, N.S.S. (1984). Phosphate solubilizing microorganisms In: Biofertilizers in agriculture. 2nd Edn., Oxford and IBH publishing Co. New Delhi, Bombay, Calcutta, India. pp. 126-132

Subbarao N.S. (1988). Phosphate solubilizing microorganism. **In**: Biofertilizer in agriculture and forestry. Regional Biofert. Dev. Centre, Hissar, India. pp. 133-142.

Sundram, K. P. (1994). Fertilizers News, Fertilizer Association of India. New Delhi.

Sundara B, Natarajan V, Hari K (2002). Influence of phosphorus solubilizing bacteria on the changes in soil available phosphorus and sugarcane and sugar yield. Field Crop Res. **77**:43-49.

Sundara Rao WVB, Sinha MK. Phosphate dissolving micro-organisms in the soil and rhizosphere. Indian J. Agric Sci 1963;**33**: 272–8.

Surange, S., A. G. Wollum, N. Kumar and C. S. Nautiyal. 1995. Characterization of *Rhizobium* from root nodules of leguminous trees growing in alkaline soils. Can. J. Microbiol. **43**:891- 894.

Suslov, T.V. (1982). Role of root-colonizing bacteria in plant growth. In: Mount MS, Lacy GH, editors. Phytopathogenic Prokariotes. London: Academic Press. pp. 187–223.

Tantaway, M.E. and Mohamed, M. (2009). Effect of inoculation with phosphate solubilizing bacteria on the tomato rhizosphere colonization process, plant growth and yield under organic and inorganic fertilization. *J. Appl. Sci. Res.* **5:**1117-113.

Tartdar, J. C, Claassen N. (1987). Organic phosphorus compounds as a phosphorus source for higher plants through the activity of phosphatases produced by plant roots and microorganisms. Biol Fertil Soils **5**:308–12.

Theodorou, M.E. and Plaxton, W.C. (1993). Metabolic adaptations of plant respiration to nutritional phosphate deprivation. *Plant Physiol* **101:** 339–344.

Tilahune, A., Endrias, G. and Takele, B. (2007). Reversing the degradation of arable land in Ethiopian highlands, Managing Africa's soil. No. 23, IIED-London.

Toal, M.E., Yeomans, C., Killham, K. and Meharg, A.A. (2000). A review of rhizosphere carbon flow modelling. *Plant and Soil* **222**:263–281.

Turan, M.A., Turkmen, N. and Taban, N. (2007a). Effect of NaCl on stomatal resistance and proline, chlorophyll, Na, Cl and K concentrations of lentil plants. *J. Agron.* **6**:378-381.

United State Department of Agriculture (USDA), (1963). Composition of Foods. USDA Agricultural Handbook No. 7.

Upendra, M., Ramdane, D. and Bharat, s. (2003). Mineral nutrition of tomato. Agricultural Research Station, USA.

Valerie,G., Hani,A.and Ressel, J.(2007). Growth simulation and fruit yield improvement of green house tomato plants by inoculation with *pseudomonas putida. soil Biology and biochemistry*. **39**:1968-1977.

Van Kauwenbergh, SJ. 2006. *Fertilizer Mineral Resources of Africa,* IFDC Publication R-16, IFDC – An International Center for Soil Fertility and Agricultural Development, Muscle Shoals, Alabama, 435 p.

Vassileva, M., Vassilev, N. and Azcon, R. (1998). Rock phosphate solubilization by *Aspergillus niger* on olive cake-based medium and its further application in a soil–*plant system World J. Microbiol. Biotech*., **14**: 281-284.

Venkatasalam, E.P. and Krishnasamy, V. (2005). Effect of macronutrients on seed yield and yield attributing characters of parental lines tomato hybrid Coth. 1. Madras Agric. J. **92** (10-12): 629- 634.

Venkatasalam, E.P. and V. Krishnasamy, (2005). Effect of macronutrients on seed yield and yield attributing characters of parental lines tomato hybrid Coth. 1. *Madras Agric. J*. **92** (10 12): 629- 634.

Verma, L. N. (1993). Biofertiliser in agriculture. In: P. K. Thampan (ed.) Organics in soil health and crop production. Peekay Tree Crops Development Foundation, Cochin, India. pp. 152-183

Viswanathan,G., Panchaksharam,T., and Kurusangu, V. (2011). Influence of AMF and Azospirulum coinoculation on the groth and yield of tomato crops in South India. *Indian journal of fundamental and Applied sciences*.**1**(4). pp 84-92.

Von Uexkull, H.R. (1979). Tomato nutrition and fertilizer requirements in the tropics. First International Symposium on Tropical Tomato. p. 65-78.

Wani S.P., Rego T.G., Rajeshwari S. and Lee K.K. (1995). Effect of legume–based cropping systems on nitrogen mineralization potential of Vertisol. Plant Soil. 175(2): 265-274.

Werner, D. (1998). Organic signals between plants and microorganisms. **In**: Pinton R, Varanini Z, Nannipieri P, eds. The rhizosphere: biochemistry and organic substances at the soil-plant interfaces. New York: Marcel Dekker Inc., 197–222.

Whitelaw M.A. (2000). Growth promotion of plants inoculated with phosphate solubilizing fungi. Adv Agron; 69: 99-151.

Xu JG, Johnson RL.(1996). Root growth, microbial activity and phosphatase activity in oil-contaminated, remediated and uncontaminated soils planted to barley and field pea. Plant Soil. 1995; 173:3–10.

Yazdani, M., Bahmanyar, M. A., Pirdashti, H. and Esmaili. M. A. (2009). Effect of Phosphatesolubilization microorganisms (PSM) and plant growth promoting rhizobacteria (PGPR) on yield and yield components of Corn (*Zea mays L.). Proc. World Acad. Science, Eng. Technol.* **37**: 90-92.

Yahya A. and Azawi S.K.A. (1998). Occurrence of phosphate solubilizing bacteria in some Iranian soils. Plant Soil. 117: 135-141.

Young, C.C. (1994). Selection and application of biofertilizers in Taiwan.Food and Fertilizer Technology Center. *Tech. Bull.* **14**:1–9.

Young, C.C., Chang, C.H., Chen, L.F. and Chao, C.C. (1998). Characterization of the nitrogen fixing and ferric phosphate solubilizing bacteria isolated from Taiwan soil. *J. Chin. Agricult. Chem. Soc.* **36**: 201–210.

Young, C.C., Chang, C.H., Chen, L.F. and Chao, C.C. (1998). Characterization of the nitrogen fixing and ferric phosphate solubilizing bacteria isolated from Taiwan soil. *J. Chin. Agricult.Chem. Soc.* **36**:201–210 (in Chinese).

Zaidi, A. (1999). Synergistic interactions of nitrogen fixing microorganisms with phosphate mobilizing microorganisms, Ph.D. Thesis, Aligarh Muslim University, Aligarh.

Zaidi, A. and M. S. Khan. (2006). Co-inoculation effects of phosphate solubilizing microorganisms and *Glomus fasciculatumon* on green gram - *Bradyrhizobium* symbiosis. Turk. J. Agric.30:223-230.