2. REVIEW OF LITERATURE
Phosphorus is a vital nutrient for plants and microorganisms next to nitrogen. Phosphorus is one of the major essential macronutrients for biological growth and for proper plant development (Ehrlich, 1990; Dave and Patel, 1999). The concentration of soluble phosphorus in soil is usually very low, normally at levels of 1 ppm or less (Mandal and Khan, 1972; Beever and Burns, 1980; Goldstein, 1994; Rodriguez and Fraga, 1999). Also phosphorus is probably one of the least available plant nutrients found in the rhizosphere (Raghothama and Kartikeyan, 2005). Phosphorus often limits plant growth and productivity (Lewis and Sale, 1993). It accounts for about 0.1% of the terrestrial matter (Sundara Rao, 1968). Bacteria and plants must often obtain their phosphorus from the external environment in a soluble ionic form has metabolic consequences since phosphorus is one of the least soluble essential nutrient ions in the environment (Goldstein and Liu, 1987).

2.1. Phosphorus in soils

Phosphorus compounds exist in soils as both organic and inorganic forms. Phosphorus occurs in soil as inorganic phosphate produced by weathering of parent rock or as organic phosphorus derived from plant, animal and microbial residues. Most inorganic phosphorus compounds in soils fall into one of the two groups viz., those containing calcium and those containing iron and aluminium.

In most agricultural soils, organic phosphorus comprises 30-80% of total phosphorus. The largest fraction of organic phosphorus approximately 50% appears to be in the form of phytin and its derivatives (Pederson, 1953; Dalal, 1978). The organic
phosphates containing compounds are derived from plants and microorganisms and are composed of nucleic acids, phospholipids and phytin. Organic phosphates accumulate in many soils and half were present as inositol polyphosphates especially \textit{myo}-inositol hexaphosphate (Anderson, 1967). Usually less than 5\% of total soil phosphorus was available to plants. Although many microorganisms can hydrolyze \textit{myo}-inositol hexaphosphate, the inositol phosphates accumulate in soil. So there must be factors preventing hydrolysis (Greaves and Webley, 1969; Cosgrove, 1970).

Water soluble phosphates present in the soil are only absorbed by plants. But, the water soluble forms are present in extremely small quantities. Though, water soluble phosphatic fertilizers are applied to the soil, they are converted into insoluble forms due to interaction between added soluble phosphates and soil constituents. Phosphorus deficiency in soil is met by the addition of phosphatic fertilizers, but its utilization efficiency of plant is very low (15-25\%) due to fixation of phosphorus as iron or aluminium phosphate in acid and alkaline soils (Tomar \textit{et al.}, 1994).

The biggest reserves of phosphorus were rocks and other deposits, such as primary apatites and other primary minerals formed during the geological age (Odum, 1986). It was estimated that there were almost 40 million tons of phosphatic rock deposits in India (Roychoudury and Kaushik, 1989) and this provides a cheap source of phosphate fertilizer for crop production (Halder \textit{et al.}, 1990). However, Biswas \textit{et al.} (1996) reported that there are nearly 260 million tons of rock phosphate deposits in India, which owing to poor quality were unsustainable for utilization (Dinesh \textit{et al.},
But the slow dissolution rate can be corrected using PSMOS (Vora and Shelat, 1998). The amount of nutrients released from RP also varies among different plants (Habib et al., 1999) and differences in mineral sources (Nahas, 1996; Reddy et al., 2002). Highly soluble phosphate fertilizers tend to quickly bind to soil iron and aluminium (Griffith et al., 2003). Besides, most agricultural soils contain large reserves of phosphorus which get accumulated as a consequence of regular applications of phosphorus fertilizers (Richardson, 1994).

Soil phosphates are rendered available either by plant roots or by soil microorganisms through secretion of organic acids. Phosphate solubilizing microorganisms (PSMOS) play an important role in correcting phosphorus deficiency of plants. They may also release soluble inorganic phosphate into soil through decomposition of phosphate rich organic compounds. Many bacteria, fungi, and actinomycetes are potential solubilizers of bound phosphates in soil, thus playing an important role in soil by solubilizing phosphorus and making it available to plants.

2.2. Phosphate solubilizing microorganisms (PSMOS)

2.2.1 Phosphate solubilizing microorganisms (PSMOS) in soils

The rate of mineralization of organic phosphorus depends largely upon the population as well as the activity of microorganisms in soil. Microorganisms capable of extensively decomposing organic and inorganic phosphorus compounds have been reported to be present in soil. The occurrence and numbers of phosphate solubilizing organisms in soils have been extensively reviewed (Kucey et al., 1989; Rokade and
Phosphate solubilizing bacteria are commonly found in most soils (Chhonkar and Tarafdar, 1984; Venkateswarlu et al., 1984). Species of Aspergillus, Penicillium, Mucor, Rhizopus (Casida, 1959; Irving and Cosgrove, 1972) produce phosphatases, the enzyme that degrades phosphorus compounds (Raghu and MacRae 1966; Bishop et al., 1994; Abd-Alla, 1994) reported the occurrence of large number of aerobic and anaerobic phosphate solubilizing bacteria (PSB) in two rice soils. They observed that P dissolving bacteria were greatest in the rhizosphere rice during transplanting stage.

PSMOS in coconut plantation soils were isolated by Thomas et al. (1985). Thomas and Shantaram (1986) also studied phosphate-solubilizing organisms from coconut plantation soils of Kerala. The importance of soil microorganisms in mobilizing soil phosphorus for utilization by plants was emphasized (Richardson, 2001, 2003).

Al–Ghazali et al. (1986) tested 51 aerobic microbial cultures isolated from Alkhair river sediments for their phosphate solubilizing ability. The isolates were identified as *Enterobacter*, *Pseudomonas* and two isolates of *Aeromonas* species and reported that all the isolates were able to solubilize both di – and tricalcium phosphates.

Patgiri and Bezbaruah (1990) isolated 46 strains of aerobic heterotrophic bacteria from tea soils in Assam. Only *Bacillus subtilis*, *B. licheniformis*, *B. cereus*, *Pseudomonas* species and *P. putrefaciens* possessed significant phosphate solubilizing and phosphatase activity. Rajarathinam et al. (1995) isolated phosphate solubilizers from soils of Kamarajar district, Tamilnadu. *Bacillus megaterium*, *B. polymyxa* and *P. stutzeri* were the most efficient P solubilizers in the soils of the district. Haque and Dave (2005) studied ecology and diversity of phosphate-solubilizing microorganisms in 20 soil samples comprising organic and non-oraganic farming, virgin and barren soils of Gujarat. Out of 40 phosphate-solubilizing microorganisms *Pseudomonas* spp. *Bacillus* spp. *Saccharomyces* spp. and *Aspergillus niger* were found to be most prevalent.
2.2.2 Phosphate solubilizing microorganisms (PSMOS) in rhizosphere soil samples

The region of the soil which is subject to the influence of plant roots is called rhizosphere. Rhizosphere is characterized by the greater microbiological activity than the soil away from plant roots i.e., non-rhizosphere area. The methods adapted to study microbial communities and their interactions in soil and rhizosphere ecosystems have been reviewed (Kent and Triplett, 2002).

There is a continuum of bacterial presence in soil → rhizosphere → rhizoplane → internal the plant tissues (Hallmann et al., 1997). Root-soil contact is an important factor for uptake of a less mobile soil nutrient such as phosphorus by crop plants. Root hairs can substantially increase root-soil contact. Identification of crop cultivars with more or longer root hairs can be useful for increasing phosphorus uptake in low input agriculture (Gahoonia et al., 1997). Bacteria living in the soil are called free-living as they do not depend on root exudates for their survival. Rhizospheric bacterial communities have efficient systems for uptake of organic compounds present in root exudates (Barraquio et al., 2000). Louw (1970) isolated efficient phosphate-solubilizing bacteria from rhizosphere and rhizoplane of wheat and lupin. Most of the bacteria isolated were Gram negative, non-spore forming, short rods.

Plant rhizosphere supports large populations of soil microorganisms and contains a wide range of plant and microbial exudates and metabolites. Thus,
Review of Literature

depending on the nature and concentrations of organic constituents of exudates and the corresponding ability of the bacteria to utilize these as sources of energy, the bacterial community develops in the rhizosphere (Curl and Truelove, 1986). In particular, organic acids were common constituents and were effective in releasing soil phosphorus through a number of mechanisms (Jones, 1998; Ryan et al., 2001).

The symbiotic associations between plant roots and mycorrhizal fungi were able to acquire phosphorus by plants (Marschner and Dell, 1994; Smith and Read, 1997). Indirect interactions between soil microorganisms and plant roots may also influence root architecture. For example, it was commonly reported that phytohormones produced by microorganisms can alter root branching and root hair development and thus has an impact on phosphorus acquisition (Holguin et al., 1999). Strains of *Bradyrhizobium* solubilized different amount of phosphates, both hydroxyapatite and tricalcium phosphate in liquid cultures (Halder et al., 1991). A considerably higher concentration of phosphate solubilizing bacteria was commonly found in the rhizosphere in comparison with non-rhizosphere soil (Katznelson et al., 1962; Raghu and MacRae, 1966).

Phosphatases play key role in transforming organic forms of phosphorus into plant available inorganic forms. They proved to be active in all the three components of rhizosphere soil (Tarafdar and Claassen, 1988), microorganisms (Tarafdar et al., 1988) and plant roots (Tarafdar and Jungk, 1987; Tarafdar et al., 2003).
Low phosphorus availability was one of the most important factors limiting plant growth in red soils across southeastern China. Many non-symbiotic microorganisms in rhizosphere can enhance phosphorus solubility. The number of phosphorus-solubilizing microorganisms and their phosphorus solubilizing ability in rhizosphere soils of 19 weed species in a citrus orchard on red soil at Changshan, Zhejiang, China, were investigated. Inorganic and organic phosphorus were used as phosphorus source to examine the phosphorus solubilizing ability of isolated microorganisms. The highest number of microorganisms was found in the rhizosphere soils (Xin et al., 2002).

During the study of phosphate solubilizing potential of microorganisms commonly encountered in the rhizosphere and non-rhizosphere soils of rye, grass, and rice, Ali et al. (1986) reported the superiority of Agrobacterium, Bacillus, Aspergillus, Penicillium, and Streptomyces in rhizosphere soils over all other non-rhizosphere cultures in phosphate solubilization. Very active microbiological process of dissolution of inorganic phosphorus by microorganisms was observed by Armor et al. (1986) in the rhizosphere of Brassica campestris. Craven and Hayasaka (1982) observed the greater inorganic phosphate solubilizing potential in the rhizosphere of actively growing Zostera marine plants than those of dormant plants. Rosa et al. (1984) isolated PSMOS able to solubilize calcium phosphates from the soil samples collected from the rhizosphere of different plants.
Katznelson et al. (1962) found that 40 to 70% of bacterial isolates obtained from seed coat surfaces of many plants showed the ability to solubilize phosphorus in agar media, but only 10% of the isolates from the rhizoplane and rhizosphere showed this ability. Baya et al. (1981) observed the rhizospheric PSB to be more active than those isolated from non-rhizosphere soil. Thakkar et al. (1993) isolated PSB from the rhizosphere of different crops such as millets, brinjal, rice, carrot, mustard and from compost and identified the bacteria. Enterobacter aerogenes was found to be the most effective tricalcium phosphate (TCP) and rock phosphate (RP) solubilizers. The greater ability to dissolve tricalcium phosphate by two strains of Pseudomonas and Achromobacter isolated from soils and the rhizosphere of lupin, oat and potato was reported by Myskow (1960).

Plants can absorb phosphorus from soils, as the major forms of phosphorus in soils are sparingly soluble. One hypothesis is that root exudates are capable of dissolving phosphate minerals. This is because a number of organic acids have been detected in root exudates of many plants grown under sterile conditions (Swenson et al., 1949; Struthers and Sieling, 1950; Bradley and Sieling, 1953; Johnston, 1952, 1954a, 1954b, 1956, 1959a, 1959b).

A greenhouse study was conducted with winter maize on ten calcareous soils collected from nine soil series of Punjab. Crop responded to both native and applied phosphorus. Ca-P was the dominant inorganic P fraction followed by Al-P and
Fe-P. Among different indices of P availability, Olsen's soil test method was found to be the most suitable (Vig et al., 2000).

The most efficient phosphate-solubilizing microorganisms belong to genera *Bacillus* and *Pseudomonas* amongst bacteria and *Aspergillus* and *Penicillium* amongst fungi (Tilak et al., 2005). The reported bacilli include *Bacillus brevis*, *B. cereus*, *B. circulans*, *B. firmus*, *B. licheniformis*, *B. megaterium*, *B. mesentericus*, *B. mycoides*, *B. polymyxa*, *B. pumilis*, *B. pulvifaciens* and *B. subtilis* from the rhizosphere of legumes, cereals (rice and maize), palm, oat, jute and chillies (Sundara Rao and Sinha, 1963; Taha et al., 1969; Barea et al., 1976; Banik and Dey, 1981; Venkateswarlu et al., 1984; Sattar and Gaur, 1985; Gaind and Gaur, 1999; Rajarathinam et al., 1995; Bhattacharya et al., 1998; Kole and Hazra, 1997, 1998). *Pseudomonas striata*, *P. cissicola*, *P. fluorescens*, *P. pinophillum*, *P. putida*, *P. syringae*, *P. aeruginosa*, *P. putrefaciens* and *P. stutzeri* have been isolated from the rhizosphere of *Brassica*, chickpea, maize, soybean and other crops (Bardiya and Gaur, 1974; Kole and Hazra, 1997; Nair and Subba Rao, 1977; Gupta et al., 1998; Pal et al., 2000). The data on abundance of PSB in rhizosphere soils of Marathwada region indicated that *Bacillus* species is the predominant bacterium in the soils of Latur, Osmanabad, and Parbhani whereas *Pseudomonas* species is the predominant one in the soils from Aurangabad to Nanded district (Bilolikar et al., 1996). A phosphobacterial strain (*Pseudomonas* sp.) isolated from rhizospheric soil of grasses growing spontaneously in Spanish soil actively solubilized phosphates *in vitro* when
bicalcium phosphate was used as a phosphorus source (Peix et al., 2003; 2004). A xylanolytic phosphate solubilizing bacterium, *Microbacterium ulmi* was isolated from sawdust of *Ulmus nigra* in Salamanca (Rivas et al., 2004).

Bacteria were isolated from the rhizosphere of cotton, wheat, alfalfa, and tomato grown in field locations in a semi-arid region of Uzbekistan. Strains were identified as *Pseudomonas denitrificans*, *P. rathonis*, *Bacillus laevolacticus*, *B. amyloliquefaciens* and *Arthrobacter simplex*. All of the bacterial strains isolated in this study have been found to increase plant growth of wheat and maize in pot experiments (Egamberdiyeva, 2005). Two isolates of *Pseudomonas corrugata*, *P. corrugata* 1, a rhizosphere associate and *P. corrugata* 7, a rhizoplane associate have been isolated and characterized from maize soils. These isolates were from the subtropical and temperate regions, respectively in Sikkim and Himalaya. The two isolates have been found to be positive for the production of antifungal compounds, phosphate solubilizing activity, nitrogenase activity, and growth at 4°C under laboratory conditions. These bacteria produce a non-fluorescent yellow pigment, particularly at low temperature (Pandey et al., 1998). Paul and Sundara Rao (1971) isolated 12 phosphate-solubilizing bacteria from the rhizosphere of four cultivated legumes from different soil regions of India. The active organisms were identified as strains of *Bacillus subtilis*, *B. brevis*, *B. pulvifaciens*, *B. pumilis*, and *B. polymyxa*. 
2.3. Extent of solubilization of phosphate by microorganisms

Microorganisms are involved in a range of processes that affect the transformation of soil phosphorus and are thus an integral component of soil phosphorus cycle. In particular, soil microorganisms were found to be effective in releasing phosphorus through solubilization and mineralization (Richardson, 2001). Many microorganisms have the potential to bring the phosphates into soluble form. This attribute was not rare since 10 to 50% of the bacterial isolates tested were capable of solubilizing calcium phosphates (Rokade and Patil, 1992). Species of *Pseudomonas, Bacillus, Micrococcus, Flavobacterium, Penicillium, Aspergillus* and *Sclerotium* and others were involved in this process.

*Bacillus subtilis, B. megaterium, B. mesentericus* and *B. mycoides* carried out solubilization of phosphates from different insoluble phosphates (Sen and Paul, 1957). Muramtsev (1958) observed that microorganisms can dissolve calcium phosphate in acid, neutral and alkaline culture media. *In vitro* screening of several forest nursery seed bed fungi revealed that *Penicillium* sp., *Aspergillus niger, A. flavus, Fusarium oxysporum, Sclerotium rolfsii* and *Cylindrocladium* were effective in dissolving phosphorus in tricalcium phosphate, hydroxyapatite and fluoroapatite (Agnihotri, 1970). Likewise, *in vitro* experiments carried out by Gostowska (1976) with 73 different strains of *Rhizobium* demonstrated that *Rhizobium meliloti, R. lupini* and *R. leguminosarum* were capable of dissolving tricalcium phosphate and ferrous phosphate. Further, solubilization of phosphatic compounds and inorganic phosphate
by *Rhizobium* sp. (Halder *et al.*, 1990a; Halder and Chakrabarty, 1993) and *Azospirillum halopraeferans* (Seshadri *et al.*, 2000) has also been reported.

Several studies were conducted to determine the extent of phosphorus solubilization by different types of microorganisms including bacteria and fungi. Phosphate solubilization by thermophilic microorganisms including bacteria, fungi and actinomycetes was also reported (Sujatha *et al.*, 2004). Although bacteria have been used in the commercial preparations of phosphate dissolving cultures to improve growth of plants, fungi seem to be better agents than bacteria (Rokade and Patil, 1992). Experiments with cultures of many bacteria and fungi such as *Bacillus*, *Pseudomonas*, *Aspergillus* and *Penicillium* showed that they are potential solubilizers of bound phosphates. But they vary in their efficiency to dissolve tricalcium phosphate.

Of 149 cultures of soil fungi tested by Mehta and Bhide (1970), 42 cultures showed potential to solubilize tricalcium phosphate ranging from 22 to 98.2%. Among the fungi tested, *Penicillium* sp., *Aspergillus fumigatus*, *A. niger*, *Pythium* sp., *Curvularia intersemnata*, *C. lunata*, *Chaetomium fumiti* and *Humicola* spp. were more efficient in solubilizing phosphorus. Phosphate solubilizing fungi (27 aspergilli, 7 penicillia and 1 *Rhizopus*) were isolated from rhizospheres of 24 crop plants, compost and garden soil using tricalcium phosphate in Pikovskaya’s medium. *Aspergillus aculeatus* was the best solubilizer among all the fungi (Narsian *et al.*, 1994). Similarly, Ostwal and Bhide (1972) observed tricalcium phosphate
solubilization of 13 to 58% in liquid medium and 8 to 37% in soil. *Pseudomonas putida* exhibited higher solubilization (58%) in liquid medium when compared to soil (37%). Reichlova (1972) reported release of 13-16% of phosphorus after 55 h by strains of *Rhizobium japonicum* isolated from soybean nodules.

Bacteria belonging to different species and genera have been found to solubilize organic and inorganic phosphate compounds (Menkina, 1950; Goswami and Sen, 1962; Sundara Rao and Sinha, 1963; Bajpai and Sundara Rao, 1971). *Bacillus megaterium*, *B. circulans* and *Escherichia freundii* were known to solubilize organic and inorganic TCP (Goswami and Sen, 1962; Sundara Rao and Sinha, 1963). The isolates of *Bacillus* sp. varied in their efficiency to solubilize tricalcium phosphate and Mussorie Rock phosphate (Srivastav *et al.*, 2004). The effect of different carbon and nitrogen sources on phosphate solubilization by *Pseudomonas fluorescens* was investigated (Dave and Patel, 2003).

Plants can absorb phosphorus from soils. Major forms of phosphorus in soils were sparingly soluble. Root exudates were capable of dissolving phosphates present in soil (Stevenson, 1967; Moghimi *et al.*, 1978). Several soil bacteria and fungi possess the ability to bring insoluble phosphates to soluble forms in soil by secreting organic acids. These organic acids lower the pH of the soil and bring about the dissolution of bound forms of phosphorus (Earnest, 1923). In general, the plants were able to use phosphorus from all the organic sources used in the study almost as efficiently as inorganic sources (Tarafdar and Claassen, 1988). Many bacteria, fungi
and a few actinomycetes were potential solubilizers of bound phosphates in soil thus playing an important role in soil solubilizing phosphorus and making it available to plants (Subba Rao, 1982). Further, information available from different sources indicate that 13 genera of bacteria, 19 genera of fungi and one genus of actinomycetes were involved in phosphate solubilization (Subba Rao, 1993).

Das (1963) tested 18 fungi isolated from paddy fields for their ability to utilize soluble phosphates. Only three of them (Aspergillus niger, Penicillium sp. and Sclerotium rolfsii) exhibited phosphate-solubilizing ability. Mikovski (1964) found that organic compounds were mainly decomposed by bacteria like Bacillus, Pseudomonas and Micrococcus while Penicillium, Aspergillus and Trichoderma decomposed TCP.

Hlmer and Schinner (1992a) isolated Penicillium sp. and Pseudomonas sp. from forest soils having high abilities in solubilizing inorganic phosphates. Gaur et al. (1973) isolated three strains of Bacillus sp and two species of Aspergillus (A. flavus, A. carbonum) from Mussoorie, Thamarkotra and Maton rock phosphates. Further they isolated A. fumigatus and A. wentii and Bacillus sp. from Delhi alluvial soil as inorganic phosphate solubilizers. The amount of solubilization differed with the organism and with the nature of phosphatic compound. The solubilization of inorganic phosphates by a basidiomycetes fungus Cyathus bulleri was observed after 14 days inoculation using Brodie’s broth (Singal et al., 1991).
The ability of different bacterial species to solubilize insoluble inorganic phosphate compounds, such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite and rock phosphate was reported (Goldstein, 1986). The bacterial genera with this capacity were *Pseudomonas*, *Bacillus*, *Rhizobium*, *Burkholderia*, *Achromobacter*, *Agrobacterium*, *Micrococcus*, *Erwinia*, *Aerobacter* and *Flavobacterium*. There were considerable populations of phosphate solubilizing bacteria in soil and in plant rhizospheres (Sperber, 1958a; Katznelson *et al.*, 1962; Raghu and MacRae, 1966; Alexander, 1977; Bowen and Rovira, 1999).

Soil contains a wide range of organic substrates, a source of phosphorus for plant growth. To make this form of phosphorus available for plant nutrition, it must be hydrolyzed to inorganic phosphorus. The PSB were reported to mobilize insoluble phosphate to soluble forms through enzymatic actions (Chhonkar and Tarafdar, 1984; Gaind and Gaur, 1991; Gaur and Gaind, 1983; Singh *et al.*, 1984; Yahya and Al Azawi, 1989). Mineralization of most organic phosphorus is carried out by means of phosphatase enzymes. The presence of a significant amount of phosphatase activity in soil has been reported (Lynch, 1990; El-Sawah *et al.*, 1993; Bishop *et al.*, 1994; Feller *et al.*, 1994; Kremer, 1994; Sarapatka and Kraskova, 1997). Important levels of microbial phosphatase activity have been detected in different types of soils (Kirchner *et al.*, 1993; Kucharski *et al.*, 1996), but the major source of phosphatase activity in soil was considered to be of microbial origin (Garcia *et al.*, 1992; Xu and Johnson, 1995). Particularly, phosphatase activity was substantially increased in the
rhizosphere (Tarafdar and Jungk, 1987). The increase in alkaline phosphatase activity can be attributed to the increased microbial population since these enzymes were not produced by plants (Chhonkar and Tarafdar, 1981).

Organic phosphates constitute 4-90% of the total soil phosphate. Therefore, organic phosphate mineralization was an important soil process because it results in release of inorganic phosphorus to the soil solution for its availability to plants and soil microbes (Alexander, 1977; Yadav and Dadarwal, 1997).

The major mechanism of mineral phosphate solubilization was the action of organic acids synthesized by soil microorganisms (Sundara Rao and Sinha, 1963; Banik and Dey, 1982; Craven and Hayasaka, 1982; Leyval and Berthelin, 1989; Salih et al., 1989; Halvorson et al., 1990; Halder et al., 1990). Organic phosphate solubilization is also called mineralization of organic phosphorus, and it occurs in soil as plant and animal remains which contain a large amount of organic phosphorus compounds. For a long time researchers have tried to increase the plant available P-fraction by means of PSMOS (Taha et al., 1969; Banik and Dey, 1983; Kucey, 1983; Dighton and Boddy, 1989; Parks et al., 1990; Yadav and Singh, 1991). Apart from fertilization and enzymatic decomposition of organic compounds, microbial mobilization of P is the possible way to increase plant available phosphorus (Illmer and Schinner, 1992). The role of microorganisms in solubilizing the insoluble soil phosphates and to make it available to crop plants is well known (Panova, 1956; Sundara Rao, 1965; Bardiya and Gaur, 1974; Gaur and Ostwal, 1972; Kabesh et al.,
1975; Gaur and Sachar, 1980). Laboratory studies have shown that several microorganisms can solubilize phosphorus from RP and other insoluble phosphatic minerals (Konig, 1961; Sundara Rao and Sinha, 1963; Agnihotri, 1970; Arora and Gaur, 1979; Reddy et al., 2002).

Many microorganisms in the soil were able to solubilize unavailable forms of calcium bound P by excreting organic acids which either dissolve RP or chelate calcium ions to bring the P into solution (Sperber, 1958b; Katznelson and Bose, 1959). Studies have shown that these microorganisms are present in the soil in different numbers (Louw and Webley, 1959; Katznelson et al., 1962; Khan and Bhatnagar, 1977) and that a large proportion of the phosphate solubilizing population was found in the rhizosphere of plants (Sperber, 1958a). Kobus (1962) reported that the numbers of PSB 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.

Vidyasekaran et al. (1973) isolated PSMOS from soil by dilution plate method using nutrient agar medium containing precipitated phosphorus. Three bacteria viz., Bacillus polymyxa, B. circulans and Pseudomonas striata and one fungus species viz., Aspergillus awamorii were isolated and they were found to be effective solubilizers of TCP. Bardiya and Gaur (1974) have suggested that the isolation of efficient RP solubilizers as an appropriate approach because they also showed the capability of solubilizing different forms of insoluble phosphates. Nine different bacteria isolated from the rhizosphere of fields of Gujarat were grown on
Pikovskaya's agar medium and incubated for 3 to 4 days at 37 ± 2° C. All the isolates formed a clear zone of phosphate solubilization (Tank and Saraf, 2003).

Out of the 4800 bacterial strains, 857 strains were able to solubilize phosphate on plates. The incidence of PSB in the rhizoplane was highest followed by rhizosphere and root-free soil (Johri et al., 1999). Several soil bacteria particularly *Bacillus*, *Pseudomonas* and fungi viz., *Aspergillus* and *Penicillium* possess the ability to solubilize insoluble phosphates to soluble form (Mane et al., 2000). The phosphorus solubilizing activity was determined by the activity of microbes to release metabolites such as organic acids, chelate the cations bound to phosphates, the latter being converted to soluble forms (Sagoe et al., 1998).

Inorganic insoluble compounds of phosphorus were largely unavailable to plants, but many microorganisms can bring the phosphates in solution. Rokade and Patil (1992) observed that 10 to 50 % of the bacterial isolates tested were capable of solubilizing calcium phosphates. Species of *Pseudomonas*, *Bacillus*, *Micrococcus*, *Flavobacterium*, *Penicillium*, *Aspergillus*, *Sclerotium*, and others were active in converting insoluble phosphorus to soluble form. Fernandez et al. (1987) observed the mineralization of organic phosphorus into available form by bacterial strains of *Bacillus cereus*.

Microorganisms were involved in a range of processes that affect the transformation of soil phosphorus and were thus an integral component of the soil P cycle. In particular, soil microorganisms were effective in releasing phosphorus from
inorganic and organic pools of total soil phosphorus through solubilization and mineralization. Microorganisms therefore were critical for the transfer of phosphorus from poorly available soil pools to plant available forms and were important for maintaining phosphorus in readily available pools. These processes were likely to be the most significant in the rhizosphere of plants.

The concept of using soil microorganisms to improve mobilization of poorly available forms of soil phosphorus is not new. It was now some 50 years since Gerretson (1948) first showed that pure cultures of soil bacteria could increase the phosphorus nutrition of plants through increased solubility of calcium phosphates. Clearly, microbial-plant interactions in the soil environments were complex and with few exceptions have proven difficult to manipulate (Richardson, 2001).

Microorganisms directly affect the ability of plants to acquire phosphorus from soil through a number of structural or process-mediated mechanisms. According to Richardson (2001) these include:

(i) an increase in the surface area of roots by either an extension of existing root systems (viz., mycorrhizal associations) or by enhancement of root branching and root hair development i.e., growth stimulation through phytoharmones.

(ii) by displacement of sorption equilibria that result in increased net transfer of phosphate ions into soil solution or an increase in the mobility of organic forms of phosphorus.

(iii) through stimulation of metabolic processes that were effective in directly solubilizing and mineralizing phosphorus from poorly available forms of inorganic and organic phosphorus. These processes include the excretion of hydrogen ions, the release of organic acids, the production of siderophores and the production of phosphatase enzyme that were able to hydrolyze soil organic phosphorus.
In particular, organic acids and associated protons were effective in solubilizing precipitated forms of soil phosphorus (viz., iron and aluminium phosphorus in acid soils and calcium–phosphorus in alkaline soils), chelating metal ions that may be associated with complex forms of phosphorus or may facilitate the release of adsorbed phosphorus through ligand exchange reactions (Jones, 1998). It was well established that plant roots effectively increase phosphorus acquisition through modified root growth and architecture and similarly produce metabolites that directly influence phosphorus availability (Raghothama, 1999). Processes such as rhizosphere acidification, exudation of organic acids and secretion of phosphatases from plant roots occur in response to phosphorus deficiency and are established mechanisms by which plants acquire phosphorus (Randall et al., 2001).

According to the results obtained by Scheffer and Schachtschabel (1992) only 0.1% of the total phosphorus from soil was available to plants. The inoculation of the soil with PSMOS may alleviate this problem (Halder et al., 1990; Illmer et al., 1995). Some scientists have described the phosphate solubilization by rhizobia (Halder et al., 1990, 1990a).

2.3.1 Factors affecting solubilization

South American soils were generally low in both total and available phosphorus and some of them absorb large amounts of applied phosphate fertilizer (Leon et al., 1986). Phosphorus deficiency was a consequence of the high weathering
rate causing a high degree of mineralogical decomposition, leaching of calcium, magnesium, potassium, sodium, and phosphorus, greater acidity, high solubilization of aluminium and iron, deep profiles of soils, decreased contents of macro and micro nutrients, absence of minerals of open structure, and therefore low cation exchange capacity (CEC). Besides, rock phosphates were of indigenous origin and present low agronomic potential (Hammond et al. 1986). The solubilizing ability of a microorganism was related to its organic acid production. However the nature of the acid produced was more important than the quantity of the acid (Agnihotri, 1970).

Microbial solubilization of insoluble mineral phosphates in soil is an important process in natural ecosystems and in agricultural soils. In spite that soils usually contain a high amount of total phosphorus, its availability to plants was very low and it was often a limiting factor of plant growth. Microbial phosphorus solubilization may increase the availability of phosphates in soils (Mikanova and Novakova, 2002). Besides, phosphorus applied to soil as mineral phosphate fertilizers was transformed from 70 to 90% to available compounds and its uptake by plants was relatively low (Goldstein, 1986; Domey, 1987). Microbial phosphorus solubilization can improve the efficacy of mineral phosphorus fertilization. A number of soil bacteria possess mineral phosphate solubilizing activity (Yahya and Al–Azawi, 1989; Mikanova and Kubat, 1994). Phosphorus solubilizing activity was also found in symbiotic nitrogenous bacteria (Mikanova and Kubat, 1999). However it was
shown that the phosphorus solubilizing activity of microorganisms was affected by the presence of soluble phosphates in the medium (Jisha, 1997).

### 2.4. Immobilization of phosphorus

Phosphorus was an essential plant nutrient which was added to the soil as inorganic phosphates. A large portion of these phosphates was immobilized after application and becomes unavailable to plants (Dey, 1988; Darmwall et al., 1989; Singh and Kapoor, 1994), although other soil characteristics (pH, soil carbon, etc.) play a role in the solubility of applied phosphorus (Peix et al., 2001). Phosphorus was the nutrient that was most limiting for plant growth (Lewis and Sale, 1993).

The microbial biomass in soil contains a significant amount of phosphorus and generally account for 2 to 5% of the total phosphorus and around 10 to 15% of the soil organic phosphorus (Richardson, 2001). Microbial phosphorus was a dynamic component of the soil phosphorus cycle and was responsive to soil fertility, seasonal conditions and management practices. While phosphorus content of microbial biomass may vary considerably in relation to microbial carbon, it was evident that significant pools were maintained even in soils considered to be phosphorus deficient for plant growth (Oberson et al., 2001). This indicates that microorganisms in soil were highly efficient in acquiring phosphorus to meet their own requirements. In addition, it has been shown that soil microorganisms were capable of rapidly assimilating phosphorus supplied from fertilizer or as plant residues. For instance,
McLaughlin et al. (1988) and Holford (1997) showed that 25% of phosphorus in labelled crop residues was incorporated into microbial biomass within seven days.

A number of studies have highlighted the potential importance of microbial phosphorus in providing available phosphorus to plants. Seasonal dynamics indicate that significant amounts of phosphorus were released from the biomass in response to soil moisture deficiency and estimated that soil microbial phosphorus was completely turned over at least annually (He et al., 1997). Highest rates of phosphorus cycling through the biomass were evident in phosphorus–deficient soil and in soils that received organic inputs, as distinct from those that were phosphorus fertilized. These observations have important implications concerning the contribution of microbial phosphorus to plant nutrition. First, the significance of phosphorus immobilization within soil microflora and its effect on short-term availability of plants was not clear. Second, processes that affect the release of phosphorus from microbial biomass and its subsequent availability to plants require further investigation. Although phosphorus in microorganisms occurs predominantly in organic forms or as polyphosphates, the phosphorus appears to be more rapidly mineralized and was readily available for uptake by plant roots (Macklon et al., 1997). However in soil environments the availability of released phosphorus will be influenced by spatial and temporal factors and also will be subjected to further immobilization and other physico-chemical reactions of phosphorus in soil. In short, while microorganisms may
directly solubilize phosphorus to meet their own requirements, subsequent benefits to plants may also occur following turnover of the microbial biomass.

2.5. Phosphate solubilizing bacteria as plant growth promoting rhizobacteria

Many agricultural soils were deficient in forms of phosphorus that are readily available to plants and, therefore, require the application of phosphorus based fertilizers to remain productive (Richardson et al., 2001). However, a large proportion of the phosphorus that was applied to soil as fertilizer rapidly becomes unavailable to plants, accumulating in inorganic phosphorus fractions, which were fixed by chemical adsorption and precipitation, and organic phosphorus fractions that were immobilized in soil organic matter (Sanyal and De Datta, 1991).

Soils contain an exceedingly diverse array of microbes (Chang et al., 2002). The interactions of the major soil components, minerals, organic matter and microorganisms have a profound effect on the biological processes in soils (Huang, 2002). PSMOS acting in unison with plant roots were responsible for solubilizing phosphate minerals. In natural soil systems, PSMOS consist of a broad class of bacteria and fungi that interact in the soil especially in the extreme microenvironments found around plant roots called the rhizosphere (Waisel et al., 1996).

Because of the enormous numbers of microbial populations and species in the soil, especially in the rhizosphere, intensive and extensive interactions have been established between soil microorganisms and various other soil organisms (including
plant roots) and plant growth promotion by rhizosphere microorganisms was well established (Bashan, 1998).

In spite of the deleterious effects of some microorganisms on plants, the beneficial effects were usually greater (Atlas and Bartha, 1998). The enhancement of the plant growth was by the inoculation of soil microorganisms and also by the solubilization of insoluble phosphates and by the phytohormone production (Ahn et al., 2002; Jeon et al., 2003; Katiyar and Goel, 2003).

Although plant growth promoting rhizobacteria occur in soil, usually their numbers are not high enough to compete with other bacteria commonly established in the rhizosphere. Therefore, for agronomic utility, inoculation of plants by target microorganisms at much higher concentration than those normally found in soil is necessary to take advantage of their beneficial properties for enhancing yields by plants (Igual et al., 2001; Igual and Rodriguez-Barrueco, 2002).

Although several PSB occur in soil, their numbers were usually high enough to compete with other bacteria commonly established in the rhizosphere. Thus, the amount of phosphorus liberated by them was generally not sufficient for a substantial increase in *in situ* plant growth. Therefore, inoculation of plants by a target microorganism at a much higher concentration than that normally found in soil was necessary for the solubilization of phosphates to increase plant yield.

Bacteria associated with plants can be harmful and beneficial. A considerable number of bacterial species, mostly those associated with the plant rhizosphere were
able to exert a beneficial effect upon plant growth. This group of bacteria has been termed "plant growth promoting rhizobacteria" (PGPR) by Kloepper and Schroth, (1978). The strains were from genera *Pseudomonas, Bacillus, Burkholderia, Azospirillum, Enterobacter, Rhizobium, Erwinia, Serratia, Alkaligenes, Arthrobacter, Acinetobacter* and *Flavobacterium*. Plant growth promoting bacteria may promote growth directly by fixation of atmospheric nitrogen, solubilization of minerals such as phosphorus, production of siderophores that solubilize iron or production of plant growth regulators or hormones (Kloepper, 1994). Therefore, they were used as biofertilizers or as control agents for agricultural improvement for a number of years (Suslov, 1982; Davinson, 1988; Lemanceau, 1992; Kloepper, 1994; Glick, 1995). Strains of *P. putida* and *P. fluorescens* have increased root and shoot elongation in canola, lettuce and tomato (Hall *et al.*, 1996; Glick *et al.*, 1997) as well as crop yields in potato, radishes, rice, sugar beet, tomato, lettuce, apple, citrus, beans, ornamental plants and wheat (Suslov, 1982; Kloepper *et al.*, 1988; Lemanceau, 1992; Kloepper, 1994).

Plant growth promoting bacteria (PGPB) were soil and rhizosphere bacteria that can benefit plant growth by different mechanisms (Glick, 1995; Vessey, 2003). Their use as natural biofertilizers was advantageous, not only from the economical, but also from the ecological point of view. A large proportion of phosphorus in soil was present in an insoluble form and therefore not available for plant nutrition. The ability of some microorganisms to convert insoluble phosphorus to an accessible
form, like orthophosphate was an important trait for a PGPB for increasing plant yields (Rodriguez and Fraga, 1999). Phosphate solubilizing bacteria were used as biofertilizers in phosphate deficient soils for enhanced crop yields. PSB were isolated on media containing TCP as the source of inorganic phosphate based on zone of clearance around the colony after 2 to 3 days incubation at 30°C and preserved.

Phosphate solubilizing bacteria were isolated from sixty soil samples of various soil classes and cropping histories in Himalayan regions of Uttar Pradesh by enrichment culture techniques. The strain was identified as Bacillus species. Seed inoculation of this bacterial strain resulted in significant increase in grain and vegetative yield of finger millet, maize, French beans, amaranth with or without phosphorus sources. The significant grain yield was maximum (quintal per hectare) with phosphate and seed inoculation was maximum in maize followed by French beans, amaranth, and finger millet (Pal, 1998). Beneficial effects of inoculation with phosphorus-solubilizing microorganisms to many crop plants have been described (Subba Rao, 1993; Tomar et al., 1996; Dubey, 1998; Pal, 1998; Rodriguez and Fraga, 1999; Sarwagi et al., 1999; Richardson et al., 2001).

Bacteria of several taxonomic classes were found in rhizosphere of crop plants and in soils. They can increase plant growth and productivity (Glick, 1995; Hassan et al., 1998; Thakuria et al., 2004; Minakshi et al., 2005). Increase in plant productivity occurs through different mechanisms such as nitrogen fixation (Boddey and Dobereiner, 1995), solubilization of mineral phosphate and other nutrients
Review of Literature

(Richardson, 2001). Rice soils contain PGPB such as nitrogen fixing, PSB, and the inocula of these bacteria enhance growth and yield of rice (Kloepper et al., 1989). Also, it has been reported that phosphate-solubilizing strains of Rhizobium and Bradyrhizobium increased growth and phosphorus content of leguminous as well as non-leguminous plants (Chabot et al., 1996, 1998; Antoun et al., 1998; Peix et al., 2001).

Wheat yield increased up to 30% with Azotobacter inoculation and up to 43% with Bacillus inoculants (Kloepper et al., 1989) and a combination of Bacillus megaterium and Azotobacter chroococcum increased the yield of the same crop up to 10-20% (Brown, 1974). Azospirillum spp. increased yield in maize, sorghum and wheat (Kapulnik et al., 1985; Baldani et al., 1987; Sarig et al., 1990) and Bacillus spp. increased yield in peanut, potato, sorghum and wheat (Broadbent et al., 1977; Burr et al., 1978; Capper and Campbell, 1986).

An experiment was conducted to study the effect of lime and phosphorus on yield and phosphorus uptake by radish (Raphanus sativus L.) during 1999 and 2000 in western Himalayan region. Lime application significantly upgraded the productivity and the uptake of phosphorus in radish crop. Like wise, the application of phosphorus also improved the yield and phosphorus uptake to a significant level (Dixit and Sharma, 2004).

Phosphate-solubilizing microorganisms when inoculated with rock phosphate increased rice yields (Gaur and Ostwal, 1972; Anthoni Raj et al., 1994;
Thamizvendan and Subramanian, 2000). Inoculation of *Vigna uniguiculata* seeds with either rock phosphate or a combination of rock phosphate and single super phosphate and *Aspergillus awamori* resulted in increased yields (Nagaraju and Nanjundappa, 1996). Similarly, concomitant inoculation of sunflower with phosphorus-solubilizing microorganisms along with rock phosphate increased grain yield (Andhani Gowda *et al.*, 1998). Efficient and economic use of phosphate fertilizer could be achieved by using phosphate solubilizing bacteria, besides obtaining extra seed yield in legumes. PSB inoculation in legumes has increased phosphorus uptake and seed yield (Ahmed and Jha, 1982; Manjunath and Suseela Devi, 1990).

*Bacillus megaterium* and *Pseudomonas fluorescens* were two of the bacteria decomposing organic phosphates and this inoculation increased crop yields (Kudzin and Yarosherich, 1962; Kvaratskheliya, 1962; Menkina, 1963; Sundara Rao and Sinha, 1963; Sundara Rao *et al.*, 1963; Kavimandan and Gaur, 1971).

Some microorganisms show consistent plant growth promotion under glasshouse and field conditions and have been developed as commercial inoculants (*Penicillium* sp.) as reported by Legget *et al.* (2001). Phosphate-solubilizing microbial inoculants include *Aspergillus, Bacillus, Escherichia, Arthrobacter* and *Pseudomonas* (Datta *et al.*, 1982; Mishra, 1985) which can add 30 to 35 kg P$_2$O$_5$ ha$^{-1}$ (Gaur *et al.*, 2004). Khalafallah *et al.* (1982) observed an increase in the PSB following inoculation of fababeans (*Vicia faba*) with a phosphate solubilizing isolate of
B. megaterium. Saber et al. (1977) found similar results for B. megaterium inoculated onto pea plants (*Pisum sativum* L.). The majority of plant growth tests on phosphorus solubilization in soil have been conducted under greenhouse conditions. Under these conditions, increased phosphorus uptake and plant growth in various crops inoculated with PSMOS have been reported (Raltson and McBride, 1976; Kundu and Gaur 1980, 1984; Khalafallah et al., 1982).

There have been a number of reports on plant growth promotion by bacteria that have the ability to solubilize inorganic and/or organic phosphorus from soil after their inoculation in soil or plant seeds (Gerretsen, 1948; Cooper, 1959; Gaur and Ostwal, 1972; Subba Rao, 1982; Datta et al., 1982; Kloeper et al., 1988; Kucey et al., 1989). Several soil microorganisms, including bacteria, improve the supply of phosphorus to plants by inorganic or organic phosphorus solubilization (Krasilnikov, 1961; Lifshitz et al., 1987; Richardson, 1994).

Phosphate solubilizing microorganisms have capability to render insoluble forms of phosphorus more available to plants (Shingte et al., 1987). PSB inoculants play an important role in making P available to crop plants as they increase the yield (Marwaha et al., 1981; Gaur, 1990; Gaur and Gaind, 1992). Keeping this in view, the efficacy of PSB was ascertained under different sources and levels of phosphorus on the production of gram (*Cicer arietinum* L.) by Tomar et al. (1996).

Both bacteria and fungi secrete organic acids into the soil and lower the pH in their vicinity to bring about solubilization of bound phosphates (Sundara Rao and
Sinha, 1963). Phosphate-solubilizing microorganisms could therefore play important roles in environments where phosphorus is not readily available as in soils of low pH (Kannaiyan, 2003). A substantial number of bacterial species, mostly associated with rhizosphere, may exert beneficial effect on plant growth (Glick, 1995). This group of bacteria has been termed “plant growth promoting rhizobacteria” or PGPR (Kloepper and Schroth, 1978).

The dual inoculation of PSMOS and VAM has synergistic effect on plant growth and P uptake as the former increase the P availability and the latter roots surface area. The increased yield and P uptake by dual inoculation of PSMOS and VAM has been observed on several crops by many workers (Azcon et al., 1976; Lee and Bagyaraj, 1986; Kucey, 1987; Sattar and Gaur, 1989; Gaur, 1990).

Synergistic interactions between vesicular arbuscular mycorrhizae and phosphate-solubilizing bacteria have been reported (Ray et al., 1981; Piccini and Azcon, 1987; Garbaye, 1994; Frey-Klett et al., 1997; Toro et al., 1997; 1998; Kim et al., 1998). Similarly, plant growth can be increased by dual inoculation with *Azospirillum* (Alagawadi and Gaur, 1992; Belimov et al., 1995) or *Azotobacter* (Monib et al., 1984; Kundu and Gaur, 1984). Inoculation with mixed cultures such as *Pseudomonas striata* and *Aspergillus awamori* increased the yield of cotton more than with either strain alone (Kundu and Gaur, 1980a). Similar increase in yield was observed after inoculation with peat based cultures of *Bacillus polymyxa* and *Pseudomonas striata* (Kundu and Gaur, 1980b). Synergistic interactions between a
slow-growing N\textsubscript{2}-fixing bacterium *Phyllobacterium* sp. and a fast-growing phosphate-solubilizing bacterium *Bacillus licheniformis*, both isolated from isolated from the rhizosphere of mangrove was reported by Rojas *et al.* (2001).

There has been longstanding interest in the manipulation of soil microorganisms to improve the phosphorus nutrition of plants with the objective of increasing overall efficiency of phosphorus use in agricultural systems. This interest stems from the fact that phosphorus deficiency is widespread in soils throughout the world, that phosphorus fertilizer represents a major cost of agricultural production and that the efficiency of phosphorus use by plants from soil and fertilizer sources were poor. Furthermore, phosphorus is a finite resource. Based on current rate of use, it was expected that the world’s known reserves of RP will be depleted in the current century (Isherwood, 2000). Beyond this time the production of phosphate based fertilizers will require the processing of low grade rock phosphates at significantly higher cost. Alternatively, the direct use of rock phosphates as fertilizers will require an effective means of solubilization. It is also imperative that management of phosphorus fertilizers in agricultural environments was improved so that many adverse environmental effects due to phosphorus losses were minimized (Tunney *et al.*, 1997).

Microorganisms were important for phosphorus mobilization in soil has led to research effort at improving plant phosphorus nutrition. Essentially, there were two major strategies for manipulating soil microorganisms. They are:
1. Management of existing microbial populations to optimize their capacity to mobilize P

Success with this approach requires detailed knowledge of how soil management practices (viz., crop rotations, soil amendments, cultivation etc.,) impact on microbial abundance, diversity and presence of various functional groups and how these relate to magnitude and availability of different soil phosphorus fractions. The manipulation of VAM in soil through crop rotations is one example of how populations might be managed to increase the availability of soil phosphorus to plants (Thompson, 1994). Increased mineralization of organic phosphorus generally occurs in response to cultivation and crop rotations which have been shown to increase the rate of phosphorus cycling through the microbial biomass. For example, incorporation of organic residues through legume rotation resulted in higher biological activity and increased microbial phosphorus uptake and release (Oberson et al., 2001).

2. The use of specific microbial inoculants to increase P mobilization

A range of soil microorganisms able to solubilize precipitated forms of phosphorus or mineralize organic phosphorus has been characterized. Typically, such organisms have been isolated using cultural procedures, with species of *Pseudomonas* and *Bacillus* (bacteria) and *Aspergillus* and *Penicillium* (fungi) being predominant. These organisms were commonly associated with the rhizosphere and when inoculated into plants, often result in improved growth and plant nutrition with responses being observed under both glass house and field conditions. Despite this,
there were a few examples of successful application of microbial inoculants. Essentially, lack of consistent performance under different environmental conditions in the field has precluded their wide use. A number of factors can be identified to explain this variable performance (Richardson, 2001).

They include:

i) Poor understandings of the actual mechanisms involved in plant growth promotion where, in fact, phosphorus mobilization may not necessarily be the primary mechanism involved.

ii) Selection of microorganisms by laboratory screening may be insufficiently rigorous when organisms were required to mobilize phosphorus in soil environments.

iii) The lack of specific association between PSMOS and host plants.

iv) Poor understanding of interactions between physical and chemical characteristics of soil and how these interact with biological phosphorus availability.

v) Poor knowledge of how to establish them as dominant components of complex microbial communities and in particular of their capability of colonizing the rhizosphere and

vi) In most instances the benefits of microbial mobilization of phosphorus may in fact be indirect.

2.6 Bioavailability

Bacterial involvement in the solubilization of inorganic phosphates was known since the first decade of the past century. Most of the studies on phosphate solubilization were done by isolating the microorganisms from soil and then studying the solubilization in vitro. For the plant, the issue of inorganic phosphate availability begins at the roots (Bieleski, 1973).
The direct application of RP ores was practiced throughout the world. Peak usage of directly applied RP in the United States occurred around 1953 where one million tons were applied annually (Maene, 2001). The inoculation of rice with diazotrophic bacteria viz., *Azospirillum lipoferum*, *Herbaspirillum seropedica* and phosphorus solubilizing bacteria *Bacillus megaterium* var. *phosphaticum* increased the shoot length, root length and grain yield over uninoculated control (Arangarasan *et al.*, 1998).

The bioavailability of nutrients contained in natural minerals was the result of a complex symbiosis, part of which was the solubilization of minerals by an exceedingly diverse group of microbes generally referred to as PSMOS. In natural soil systems, PSMOS consists of bacteria and fungi that interact in extreme microenvironmements found around plant roots called the rhizosphere. Colloidal clays and carbon based humic substances were also critical components of these systems (Huang and Schnitzer, 1986).

The bioavailability of nutrients contained in naturally occurring minerals such as apatite was strongly dependent upon the presence of plant root exudates (Bar–Yosef, 1996), the speciation (Van Stratten, 2002), and the crystalline structure of minerals (Banfield and Hamers, 1997; Barker *et al.*, 1997).

Complex minerals which were insoluble were converted to plant available nutrients through interactions among plant roots, microbes, organic substances and clays (Huang and Schnitzer, 1986). Natural phosphate minerals were some of the
most complex organic compounds known with over 300 phosphate minerals identified so far. Virtually every known element has been found in phosphate minerals (Nriagu, 1984). There may be as many as $10^6$ different types of bacteria and fungi that solubilize mineral phosphates (Nahas, 1996).

Phosphorus for agricultural applications was available to growers in two distinctly different forms for entirely two different approaches to plant nutrition. One form usually referred to as ‘conventional’ fertilizer uses synthetic solutions of water soluble phosphates that were supposed to provide a source of ‘available’ phosphorus for plants.

The use of phosphorus solubilizers as inoculants has been found to increase growth, yield and phosphorus uptake by many crop plants. The field and pot trials with PSMOS with or without phosphatic fertilizers, TCP, pyrites or hydroxyapatites showed increase in yield and P uptake from marginal to significant levels (10 to 27%). The effect of PSMOS on plants were attributed to the phosphorus solubilization plus other factors like release of phytohormones, supporting nitrogen fixation, mineralization and mobilization of other nutrients, promotion of PGPR microorganisms which were equally responsible for increased crop yield (Sattar and Gaur, 1984; Altomore et al., 1999). A green house and field trials of wheat seed with a phosphate-dissolving isolate of *Penicillium radicum* increased grain yield by 14% in the field and 9% in the green house (Whitelaw et al., 1997).
Biological and organic farmers seeking maximum production take advantage of the tons of microbes in soils to release "tied-up" phosphates. The release of bioavailable phosphates from organic sources such as compost supplies immediate phosphate where as soil corrections were achieved by the direct application of natural rock minerals (Zimmer, 2000).

Bioavailability or biological availability was that portion of a chemical compound or element that can be transferred from a soil component to a living organism. Bioavailable nutrients result from the complex symbiotic interactions among microbes, higher plants, particulate and colloidal soil components in the extreme microenvironment of the rhizosphere.

In most soils, inorganic phosphorus occurs at fairly low concentrations in the soil while a large proportion of it was more or less strongly held by diverse soil minerals. The uptake activity of plant roots can affect the concentration of phosphorus in the soil and ultimately, the bioavailability of soil inorganic phosphorus to plants. The release of root exudates such as organic ligands is another activity of the root that can alter the concentration of phosphorus in the soil solution. These various processes and their relative contributions to the changes in the bioavailability of soil inorganic phosphorus that occur in the rhizosphere can considerably vary with plant species, plant nutritional status and ambient soil conditions (Hinsinger, 2001).