Chapter 1

Introduction
INTRODUCTION

Vedas and Upanishad as well as other ancient Indian documents mentioned soil as synonymous with land, the mother supporting and nourishing life on the Earth. Soil is the habitat of a variety of biological materials, viz. plants, animals and microorganisms. Soil is composed of organic and inorganic matrices formed by combined action of biotic and abiotic processes. Organic components in combination with the mineral particles together form the soil body. Living organisms contribute to the biological activity of the soil. Thus, soil is not a dead body, but it is the site of tremendous activity of billions of soil organisms. Soil is considered as a storehouse of microbial activity, though the space occupied by living microorganisms is estimated to be less than 5% of the total space. These organisms play a major role in supporting plant life (Lynch, 1990).

Some amounts of salts are always present in soil. Soluble salts are released from rocks during weathering and in course of soil formation. When the concentration of these salts is low, it is not harmful for the growth of plants, but with the increase in salt content of the soil to high level, plant growth is adversely affected which in turn, decreases the productivity of agricultural crops. From agricultural point of view, soils in which concentration of the salts is so high as to adversely affect plant growth and crop productivity are called salt-affected soils. The major salts, which lead to the formation of salt-affected soils, include chlorides, sulphates, carbonates and bicarbonates of calcium, magnesium and sodium. The content of potassium salt is generally low (Sharma, 1998).

Origin of Salt-Affected Soils:

The salt affected soils originated as a result of following factors:

- **Continental cycles**: related with the movement, redistribution and accumulation of carbonates, sulphates and chlorides in the inland regions.
• **Marine cycles**: accumulation of NaCl from the ocean/sea in lowlands of coastal regions.

• **Deltaic cycles**: movement and accumulation due to evaporation of deep ground waters wedged up to the surface through tectonic features or other processes.

• **Anthropogenic cycles**: salt accumulation due to erroneous human activities.

There are definite zones with respect to the accumulation and distribution of salts in the soil. This depends upon the mobilities of different salts. Highly leachable salts (NaCl, Na₂SO₄, Na₂CO₃, NaHCO₃, MgCl₂, MgSO₄, and CaCl₂) mainly contribute towards soil salinization. Consistent precipitation and dissolution reaction under varying geochemical and hydrological processes sustain and replenish the reserves of salts in the soil.

Therefore, the climatic, geomorphologic, topographical, hydrological and biological conditions, which favour excessive evaporation of water, facilitate the process of soil salinization. Climatically, salt affected soils mostly occur in hot and dry arid regions where evaporation of ground water is high. Geomorphologically salt-affected soils are associated with low lands, i.e. flood plains, deltas, troughs, low river terraces, lakes and coastal regions. Hydrologically, development of inland salinity is related to regions of high or rising water-table conditions in the soils where the ground water containing dissolved salts is under the direct influence of excessive evaporation and transpiration.

**Extent of the Problem:**

Salt-affected soils exist in all continents of the world. Based on Food and Agricultural Organization/United Nations Educational, Scientific and Cultural Organization soil map, about 952 million hectares of land in the world is under varying degree of deterioration due to excessive accumulation of salts in the soil profile (Bajwa, 1999). The approximate about the area of salts affected soil in the different states of India made by the Central Soil Salinity Research Institute, Kanal is given in Table 1.
Table 1: Area of Salt Affected Soils (Soil Salinity plus Alkalinity) in India

<table>
<thead>
<tr>
<th>State</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Andaman and Nicobar</td>
<td>1</td>
</tr>
<tr>
<td>Andhra Pradesh</td>
<td>394</td>
</tr>
<tr>
<td>Bihar</td>
<td>85</td>
</tr>
<tr>
<td>Delhi</td>
<td>0.6</td>
</tr>
<tr>
<td>Goa</td>
<td>17</td>
</tr>
<tr>
<td>Gujarat</td>
<td>1649</td>
</tr>
<tr>
<td>Haryana</td>
<td>555</td>
</tr>
<tr>
<td>Jammu and Kashmir</td>
<td>80</td>
</tr>
<tr>
<td>Karnataka</td>
<td>179</td>
</tr>
<tr>
<td>Kerala</td>
<td>45</td>
</tr>
<tr>
<td>Madhya Pradesh</td>
<td>242</td>
</tr>
<tr>
<td>Maharashtra</td>
<td>127</td>
</tr>
<tr>
<td>Orissa</td>
<td>135</td>
</tr>
<tr>
<td>Pondicherry</td>
<td>0.3</td>
</tr>
<tr>
<td>Punjab</td>
<td>480</td>
</tr>
<tr>
<td>Rajasthan</td>
<td>1183</td>
</tr>
<tr>
<td>Tamil Nadu</td>
<td>470</td>
</tr>
<tr>
<td>Uttar Pradesh</td>
<td>958</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>7421</strong></td>
</tr>
</tbody>
</table>

| Courtesy: Sharma, R.C (1998), Agricultural Salinity Management in India |

In India 12 million hectares of agricultural land is under salinity (Gaind and Gaur, 1991). Groundwater Survey and Development Agency (GSDA, 1991) have reported that in Maharashtra state mainly 2 types of alluvial soils do exists. Tapi river alluvial soil includes Dhule and Jalgaon district. Purna river alluvial soil includes three districts Amravati, Akola and Buldana. Purna river, a major tributary of river Tapi, takes birth at an elevation of 900 m in the Gawilgarh hills of Satpuda near Bhainsdehi in Betul district of Madhya Pradesh (M.P.).

The river Purna occupies the lowest level of alluvial fill. The average rainfall in Purna river basin (PRB) is 767 mm. The PRB comprises of alluvial deposits with the maximum thickness of 400 m. Based on exploration, colour, texture, composition and its nature, alluvium is divided as younger and older alluvium. The thickness of younger alluvium is 700 m and includes angular to sub rounded coarse sand, clays and basaltic gravels. The older alluvium is more than 250 m thick, consists of brown clay (GSDA, 1991).
Hydro geologically, the Puma river basin is one among the worst alluvial tract of India though its water holding capacity is good. Secondly, Puma river remains dry throughout the year except for the rainy season. Even the major tributaries viz., Pedhi, Chandrabhaga, Shahanur, Wan, Katepurna, Nalganga and Morna remain dry for most of the time. As such there is no other water holding body in the area, therefore the time span of natural recharge is only rainy period with an average value of 42-45 days with a precipitation rate of 800 mm/YT (Sonule and Srivastava, 1998).
Fig. 2: Location of Purna River Basin

Map of India

Map of Maharashtra

Sampled Area

Madiya Pradesh

Bhainsdabhi

Amarwadi

Akola

Anjg some Sury
Saryapur
Bhujbal

Purna River

District Boundary
The total catchments area of Purna river basin is around 18929 sq. km. Maximum salinity of 19,000 microchips/cm is present in PRB. Population in PRB is 3.92 million. Out of which approximately 350 villages (population 0.35 million) are severely affected and need solution. Rain fed agriculture is the traditional practice and mainstay of the population in the area. Agricultural development in saline tract was not thought of because of existence of salinity in ground water.

Due to high levels of salinity and alkalinity, barren spots appear in the field or even the entire field may look barren. Increase in salinity and alkalinity in the soil can also adversely affect soil biology, mineralization of organic matter and transformation of applied fertilizer nutrients. Saline soils are usually low in organic matter (Waisel, 1972); phosphate solubilization is poor (Surange and Kumar, 1993; Thakkar, 1993; Patil et al., 2003) and often leads to its deficiency in plants.

Soil mainly contains the elements carbon, nitrogen, phosphorus, sulphur, out of which phosphorus (P) is the second vital nutrient next to nitrogen required for growth of microorganisms and plants. The inorganic forms are the compounds of calcium, iron, aluminium and fluorine (Subbarao, 1993; Dubey, 1999). Most Indian soil contains insoluble inorganic phosphate but it is of no use to the crops unless it is solubilized. In acidic soil, phosphorus is rapidly converted into iron and aluminium phosphate while in neutral and alkaline soils into insoluble calcium phosphate (Gaur and Gaind, 1983; Tambekar, 1998). These insoluble compounds render P unavailable to crops. Also though soil contains organic phosphorus, crops can use it only if it is mineralized.
A survey of Indian soils revealed that 98% of these soils need phosphorus either in the form of chemical or biological fertilizer (Singh and Kapoor, 1984). Based on current rate of use, it is expected that the world's known reserves of high-quality rock phosphate will be depleted within the current century. The ultimate source of phosphorus is in the form of mineral, which is made available in the biosphere through transformations. The process of P-cycling or transformation can occur through inorganic or biological processes. Inorganic processes generally include physico-chemical reactions such as precipitation, dissolution, and sorption/desorption. The biological process is basically through mineralization followed by immobilization via uptake by phosphate-solubilizing microorganisms (PSMs) (Isherwood, 2000).

Traditionally agriculture was dependent on natural inputs like farmyard manure, green manure, or on microbial activity. However, to meet the phosphorus demand, farmers in PRB used to apply chemical fertilizers and the use of organic inputs has continued to decline. Over use of chemical
fertilizers totally replaced the organic inputs with inorganic inputs. It resulted in decreased organic matter (humus) in soil, which severely affected soil fertility.

Chemical fertilizer can create hostile environment for soil microorganism, especially in the zone close to the fertilizer particle or granule. This hostile environment could be caused by increased salt concentration in the soil, imbalances of ionic nutrients, pH or high concentration of nitrite. Fertilizer granules of potassium chloride dissolve the local soil solution concentration of salt which may be high enough to affect microbial activity adversely (Gadagi and Alagawadi, 2003).

**Microbial Diversity in Soil**

A gram of soil contain as many as $1 \times 10^{10}$ organisms, representing at least 4000 different microbial species (Torsvik et al., 1990). As a result the potential biodiversity harbored in the soil is staggering. The importance of microbes as a component of the Earth’s terrestrial biomass and their power to influence terrestrial ecosystem is derived from their sheer numbers and from the diverse array of biochemical reactions they catalyze in soil. Microbes are abundant in the soils of the Earth and their activities have far reaching consequences for all of the terrestrial ecosystems, but the growth and physiological activity of microbes may be affected with the change in the soil conditions such as salinity, pH, moisture and carbon and nitrogen sources.

Microorganisms are nature’s original recyclers, converting toxic organic compounds to harmless products, often carbon dioxide and water. They play key role in essential soil processes including organic matter decomposition, nutrient cycling and mineral transformation (Axelrood et al., 2002). Without microbes the recycling of essential nutrients on the Earth would halt (Cornrad, 1996). Microbes communicate and some generate the signals for the formation of metabolically diverse communities. Because of long evolutionary history of microbes, their manifold metabolic capacities and their divergent physiologic lifestyle, microbial life on the Earth is exceedingly diverse (Woese, 1998). The early evolution and exploitation of chemical resources in diverse environments have played a major role in determining
their unparalleled biodiversity. Thus the microbial diversity acts as an indicator of soil quality and plant productivity (Kapoor and Jain, 2005).

On the other hand, rainfed farming is becoming less profitable due to ill distribution of rainfall, increased pest incidence and unpredictable prices of chemical fertilizers (Hallikeri et al., 2003). Worldwide increase in the prices of chemical fertilizers as well as its adverse effects on porosity and fertility of soil has attracted the use of bioinoculant and their influence on soil health. Hence, the current trend throughout the world is to explore the possibility of using alternate nutrient sources or increasing the efficiency of fertilizers by supplementing them with organic fertilizers and microbial inoculants. In such cases of soil, which are low in phosphates, PSM can be added with low-grade phosphates, slag or bone meal and a modest application of rock phosphate may result in enhanced ‘P’ availability.

Biofertilizers are gaining importance in recent years due to increase in the cost of inorganic fertilizers and reduction in the benefit to cost ratio of crop cultivation. The importance of phosphorus biofertilizer (PBF) is well established (Gaur, 1990). The PBF preparation contains microorganisms, which solubilized insoluble phosphate (Dey et al., 1976; Banik and Dey, 1981; Dubey, 1996; Vaishya et al., 1996, Bhattacharya et al., 1997). The PBF hold vast potential for the future and not only that, the biological system is a renewable one, environmentally safe, economically viable and suitable in sustainable agriculture. Phosphorus solubilizing bacteria (PSB), Vesicular Arbuscular Mycorrhiza (VAM) etc. could be regarded as broad-spectrum biofertilizers.

**Phosphate Solubilizing Microorganisms:**

Several heterotrophic and chemoautotrophic bacteria have the capacity to solubilize insoluble mineral phosphates. Many genera of bacteria such as *Achromobacter*, *Agrobacterium*, *Bacillus*, *Pseudomonas*, *Serratia* and several others have been reported to solubilize varying quantities of phosphorus depending on the efficiency of strains. The most dominant PSB belong to genera of *Bacillus* and *Pseudomonas* as listed below:

**Pseudomonas spp.**: *Pseudomonas fluorescence*, *Ps. striata*, *Ps. rathonis*, *Ps. syringae*, *Ps. aeruginosa* (Myskow, 1960; Ostwal and Bhide, 1972; Bardiya and Gaur, 1974; Barea et al., 1976; Nair and Rao, 1977; Sattar and Gaur, 1984; Venkateshwarlu et al., 1984; Illmer and Schinner, 1992).

Fungi are the strong group of mineral phosphate solubilizing organisms, which also solubilize rock phosphate and bone meal besides *Ca_3(PO_4)_2*, *FePO_4*. Among the phosphate solubilizing fungi, many species of the genera of *Aspergillus* and *Penicillium* have been reported as efficient phosphate solubilizers which are listed as below:


**Penicillium spp.**: *Penicillium bilaji*, *P. digitatum*, *P. fumiculosum*, *P. pinophilum*. In addition to these fungi, others such as *Chaetomium*, *Sclerotium rolfsii*, *Cladosporium*, *Paecilomyces* and *Trichoderma* were reported by several workers. PSM can be isolated from soil, rhizosphere, rock phosphate and manure (Banik and Dey, 1981; Gaur, 1990; Kole and Hazra, 1997). These microorganisms solubilize and assimilate phosphorus for their own need and release sufficient quantities in excess of their requirements in the growing system.

**Factors Affecting Phosphate Solubilization:**

The important factors influencing P-solubilization under field conditions include soil type and conditions, nature of phosphorus, PSB and plant genotype. The physical conditions of soil, pH, organic matter and plant nutrients directly and indirectly influence the process of solubilization. The environmental conditions such as temperature, moisture, aeration, humic
acids etc. control the growth and activity of phosphate solubilizing microorganisms. Optimum conditions are required for the improved rate of phosphate solubilization (Gaind and Gaur, 1991; Illmer et al., 1995; Nahas, 1996; Narsian and Patel, 1997; Johari et al., 1999; Nautiyal et al., 2002). The other aspects such as nature of insoluble phosphorus is also important. Plant genotypes particularly the rhizosphere microorganisms and root exudates play an important role in ‘P’ solubilization (Kundu et al., 2002).

**Mechanism of Phosphate Solubilization and Mineralization:**

**Production of Organic Acids:**

Most phosphate solubilizing microorganisms produce organic acids but their nature varies with the strain (Gaur and Sachar, 1980; Venkateshwarlu et al., 1984). The PSM dissolve hydroxyapatite by producing organic acids of both volatile and non volatile (citric acid and lactic acid). Organic acids act as chelating agents as well as have acidifying effect.

**Chelation:** Production of chelators by PSM, such as 2-ketogluconate (Duff et al., 1963; Banik and Dey, 1982), citrate, oxalate and lactate. All of which can complex the cationic portion of insoluble phosphate, salts and thus force the dissociation of phosphates.

**Inorganic Acids:**

Along with organic acids, inorganic acids such as sulphuric acid may sometimes take part in the process of phosphate solubilization (Asea et al., 1988).

**Mineralization of Organic Phosphates:**

Bacteria, fungi and actinomycetes by their enzymatic action make the bound organic phosphates available to plants. Organic phosphorus consists mainly of phytins, nucleic acids and lecithins, which are mineralized by enzyme phytase, nucleases and lecithinase respectively. Phospholipase and nucleases have di-esters as their substrate. The enzyme catalyzing the
hydrolysis of the monoesters act either in acidic and alkaline pH and called as acid or alkaline phosphatases (Narsian et al., 1989).

**Hydrogen Sulphide Production:**

*Aspergillus niger, Desulfovibrio spp.* and *B. megatherium* produces hydrogen sulphide (H$_2$S), which can react with iron in FePO$_4$ and precipitate it as iron sulfide there by liberating phosphate. However, inorganic phosphate solubilization by most microorganisms is attributed mainly by acidification, chelation, H$_2$S production and exchange reaction (Sperber, 1958; Halder et al., 1992; Tambekar, 1998).

**Biochemical Basis for P-Solubilization in Bacteria**

Many rhizobacteria are known to solubilize calcium phosphate complexes by secretion of gluconic acid (Kim et al., 1997; Gyaneshwar et al., 1998) and 2-ketogluconic acid (Duff et al., 1963). The biochemical basis of gluconic and 2-ketogluconic acids is well studied (Goldstein, 1995). These bacteria oxidize glucose in the periplasm to gluconic acid by glucose dehydrogenase (Gcd) resulting in the solubilization of mineral phosphates. Some bacteria can further convert gluconate to 2-ketogluconate by gluconate dehydrogenase (Gadh). The 2-ketogluconic acids is stronger acid than gluconic acid. Gcd requires cofactor pyrroloquinoline quinone (PQQ) that transfers the electron from glucose to electron transport components that are present in the plasma membrane (Nareshkumar, 2002).

Thus, in many bacteria gluconic acid formation is associated with membrane potential rather than phosphate solubilization. However, in some bacteria gluconic acid secretion is dependent on phosphorus deficiency (Gyaneshwar et al., 1998). Understanding the biochemical basis for secretion of gluconic acid in large quantities by efficient PSMs will help to modify natural rhizobacteria to attain 'P' solubilizing ability. Incorporation of PQQ synthase gene from *Erwinia herbicola* to *Burkholderia cepacia* IS 16 and *Pseudomonas* spp. resulted in 'P' solubilizing ability (Rodriguez et al., 1999).

Various groups of microorganisms viz., ammonifiers, nitrifiers, sulfur oxidizers, nitrogen fixing bacteria, cyanobacteria, vesicular arbuscular
mycorrhiza etc. which are present in the natural environment of soil have been found to vary in their tolerance to salinity ranging from strongly intolerant to strongly obligate. It is also possible to train an intolerant organism to tolerant by passing through a graded level of increasing salinity. The interval solute concentration of the cells is more or less the same to that of the environment irrespective of the group to which it belongs. The halophilic organisms survive under high salt environment by adaptation, salt tolerance, salt avoidance or salt resistance. The various theories advanced for halophilism are changes in colloidal properties of protoplasm, adaptation of intracellular enzymes and changes in isoelectric point and presence of cysteine etc. (Kessler, 1958).

The possibility of isolation and training of soil microorganisms to varying concentrations and combinations of ions indicate the possibility of managing salt-affected soils by inoculating such strains. The ability of microorganisms to resist saline conditions could be due to salt avoidance and salt tolerance abilities. It can avoid salt stress of its environment either by diluting the entering salts or by excluding the salts. Hence there is a need to isolate and identify salt resistant strains of PSB for efficient nutrient transformation and enhanced plant growth under salt affected situations (Somani et al., 1998).

According to Gaur (1990) the most efficient and dominant phosphate solubilizers belong to genera Bacillus and Pseudomonas. Bacillus subtilis is a ubiquitous, saprophytic soil bacterium that is the most dominating member of phosphate solubilizing bacteria, due to its ability to produce a wide variety of enzymes. Members of the genus are capable of producing endospore that is highly resistant to unfavorable conditions (Logan and Berkeley, 1986). This feature of the organism has been commercially exploited for biofertilizer production. On the other hand, use of Bacillus subtilis in an industrial setting does not pose any unreasonable risk to human health or the environment; the number of microorganisms released from the fermentation facility is low. The laboratory studies of microbial physiology have focused mainly on organisms
such as *Escherichia coli* and *Bacillus subtilis*. In addition, much is now known about the biochemistry, physiology and genetics of *B. subtilis* and other species that facilitates further development and greater exploitation of this organism in agriculture and industrial processes (Pace, 1997).

**Biodiversity of Microorganisms:**

Man and microbes have had a longstanding love and hate relationship on this planet. The microbes touch human life in many ways and play a paramount role in shaping man's destiny. One of the aspects of this interplay is biodiversity.

Depending on the audience, biodiversity seems to have different meanings. To the general public, biodiversity has become synonymous with the conservation of species, usually centered on charismatic mega fauna, which may or may not be endangered, or on tropical biomes, especially rainforests. To the academic research community, biodiversity is frequently treated as though it were a synonym of diversity, without the precision of meaning that diversity has in a strict ecological sense. In microbial terms, diversity is expressed as the total number of species (species richness) and relative abundance of these species (species evenness) in a given habitat. In molecular ecological terms, it can be defined as the number and distribution of different sequence types present in the DNA extracted from the community in the habitat.

Biodiversity is a property of set of organisms. Although the origin of term biodiversity is unclear, what is clear is that biodiversity became the subject of considerable interest in both the popular and scientific literature during the last decade. It is a nonspecific term that is generally agreed to indicate the variability of all living organisms and at all taxonomic levels, from the species to the ecosystem (Kate and Laird, 1999). The term biodiversity according to Erwin (1991) is related to the number of species or species richness, along with the richness of activity each species undergoes during its existence through events in the life of its members, plus the non-phenotypic
expression of its genome. Thus to study microbial diversity, it is necessary to understand interaction between and among species in a given habitat.

The most useful definition of biodiversity is that given by the international union for conservation and natural resources. Biodiversity encompasses all life forms, ecosystems and ecological processes and acknowledges the hierarchy at genetic, taxon and ecosystem levels. Biological diversity is usually considered at three different levels: genetic diversity, species diversity and ecosystem diversity.

Genetic diversity refers to the variety of genetic information contained in all of the individual plants, animals and microorganisms. Genetic diversity occurs within and between populations of species as well as between species. The genetic diversity has been defined in various ways (Magurran, 1988).

Cultivation of microorganisms is the major limitation in the genetic diversity studies because 1% of the total microorganisms are culturable. Cultivation of microorganisms is necessary to get pure culture, which is required for comprehensive understanding of microbial physiology, or to assess microbial metabolic pathways. Molecular methods does not require cultivation of microorganisms and therefore, applicable to both culturable and unculturable microorganisms (Sharma et al., 2005). New genetic variation is produced in population of organisms that can reproduce sexually by recombination and in individuals by gene and chromosome mutations. The pool of genetic variation present in an interbreeding population is shaped by selection. Selection leads to certain genetic attributes being preferred and results in changes to the frequency of genes within this pool. In particular, those genes, which control fundamental biochemical processes, are strongly conserved across different species and generally show little variation. Other more specialized genes display a greater degree of variation.

Species diversity refers to the variety of living species. Aspects of species diversity can be measured in a number of ways. Most of these can be classified into three groups of measurement: species richness, species
abundance and taxonomic or phylogenetic diversity. Measures of species richness count the number of species in a defined area. A typical sample may contain several very common species, a few less common species and a numerous rare species. Measures of species diversity that simplify information on species richness and relative abundance into a single index are in extensive use. Another approach is to measure taxonomic or phylogenetic diversity, which consider the genetic relationship between different groups of species. Different measures of taxonomic diversity emphasis various taxonomic characteristics and relationships. The species level is generally regarded to be the most appropriate to consider the diversity between organisms. This is because species are primary focus on evolutionary mechanisms and therefore are relatively well defined. Microorganisms constitute major component of the total biodiversity on the earth with an estimated number of 23 million (working estimate) to 151 million (highest estimate) species. India has nearly 1/5th of global diversity of these organisms (Rai, 2004).

Ecosystem diversity relates to the variety of habitats, biotic communities and ecological processes, as well as the tremendous diversity present within ecosystem in terms of habitat differences and the variety of ecological processes (Magurran, 1988).

Due to their physiological diversity, microorganisms play a major role in the cycling of chemical elements within the biosphere, but this relevance for environmental process is only fragmentarily reflected in our current knowledge about microbial diversity because the small size and morphological simplicity of microorganisms have hampered the study of their diversity (O’Donnell et al., 1995; Pace, 1997). In the past few years, due to advances in molecular methods and techniques, the knowledge of microbial diversity has increased dramatically not only from a phylogenetic and taxonomic perspective but also from an ecological basis.

This metabolic wealth has traditionally been exploited by man in processes such as fermentation, production of antibiotics, vitamins etc.
More recently, this largely unexplored reservoir of resources has begun to be harnessed for innovative applications useful to mankind. These include the use of microorganisms for production of biofertilizers. Culture-dependent and culture-independent (molecular) methods have been employed for understanding the diversity of microbes in these environments. Extensive global research efforts have revealed the novel diversity of extremophilic microbes. These organisms have evolved several structural and chemical adaptations, which allow them to survive and grow in extreme environments. The enzymes of these microbes, which function in extreme environments (extremozymes), have several biotechnological applications. They have adapted to extremely diverse environments and have developed an extensive range of metabolic pathways. Extreme environment include high temperature, pH, salt concentration, nutrient concentration and water availability. Both biochemical and molecular genetic techniques have been used to explore microbial diversity. Therefore, microbiologists turned to metabolic properties such as utilisable source of nutrition, especially carbohydrate fermentation, source of carbon, nitrogen and energy (Ogunseitan et al., 2000).

Many studies carried out to investigate the dynamic of a microbial population. Studies on the dynamic of the indigenous community are much more difficult because of their complexity and lack of knowledge of what to expect. If the ecological characteristics of soil microorganisms are to be investigating, then it is necessary to investigate the influence of various physical parameters, biological activities and interaction with other microorganisms and with plants as well as to ascertain the degree of spatial and temporal heterogeneity. These methods, which allow fast, accurate, and comprehensive analysis of a microbial community within a soil sample, need to be developed.

Microorganisms have immense genetic and metabolic diversity. They are key players in the environment and have practical importance in agriculture, disease prevention, animal nutrition, waste management and biotechnology.
The diversity of naturally occurring microorganisms especially, PSM is enormous and represents a largely untapped resource with almost unlimited potential for novel, beneficial products. Microorganisms namely PSM represents largest reservoir of undescribed biodiversity. Insufficient organic matter in soils, alkaline pH, high temperature, low fertility, salinity of soils seems to restrict the phosphate solubilizing bacterial density and diversity. PSM have been reported to solubilize varying quantity of phosphorus depending on the efficiency of the strains. Interest in the ability of these microorganisms to solubilize ‘P’ minerals is based on the enhancement of ‘P’ availability to plants and reduction of fertilizer ‘P’ requirements. These microorganisms may also have root growth enhancing properties as well as capabilities to solubilize other plant nutrients such as Cu and Zn. Thus Phosphate solubilizing microorganisms play a major role in making the nutrient available to plants, especially in alluvial soils (Kapoor and Mishra, 1989).

Vidarbha region, in Maharashtra offer many such unexplored sites, which need to be assessed. Hence it is imperative to search for microorganisms, which are compatible with specific crop rhizosphere and can mobilize phosphate in soil. The study allows the identification of a greater diversity of microorganism and have identified the new bacteria, which are abundant in soil, but not known earlier. As phosphate biofertilizers are being largely used at Vidarbha region recently, the dire necessity to have a comparative study on the potentiality of native phosphate solubilizing microorganisms will help to diagnose the need of phosphate biofertilizer application in Vidarbha region in future. In this context, the present review deals with microbial diversity of *Bacillus subtilis* with specific emphasis on
phosphate solubilizing activity. Hence aims and objectives behind this project were:

- Isolation and Identification of local phosphate solubilizing *Bacillus subtilis* from salinity affected area, which was ecologically adapted.
- Studies on metabolic and physiological diversity of isolated phosphate solubilizing *Bacillus subtilis*.
- Concentration on geographical diversity of phosphate solubilizing *Bacillus subtilis* in relation to salinity.
- Biochemical examination of phosphate solubilizing *Bacillus subtilis*.
- Studies on the effect of some carbon and nitrogen sources as well as the effect of temperature and pH on phosphate solubilizing activity of phosphate solubilizing *B. subtilis*.
- Comparison of isolated *Bacillus subtilis* strains for their similarity and dissimilarity with reference to biodiversity.

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