Potassium-Solubilizing Microorganism in Evergreen Agriculture: An Overview

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Abstract

Increasing cost of the fertilizers with lesser nutrient use efficiency necessitates alternate means to fertilizers. Soil is a storehouse of nutrients and energy for living organisms under the soil-plant-microorganism system. These rhizospheric microorganisms are crucial components of sustainable agricultural ecosystems. They are involved in sustaining soil as well as crop productivity under organic matter decomposition, nutrient transformations, and biological nutrient cycling. The rhizospheric microorganisms regulate the nutrient flow in the soil through assimilating nutrients, producing biomass, and converting organically bound forms of nutrients. Soil microorganisms play a significant role in a number of chemical transformations of soils and thus, influence the availability of macroand micronutrients. Use of plant growth-promoting microorganisms (PGPMs) helps in increasing yields in addition to conventional plant protection. The most important PGPMs are Azospirillum, Azotobacter, Bacillus subtilis, B. mucilaginosus, B. edaphicus, B. circulans, Paenibacillus spp., Acidithiobacillus ferrooxidans, Pseudo-Burkholderia, potassium, phosphorous, zinc-solubilizing monas,

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microorganisms, or SMART microbes; these are eco-friendly and environmentally safe. The rhizosphere is the important area of soil influenced by plant roots. It is composed of huge microbial populations that are somehow different from the rest of the soil population, generally denominated as the "rhizosphere effect." The rhizosphere is the small region of soil that is immediately near to the root surface and also affected by root exudates.

Keywords

- Potassium-solubilizing microorganisms (KSMs) Nutrient use efficiency
- Soil-plant-microorganism system Rhizosphere Evergreen agriculture

1.1 Introduction

Among nitrogen (N), phosphorus (P), and potassium (K), potassium is the third important macronutrient element of plant nutrient that plays significant roles in the activation of several metabolic processes, including photosynthesis, protein synthesis, and enzymes, as well as in resistance to diseases, insects, etc. (Rehm and Schmitt 2002). Potassium though is present as an abundant element in the soil or is applied to fields as natural or synthetic fertilizers, only 1-2 % of this is available to plants, the rest being bound to other minerals and therefore unavailable to plants. The most common soil components of potassium, 90-98 %, are feldspar and mica (McAfee 2008). Soil microorganisms influence the availability of soil minerals, playing a central role in ion cycling and soil fertility (Lian et al. 2008). Their uses as efficient biofertilizer agents for agriculture improvement, environmental protection, and soil fertility have been a focus of recent research. Certain bacteria are capable of decomposing aluminosilicate minerals and releasing a portion of the potassium contained therein (Biswas and Basak 2009). A detailed understanding of how microorganisms affect mineral dissolution rates is essential to quantify mineral weathering on global element cycling (Xiufang et al. 2006). Plants absorb potassium only from the soil; its availability in the soil is dependent upon the K dynamics and total K content. Out of the three forms of potassium present in the soil, only soil minerals make up more than 90-98 % of soil potassium (Sparks 1987) and most of it is unavailable for plant uptake. The second non-exchangeable form of potassium found approximately 1-10 % of soil potassium and consists predominantly under the interlayer K of non-expanded clay minerals such as illite and lattice K in K-feldspars that play an important role to the plant uptake (Sharpley 1989). Release of non-exchangeable K to the third exchangeable form occurs when the exchangeable level and solution K is decreased by crop removal, runoff, erosion, or leaching (Sheng and Huang 2002; Meena et al. 2013; Maurya et al. 2014). With the introduction of high-yielding crop varieties and the progressive intensification of sustainable agriculture, the soils are generally getting depleted in the potassium reserve at a faster rate. Moreover, due to imbalanced fertilizer application, potassium deficiency is becoming one of the major constraints in the crop production (Meena et al. 2015a, b; Singh et al. 2015).

This emphasized the search to find an alternative and effective indigenous source of K for plant uptake and also to maintain K status in soils for sustaining crop production (Meena et al. 2015b). Identification of efficient microbial strains that have the capability to solubilize potassium minerals quickly that can conserve our existing resources to avoid environmental pollution hazards caused by the heavy use of chemical fertilizers. A wide range of bacteria, namely, Acidithiobacillus ferrooxidans, Pseudomonas, Burkholderia, Bacillus mucilaginosus, B. circulans, B. edaphicus, and Paenibacillus spp., have been reported to release potassium in accessible form from potassium-bearing minerals in soils (Sheng 2005; Liu et al. 2012; Meena et al. 2014a, b; Kumar et al. 2015). These potassium-solubilizing bacteria (KSB) microorganisms were found to dissolve potassium, aluminum, and silicon from insoluble K-bearing minerals such as illite, micas, and orthoclases through excreting organic acids, by either directly dissolved rock K or via chelated silicon ions to bring K into the solution (Zhang and Kong 2014). Inoculation with potassiumsolubilizing microorganisms (KSMs) has been reported to exert beneficial effects on growth of cotton and rape (Sheng 2005), cucumber and pepper (Han et al. 2006), wheat (Sheng and He 2006), and sudan grass (Basak and Biswas 2010). Similarly, inoculation of wheat and maize plants with Azotobacter chroococcum, Bacillus mucilaginosus, and Rhizobium resulted in significantly higher mobilization of potassium from waste mica, which in turn acted as a source of potassium for plant growth (Singh et al. 2010). Therefore, potassium-solubilizing bacteria are extensively applicable as biofertilizers in China and Korea as significant cultivated areas of soils in these countries the soil-available K are deficient (Zhang and Kong 2014). Thus, application of K-solubilizing bacteria as biofertilizer for agricultural improvement can reduce the use of agrochemicals and support eco-friendly crop production (Sindhu et al. 2010).

Currently, very little information is available on potassium solubilization through bacteria, mechanisms of solubilization, and the effect of KSM inoculation on nutrient availability in soils and growth of different crops. Potassium is available in soil in four forms, which are an exchangeable cation form, as K ions (K⁺) in the soil solution, tightly bound on the surfaces of clay minerals and organic matter, tightly held or fixed by weathered micaceous minerals, and present in the lattice of few K-containing primary minerals. There are various processes which help to the availability of potassium in the soil. Soil solution potassium is present in the available form in the soil for plant uptake; however the concentration of potassium is affected by cropping history, soil weathering, and fertilizer use. Thus, the amount present is not sufficient to meet the crop requirement. Then, an indicator of soil potassium status, such as exchangeable potassium, has a rapid equilibrium with the soil solution potassium and is considered as readily available. Fixed and lattice potassium that can be grouped together make up the pool of non-exchangeable inorganic potassium in the soil.

1.1.1 Potassium and Its Importance

Potassium is an essential macroelement for all living organisms. In plant physiology it is the crucial cation in regard to its content in plant tissues and with respect to its biochemical and physiological functions (Zhang and Kong 2014). The quantity of potassium absorbed by roots is only second after that of nitrogen for most cultivated plants; however the demand for potassium is higher than that for nitrogen in banana, cotton, and some other species (Mora et al. 2012). As the cation K^+ , it is dissolved in the soil solution as well as adsorbed to clay and organic colloids. But it can also be part of more complex chemical compounds (Sheng et al. 2003; Zheng et al. 2009; Zandonadi et al. 2010).

Potassium is absorbed by roots and translocated inside the plant as the positive cation K⁺. It is characterized by a high mobility at all levels inside the plant in individual cells, tissues, and in long-distance transport through the xylem and phloem, although no structural role of potassium has been found. This is in contrast to calcium and magnesium, which have important structural functions, but only limited mobility in plants. Control of plant water status is an important potassium function. It promotes water absorption by the roots, keeps osmotic tension and turgor in cells and plant tissues, and regulates the activity of stomata cells to prevent unnecessary water loss by transpiration. Potassium has a role in photosynthesis and in the production and translocation of carbohydrate to areas of meristematic growth, fruit development, and storage.

The carbohydrate production and transport function is very important in vegetables and fruit production as it directly affects sugar and starch accumulation. Early stages of potassium deficiency are reflected to decreases in the yield production. This stage of potassium deficiency is called hidden hunger as no specific symptoms appear on the plants. As the intensity of the deficiency increases, symptoms consisting in yellowing and eventual necrosis of the border in older leaves do appear, beginning from the tip and progressing backward over the leaf. Because it is a mobile element, when the potassium deficiency occurs, the element is transferred from old leaves to the young growing points. A slowdown of the growth rate is also present at this level of potassium deficiency. The main internal consequences of potassium deficiency are general reductions of the strength of plant structures, loss of vigor; slowdown of carbohydrate transport, and less resistance to low water availability and to diseases.

Potassium deficiency decreases the stunting of growth with shortening of internodes. Its deficiency caused a reduction in photosynthesis; blackening of tubers in the case of potato and tips or margin of lower leaves of legumes, cotton, maize, and tobacco; and either scorching or burning of all small grains (Ashley et al. 2005). Potassium plays a significant role in the maintenance of cellular organizations for regulating permeability of cell membranes and also keeping the protoplasm in a proper degree of hydration. It activates the certain essential enzymes in protein and translocation of carbohydrates and carbohydrate metabolism and imparts resistance to plants against bacterial and fungal disease. Potassium is an integral part for the development of chlorophyll. It plays an important role in photosynthesis, which is converting carbon dioxide and hydrogen into sugars, for translocation of sugars, and for starch formation, and also metabolic activities of plants (www.ikisan.com).

Adequate quantities of potassium are essential for a plant to achieve its full yield potential and also for various aspects of product quality such as grain size and appearance, oil content, tuber size, dry matter and percentage sugar, starch content, and fruit ripening and quality. The different functions of potash in the plant are also related to physiological conditions and various stresses. These functions are diverse and include efficient nitrogen and drought tolerance, water use, frost resistance, and resistance to pests and diseases. It is therefore not surprising that the lack of plantavailable potash in the soil results in weaker, less vigorous crops that suffer major growing seasons. In years when growing conditions and yield production are good, the response to potash may be modest, especially for cereal crops, but during adverse years, its contribution to optimum yields will be substantial. Adequate potassium is crucial for which it provides some "insurance" against adverse conditions in difficult growing seasons.

1.1.2 Global Potassium Demand

Potash fertilization started during in the nineteenth century, when Justus v. Liebig discovered that the plants required it in different proportions and quantities. During the past 40 years, world potassium consumption has increased up to 2.5fold. Between 1960 and 2000, the world use of potassium fertilizers rose from 9 to 22 Mt and potassium fertilizer use accounts for 16 % of total fertilizer usage. In the developed countries, potash consumption has increased 1.25-fold in the past 40 years, while in the developing countries its demand expended 22-fold from 0.5 Mt in 1960 to 11.3 Mt in 2000. The world potash production had been increased up to 37 Mt (USGS Mineral Commodity Summary 2012) as well as the price of potash \$470/per tons since 2011 (www. infomine.com, 2013). However, K fertilizer cost has not stopped enhancing every year; this has led to the increased cost of rice production and should reduce farmer's income.

1.1.3 Potassium Status of Soils in India

In India, nutrient removal continues to exceed 10 Mt of $N + P_2O_5 + K_2O$ every year. Clearly, expansion in fertilizer application (input) continues to fall short of nutrient removals

States	Additions	Removal	Balance
Andhra Pradesh	411.3	708.6	-297.3
Karnataka	330.3	734.4	-404.0
Madhya Pradesh	168.9	943.9	-775.0
Kerala	72.3	278.6	-206.3
Uttar Pradesh	863.1	1842.1	-979.0
Gujarat	146.1	1137.4	-991.3
Haryana	16.6	669.1	-652.5
Maharashtra	420.8	1482.9	-1062.1
Rajasthan	20.9	1014.8	-993.9
Punjab	38.4	1022.8	-984.5
Jharkhand	9.8	169.6	-159.9
Assam	56.0	255.8	-199.8
Tamil Nadu	304.2	726.1	-421.9
Orissa	63.0	383.6	-320.6
West Bangal	304.4	972.8	-668.4
Total (15 states)	3226.1	12,342.5	-9116.5
		011	

 Table 1.1
 Nutrient balance (NPK) under major states of India

Adapted from Srinivasrao et al. 2011

(output) resulting in the depletion of soil fertility and negative nutrient balance sheet. Out of 371 districts for which more than 11 million soil test data are available, 76 districts are in low, 190 in medium, and 105 districts are in the high category. Thus, 21 % districts are in the low, 51 % in the medium, and 28 % in the high category of potassium fertility status (Table 1.1). All the low and medium K soils which constitute 72 % of the total need K fertilization for optimum yield and balanced soil fertility (Hasan 2002; Ramamurthy and Bajaj 1969).

About 70-75 % of the K absorbed is retained by leaves, straw, and stover. The remainder is found in harvested portions such as grains, fruits, nuts, etc. Whenever the soil cannot adequately supply the K required to produce high yields, farmers must supplement soil reserves with fertilizer K. Improvements in both quantity and quality will add to export earnings. The information presented here is based on more than 11 M soil samples made available by soil testing laboratories run by state departments of agriculture and the fertilizer industry. Though 11 M soil tests are not sufficient to comprehensively cover a country which has >140 Mha cultivated land, they reflected changing K-fertility status of soils in different parts of the country and provide some



Fig. 1.1 The graphical representation of soil potassium status of India

measure of the need for scientific use of K fertilizers (Fig. 1.1).

1.1.4 Potassium Fixation in Soil

In addition to releasing K, soil minerals can also fix K, significantly affecting K availability. This involves the adsorption of K ions on two sites in the interlayers of weathered sheet silicates, such as illite and vermiculite. The degree of K fixation in soils depends on the type of clay mineral and its charge density, moisture content, competing ions, and soil pH. Montmorillonite, vermiculite, and weathered micas are the major clay minerals that tend to fix K (Sparks 1987). Additionally, soil wetting and drying also significantly affect the K fixation. The fixation process of K is relatively fast, whereas the release of fixed K is very slow due to the strong binding force between K and clay minerals (Oborn et al. 2005). Whether a soil fixes or releases K highly depends on the K concentration in the soil solution (Schiavon et al. 2010). In addition to organic acids, the H+ concentration in the soil solution (via soil pH) seems to play a key role in K release from clay minerals.

Optimization of soil pH may be a means of enhancing K release. For optimized K fertilizer management practices, it is crucial to understand the factors that regulate K release from soil non-exchangeable pool. Recent investigations have raised awareness of the impact of K on the soil structure and its ability to capture water. It has been reported that the application of mineral K fertilizers enhances the water-holding capacity of soils and also improves the structural stability of sandy soil in particular (Holthusen et al. 2010). Higher water retention plays a key role in securing the soil productivity in water-limited areas. Therefore, more information is needed in order to understand the effect of K fertilization on the soil's physical properties and soil water-holding capacity (Fig. 1.1).

1.2 Need for Fertilizers

Diffusion of fertilizer consumption in Indian agriculture field has been quite widespread. The uses of imbalanced NPK in the agriculture field have become highly conspicuous. The efficiency of fertilizer application has gradually gone up to 3 kg/ha. It is universally accepted that the application of chemical fertilizers is an integral part of raising the agricultural production to a higher place. The Food and Agricultural Organization of the United Nations (FAO) studied and have established beyond doubt that there is a close relationship between the fertilizer consumption level and average crop yields. The advantages of using nutrients or inorganic fertilizers are immediately available to plants and the exact amount of a given element that can be measured before feeding plants. However, commercial fertilizer, particularly nitrogen, has been easily leached out through rain or irrigation.

1.2.1 Potassic Fertilizers

The potassium content of potassic fertilizers is usually expressed as potassium oxide, K_2O , also referred to as potash. These fertilizers are manufactured from minerals and ores. The commercial fertilizers are salts of potassium usually chlorides and sulfates which are soluble, hence readily available to the plants (www.incitecpivot. com).

The entire requirement of potash is met via imports as there are no exploitable reserves of potassic minerals in the country. The total import of MOP during the current year was 30.40 lakh Mt (www.sobip.com). Studies also have shown that building up readily available potassium reserves in the soil ensures the best opportunity for plants to achieve their optimum economic yield. The application of large amounts of potassium fertilizer to agriculture soil with little readily available potassium will not always enhance yields to the equal amount as those in enriched soil. This is due to the enriched soil reserves that potassium is equally and uniformly distributed throughout the layer of soil in which most of the roots grow. The crop uptake of the depleted potassium in the soil solution as a result is rapidly replenished from the reserves. The benefits are somewhat more with those crops that have a short growing season. Such crops do not have extensive root systems and as a result they must acquire quickly nutrients to optimize growth. But the emergence of potassium fertilizers has not given the ultimate solution for developing countries like India because it lays a major economic constraint since large sums are spent on potassium fertilizers alone (The Economic Times 2006).

1.2.2 Fertilizers and Environmental Pollution

Fertilizers are generally safer in comparison to the pesticides that exhibit toxic properties of living systems. However, all doses of fertilizers that have been applied to the soil are not fully utilized by plants. About 50 % of applied fertilizers to plants are left behind as residues. Generally, inorganic fertilizers are not directly toxic to humans and other life forms; they have been present to upset the existing ecological balance. The nutrients escape from the agriculture or other fields that have been found in excessive quantities in lakes, rivers, and coastal waters. Algae blooms found where the nutrient load is high and these smother other aquatic vegetation in the water bodies. These phenomena may lead to death of the many aquatic dwellers. Environmental contamination arises because not all the fertilizer applied is taken up by the crop and removed at harvest.

1.2.3 **Availability of Potassium in Soil**

Only little quantities of K are maintained in the soil solution 5-20 kg/ha K (6-24 kg K₂O). The majority of the reserves of potassium in the soil are held through negative charges in organic matter and clay minerals. The potassium may be held weakly or strongly depending upon the position in the clay lattice. Plant uptake K⁺ which is loosely bound (exchangeable K) is quickly released, while the strongly bound reserve ("non-exchangeable K") is very slowly released. Individual soils have various intensities to hold potassium according to the clay type and content and also the amount of the soil organic matter. The sandy soils have a very limited amount of the reserves of exchangeable K. Clay minerals themselves contain potassium (Fig. 1.2).

1.2.4 Forms and Availability of Potassium

Fig. 1.2 Different

Potassium is the seventh most abundant element in the Earth's crust and makes up about 2.4 % by weight of the earth's crust. Yet only 1-2 % is available to plants. Potassium exists in the soil as exchangeable K, dissolved K⁺ ions (solution K), mineral K, and non-exchangeable K. Plants can take up solution K and exchangeable K from the soil, but the non-exchangeable K and mineral K are unavailable to plants. Depending on the soil type, ~98 % of total soil K is found in unavailable form. Most potassium minerals are insoluble. Feldspars and micas are minerals that contain most of the K and common parent materials for most soils (Foth and Ellis 1997). The crops cannot use the K in this crystalline (insoluble form). Over very long periods of time (Fig. 1.2), these minerals weather (break down) and K is released; this process is too slow to supply the full K needs of field crops.

1.3 **Environmental Factors** Affecting Potassium Solubilization

In soil, K mobilization is affected by many biotic and abiotic environmental factors like soil properties (physicochemical characteristics, aeration, and pH), the presence of mycorrhizae fungi and rhizosphere bacteria, and the composition of plant root exudates. These parameters may also influence the K mobilization ability of bacteria. Gahoonia et al. (1997) explained three



hypothetical processes:(1) Mineral fragmentation caused by the activity of the root increases the bacterial effect on mineral mobilization due to increased surface area for reactivity. (2) Root exudates help by indirectly providing the substrates for the production of weathering metabolites by bacteria. (3) Besides producing weathering agents, bacteria produce phytohormones which stimulate the development of root, modify root exudation and physiology, and help improve nutrient uptake and mobilization of minerals. Potassium concentration in various plant parts is not similar as it may vary from different climate conditions. In case of feldspar, illite, and muscovite, more K is released under aerobic conditions as compared to anaerobic conditions (Badar 2006). In liquid medium Bacillus edaphicus showed better growth and greater K-releasing ability with illite as compared to feldspar (Sheng and He 2006).

1.3.1 Potassium Functions in Plants

Potassium has many functions in plant growth, such as to smooth the progress of cell division and growth, to increase disease resistance and drought tolerance, and to regulate the opening and closing of stomata required for osmotic regulation. Besides, this potassium is essential for photosynthesis process and acts as key to activate enzymes to metabolize carbohydrates for the manufacture of amino acids and proteins. Furthermore, potassium assimilates transport during plant ontogeny, and one of the most important influences is improving the oil content of plants. Since the use of potassium has covered a lot of plant activities, depletion in potassium uptake can cause problems for plant growth. A symptom of potassium deficiency is chlorosis along the leaf margins. Then, in severe cases, the leaf will turn into yellow color and eventually will fall off. It also affects plant growth and canopy photosynthesis process. There are several factors that lead to this problem, for instance, low soil potassiumsupplying capacity, insufficient application of mineral potassium fertilizer and biofertilizer, complete removal of plant straw, leaching losses,

and phosphorus and nitrogen deficiency (Das and Sen 1981). Fundamentally, K^+ is highly water soluble and highly mobile and transported in the plants' xylem (Lack et al. 2005). Membrane transport of the potassium can be mediated either through potassium channels, utilizing the membrane potential to easy transport of potassium down its electrochemical gradient, or through secondary transporters. In plants, potassium acts as a regulator since it is constitutes 60 different enzyme systems of drought tolerance and water use efficiency.

Plants' K concentrations of crops vary widely with the site, year, crop species, and fertilizer input. It ranges from 0.4 % to 4.3 % (Askegaard et al. 2004). Oborn et al. (2005) concluded that crop K concentrations are often well below (~3.5 %). For many crops, the critical K concentration is in the range of 0.5-2 % in dry matter (Leigh and Jones 1984). With the exception of the cytosol and the vacuole, the subcellular distribution of K is largely uncharacterized. The concentration of K in the cytoplasm is kept relatively constant at around 50-150 mM, while its concentration in the vacuole varies substantially depending on supply status. Together with accompanying anions (NO⁻³, Cl⁻, malate⁻), vacuolar K largely determines the osmotic potential of the cell sap. In the agronomic literature, high K concentration in crops has often been termed luxury consumption. However, as outlined below ("Potassium Nutrition and Crop Stress Resistance" section), high accumulation of K by crops during optimal growing conditions may be considered as an *insurance strategy* to enable the plant to better survive a sudden environmental stress (Kafkafi 1990).

Plant species are known to differ in their K requirement and in their ability to take up K. The differences in absorption of K among different plant species are attributed to variations in root structure, such as root density, rooting depth, and root hair length (Nieves-Cordones et al. 2014). All crops require potassium, especially high-carbohydrate plants such as maizes, bananas, and potatoes (Hillel 2008). KSB is a hetero-trophic bacterium which obtains their energy and carbon source from the buildup of dead organic

materials. Besides these, KSBs are aerobic bacteria which play a significant role in maintaining the soil structure through contribution in the formation and stabilization of water-stable soil aggregates. In addition, this gram-positive bacterium can produce a substance that stimulates plant growth or inhibits root pathogens (Egamberdiveya 2006). Moreover, KSB specifically are well known for its capability to solubilize rock potassium mineral such as illite, orthoclases, and micas. This is done through the production and excretion of different organic acids (Subhashini and Kumar 2014; Maurya et al. 2014). Therefore, KSB increases potassium availability in soils and increases mineral contents in plants.

1.3.2 Quantification of Potassium Solubilization

Studies on the dynamics of potassium solubilization by microorganisms are best carried out in vitro based on their measurement of K released into culture broth from cultures developed by using an insoluble mineral compound as the only K source. The rate of potassium solubilization is often estimated by the subtracting of the final K concentration (minus that of a non-inoculated control) from the initial theoretical K supplied through the K-bearing substrates. This estimate has the limitation of not taking into account the K utilized by the cells during growth. The efficiency of potassium solubilization by different microorganisms varies with the nature of potassium-bearing minerals and more specifically it depends on the structure and chemical composition of minerals. It was observed that bacteria degraded kietyote and pegatolite and released 47 and 44.4 mg soluble potassium, after 38 h of incubation respectively, (Yakhontova et al. 1987). The easy release of the potassium from the minerals follow in order as illite > feldspar > muscovite (Sheng et al. 2002; Badar 2006).

Besides, the releases of K from minerals also get affected by pH of the medium, dissolved oxygen, and the microbial strain used. It was estimated that with the increase in pH from 6.5to-8.0, the soluble K content increased from 490 to 758 mg/L. Instability of the potassiumsolubilizing character of a few strains after several times of inoculation has been reported. However, the traits seem to remain stable in most of the isolates. Although no exact quantitative comparison can be made from experiments with different sources of insoluble K, the review of literature suggests that fungi such as *Aspergillus* spp. and *Penicillium* spp. are more effective solubilizers than bacteria such as *Bacillus* spp., *Paenibacillus* spp., *Pseudomonas* spp., etc.

1.4 Potassium-Solubilizing Microorganisms (KSMs)

Diverse groups of soil microflora were reported to be involved in the solubilization of insoluble and fixed forms of K into available forms of K which are easily absorbed by plants (Zarjani et al. 2013; Gundala et al. 2013). Microbial inoculants which are able to dissolve K from mineral and rocks that enhanced plant growth and yield are also economically viable and eco-friendly. The first evidence of solubilization of rock potassium by microbial involvement had been shown by Muentz (1890). A wide range of KSMs. namely, Bacillus mucilaginosus, B. edaphicus, B. circulans, Paenibacillus spp., Acidithiobacillus ferrooxidans, Pseudomonas, and Burkholderia (Sheng et al. 2008; Singh et al. 2010; Basak and Biswas 2012), have been reported to release potassium in an accessible form from K-bearing minerals in soils. Several fungal and bacterial species, popularly called KSMs that assist plant growth by mobilization of insoluble forms of K. KSMs are ubiquitous whose numbers vary from soil to soil. The rhizosphere microorganisms widely contribute in the solubilization of bound form of soil minerals in the soil (Supanjani et al. 2006; Sindhu et al. 2009). A variety of soil microorganisms have been found to solubilize silicate minerals (Sheng et al. 2001).

Many microorganisms like fungi, bacteria, mycorrhizae, and actinomycetes colonized even on the surface of mountain rocks (Groudev 1987;

Gundala et al. 2013) and it has been reported that the B. mucilaginosus sub spp. siliceus is silicatesolubilizing bacteria that liberates K from aluminosilicates and feldspar. According to Aleksandrov et al. (1967) that were isolated from agricultural land at different locations and found that various bacterial species like silicate bacteria were found to dissolve K, silicates, and aluminum from insoluble minerals, it also helps in the decomposition of organic matter, crop residues etc., and suggested that they play a major role in nutrient cycling in the soil-plant system. B. mucilaginosus stain CS1 is a silicate bacterium which exhibited inhibitory effect on the growth of gram-negative bacteria. It has also been reported as silicate-solubilizing bacteria present in rhizosphere as well as non-rhizosphere soil (Lin et al. 2002; Liu 2001). K-solubilizing rhizosphere bacteria were isolated from the roots of cereal crops which are grown in potassium and silicate-amended soil (Mikhailouskaya and Tcherhysh 2005).

1.4.1 Potassium-Solubilizing Bacteria (KSB)

A wide range of rhizospheric microorganisms reported as K-solubilizers include B. mucilaginosus (Zarjani et al. 2013), B. edaphicusand (Sheng 2002), B. circulans (Lian et al. 2002), Burkholderia, Acidithiobacillus ferrooxidans, B. mucilaginosus (Zhang and Kong 2014), Bacillus edaphicus (Sheng and He 2006), Arthrobacter spp. (Zarjani et al. 2013), Enterobacter hormaechei (Prajapati et al. 2013), Paenibacillus mucilaginosus (Liu et al. 2012; Hu et al. 2006), P. frequentans, Cladosporium (Argelis et al. 1993), Aminobacter, Sphingomonas, Burkholderia (Uroz et al. 2007), and Paenibacillus glucanolyticus (Sangeeth et al. 2012). These microbial strains have the ability to solubilize K from K-bearing minerals, but only a few bacteria, such as B. edaphicus and B. mucilaginosus, have high capacity for mobilizing and solubilizing of K from minerals (Zhao et al. 2008; Rajawat et al. 2012). Bacteria have wide applications in mining, metallurgy, microbial fertilizer, and feed (Maurya et al. 2014; Meena et al. 2014a; Zhang and Kong 2014).

1.4.2 Potassium-Solubilizing Fungi (KSF)

Arbuscular mycorrhiza can increase the solubility of the mineral form of potassium by releasing protons, H⁺, or CO₂ and organic acid anions such as citrate, oxalate, and malate. This also increased the nitrogen, potassium, calcium, and iron in the plant leaves and fruits (Veresoglou et al. 2011; Yousefi et al. 2011). The inoculant of the two arbuscular mycorrhizal fungi (AMF) species G. intraradices and G. mosseae were applied in soil on a weight basis and recorded the increaspotassium ing uptake by maize crop (Wu et al. 2005) Information on the uptake of the macronutrient cations through AM plants has been relatively inconsistent in that increases, decreases, and no effects have been reported (Clark and Zeto 1996), and in the case of potassium, it depends on the soil condition as well as nature of plant growth and other conditions (Clark and Zeto 2000). Alves et al. (2010) reported that after 90 days, the plant height, root length, shoot dry weight potassium and phosphorus contents, and mycorrhizae colonization were increased in comparison to control. Potassium uptake compared to Mg and Ca was especially enhanced in AM switch grass grown in acid soil (Clark et al. 1999). Ectomycorrhizal fungi particularly isolated UFSC-Pt22 and UFSC-Pt186 and contributed to the increase of the efficiency of alkaline breccias as a source of P and K to the plant growth of Eucalyptus dunnii seedlings, respectively (Alves et al. 2010).

Prajapati et al. (2012) reported that potassiumsolubilizing fungi (KSF) strains such as *Aspergillus terreus* and *Aspergillus niger* were isolated from various K-rich soil samples and observed that *A. terreus* and *A. niger* could solubilize insoluble potassium and showed the highest available potassium in liquid medium by using two various insoluble sources of potassium, i.e., feldspar and potassium aluminum silicate, based on their colonies and morphology characters. A. terreus shows highest solubilization as well as acid production on both insoluble potassium sources. The concentration of trace elements is another relevant factor in the context of rock solubilization by fungi (A. niger) also reported by production of acids (Mirminachi et al. 2002). Furthermore, symbiotic nitrogen-fixing rhizobia and *Pseudomonas*, which fix atmospheric nitrogen into ammonia and that export the fixed nitrogen to the host plants, have also shown K- and P-solubilizing activity. For instance, Aspergillus spp., Aspergillus terreus (Prajapati et al. 2013), Aspergillus niger (Prajapati et al. 2012), and Penicillium spp. (Sangeeth et al. 2012) enhanced K-solubilization by mobilizing inorganic and organic K and release of structural K from rocks and minerals.

1.4.3 Mechanisms of K-Solubilization

Currently, little information is available on K-solubilization by rhizospheric microorganism, in which the mechanisms of K-solubilization by production of organic acid to provide potassium nutrients as well as other nutrients for enhancing crop growth. Sheng and Huang (2002) found that K release from the minerals was affected by oxygen, pH, and the bacterial strains used. The efficiency of the K-solubilization by various microorganisms was found to vary according to the nature of potassium-bearing minerals and aerobic conditions (Uroz et al. 2009). The extent of potassium solubilization of B. edaphicus in the liquid media was more growth on illite than feldspar (Sheng and He 2006). Indigenous rhizospheric microorganisms have the greater to absorb and mobilize the fixed form of nutrients (potassium) from trace mineral sources. Silicate bacteria were found to dissolve aluminum, potassium, and silica from insoluble minerals. Hydrogen ion of soil or soil solution is directly related to releases of K from minerals. The content of was potassium solubilization enhanced 84.8–127.9 % in microbial inoculated than un-inoculated treatment. The extent of potassium solubilization was reported higher in illite by *B. edaphicus* in the broth culture compared to feldspar (Sheng and He 2006). Badar (2006) reported that the extent of potassium solubilization by silicate-solubilizing bacteria were recorded as 4.90 mg/L at pH 6.5–8.0. *B. mucilaginosus* was solubilized the 4.29 mg/L K-solubilization in media supplemented with muscovite mica (Sugumaran and Janarthanam 2007). The K-releasing affected by pH, soil mineral properties, and aerobic conditions (Chen et al. 2008; Bin et al. 2010).

Mechanism of potassium solubilization means by which the insoluble potassium and structurally unavailable form of potassium compounds are mobilized and solubilized due to the production of various types of organic acids which are accompanied by acidolysis and complexolysis exchange reactions, and these are key processes attributed to the conversion in a soluble form (Uroz et al. 2009). The organic and inorganic acids convert insoluble K (mica, muscovite, biotite feldspar) to the soluble form of K (soil solution form) with the net result of increasing the availability of the nutrients to the plants. The various types of organic acid produced by KSMs were differing with different organisms. Organic acids were detected in the microbial suspension (Verma et al. 2014; Zhang and Kong 2014; Maurya et al. 2014). KSMs have the ability to weather phlogopite through acidic dissolution and aluminum chelation of the crystal network (Leyval and Berthelin 1989; Abou-el-Seoud and Abdel-Mageed 2012: Meena et al. 2014b).

The release of various types of organic acids were reflected by microorganisms to solubilized the insoluble K to an available form of K which is easily uptaken by the plant. Researchers suggested that the plant growth promotion activities were related to K-solubilization as well as the release of organic acids by the K-solubilizing strains. Sheng and He (2006) reported that solubilization of feldspar and illite and via rhizospheric microorganisms is due to the production of organic acids like citric acid, tartaric acids, 2-ketogluconic acid, oxalic acid, gluconic acid, malic acid, propionic, fumaric, glycolic, and succinic acid seems to be the most frequent agent of K-solubilization mineral (Zarjani et al. 2013; Prajapati et al. 2012; Prajapati and Modi 2012; Wu et al. 2005).

The solubilization of structural K compounds by naturally-abundant KSMs is common under in vitro (Meena et al. 2013; Maurya et al. 2014), field, and greenhouse conditions (Prajapati et al. 2013; Parmar and Sindhu 2013). Indigenous, rhizospheric microorganisms are very effective in releasing K from structural K through solubilization and from exchangeable pools of total soil K by acidolysis, chelation, and solubilization by KSMs (Uroz et al. 2009). Biomass of rhizospheric microorganism in the soil also contains a major quantity of fixed K which is potentially available to plants (Girgis 2006; Subhashini and Kumar 2014).

Mechanisms for KSMs to solubilization of K are by: (i) lowering the pH or (ii) through increasing the chelation of the cations bound to K and (iii) acidolysis of the surrounding area of microorganism. The lowering in pH of the medium suggests the release of different organic acids and protons by the K-solubilizing microorganisms (Zarjani et al. 2013; Parmar and Sindhu 2013). Such acidolysis by organic acids produced by the rhizospheric microorganisms can either directly dissolve the mineral K as a result of slow releases of exchangeable K or readily available exchangeable K or can chelate by both Al and Si ions associated with K mineral (Romheld and Kirkby 2010). Thus, the synthesis and discharge of organic acids through microorganisms into the surrounding environment acidify the microbe's cells and their surrounding environment that ultimately lead to the release of K ions from the mineral K by protonation and acidification (Goldstein 1994). Of the various organic acids involved in the solubilization of insoluble K, gluconic, oxalic acids a-ketogluconic and succinic citric are the most efficient acids released by microbial strains (Table 1.2). Figure 1.3 showed the direct and indirect mechanisms of plant growth-promoting properties of potassium-solubilizing microorganism (KSMs) and their K-solubilizing ability of mica (K- bearing mineral) on Aleksandrov medium (this figure modified from Meena et al. 2014b).

Organic acids produced by KSMs can be detected through enzymatic and highperformance liquid chromatography methods (Archana et al. 2013; Zhang et al. 2013). However, the acidification does not seem to be the only mechanism of solubilization, as they have ability to reduce the pH in some cases which did not correlate with the ability to solubilize mineral K (Zhang and Kong 2014; Subhashini and Kumar 2014). Furthermore, the chelating ability of the different organic acids is also very important, as it has been shown that the addition of 0.05 M EDTA into the medium which has the same solubilizing effect as compared to the inoculation with Penicillium bilaii (Sheng and Huang 2002; Liu et al. 2006).

1.5 Morphological and Biochemical Characterization

Cell morphologies of the KSB isolate were determined through using an optical microscope after stained with the phenol red. Physiological tests, including Proskauer (VP) and Voges-Methyl red (MR) reactions, utilization of organic acids, anaerobic growth, production of acid from carbohydrates, were observed by the method of Claus and Berkeley (1986). Subhashini and Kumar (2014) studied the substrate utilization patterns along with salt tolerance and temperature, pH, and salt.

1.6 Molecular Biology of Potassium-Solubilizing Microorganism

For maintaining turgid pressure of microbial and plant cells, potassium (K^+) is one of the important elements. Stimulation of potassium acquisition is one of the most rapid responses to an osmotic up-shock in bacteria. Potassium (K) is mostly present as intracellular cations, which has

	Potassium status (kg K ₂ O/ha)		
States	<130	130–335	>335
Andhra Pradesh	2	14	3
Delhi	0	1	0
Himachal Pradesh	6	4	3
Bihar and Jharkhand	1	24	2
Assam	7	3	0
Dadra and Nagar Haveli	0	1	0
Chandigarh	0	1	0
Goa	1	0	0
Gujarat	0	3	16
Manipur	1	0	0
Kerala	4	6	0
Jammu and Kashmir	5	5	0
Meghalaya	1	0	0
Haryana	0	2	9
Nagaland	5	0	0
Maharashtra	0	12	13
Karnataka	3	10	7
Punjab	0	9	3
Mizoram	1	0	0
Rajasthan	0	23	0
Orissa	2	11	0
Tamil Nadu	0	6	7
Pondicherry	1	0	0
Madhya Pradesh and Chhattisgarh	3	10	31
Sikkim	0	4	0
Arunachal Pradesh	2	3	0
Tripura	3	0	0
Uttar Pradesh and Uttaranchal	26	23	7
West Bengal	2	13	1
Total districts, (%)	76 (21)	190 (51)	105 (28)

 Table 1.2
 The categorization of districts as K-fertility status of India

a major role in maintaining the turgor pressure of the cells and also plays an important role in bacterial osmoadaptation, gene expression, pH regulation, and activation of cellular enzymes (Epstein 2003). Three different types of K transporters (Trk, Kdp, and Kup) have been involved for the uptake of K. Trk is a multicomponent complex widespread in bacteria and archea and it has a moderate affinity to the K uptake. Trk consist of a trans-membrane protein named TrkH or TrkG, which is the actual Ktrans-locating subunit, and TrkA is a cytoplasmic membrane surface protein, which binds to NAD-binding protein that is required for the system's activity (Sleator and Hill 2002). Kdp is an inducible system with greater affinity and

specificity for K, found in Escherichia coli and other bacteria. Kdp is the only bacterial K acquisition system whose expression is significantly regulated at the transcriptional level through KdpE response regulator and KdpD sensor kinase (Epstein 2003; Domínguez-Ferreras et al. 2009). Bacillus subtilis has the Ktr gene which is involved in K uptake. HAK/KT/KUP family, the genes of these families are homologous to bacterial KUP (TrkD) potassium transporters. The KUP transporter from E. coli is characterized by a midrange (0.37 mM) KM for K^+ and a similar affinity to the Cs^+ and Rb^+ . Complementation of TK2463 cells by AtKUP1. The E. coli TK2463 mutant is defective in three K1 uptake transporters (Trk, Kdp and Kup)



Fig. 1.3 Direct and indirect mechanisms of potassium solubilizing microorganism (KSMs) and their K-Solubilizing ability of mica (K-bearing mineral) on Aleksandrov medium

(Epstein and Kim 1971) and was transformed through empty vector or with plasmids containing the AtKUP1 gene. The production of different organic acids is considered as the principal mechanism for solubilization of mineral phosphate and potassium by microorganism, this assumption has been corroborated by the cloning of two genes which is involved in gluconic acid production, viz., pqq, and gab Y. Gluconic acid is the principal organic acid produced by Bacillus spp., Pseudomonas spp., Arthrobacter spp., Aspergillus spp., and Penicillium spp. Chelating substances and inorganic acids such as sulphydric, carbonic, and nitric acid are considered as other mechanisms for potassium and phosphate solubilization. Xiufang et al. (2006) identified and characterized potassium-solubilizing bacteria by 16S rDNA gene sequencing analysis using universal primers, the forward primer and the reverse primer; the recombinant plasmids were developed from the cloned products and the relatedness between the strains and were estimated from phylogenetic analysis (Xiufang et al. 2006).

1.7 Effect of Potassium-Solubilizing Microorganisms on Plant Growth and Yield

Inoculation of seeds and seedling treatments of plants with KSMs generally showed significant enhancement of germination percentage, seedling vigor, plant growth, yield, and K uptake by plants in particular, under glasshouse conditions (Singh et al. 2010; Awasthi et al. 2011; Zhang et al. 2013; Zhang and Kong 2014; Subhashini and Kumar 2014). The application of organominerals with a combination of silicate bacteria for enhancing plant growth and yield of maize and wheat was first reported by Aleksandrov (1958). More importantly, research investigation conducted under field level test crops such as wheat, forage crop, maize, and sudan grass crops has revealed that KSMs could drastically reduce the usage of chemical or organic fertilizers (Xie 1998). As reported by previous researchers (Singh et al. 2010; Sindhu et al. 2012; Zeng et al. 2012), the enhancement of

plant K nutrition might be due to the stimulation of root growth or the elongation of root hairs by specific microorganisms, thus no direct increase in the availability of soil solution K is expected.

KSMs have been isolated from rhizospheric soil of various plants and from K-bearing mineral (Parmar and Sindhu 2013; Zhang et al. 2013), feldspar (Sheng et al. 2008), potato-soybean cropping sequence (Biswas 2011), Iranian soils (Zarjani et al. 2013), ceramic industry soil (Prajapati and Modi 2012), mica core of Andhra Pradesh (Gundala et al. 2013), common bean (Kumar et al. 2012), biofertilizers (Zakaria 2009), sorghum, maize, bajra, chili (Archana et al. 2013), cotton, tomato, soybean, groundnut, and banana (Archana et al. 2012), soil of Tianmu Mountain, Zhejiang Province (China) (Hu et al. 2006), rice (Muralikannan 1996), tea (Bakyalakshmi et al. 2012), Valencia orange (Shaaban et al. 2012), black pepper (Sangeeth et al. 2012), potato (Abdel-Salam and Shams 2012), thyme (Yadegari et al. 2012), eggplant (Han and Lee 2005), peanut and sesame (Youssef et al. 2010), and tobacco (Subhashini and Kumar 2014). Better crop performance was reported to be achieved from several horticultural plants, vegetables, and cereals, which were successfully inoculated with KSMs (Singh et al. 2010; Basak and Biswas 2012; Prajapati et al. 2013). K-use efficiency in agricultural lands could effectively be improved through the inoculation of relevant KSMs, which is, in fact, an integration and sustainable means of nutrient management of crop production. Enhancement of plant growth by improving N-fixer and P and K-solubilizers is another beneficial effect of microorganisms with K-solubilizing potential (Verma et al. 2010; Basak and Biswas 2012; Meena et al. 2014a; Subhashini and Kumar 2014). A hydroponics study was carried out by Singh et al. (2010) to evaluate the effect of В. mucilaginosus, Rhizobium spp., and A. chroococcum on their capacity to mobilize K from waste mica using wheat and maize as the test crops under a phytotron growth chamber. The significant K assimilation was recorded in wheat and maize, where waste mica was the sole source of K; this has been translated into higher biomass accumulation, K content, and acquisition through plants as well as crude protein and chlorophyll content in plant tissue. Among the rhizobacteria, *B. mucilaginosus* showed significantly greater mobilization of potassium over *Rhizobium* and *A. chroococcum* inoculation. According to Sheng and He (2006), in the investigation of K mobilization by the wild-type strain NBT of *B. edaphicus*, in a pot experiment, wheat has been grown in a yellow-brown soil that contained low available K. After inoculation with bacterial strains, the root growth and shoot growth of wheat were significantly increased and higher NPK contents of plant components as compared to un-inoculated.

Inoculation with KSMs have been reported to exert beneficial effects on growth of cotton and rape (Sheng 2005), pepper and cucumber (Han et al. 2006), khella (Hassan et al. 2010), sorghum (Badr et al. 2006), wheat (Sheng and He 2006), tomato (Lin et al. 2002), chili (Ramarethinam and Chandra 2005), sudan grass (Basak and Biswas 2010), and tobacco (Zhang and Kong 2014). Similarly, Zahra et al. (1984) reported that the effect of soil inoculation of the silicate bacteria B. circulans for solubilization of Si and K from various minerals and soil showed significant increase of organic matter and 17 % yield of rice (Muralikannan 1996). Increased wheat yield up to 1.04 t/ha reported by Mikhailouskaya and Tcherhysh (2005) with inoculation of KSMs on several eroded soils and comparable with yields on moderately eroded soil without bacterial inoculation and dry matter production also increased. According to Badar et al. (2006), the co-inoculation of KSMs with P- and K-bearing minerals on sorghum was recorded to enhance dry matter yield (48 %, 65 %, and 58 %), P (71 %, 110 %, and 116 %) and K (41 %, 93 %, and 79 %) uptake in three different soils: sandy, calcareous, and clay soils, respectively. Archana et al. (2008) reported that the KSMs was isolated from rock and rhizosphere soils of greengram (Vigna radiata) and reported that these KSMs enhanced the solubilization of K in acid-leached soil as well as increased seedling growth and yield of greengram. Sugumaran and Janarthanam (2007) reported that increased in the oil content 35.4 % and dry matter by 25 % in groundnut crop and available K and P increased from 86.57 to 99.60 mg/kg and 6.24 and 9.28 mg/kg, respectively, in soil by inoculation of *B. mucilaginosus* as compared to un-inoculated control.

According to Archana et al. (2012), the efficient K-solubilizing bacteria Bacillus spp. showed increase in growth and yield of maize. It indicates that the KSMs significantly increased yield, plant growth, and nutrient uptake component over absolute fertilizer control. Supanjani et al. (2006) reported that integration of P and K rocks with inoculation of K- and P-solubilizing bacteria increased K availability from 13 % to 15 % and P availability from 12 % to 21 %, respectively. Soil application of KSMs plant has ~16 % photosynthesis and 35 % higher leaf area to control. The overall results of this experiment is the treatment of P and K rocks with P- and K-solubilizing bacterial strain were sustainable and alternative of chemical fertilizer for crop production. Bagyalakshmi et al. (2012) reported that the K-solubilizing stains were isolated from rhizosphere of tea and used as biofertilizers of K in tea that have a solubilizing capacity of muriate of potash (MOP) was increased as compared to mineral K sources. Supplementation of glucose and ammonium nitrate was found to be highly effective in solubilization of MOP as compared to the other sources which should be considered prior to the application of these strains in tea soils as bio-inoculants.

K-solubilizing bacteria are extensively used as biofertilizers in China and Korea in the significant areas of cultivated soils in both of the countries are deficient in plant-available K is considered to be a major limiting factor for food production in many agricultural soils (Xie 1998). Thus the application of K-solubilizing bacteria as biofertilizers for agriculture improvement that can reduce the application of agrosupport chemicals and eco-friendly crop production (Prajapati et al. 2013; Maurya et al. 2014; Meena et al. 2014a; Zhang and Kong 2014). Therefore, it is imperative to isolate more species of mineral-solubilizing bacteria to enrich the pool of microbial species and genes as microbial fertilizers, which will be of great benefit to the ecological development of agriculture (Liu et al. 2012).

1.8 Future Prospect of Potassium-Solubilizing Microorganisms

Potassium-solubilizing microorganisms play an important role in plant nutrition that enhance the K acquisition of plants through soil which increase plant growth promotion activities, these KSMs contribution important role as to bio-fertilization of agricultural crops. Accordingly, further investigation is required to improve the performance and use of potassium-solubilizing microorganism as efficient microbial bio-inoculants. The greater attention is needed for studies and application of efficient combinations of potassiumnew solubilizing microorganisms and other plant growth-promoting microorganisms for improved results. The mechanisms explaining the synergistic interaction among KSMs required further research to elucidate the biochemical basis of these interactions. On the other hand, the application of biotechnological tools for genetic manipulation of potassium-solubilizing microorganism that increase their potassium-solubilizing efficiency/ability/capabilities and/or the insertion of this trait into other strains of plant growthpromoting effects is not only crucial but also causes it to be practically feasible. In addition to application of classical genetic methods to mutants, the strains that increased the organic acids production which could constitute an effective approach that cannot be underestimated. The genetic manipulation through recombinant DNA technology use to a feasible approach for enhanced the strains efficiency. Gene cloning approaches involved in mineral potassium solubilization, such as those influencing the synthesis of different organic acids, would be the first step in such a genetic manipulation program.

Future research work should also investigate or improve the performance and stability of the potassium solubilization trait once the microorganisms have been inoculated in soil as in both genetically modified and natural strains. The survival efficiency and their establishment of the inserted strains can be affected by little competitiveness as a result limiting the effectiveness of application. However, the putative risk rose during the release of genetically modified microorganisms in soil which is an important matter of controversy, in this regard to the possibility of horizontal transfer of the introduced DNA to many other soil microorganisms. For these reasons, the application of genetic reporter systems, such as green fluorescent protein genes or bioluminescence genes, are important in studying the survival and fate of the strain in soil. The genetic engineering of the potassium-solubilizing character must be eventually required for the chromosomal integration of the gene for greater stability of the character and also to avoid horizontal transfer of the introduced gene in soil. This strategy would also decrease/prevent the risk of metabolic load that showed the presence of the plasmid in the bacterial cell. The chromosomal integration may also have disadvantage for little expression activity, due to the small copy number of the gene as compared to the plasmid-harbored genes. An alternative approach to this situation might be due to the integration of multicopies of the target gene. In addition to these, the application of major and species-specific promoters, which have been activated under certain specific environmental conditions of soil and another interesting approach for successful gene expression, is engineered strain.

Acknowledgment Authors are thankful to the Head of the Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, for providing all the necessary facility to conduct this experiment. VSM is thankful to the University Grants Commission (UGC), New Delhi, for the fellowship during his Ph.D. work and SKM is thankful to the Indian Council of Agricultural Research (ICAR), New Delhi, and Government of India (GOI) for the junior research fellowship (JRF) during her study.

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