Phosphate-Solubilizing Microorganisms in Sustainable Production of Wheat: Current Perspective

3

Mohammed Saghir Khan, Asfa Rizvi, Saima Saif, and Almas Zaidi

Abstract

In terms of global production, wheat among cereals ranks third after rice and maize, contributing about 35% of the total food grain production. Wheat due to high nutritional value is considered one of the important dietary constituents and, hence, has become one of the better food choices around the world. For growth and development, wheat requires large amounts of major plant nutrients especially phosphorus (P). Application of sufficient amounts of P has many beneficial impacts on wheat including its role in growth, grain formation, and development, and in straw yield. Phosphorus deficiency, however, may adversely affect the growth and, therefore, hampers the physiological processes leading eventually to overall stunting of the plant. In order to circumvent the phosphorus problems and hence to achieve optimum yields, wheat growers usually apply excessive amounts of chemical phosphatic fertilizer which is both expensive and destructive to soil fertility. To overcome these problems, a physiologically versatile array of microorganisms especially belonging to phosphate-solubilizing group has been introduced into the agricultural system for improving wheat production. The P-solubilizing microorganisms (PSM) solubilize unavailable soil P and make it available for uptake by plants. The use of microbial phosphatic fertilizer (microphos) in wheat production system is considered an eco-friendly strategy without adversely affecting the soil health. Despite numerous informations available on the impact of P-solubilizing microorganisms on various plants, literature suggesting the use of PSM in wheat production is limited. Realizing the importance of PSM in enhancing the overall performance of wheat, attempt has been made to better understand as to how the PSM affects wheat production in variable agricultural practices. Also, efforts will be made to find PSM which could be

M.S. Khan (✉) • A. Rizvi • S. Saif • A. Zaidi
Department of Agricultural Microbiology, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh 202002, Uttar Pradesh, India
e-mail: khanms17@rediffmail.com

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applied to facilitate the growth and development of wheat grown in different agroecological niches. Constant and sustainable application of PSM is expected to decrease the use of fertilizers in wheat production strategies.

3.1 Introduction

Wheat (*Triticum aestivum*) is one of the important cereal crops which is extensively cultivated and used as food worldwide. Wheat is cultivated in variable climatic conditions that range between 47°S and 57°N latitudes on different soils including sandy and clayey soil and has the highest adaptation among all the crop species (Naresh et al. 2014). Globally, about 680 million tons of wheat was produced during 2008–2012, while in 2011, the production rate reached to almost 700 million tons (FAO Stat http://faostat.fao.org/site/291/default.aspx). Moreover, the statistics reveal that more than 600 million tons of wheat is harvested annually. Among various wheat-producing countries, India is the second largest wheat-producing country with 11.9% production from approximately 12% of total area (USDA 2010). In India, wheat is cultivated in about 30 million hectares land resulting in 93 million tons yield. The national productivity is estimated as 2.98 tons ha^{-1}. However, despite being an important food crop of the country, the average production of wheat is slowly dwindling due to several reasons (Ray et al. 2012). Chief among them has been the declining cultivable lands, fluctuating environmental conditions (global warming) and excessive usage of chemical fertilizers in order to obtain maximum yields.

The consistently increasing cost of chemical fertilizers and their deleterious impact on soil fertility and human health (via food chain) are some of the vital problems of farmers growing wheat across different regions (Eman et al. 2008; Singh et al. 2008). In order to reduce the use of fertilizers in wheat production, scientists require searching for some other inexpensive alternatives. In this regard, biofertilizers, defined as a biological product containing living microorganisms which, when applied to seed, plant surfaces, or soil, colonize the rhizosphere or the interior of the plant and facilitate growth by enhancing the supply or availability of primary nutrients to the host plant (Vessey 2003), involving PSM that have offered a better alternative to the expensive and environmentally unfriendly fertilizers in wheat production (Sarker et al. 2014; Sharma et al. 2013). The chief benefit of applying PSM in wheat cultivation is their ability to better colonize and establish in the rhizosphere and to make P available to plants constantly (Sharma 2002). Apart from P, PSM also facilitates the growth of plants by providing gibberellins, cytokinins, and IAA (Priya et al. 2013; Sharma et al. 2012), improves uptake of water and nutrients (Abbasniayzare et al. 2012; Khan et al. 2007), secretes antibiotics and other toxic products (Shanmugam et al. 2011; Lipping et al. 2008), and supplies vitamins belonging to β-group (Revillas et al. 2000). Also, PSM improves the wheat growth by inhibiting plant pathogens (Salma et al. 2014). For instance, P-solubilizing bacteria like *Pseudomonas* and *Bacillus* species when used alone or in combination profoundly increased grain yield, tiller formation, and seed P of wheat (Afzal et al.

2005). Similarly, the pre-sowing application of PSM inoculated wheat resulted in a considerable increase in yield relative to uninoculated wheat (Dwivedi et al. 2004). These and other similar studies suggest that the sole or combined application of PSM could be used to enhance the overall performance of wheat.

3.2 Nutritional Importance of Wheat

Wheat is one of the main staple food crops for majority of the world population and is the major staple food in many Asian countries. Wheat is an important cereal crop of human dietary systems all over the world. Nutritionally, it is highly rich in carbohydrate and serves as a good source of energy. Interestingly, proteins and fibers are also the major constituents of wheat composition. In addition, wheat also contains significant amounts of vitamins, minerals, lipids, and a few phytochemicals. The nutrient composition of wheat is represented in Table 3.1.

Table 3.1 Dietary components of winter wheat

Energy	1368 KJ (327 k Cal)
Carbohydrates	71.18 g
Sugars	0.41
Dietary fiber	12.2 g
Fat	1.54 g
Proteins	12.6 g
Vitamins	
Thiamine (B1)	33% (0.383 mg)
Riboflavin (B2)	10% (0.115 mg)
Niacin (B3)	36% (5.464 mg)
Pantothenic acid (B5)	19% (0.954 mg)
Vitamin B6	23% (0.3 mg)
Folate (B9)	10% (38 μg)
Choline	6% (31.2 mg)
Vit. E	7% (1.01 mg)
Vit. K	2% (1.9 mg)
Minerals	
Calcium	3% (29 mg)
Iron	25% (3.19 mg)
Magnesium	35% (126 mg)
Manganese	190% (3.985 mg)
Phosphorus	41% (288 mg)
Potassium	8% (363 mg)
Sodium	0% (2 mg)
Zinc	28% (2.65 mg)
Other constituents	
Selenium	70.7 μg

Source: USDA nutrient database

3.3 Role of Phosphorus in Promoting Wheat Growth

Plants in general require variety of nutrient elements for survival and growth. These elements are categorized as macro- and micronutrients depending upon the requirement of various crop plants. Phosphorus among plant nutrients is required in larger quantities by plants, the deficiency of which severely affects the whole metabolism of many plants including wheat. Phosphorus plays an important part in photosynthesis, energy transfer, utilization of sugar and starch, nucleus formation and cell division, signal transduction, macromolecular biosynthesis and respiration (Khan et al. 2010), and N_2 fixation (Wibisono et al. 2015). It also initiates root growth and development and maintains the overall health of the plants.

In wheat, P plays a prime role in strengthening of the straw and results in better fruit production (Anonymous 1988). Presence of P also accounts for a better tillering in wheat, promotes early maturation of plant, and assists seed formation. For example, in a study, the combined effect of irrigation and phosphorus demonstrated a positive impact on the developmental stages of wheat. Furthermore, an increase in the number and weight of the grains was recorded. Topical application of P further increased the size of wheat grains (Hossain et al. 1996; Turk and Tawaha 2002). Phosphorus fertilization is therefore a major input in crop production across different regions, because some soils lack sufficient P to optimize crop quality and yields (Griffith 2009). Effective nutrient management, hence, requires that P be available in adequate amounts when needed by the plant (Sarfraz et al. 2009). In order to supply available and soluble form of P to wheat plants, a group of soil microorganisms collectively called as phosphate-solubilizing microorganisms (PSM) have been applied. To substantiate this further, Islam and Hossain (2012) proposed that the use of synthetic phosphatic fertilizer could be reduced if the insoluble soil P is solubilized naturally by PSM and is made available to plants. In some other experiments, P-solubilizing bacteria (PSB) when applied as bioinoculants have been shown to solubilize the fixed soil P, thereby improving the crop yield (Gull et al. 2004) including those of wheat (Afzal and Asghari 2008). In addition to the sole application of PSM, there has been considerable reports where PSM in synergism with other organisms, for example, AM fungi, has been found to enhance uptake of solubilized soil P (Barea et al. 2002) and concurrently increased nutrient uptake and yield of wheat and maize (Raja et al. 2002).

3.4 Rationale for Using Phosphate-Solubilizing Microorganisms in Wheat Production

Phosphorus, one of the major plant nutrients, affects many stages of plant growth and enhances grain yield and yield components. On the contrary, in many agricultural production systems, P has been identified as the most deficient essential nutrient after N. And hence, nutrient supply to agronomic production systems has increased to achieve optimum yields in order to sustain the growing populations demand around the world. When soil is deficient in available P, phosphatic fertilizers are applied to soils. Although inorganic fertilizers are readily available, they are slowly converted to unavailable forms due to precipitation/complexation. Among cereals, wheat requires more nutrients than other crops. Worldwide, the commercial production of fertilizers needs substantial amounts of energy, and, hence, it becomes costly. Moreover, phosphatic fertilizers are consistently used in wheat production to achieve higher yields. The excessive and continued application of phosphatic fertilizers, however, destruct the soil fertility (Younis et al. 2013). High dose of P fertilizers causes abrupt shoot growth, while it limits root growth. Also, following accumulation within soils, P can pollute the ground water resources. Protein digestion inhibitors deposited in plant cell vacuoles were not taken up by sucking herbivores but hampered the chewing herbivores (Mattson 1980). Moreover, the uptake of P by plants is considerably low due to its rapid fixation with Fe and Al oxides in acidic soils (Goldstein 1986; Norrish and Rosser 1983) and calcium in neutral or calcareous soils (Lindsay et al. 1989). Due to these, approximately 75–90% of the P fertilizers applied to soil are lost, and, hence, plants generally suffer from P deficiency. The combined use of the phosphatic fertilizers to maximize the wheat production without experiencing any toxicological hazards is therefore urgently required. In order to overcome the cost of production, abolish the toxic effect of fertilizers, and fulfill the P requirements of wheat, it has become imperative to search for some newer and inexpensive option that could solve such difficulties. To address such problems, the focus is shifted toward the use of PSM both singly (Kumar et al. 2014a) and as mixture with fertilizers (Babana et al. 2013) or as coculture (Upadhyay et al. 2012) to improve soil fertility leading to increase in wheat yields (Zaidi and Khan 2005). When applied properly, PSM in agricultural practices has been found to decrease the use of costly phosphatic fertilizers (Ali et al. 2014; Dalve et al. 2009). For example, Ramlakshmi and Bhrathiraja (2015) in a study conducted for marigold production have suggested that the mixture of *Paenibacillus polymyxa* (phospho-bacterium) and *Glomus fasciculatum* (AM fungi) could decrease the application of P fertilizer by 25%. The integrated nutrient management (Chaitra and Patil 2007) which involves the use of PSM carrying variable characteristics has, therefore, motivated wheat growers worldwide (Naqvi and Ahmad 2012; Goes et al. 2012). Phosphate-solubilizing microorganisms also act as biological control agents (Zaidi et al. 2014) and by limiting the phytopathogens increase the performance of plants (Basharat et al. 2011).

3.5 PSM Improves Wheat Production

3.5.1 PSM: Definition, Origin, and Selection of Phosphate-Solubilizing Microorganisms

Phosphate-solubilizing microorganisms are a group of useful microorganisms which hydrolyze organic and inorganic P (Chen et al. 2006b). Numerous PSM have been recovered from non-rhizosphere (Onyia and Anyanwu 2013) and rhizosphere soils (Qiao et al. 2013), rhizoplane (Sarkar et al. 2012), phyllosphere (Mwajita et al.

2013), rock phosphate (RP) deposit area soil (Mardad et al. 2013), marine environment (Mujahid et al. 2014), and polluted soils (Susilowati and Syekhfani 2014). Some of the important P-solubilizing bacteria belongs to genera *Achromobacter* (Ma et al. 2009), *Acinetobacter* (Gulati et al. 2010), *Sphingomonas* and *Burkholderia* (Panhwar et al. 2014; Song et al. 2008), *Bacillus* (Tallapragada and Usha 2012), *Serratia* (Selvakumar et al. 2008), *Enterobacter* (Frank and Julius 2012), *Micrococcus* (Reena et al. 2013), *Pseudomonas* (Mehnaz et al. 2010), rhizobia (Kumar et al. 2014; Kenasa et al. 2014), and actinomycetes (Saif et al. 2014). The important P-solubilizing fungi belong to genera *Penicillium* (Reena et al. 2013), *Aspergillus* (Coutinho et al. 2012), and *Trichoderma* (Yasser et al. 2014). However, among various phosphate solubilizers, P-solubilizing fungi (PSF) in general have been found superior P solubilizer compared to PSB (Venkateswarlu et al. 1984). Like any other plant, wheat too represents a habitat for diverse PSM, which colonize the (i) rhizosphere (Majeed et al. 2015; Kundu et al. 2009), (ii) the phyllosphere (Verma et al. 2016a), (iii) PSM living inside tissues (endophytes) (Verma et al. 2016a), and (iv) stressed environment.

Rhizosphere PSM The region of soil that is directly influenced by root exudates and associated soil microbiota is generally termed as rhizosphere. The term rhizosphere (Greek word "rhizo" meaning root and "sphere" is one field of action, influence, or existence) was introduced by Hiltner in 1904. The rhizosphere is generally rich in rhizodeposition (sloughed-off plant cells), proteins, and sugars released by roots. These exudates support the growth of various microbial communities including PSM. Like many other microbial communities, PSB have been recovered from many crop rhizospheres including those of wheat. Some of them have been identified as *Pseudomonas aeruginosa* (Kumar et al. 2015), *P. fluorescens*, *P. putida* (Zabihi et al. 2011), *P. stutzeri* (Venieraki et al. 2011), *Bacillus* (Ogut and Er 2016; Ogut et al. 2011), *Lysinibacillus sphaericus*, *Paenibacillus polymyxa*, *Staphylococcus succinus*, *Sporosarcina* sp. (Verma et al. 2016b), *Azotobacter chrococcum* (Kumar and Narula 1999), *Thiobacillus* sp. (Babana et al. 2016), *Vibrio splendidus* (Babana et al. 2013), *Proteus* sp. (Billah and Bano 2015), *Azospirillum brasilense* (Venieraki et al. 2011), *Acinetobacter* (Ogut et al. 2010), *Stenotrophomonas* sp. AJK3 (Majeed et al. 2015), *Enterobacter* sp., *Arthrobacter chlorophenolicus* (Kumar et al. 2014a), and *Serratia marcescens* (Lavania and Nautiyal 2013). Of the filamentous fungi involved in solubilization of insoluble P, *Aspergillus niger* (Shrivastava and D'Souza 2014), *Penicillium bilaii* (Ram et al. 2015), *Penicillium oxalicum* (Xiao et al. 2013), and *Mucor ramosissimus* (Xiao et al. 2009) are the most important PSF, while strains of *Candida krissi* (Xiao et al. 2009) have also been identified as P solubilizer which solubilized insoluble P by secreting organic acids.

Phyllosphere PSM The term phyllosphere refers to the total aboveground portions of plants inhabited by microorganisms (Last 1955; Ruinen 1956). Phosphate-solubilizing microbes in the wheat phyllospheres have been reported and identified using PCR technique. For instance, Verma et al. (2014) isolated wheat-associated epiphytic bacteria from five locations in central zone (one of the wheat agroecological zones) in India. The phosphate-solubilizing bacteria isolated from phyllosphere (N = 89) belonged to genera *Arthrobacter*, *Bacillus*, *Corynebacterium*, *Methylobacterium*, *Paenibacillus*, *Pseudomonas*, and *Psychrobacter*. Of these, *Arthrobacter humicola* showed the highest P-solubilizing activity. Other genera identified to species level using 16S rRNA gene sequencing and subsequent molecular phylogeny analysis included *Paenibacillus amylolyticus*, *Bacillus aryabhattai*, *Methylobacterium extorquens*, *Methylobacterium mesophilicum*, *Methylobacterium radiotolerans*, *Psychrobacter fozii*, and *Pseudomonas fuscovaginae*.

Endophyte PSM An endosymbiont (bacterium or fungus) often called an endophyte resides inside plant tissues (Hardoim et al. 2008) for longer periods of its life cycle but causes no diseases to plants (Vijayabharathi et al. 2016; Puri et al. 2015; Hardoim et al. 2015). Also, the endophytic bacteria improve plant growth and nutrition more efficiently compared to rhizospheric bacteria because they show more intimate relationship with plant tissues. The endophytes have an ecological advantage over epiphyte microbes because they are shielded from unfavorable environmental conditions such as high temperature, salinity, drought, pH, osmotic potentials, and ultraviolet radiation (Seghers et al. 2004). The endophytes generally adhere to root hair zone of apical roots and enter through a crack or damage. Following entry inside, they colonize the differentiation zone and intercellular spaces in epidermis (Raven et al. 2009). After crossing the exodermal barrier, they colonize different regions such as point of entry, deep inside cortex, and the cortical intercellular spaces. The plant tissue type, plant growth stage, and soil fertilizer treatment all contribute to composition of endophyte bacterial community in wheat (Robinson et al. 2015). Like rhizosphere/phyllosphere microbial communities, endophytes also facilitate the growth of plants by various mechanisms (Gaiero et al. 2013) including P-solubilization (Wakelin et al. 2004). There are other studies also which suggest that soil inoculation with P-solubilizing *Bacillus* spp. can solubilize unavailable soil P and applied P, leading to a better plant development and greater yields (Canbolat et al. 2006). The root endophytes Bacillus, *Enterobacter*, *Micrococcus*, and *Pseudomonas* genera identified by Mbai et al. (2013) have also shown to have potential to solubilize P. In other study, Jha and Kumar (2009) isolated a diazotrophic bacterium identified as *Achromobacter xylosoxidans* WM234C–3 from surface-sterilized roots and culms of wheat variety Malviya 234 which had a significant P-solubilizing activity. Zinniel et al. (2002) also isolated diazotrophic endophytic bacteria from wheat, whereas the filamentous *Actinobacteria* and some fungi were observed in wheat plants by Coombs and Franco (2003). Recently, Oteino et al. (2015) reported that majority of the endophytic *Pseudomonas* strains produced gluconic acid (GA) (14-169 mM) and demonstrated moderate to high P-solubilizing activity (400–1300 mgl^{-1}). Thus, the study of endophytes is important primarily for two reasons – (i) it helps to better understand its ecology and (ii) the bioactive molecules secreted by endophytes facilitate the growth of plants in sustainable agricultural practices. Therefore, concerted efforts should be directed to find some new and potentially exciting endophytes for ultimate use in different agricultural production systems across different ecological niches.

Stress-Tolerant PSM Phosphate-solubilizing microorganisms in general have regularly been isolated from conventional environment. However, the isolation of PSM from derelict/stressed environment would be advantageous because such stress-tolerant PSM could be beneficial for crops growing in stressed/polluted soils. In this context, various P-solubilizing bacteria have been recovered from wheat growing in disturbed environments, for example, low temperature (Mishra et al. 2011; Verma et al. 2015a), drought (Verma et al. 2014), acidic soil (Verma et al. 2013), and salinity (Egamberdieva et al. 2008; Tiwari et al. 2011) using culture-dependent techniques. Verma et al. (2016a) in a recent investigation assessed the diversity and functional attributes of thermotolerant bacteria hosting leaves, shoots, roots, and rhizospheric soils of wheat growing in the peninsular zone of India. Majority of the isolated genera demonstrated P-solubilizing activity and belonged to genera *Achromobacter*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Methylobacterium*, *Pseudomonas*, *Rhodobacter*, *Salmonella*, and *Staphylococcus*. Verma et al. (2016b) in a follow-up experiment recovered the epiphytic bacterial strains identified as *B. amyloliquefaciens*, *A. faecalis*, and *P. poae* from the wheat phyllosphere. In a similar study, *A. faecalis* and *P. poae* were isolated from wheat growing at arid land and high-temperature regions and were identified for the first time as epiphytic PGPB (Joo et al. 2005). Similar studies were carried out by Verma et al. (2013) to reveal acidotolerant P-solubilizing bacteria exhibiting other PGP activities from phyllosphere of two varieties of wheat growing in acidic soil in the southern hills zone of India. Among these PSB, *Variovorax soli* (21.52 ± 1.3 μg P mg^{-1} day^{-1}) was isolated from wheat var. HD2833 and *Methylobacterium* sp. (36.35 ± 1 μg P mg^{-1} day^{-1}) and *M. radiotolerans* (21.35 ± 1 μg P mg^{-1} day^{-1}) from wheat var. HW3094. Apart from bacteria and fungi, the P-solubilizing actinomycetes can also survive in extreme environments (e.g., drought, fire), and through their ability to secrete antibiotics and phytohormone-like compounds, they can enhance plant growth. *Micromonospora aurantiaca* and *Streptomyces griseus*, for example, have shown the greatest stimulatory effect on wheat due to their P-solubilizing efficiency and plant growth-promoting activities (Hamdali et al. 2008, Jog et al. 2012). This ability of actinomycetes of surviving under extremes of environmental conditions has therefore attracted greater attention toward their use as biological agents in stressful conditions. Stress-tolerant microbial inoculants are required for inoculation under extreme environments like high temperature so that such organisms could survive under such adverse environment while maintaining their plant growth-promoting activities. And hence, the selection of thermotolerant P-solubilizing microorganisms carrying numerous PGP traits could be used to produce inoculants for crops grown in the arid, subarid, high-plateau, and high-temperature zones. Furthermore, considering the variety of PSM's widely spread in different habitats, there is ample scope to find many more prospective microorganisms from variable environments for eventual transfer to end users/farmers.

3.6 Selection of Phosphate Solubilizers

P-solubilizing microorganisms have been recovered from conventional (Surapat et al. 2013) to derelict soils (Susilowati and Syekhfani 2014) and from rhizosphere (Ranjan et al. 2013) to endophytic (Resende et al. 2014) environment. They have subsequently been used in various agronomic practices with greater positive impact on different crops (Sonmez and Tufenkci 2015) including wheat (Sial et al. 2015) under different production systems. And hence, the use of P-solubilizing organisms in crop production is increasing which is likely to substitute or/decrease the use of phosphatic fertilizers considerably (Adesemoye et al. 2009). Considering the importance of PSM in sustainable crop production, many workers have isolated such beneficial microbes (Ahemad and Khan 2012) employing serial plate dilution technique or enrichment culture method. Generally, the PSM are isolated using media containing insoluble tricalcium phosphate (TCP), and the best suitable and most widely used medium for this purpose has been the Pikovskaya medium (Pikovskaya 1948) (g/l: glucose 10, Ca_3 $(PO_4)_2$ 5, $(NH_4)_2$ SO_4 0.5, NaCl 0.2, $MgSO_4.7H_2O$ 0.1, KCl 0.1, yeast extract 0.5, $MnSO_4$ and $FeSO_4$ trace, and pH 7). Rhizospheric or non-rhizospheric soil samples are diluted serially and spread plated (100 μl) or streaked or spot (10 μl) inoculated on Pikovskaya agar plates or any plates having insoluble P and properly incubated for 5–7 days (bacteria) and 3–5 days (fungi and actinomycetes) at 28 ± 2 °C. Organisms showing PS activities are identified by the appearance of zone of solubilization (clear halo) near microbial growth (Plate 3.1) on TCP/insoluble P supplemented plates. The consistency of this technique is, however, not

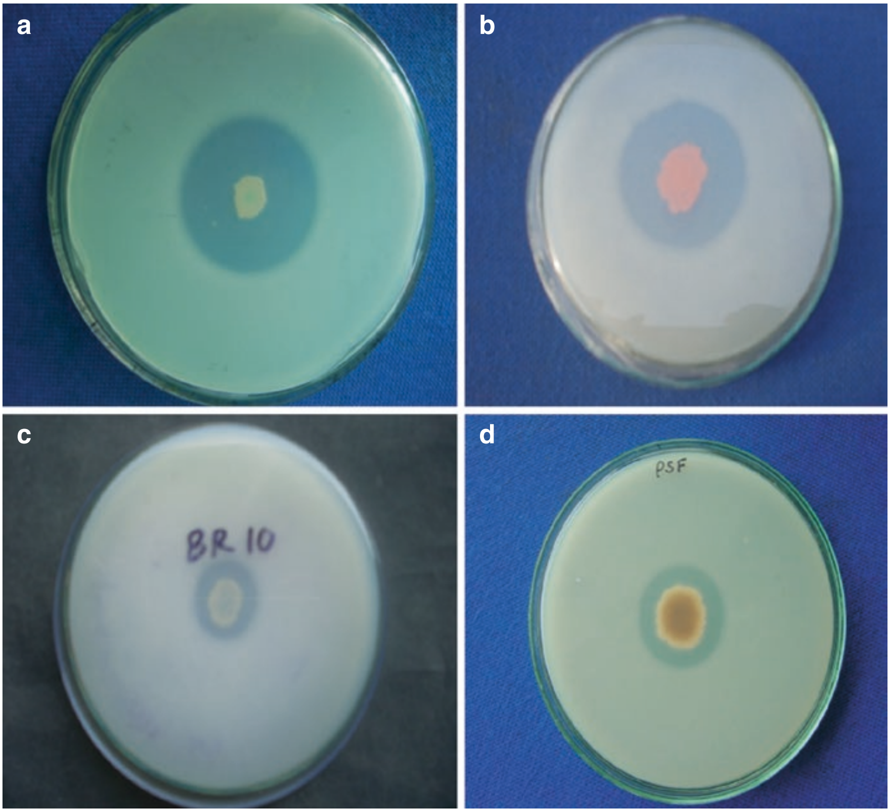

Plate 3.1 Tricalcium phosphate solubilization on Pikovskaya plates by (**a**) *Pseudomonas* sp., (**b**) *Serratia* sp., (**c**) *Bacillus* sp., and (**d**) fungal species

accepted by many workers since numerous bacterial isolates in other studies have even though failed to yield any visible zone of P-solubilization on agar plates but instead solubilized insoluble P in culture medium. However, considering halo formation as a positive indicator of P-solubilizers, the organisms (colonies) showing halo on plates are picked up and used to determine their capability to solubilize insoluble P under liquid medium. After evaluating their P-solubilizing ability on agar plates and in liquid medium, the PSM are assessed for their in vitro potential to secrete plant growth-promoting bioactive molecules. The most putative strains are then identified to species level employing molecular techniques, for example, 16S rDNA sequencing and phylogenetic method. The organisms identified by biochemical (identified up to genus level only) or molecular methods (identified up to species level) and showing single or many plant growth-promoting activities apart from their intrinsic P-solubilizing activity are selected and checked in pots and fields using seed treatment, seedling dipping, or soil application methods for their final transfer to growers for consequent application in agronomic practices as an inexpensive phosphatic option.

3.7 Phosphate Solubilization: How It Occurs?

The heterogenous microbial populations in different habitat include PSM also. Out of the total P-solubilizing microorganisms, 1–50% is contributed by PSB, whereas only 0.1–0.5% populations account for PSF (Chen et al. 2006a). Both phosphate-solubilizing bacteria and fungi convert the insoluble form of phosphorus (inorganic and organic) into soluble and available form of P which is taken up as a source of P by plants. Broadly, the solubilization/mineralization of inorganic/organic P occurs by one of the three mechanisms: (a) production of organic acids, (b) excretion of H^+ions, and (c) synthesis of enzymes (Kapri and Tewari 2010; Arcand and Schneider 2006). Of these, solubilization of inorganic P through the organic acids secreted by PSM (Khan et al. 2014) is one of the most extensively accepted theories of P-solubilization. The organic acids produced by PSM largely include gluconic acid, oxalic acid, ketogluconic acid, citric acid etc. (Table 3.2). The release of organic acids is directly associated with the lowering of pH of the medium (Mardad et al. 2013; Whitelaw 2000; Maliha et al. 2004). The effectiveness of solubilization/mineralization, however, depends on the types and amounts of organic acids/enzymes secreted into the liquid medium. Also, the inherent properties of the organic acids are vital than the whole amount of acids released by P-solubilizers (Scervino et al. 2010). Moreover, the insoluble P is also converted into soluble P without the secretion of OA by microbes (Illmer and Schinner 1992). For example, ammonia assimilation resulting in proton extrusion has been found as an alternative mechanism for P-solubilization (Parks et al. 1990; Ogut et al. 2011). Phosphate solubilization by PSM also occurs by enhancing the process of chelation of cations that are bound to the soil P or by generating soluble compounds with cations linked with insoluble soil P so as to discharge the P into the soil system. Another mechanism of P-solubilization includes the production of enzymes

Table 3.2 Organic acids produced by phosphate-solubilizing microorganisms

P-solubilizing microorganisms	Organic acids produced	Initial pH of medium	Final pH of medium	Amount of P solubilized (mg/l)	Reference
Aspergillus sp.; *Penicillium* sp.	Citric, gluconic, oxalic, succinic, glycolic, malic	7.0; 7.0	3.2; 3.3	392 ; 381	Sane and Mehta (2015)
Aspergillus niger	Oxalic, lactic			3640	Padmavathi (2015)
Trichoderma harzianum	Lactic, succinic, tartaric, citric	5.4	4.3	ND	Li et al. (2015)
Rhizobium tropici, *Paenibacillus kribbensis*, *Acinetobacter* sp.	Malic, 2-ketoglutaric, lactic, succinic, tartaric, propionic, gluconic	5.0; 7.0	5.0; 4.0	70 ; 75; 75 (approx.)	Marra et al. (2015)
Pseudomonas sp. *R7*	Lactic, isocitric, tartaric, pyruvic	7.03	4.92	19.5	Mihalache et al. (2015)
Pseudomonas fluorescens L228	Gluconic	7.0	4.06	1312	Oteino et al. (2015)
Rhizobium sp. *VM-2*	Organic acids	7.0	4.93	799	Satyanandam et al. (2014)
Rhizobium sp. *RASH6*	Succinic, gluconic	7.0	3.4	275	Singh et al. (2014)
Bacillus megaterium	Malic, quinic	7.0	4.0	ND	Kang et al. (2014)
Trichoderma harzianum	Citric, lactic, succinic	7.2	4.68	9.31	Promwee et al. (2014)
Pantoea agglomerans, *Burkholderia anthina*, *Enterobacter ludwigii*	Gluconic, oxalic, citric	7.0	3.2; 3.5; 4.0	575.16; 384.28; 600	Walpola and Yoon (2013a, b)
Enterobacter hormaechei sub sp. *steigerwaltii*	Gluconic, succinic, malic, glutamic	7.0	3.5	505	Mardad et al. 2013
Azospirillum, *Bacillus*, *Enterobacter*	Acetic, citric, gluconic	7.0	Reduced pH	218.1; 298.3; 258.6	Tahir et al. (2013)
Burkholderia ambifaria KS 01, *B. Tropica* KS 04	Acetic, citric, gluconic, lactic, succinic, propionic	6.6	4.86; 4.05	433.81; 499.85	Surapat et al. (2013)
Penicillium sp.	Gluconic, citric	6.25	3.22	39.2–86.1	Nath et al. (2012)
Acinetobacter sp. *WR 1222*	Gluconic	7.0	4.21	414	Ogut et al. (2010)

ND not determined

like (a) phosphatases (meant for dephosphorylation of phospho-ester bonds), (b) phytases (responsible for the release of P from phytic acid), and (c) phosphatases (enzymes that cleave the C-P linkage in organophosphonates). The phosphatases and phytases together mediate the P mineralization process. The phosphatases play an important role in releasing the inorganic phosphates through scavenging of phospho-ester bonds, whereas most of the phytases are involved in the cleavage of C-P bonds. Another interesting role attributed to these enzymes is the degradation of phytate, thereby leading to mineralization of organic P present within the soil (Behera et al. 2014).

3.8 How Phosphate Solubilizers Facilitate Wheat Growth

Indeed, the plant growth enhancement by P-solubilizing microorganisms in P-deficient soil occurs greatly through solubilization of insoluble P. The soluble P is then taken up as a major nutrient element by plants. Apart from making soluble P available to plants, the PSM also secretes some important active biomolecules (Table 3.3) that directly or indirectly enhance the growth and productivity of crop plants (Fig. 3.1). Chief among them is the release of siderophores (Ghosh et al. 2015), indole acetic acid (Chitraselvi et al. 2015), and gibberellic acid (Jha and Subramanian 2014). Synthesis of siderophores, an iron-chelating substance by PSB, for instance, *Pantoea agglomerans* and *Burkholderia anthina*, may indirectly affect the growth of plants (Datta and Chakrabartty 2014; Walpola and Yoon 2013b, c). Siderophores released by PSB form a complex with iron (Fe^{3+}) in the rhizosphere and limit its availability to the phytopathogens and concomitantly prevent phytopathogens from causing damage to plants. Thus, PSB due their ability to secrete siderophores could be developed as biocontrol agent as well. Secretion of IAA by phosphate solubilizers, for example, *Azospirillum*, *Bacillus*, and *Enterobacter*, (Tahir et al. 2013) is yet another microbiological trait that has shown greater positive impact on overall performance of wheat plants. IAA secreted as a secondary metabolite due to rich supply of substrates by PSB control cell elongation and division, phototropism, and apical dominance in plants (Remnas et al. 2008; Ali et al. 2009). Also, IAA helps in the expansion of roots and increases the number of root hairs and lateral roots which participate in uptake of nutrients from soil (Datta and Basu 2000). Indole acetic acid also inhibits or impedes the abscission of leaves inducing flowering and fruiting (Zhao 2010). Interestingly, phosphate-solubilizing organism, for example, *Bacillus*, also secretes cyanogenic compounds (Agrawal and Agrawal 2013), and *Burkholderia tropica* (Tenorio-Salgado et al. 2013) and phosphate-solubilizing actinomycetes *Streptomyces* spp. (Jog et al. 2014) exhibited antifungal activity which suppress the fungal phytopathogens and indirectly promote the growth of plants (Singh et al. 2014). Among various P-solubilizing bacteria, some bacterial strains, like *Stenotrophomonas rhizophila*, *Enterobacter cloacae* etc., have been

Table 3.3 Examples of plant growth regulators synthesized by phosphate-solubilizing microorganisms with reference to wheat

Phosphate-solubilizing microorganisms	Source	PGP activities	Reference
Pantoea sp.	Wheat seeds	IAA, siderophore, N_2 fixation	Herrera et al. (2016)
Burkholderia sp. *Enterobacter* sp.	Wheat rhizosphere	IAA, siderophore	Moriera et al. (2016)
Pseudomonas fluorescens	Wheat rhizosphere	Siderophore, IAA	Safari et al. (2016)
Serratia marcescens *P. aeruginosa*	Vegetables rhizosphere	IAA NH_4 and HCN	Kumar et al. (2015)
Psychrobacter maritimus *Serratia proteamaculans* *Bacillus anthracis*	Wheat rhizosphere	IAA, siderophore	Amara et al. (2015)
Serratia grimesii *Serratia marcescens*	Wheat rhizosphere	N_2 fixation, zinc solubilization, EPS activity, ACC deaminase, biocontrol activity, IAA production	Abaid-Ullah et al. (2015)
Stenotrophomonas rhizophila	Wheat rhizosphere	N_2 fixation, IAA, catalase and cytochrome oxidase	Majeed et al. (2015)
Bacillus sp.	Wheat rhizosphere	Catalase and cytochrome oxidase production	
Enterobacter cloacae subsp. Dissolvens	Soyabean rhizosphere	IAA production, siderophore production, ammonia production, potassium and zinc solubilization	Ramesh et al. (2014)
Pseudomonas fuscovaginae	Wheat phyllosphere	N_2 fixation, biocontrol activity, IAA, siderophore production, and NH_4 production	Verma et al. (2014)
Psychrobacter fozii	Wheat phyllosphere	ACC deaminase activity, biocontrol activity, IAA, siderophore production, Gibberellic acid production, HCN and NH_4 production	
Streptomyces sp.	Wheat rhizosphere	Chitinase, phytase, siderophore production, IAA production	Jog et al. (2014)
Planococcus rifietoensis	Wheat rhizosphere	IAA production, ACC deaminase activity	Rajput et al. (2013)

(continued)

Table 3.3 (continued)

Phosphate-solubilizing microorganisms	Source	PGP activities	Reference
Providencia sp.	Wheat rhizosphere	NH_4 production, siderophore, HCN, indolic compound, antifungal activities, Zn solubilization, antibacterial activity	Rana et al. (2012)
Azospirillum isolates	Wheat rhizosphere	N_2 fixation, IAA production	Venieraki et al. (2011)
Pseudomonas aeruginosa	Wheat endorhizosphere	Siderophore, ACC Deaminase, IAA production, NH_4 production, antifungal enzyme production as cellulase, protease, pectinase	Sharma et al. (2011)

Abbreviations used in this table are: *IAA* indole acetic acid, *HCN* hydrogen cyanide, *NH4* ammonia, A*CC* 1-aminocyclopropane-1-carboxylate, and *EPS* exopolysaccharide

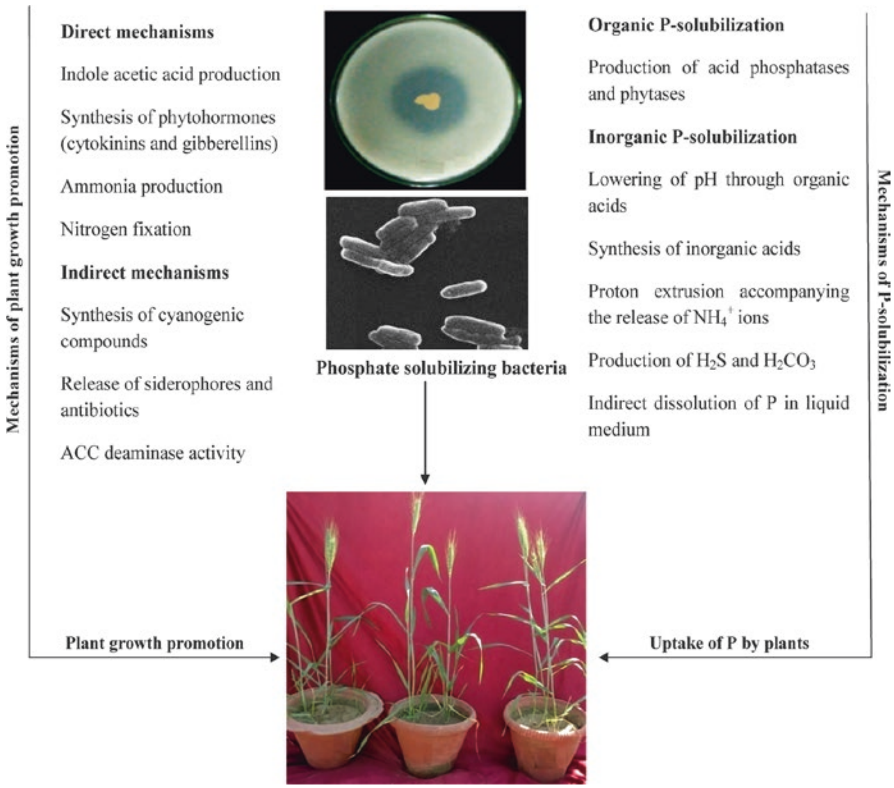

Fig. 3.1 Mechanism of phosphate solubilization and wheat growth enhancement by phosphate-solubilizing microorganisms

reported to fix atmospheric nitrogen (Majeed et al. 2015; Singh and Jha 2015). This property of N_2 fixation by PSB could be of special interest for growers for soils deficient in both N and P since application of single PSB expressing dual activity of P-solubilization and N_2 fixation together are likely to overcome the N and P deficiency in P- and N-deficient soils. Among plant growth regulators, ACC deaminase is an important plant growth regulator that induces metabolic changes and, hence, increases the growth of plants indirectly by hindering/reducing ethylene secretion (Glick et al. 2007; Bal et al. 2013; Magnucka and Pietr 2015). In a recent study, Singh and Jha (2015) isolated a phosphate-solubilizing bacterium *Enterobacter cloacae* from rhizospheric roots of *Aerva javanica* carrying various plant growth-promoting activities such as the isolate was able to produce ACC deaminase, display nitrogen fixation ability, synthesize IAA, and secrete ammonia. Considering the multifarious traits of this bacterium, it was suggested that such bacteria could be used as biofertilizers for increasing the production of crops including those growing in salt-stressed environment.

3.9 Examples of Sole and Composite Effects of PSM on Wheat

With ever increasing human populations, there is greater pressure on agriculture to satisfy human food demands across different regions. Indeed, modern agriculture especially green revolution has reduced some of the human problems by producing more and more foods. To achieve optimum yields, practitioners have, however, extensively used agrochemicals including fertilizers and pesticides in agronomic practices. The expensive and injudicious applications of agrochemicals have undoubtedly increased food production, but their use over the years has backfired as well. The excessive application of agrichemicals has resulted in destruction of microbial diversity and consequently the loss of soil fertility. To obviate such threatening problems of soil pollution and to restore soil fertility, the use of inexpensive natural resources, for example, PSM, has been practiced in different production systems in recent times. Wheat is a high P-demanding cereal crop, and at global scale, wheat production suffers from certain problems; one of the key limitations in enhancing wheat production is the inappropriate use of plant nutrients, especially P and K, and the mean P uptake of wheat is about 3.8 kg P/t of grains (Timsina and Connor 2001). The recovery of P by wheat from fertilizers is quite low, and it is estimated that about 15–20% of the applied P is recovered, while the 80–85% P is fixed as insoluble soil P (Rodríguez and Fraga 1999). It is reported that only 0.1% of the total P remains in soluble form which is available for uptake by plants. Constantly increasing costs of synthetic phosphatic fertilizers together with its high complexation ability in soil have warranted the search for alternative and viable means of P nutrition of wheat. In this context, the sole (Agrawal and Pathak 2010) or composite (Saxena et al. 2014) application of PSM have been considered as a suitable and practicable choice for providing soluble P to wheat while reducing dependence on chemical fertilizers (Table 3.4).

Table 3.4 Influence of single and multiple applications of phosphate-solubilizing microorganisms on biological and chemical characteristics of wheat

PSM inoculants	Growth parameters of wheat	References
Single microorganism		
Penicillium bilaii	Grain yield, spike density	Ram et al. (2015)
Pseudomonas fluorescens	Growth traits and yield	Zia-ul-Hassan et al. (2015)
Pseudomonas sp.	Nutrient uptake and seedling growth	Sarker et al. (2014)
Bacillus megaterium var. phosphaticum	No. of kernels/spike, grain yield, grain protein ratio	Bulut (2013)
PSB strain MR1	Grain and straw yield	Haque et al. (2013)
Aspergillus awamori	Dry matter accumulation at tillering and ear emergence, grain and straw yield	Sharma et al. (2012)
Pseudomonas sp.	Straw yield and P uptake	Babana et al. (2012)
Penicillium oxalicum	Shoot and root dry weight, grain yield, P accumulation	Singh and Reddy (2011)
Azotobacter chroococcum	Grain and straw yield	Narula et al. (2005)
Composite culture		
Azotobacter sp. +mycorrhiza	Seed protein, NPK in seeds and root colonization	Amraei et al. (2015)
Bacillus megaterium BHU1+ *Arthrobacter chlorophenolicus* BHU3	Plant height, grain yield, straw yield and nutrient acquisition	Kumar et al. (2014)
P. fluorescens + *B. cepacia* + *G. etunicatum*	Growth, yield and nutrient uptake	Minaxi et al. (2013)
B. lentus + *P. putida*	Tiller number, grain yield, total biomass	Saber et al. (2012)

3.10 Inoculation Impact of Phosphate-Solubilizing Bacteria on Wheat

Numerous bacterial species identified as PS bacteria have been used as biofertilizer (microbial inoculants) in agricultural practices largely because of their immense ability to improve the availability of applied and soil P (Vessey 2003). In general, the valuable effects of PSB on crop production have been extensively reported (Khan et al. 2007; Zaidi et al. 2009), but the application of PSB as microbial inoculants (biofertilizer) in wheat cultivation is limited. Considering the importance of PSB and lack of sufficient information on the role of PSB in wheat productivity, an attempt is made here to highlight the impact of single or dual culture of PSB in the improvement of wheat grown under different agroecological niches.

Application of PS bacterium *Bacillus megaterium* var. phosphaticum [M-13] in the presence of P fertilizers greatly increased the grain and straw yield of wheat when grown in pots. Also, an increase of 27.3%–53.3% in number of spikes per square meter was observed in the presence of inoculated PSB strain over control treatment (Bulut 2013). In a follow-up study, Hossain and Sattar (2014) investigated the effect of mineral P fertilizer and phosphate-solubilizing bacteria (*Pseudomonas* sp. and *Klebsiella* sp.) used singly or as mixture on the growth, yield, nutrient uptake, and P use efficiency of wheat grown in field soils treated with varying levels of inorganic phosphorus (triple super phosphate) fertilizer. The highest grain and straw yield (2.13 and 2.84 t ha^{-1}) were observed when *Pseudomonas* sp. and *Klebsiella* sp. were applied with 15 kg P ha^{-1} at Pabna and Rajshahi, respectively. Inoculation of *Pseudomonas* sp. for Pabna and *Klebsiella* sp. for Rajshahi in the presence of triple super phosphate resulted in better yield and nutrient uptake of wheat and quality of soil compared to other treatments. When used alone, PS bacteria increased the efficiency of P during crop production, and a positive significant correlation was found between yield contributing characters and grain yield of wheat. This study clearly indicated that PSB could solubilize unavailable P to available form and made it available to crops resulting in greater nutrient uptake and yield of wheat.

Similarly, Afzal et al. (2005) found a significant enhancement in grain and biological yield of wheat grown in presence of PSB (*Pseudomonas* and *Bacillus* species) used either alone or in combinations. Moreover, a statistically significant improvement in seed P content and tillers per m^2 over control was recorded. It was concluded from this study that P-solubilizing microorganisms when used singly or jointly with other organisms showed a significant impact on grain and biological yield, tillers per m^2, and seed P content. An increase in straw and grain yields of wheat following interaction between levels of phosphatic fertilizers and PSB inoculations have been reported (Dwivedi et al. 2004). In a similar study, a synergistic relationship between P-solubilizing microorganisms, for example, *Pseudomonas striata* and *Penicillium*, and asymbiotic N_2 fixer *A. chroococcum* facilitated a better uptake of poorly soluble P and, consequently, enhanced dry biomass, grain yield, and P uptake of wheat plants (Zaidi and Khan 2005). Later on, Sarker et al. (2014) observed a considerable increment in growth and nutrient uptake of *Pseudomonas* inoculated wheat plants. Following inoculation with *Pseudomonas* sp., the dry biomass of shoots increased significantly over uninoculated control. Additionally, the concentration of macronutrients like, N, P, and K in root and shoot tissues were found maximum in inoculated wheat plants. Kumar et al. (2001), in a pot experiment carried out in greenhouse, assayed the survival of P-solubilizing strains of *A. chroococcum*, including soil isolates and their mutants, in the rhizosphere, and their influence on biological characteristics (growth and root biomass) of three genetically diverse wheat cultivars. Wheat plants inoculated with/without microbial cultures were grown in soils treated with different dose rates of N and P fertilizers. Seeds of wheat bacterized with P-solubilizing and plant hormone producing *A. chroococcum* displayed superior response relative to uninoculated controls. Furthermore, grain and straw yields were increased significantly by 12.6% and

11.4%, respectively, following inoculation of mutant strains of *A. chroococcum* over control. The survival of mutant strain of *A. chroococcum* in the rhizosphere was enhanced by 12–14% as compared to parent soil isolate. Of the mutant strain, strain M37 was found superior for all three varieties and significantly increased grain yield and root biomass by 14% and 11.4%, respectively, over control. In an experiment, the application of P-solubilizing bacteria (*Thiobacillus thiooxidans*) in combination with fertilizers (Tilemsi rock phosphate) has resulted in a tremendous increase in wheat yields. Formulation of RP fertilizers along with *T. thiooxidans* AHB411 and *T. thiooxidans* AHB417 increased the yield up to 33.3% and 11.9%, respectively. Other biological parameters like number of tillers per plant and length of panicle and seed characteristics such as grains per panicle and 1000 grain weight were significantly improved. Mixed inoculation of *T. thiooxidans* and Bio TRP1 increased the grain yield of wheat by 46%, whereas straw yield was enhanced by 74% relative to control (Babana et al. 2016).

3.11 Response of Wheat to PSF Inoculations

Apart from phosphate-solubilizing bacteria, fungi have also been found as a better P-solubilizing organism (Khan et al. 2010; Yasser et al. 2014), and upon inoculation, they have shown considerable improvement in wheat production (Wahid and Mehana 2000). For instance, Ram et al. (2015) in a recent field experiment determined the effect of seed treatment with PSF, *Penicillium bilaii* at varying levels of P on growth, P concentration in leaves, and production of wheat. In the absence of P, the single application of PSF profoundly enhanced grain yield by 12.6% over uninoculated control. On the contrary, PSF in the presence of 50% P fertilizer augmented wheat yield which was equal to the application of 100% P but without PSF inoculation. The interaction between PSF inoculation and P levels affected the spike density significantly. When the P levels were 0 and 50%, the spike density increased significantly to about 7% as compared to control, without PSF application. The PS fungus *Penicillium bilaii* was capable of enhancing the number of grains per spike and grain yield of wheat remarkably when compared with uninoculated treatments. A 3.7% increase in the 1000 grain weight was recorded following PSF application in wheat relative to control. Also, the application of *P. bilaii* and phosphatic fertilizer together increased the concentration of P both in grains and straw of wheat plants. When measured at 30 DAS, the P content in the leaves of *P. bilaii* inoculated wheat plants was found to increase. The study in general reflected a growth enhancement in wheat as a result of PSF inoculation as well as application of phosphatic fertilizer (Ram et al. 2015). In a similar experiment performed by Singh and Reddy (2011), the growth of wheat plants was enhanced due to inoculation with *Penicillium oxalicum*. *Penicillium oxalicum* in combination with rock phosphate (RP) increased the shoot length by 1.5 times compared to uninoculated plants. Moreover, the dry biomass of shoots and roots of inoculated plants grown in soil treated with rock phosphate was comparatively higher than control. The mixture of *P. oxalicum* and RP, however, also increased the yield by 42%. The total P content of wheat plants also increased in the presence of *P. oxalicum*. Generally, the P accumulation within various plant organs like shoot, root, and grains of wheat plants inoculated with mixture of *P. oxalicum* and RP was almost three times higher than the P accumulated in untreated plants. Apart from increase in P, the phosphatase and phytase activity were also enhanced following *P. oxalicum.* The overall improvement in the performance of wheat plants was, therefore, attributed to the inoculation of phosphate-solubilizing fungus *P. oxalicum* which increased the soil available P and concurrently facilitated the growth of wheat plants.

3.12 Influence of Composite Inoculations on Wheat Production

Wheat crop requires a larger quantity of some essential plant nutrients, such as N and P. The deficiency of such nutrient elements restricts the growth of plants severely. And hence, to supply such plant nutrients, inoculation of inexpensive and favorably interacting microorganisms have been found effective and viable. Also, where P is limited, it has been reported that plants inoculated with AM-fungi, either singly or as co-culture with PSM enhanced the uptake of P by wheat plants (Raja et al. 2002). In view of this, Saxena et al. (2014) studied the interactive effect of an AMF, for instance, *Glomus etunicatum*, and a PSB, *Burkholderia cepacia* BAM-6, on wheat plants sown in pots having low available P in order to find bio-inoculants for semiarid regions. The composite application of *G. etunicatum* and *B. cepacia* increased the growth and yield in comparison to the single application of *G. etunicatum* and *B. cepacia*. Crop yield was increased by more than 50%, while N concentration was enhanced by 90%, due to the co-inoculation. The root colonization infected by AMF and population of PSB in rhizosphere also increased with time in soil. This study suggested that *B. cepacia* BAM-6 interacted synergistically with AMF and enhanced the growth and nutrient uptake of wheat plants. Therefore, the mixture of two unrelated organisms could be used as biofertilizer for wheat crop grown in arid to semiarid regions. In other study, Tomar et al. (1998) used various combinations of *Azotobacter*, AM fungi, PSB, and NPK fertilizers in wheat production practices. The highest (3.80 tons ha^{-1}) grain yield was recorded with dual inoculation of AM fungi and P-solubilizing bacteria in the presence of NPK which was followed by 3.41 tons ha^{-1} with NPK only and 2.63 tons ha^{-1} for control. In a similar experiment, the synergistic effects of plant growth-promoting rhizobacteria and an AM fungus *G. fasciculatum* on plant growth, yield, and nutrient uptake of wheat plants grown under field conditions were assayed by Khan and Zaidi (2007). The tripartite combination of asymbiotic nitrogen fixer *A. chroococcum* with PS bacterium *Bacillus* and *G. fasciculatum* significantly augmented the dry biomass by 2.6-fold compared to control. At 135 days after sowing (DAS), the grain yield of wheat plants bacterized with *A. chroococcum*, *Bacillus* sp., and *G. fasciculatum* was twofold greater in comparison to non-inoculated plants. Grain protein (GP) was maximum (255.2 mg g^{-1}) in plants treated with four cultures namely, *A. chroococcum*, *Bacillus* sp., *G. fasciculatum*, and *Penicillium variabile* (PSF), while the minimum GP (113.7 mg g–1) was obtained with sole application of *G. fasciculatum*. The N and P contents were maximum (33.6 and 67.8 mg plant^{-1}, respectively) in wheat plants co-inoculated with *A. chroococcum*, *Bacillus* sp., and *G. fasciculatum*. However, the N and P contents of soil measured at 135 DAS varied among treatments. Use of *P. variabile* along with single or dual cultures had negative impact on the measured parameters. Percentage root infection, spore density of the AM fungus, populations of *A. chroococcum*, and P-solubilizing microorganisms were enhanced at 80 DAS. This result demonstrated that the various combinations of PGPR constantly amplified the growth and yield, N and P contents, and grain quality of wheat. In a field study conducted consecutively for 2 years, Kaur and Reddy (2015) used two phosphate-solubilizing bacteria, *Pantoea cypripedii* (PSB-3) and *Pseudomonas plecoglossicida* (PSB-5), which were applied singly or as mixture with RP against maize and wheat crops, and their impact was compared with chemical P fertilizer (diammonium phosphate, DAP). Application of PSB along with RP improved the shoot height, shoot and root dry matter, grain yield, and total P concentration in both maize and wheat crops in comparison to the other treatments. Available soil P, enzyme activities, and PSB populations in both maize and wheat rhizosphere were significantly increased due to inoculation of PSB X RP fertilization relative to DAP application. The mixed application of PSB and RP was found more economical, and, hence, it was suggested that the composite application of PSB and RP would be a suitable alternative to phosphatic fertilizer in sustainable production of wheat. The composite culture of phosphate-solubilizing bacterial strains *Pseudomonas fluorescens* (BAM-4) and *B. cepacia* (BAM-12) and *Glomus etunicatum* enhanced the shoot and root dry biomass and grain yields of wheat plants relative to the uninoculated plants (Saxena et al. 2013). The solubilization of insoluble P is carried out effectively by PSB, whereas the process of P uptake by plant roots is attributed to AM fungi which assist the transportation of solubilized P through plant roots. A composite inoculation of PSB and AM fungus showed better growth and yield of wheat plants in comparison to the plants inoculated with single microbial culture (Minaxi et al. 2013). Also, the AM fungi enhance the P uptake of plants by enhancing the contact surface and volume of soil (Clark and Zeto 2000).

3.13 Inoculation Effects of Immobilized Culture on Wheat Production

Immobilization of bacterial cells has commonly been used in agriculture, pharmaceutical, food, and other industries to obtain a defensive structure or a capsule that could allow immobilization, protection, release, and function of active ingredients. And hence, bacterial cells face little challenge from adverse environmental conditions since encapsulation helps bacterial cells to stabilize and enhance their viability and stability during production, storage, and handling of cultures. Also, encapsulation provides extra protection to bacterial cells during rehydration and lyophilization. In addition, the use of microbial cultures into soil has shown that some microbial inoculants can augment plant uptake of nutrients and consequently increase the use efficiency of applied chemical fertilizers (Adesemoye and Kloepper 2009). In this context, rhizobacteria can play an important role in creating a suitable system for crop production. However, the application of free-living PSB into soil is difficult because it is not easy to maintain the survivability of cells around plant roots largely because they are highly susceptible to environmental variables, for example, temperature, humidity, and stressor molecules. The variation in PSB effect on plants is chiefly due to the differences in the quality of microbial inoculants. Due to these, the efforts should be directed to find an adequate formulation that could be developed as a commercial inoculants product. Considering these, Schoebitz et al. (2013) evaluated the P-solubilizing ability of rhizobacteria using RP as insoluble P and the assimilation of soluble P by wheat plants in quartz sand potted experiments. For this, two P-solubilizing bacteria such as *P. fluorescens* and *Serratia* sp. were encapsulated in sodium alginate and potato starch beads. They were further tested for enzyme activity (alkaline and acid phosphatase) and P-solubilization in Pikovskaya liquid medium. A considerable decrease in pH was obtained following P-solubilization. A total of 89 and 93 µg P ml^{-1} was solubilized by immobilized P-solubilizing bacteria, which was significantly greater than those observed for autoclaved alginate-starch beads. An appreciable increase of 64% in P uptake by wheat plants was observed after 60 days of growth when wheat plants were treated with immobilized *P. fluorescens* + 3.25 ppm of P. This finding suggests that use of the immobilized rhizobacteria could be a viable option for increasing the P level in wheat grown in different agroecological niches.

Conclusion

The phosphate-solubilizing microorganisms are a boon to the agricultural system. It is indeed an inexpensive and an environmentally friendly strategy to reduce the use of chemical fertilizers in farming practices. The enhancement in biological and chemical properties of wheat plants has been reported due to inoculation with variety of phosphate solubilizers including bacteria, fungi, actinomycetes, and mycorrhizae etc. The yield and other growth parameters of wheat have been enhanced, in general, following inoculation with PSM when used singly or as mixture with other free-living PGPR/AM fungi. Another positive aspect of using these microorganisms is that the health of soil is not compromised at any stage of plant growth. Thus, the phosphate-solubilizing microorganisms in general are considered a useful soil microflora which could be developed at commercial scale as bioinoculants for enhancing the production of wheat while reducing dependence on chemical fertilizer.

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