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Review

Sustainable Agriculture and the Role of Biofertilizers

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Abstract

Day today the population of the world is escalating but on the other hand the agriculture production is not growing in the same proportion to meet the growing demand for agriculture products. Chemical fertilizers are used to supplement nutritional demands of the plants as many soils are deficient in essential nutrients like nitrogen (N), phosphorus (P) and potassium (P). But these synthetic fertilizers are expensive and hazardous to the environment. Hence microbial fertilizers such as nitrogen fixing bacteria, phosphate solubilizing bacteria (PSB), potassium solubilizing bacteria (KSB) and plant growth promoting rhizobacteria (PGPR) attracted the attention of many workers for their commercial utilization in agriculture to correct the deficiency of N P K in the soils. These microorganisms contribute directly and indirectly to the soil health through their metabolic activities. In this review, efforts were done to recapitulate research findings of several workers which established the beneficial effects of these biofertilizers on different crops in various locations.

Keywords: Biofertilizers, nitrogen fixation, potassium solubilizing bacteria, PGPR, sustainable agriculture.

Introduction

Agriculture nowadays is becoming expensive with the use of chemical fertilizers which are used continuously and indiscriminately which certainly hamper the soil environment. Moreover these fertilizers are not utilized completely by the plants and a little amount of these fertilizers is utilized by the plants and the rest remains unused in the soil. Generally, 60-90% of the total applied fertilizer is lost and the remaining 10-40% is taken up by plants (Adesemoye and Kloepper, 2009). Hence there is every need to make this unavailable fertilizer available by the use of microorganisms as biofertilizers, especially bacteria which can solubilize the phosphates, potassium and make them available to plants and those which are involved in nitrogen fixation along with the production of growth promoting substances such as auxins, gibberellins, production of antibiotics and siderophores, the iron chelating compounds. Biological Nitrogen Fixation (BNF) is an important component of sustainable agriculture and the nitrogen fixers like *Azospirillum* spp., *Rhizobium* spp., *Azotobacter* spp. etc., are being used as biofertilizers frequently which contribute to the nitrogen economy of the plants. There are encouraging reports on the use of efficient nitrogen-fixing bacteria (Mane *et al*., 2000; Rao, 2003; Tyagi *et al*., 2003; Egamberdiyeva *et al*., 2004; Guggari and Kalaghatagi, 2005; Govindan and Thirumurugan, 2005; Sutaliya and Singh, 2005; Sinha *et al*., 2014; Fabricio and Martín, 2016).

Moreover with increased anxiety about the production of adequate amount of food to feed the constantly increasing human population which is going to touch a mark of 9 billion by 2050 has forced us to reinforce the importance of sustainable increase in crop productivity. One of the methods for sustainable agricultural includes the use of biofertilizers because of aforesaid benefits by these microorganisms (Wadhwa *et al*., 2017). Biofertilizer can be define as formulations of live microorganisms, when inoculated to seeds, on plant foliage, or to soil establishes in the rhizosphere or become endophytic and promotes growth of the host plants by increasing availability of primary nutrients. The use of biofertilizers can reduce the use of chemical fertilizers which is beneficial for farmer community. The biofertilizers refurbish natural nutrient cycles in the soil and build soil organic matter. Biofertilizers are ecofriendly, non-hazardous and more cost effective than chemical fertilizers. They improve physico-chemical properties of soil like soil texture, pH, and other properties. In terms of N: P: K, it was found to be superior to farmyard manure and other type of manure (Mukhopadhyay, 2006). These are also considered as good plant growth promoting microorganisms (PGPMs**)**. Biofertilizers could be classified as those involved in nitrogen fixation, phosphate solubilization, potassium mobilization and plant growth promotion.

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Role of nitrogen fixing microorganisms as biofertilizers

Nitrogen is an essential and key element in improving crop productivity throughout the world. About 42 million tons of fertilizer N is being used annually on global scale for the production of three major cereal crops viz., wheat, rice and maize. Crop plants are able to use about 50% of the applied fertilizer N while 25% is lost from the soil-plant system through leaching, volatilization and denitrification (Saikia and Jain, 2007). Although increasing nitrogen demand of crops is mainly satisfied by the application of mineral fertilizers, biological nitrogen fixation, a process involving the reduction of atmospheric nitrogen to ammonia by microorganisms, which accounts for nearly 60% of earth's newly fixed nitrogen (Postgate, 1982), has assumed great importance in maintaining soil fertility status. The well-known asymbiotic diazotrophic bacteria belong to the genera like *Acetobacter, Azotobacter, Azospirillum, Azoarcus, Burkholderia, Enterobacter, Herbaspirillum, Pseudomonas, Klebsiella* etc., which are able to exert positive effect on plants by producing and secreting plant growth regulators (PGRs) and/ or by supplying biologically fixed nitrogen (Bazzicalupo and Okon, 2000) and symbiotic nitrogen fixing bacterial belongs to genera of *Rhizobium*.

Azospirillum

Azospirillum is a genus of bacteria from α-Proteobacteria, Ord. Rhodospirillales, fam. Rhodospirillaceae, which contains nitrogen-fixing bacilli, Gram-negative, aerobes, motiles, peritrichous, catalase and oxidase positives, containing Q-10 isoprenoid quinones as chemotaxonomic markers and C18:1ω7C as predominant fatty acid (Zhou *et al.* 2009, Fendrihan *et al*., 2017. *Azospirillum* is an associative microaerophilic diazotroph isolated from the roots and above ground parts of a variety of crop plants (Bulow and Dobereiner, 1975; Dobereiner *et al*., 1976; Lakshmi Kumari *et al*., 1976; Cohen *et al.*, 1980; O'Hara *et al*., 1981; Tilak and Murty, 1981; Patriquin *et al*., 1983; Pacovsky *et al*., 1985; Agarwala and Tilak, 1988; 1988a; Indira and Bagyaraj, 1997, Xie and Yokota, 2005; Mehnaz *et al*., 2007; Lin *et al.,* 2016). These organisms are curved rods of variable sizes which exhibit spirillar movement and polymorphism. The cells contain poly-β-hydroxy butyrate (PHB) granules and fat droplets. An extreme colonization by azospirilla to root hairs, epidermal cells, cortical intracellular spaces have also been reported (Das, 1993). *Azospirillum* sp. is one of the best studied genus of plant growth promoting rhizobacteria at present. This microorganism is able to colonize more than one hundred plant species and significantly improves their growth, development and productivity under field conditions (Bashan and De-Bashan, 2010). One of the principal mechanisms proposed for *Azospirillum* sp. to explain plant growth promotion of inoculated plants, has been related to its ability to produce and metabolize several phytohormones and other plant growth regulation molecules (Cassan, and Diaz-Zorita, 2016). It is also found to increase the formation of root hairs and lateral roots and results in higher biomass of plants. It also produces growth- promoting phytohormones and hence acts as plant growth-promoting rhizobacteria (PGPR), which colonize the rhizosphere of numerous crop plants in tropical and subtropical regions. The bacteria affect several metabolic activities of plants including cell membrane activity, increasing root system, enhances better minerals and water absorption as reviewed by Bashan *et al*. (2004). *Azospirillum* spp. increased yields of cereal crops and forage grasses by improving root development increasing the rate of water and mineral uptake from the soil and by biological nitrogen fixation (Okon, 1985). Use of *Azospirillum* has been found to enhance the yield of different cereal crops; increase in yield in maize crop in the range similar to 60 kg urea N ha^{-1} was reported by Fulcheri and Frioni (1994), cereal inoculation with *Azospirillum brasilense* contributed to yield increases of up to 27% in wheat and 6% in oats (Swędrzynska, 2000). *Azospirillum brasilense* strains Ab-V5 and Ab-V6 increased maize and wheat yields by 27% and 31% respectively (Hungria *et al.,* 2010). Similar effect was reported in rice (Banayo *et al*., 2012). An enhanced grain yields was observed in wheat, barley and oats inoculated with *Azospirillum* sp. RAM-7 strain along with nitrogen fertilizer (Dalla Santa *et al.,* 2004). *Azospirillum* application improved the growth of wheat (Arzaneh *et al*., 2011); *Rhizobium tropici* strain CIAT 899 lipo-chitooligosaccharides showed an increase in grain yield and shoot dry yield and N yield in maize (Marks *et al*., 2015; Fendrihan *et al*., 2017).

The effect *of Azospirillum* and *Azotobacter* on cereal crops has been reviewed by Abd El-Lattief, (2016). An increase in total plant and grain dry weight of maize was observed when plants were inoculated with *Azospirillum brasilense* and *H. seropedicae* (Riggs *et al*., 2001). Inoculation of rice plants with *Azospirillum* has been found to cause significant increases in growth and yield which is equivalent to that is attainable by application of 15-20 kg N/ha (Rodrigues *et al*., 2008). Inoculation of wheat with *Azospirillum* produced significantly higher grain yield by 29% (Askary, *et al*., 2009). Similarly, inoculation of *Azospirillum* spp. significantly increased different growth parameters and yield of diverse vegetables crops like tomato (Subbiah 1990), Kumaran *et al.* (1998), okra (Subbiah 1991), Cauli flower (Kalyani *et al.,* 1992). Cabbage (Jeeva Jothi *et al*., 1993, chilli (Paramaguru and Natarajan, 1993; Deka *et al*., 1996), in brinjal (Naidu *et al*., 2002), in onion (Yadav *et al*., 2004; Mahanthesh *et al*., 2005). Production of growth promoting substances like phytohormones, vitamins, antimicrobial and antifungal substances by *Azospirillum* is well established. The most extensively reported growth promoters are Indole acetic acid (IAA), gibberellins, vitamins and siderophores. Multiple biosynthetic pathways of IAA in *Azospirillum* were reported

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by using radiolabelled precursors (Prinsen *et al*., 1993). A cell-free supernatant of *A. brasilense* applied to soybean plants induced the highest number of roots and increased root length (Molla *et al*., 2001). Somers *et al.* (2005) identified phenyl acetic acid (PAA) from *A. brasilense* supernatant which is an auxin-like molecule with antimicrobial activity. According to Roesti (2005), the stimulatory effect of the bio inoculants in plant growth might not only result from a direct plant growth promotion effect but also from an indirect modification of bacterial community. Seshadri *et al.* (2000) reported P-solubilizing capacity of three different strains (LMG 7107, LMG 7108 and LMG 7109) of *A. halopraeferans* in Sperber's medium.

***Rhizobium*:**

Rhizobia are agriculturally important soil bacteria capable of fixing atmospheric nitrogen symbiotically in root and shoot nodules of leguminous plants. They are Gram-negative, motile, non-spore forming, aerobic chemo-organotrophs. Optimum growth temperature range is 25-30°C and a pH of 6.0-7.0 (Somasegaran and Hoben, 1994; Mia *et al*., 2012) Inoculation of legumes with efficient rhizobial can improve nitrogen (N_2) fixation in legume crops especially when native rhizobial strains are fewer or ineffective from soils. Inoculation of legumes with efficient strains of the rhizobia has significant economic and ecological benefits. However it has been suggested that 90% of the rhizobial inoculants applied globally are of no practical benefit to the productivity of legumes due to defective inoculant technology, substandard inoculants and poor decision making polices in their usage (Brockwell and Bottomley 1995, Moumita, 2013). According to current classification rhizobia belong to the alpha subdivision of protobacteria, that were first classified into two genera, the genus *Rhizobium* including the fast growing strains and the new genus Bradyrhizobia, created for the slow growing ones (Jordan, 1984). Six genera; *Rhizobium*, *Bradoyrhizobium*, *Ensifer* (*Sinorhizobium*), *Azorhizobium*, *Mesorhizobium*, *Allorhizobium*, and 28 species have been recognized (Dudeja and Duhan, 2005, Melchiorre *et al.,* 2010, Tariq *et al.,* 2012**).** There are several reports which support the beneficial effects of rhizobia on crop production and yield**.** Indigenous *Rhizobium leguminosarum* BHURC04 (*Cicer* sp.) was found significantly better for nodulation, plant growth and yield of chickpea over control in a field trial (Yadav and Verma, 2014). Ogutcu *et al.* (2010) reported that inoculations with *R. leguminosarum* bv. *Ciceri* strains significantly increased dry weights of root and shoot, number and dry weights of nodules, chlorophyll and N content of chickpea plants, and amounts of total and fixed N_2 parameters compared with the uninoculated control treatment in saline and non-saline soils. Soils treated with *Rhizobium* increased growth parameters in chickpea (Zaman *et al*., 2011).

In a study conducted on the effect of *Rhizobium japonicum* on *Vigna mungo* and *Vigna radiata* showed that the inoculated plants possessed greater height, greater fresh weight, greater number of roots, nodules, greater number of leaves, shoots, pods, greater length of pods, greater seed weight, over their respective controls (Ravikumar, 2012). Flores-Felix *et al.* (2013) analyzed the *in vitro* plant growth promotion mechanisms of a *Rhizobium leguminosarum* strain PEPV16 isolated from *Phaseolus vulgaris* nodules which produced siderophores, indole acetic acid and solubilized phosphate and increased the dry matter of shoots and roots of lettuce and carrots, respectively supporting the suitability of *Rhizobium* as biofertilizer for nonlegumes. According to Agarwal and Ahmad (2010) the yield characteristics had registered maximum yield in *Rhizobium* inoculated sets of Berseem (*Trifolium alexandrinum* L.) Cv. Wardan (*Trifolium alexandrinum* L.). Nodule weight per plant of *Hedysarum coronarium* L. cultivated on calcareous soil in Northern Tunisia was high in *Rhizobium* inoculated plants (Soumaya *et al*., 2016). *Rhizobium* strains NSCBR-14 and NSCBR-(25) inoculated to common bean increased total biomass yield and grain yield significantly (Argaw and Tesso, 2017). Korir *et al.* (2017) reported that inoculation of common bean with the two rhizobia strains, i.e., IITA-PAU 983 and IITA-PAU 987 strains performed at par with inoculation with the reference strain, CIAT 899, in enhanced nodulation, shoot and root dry weight over the control treatment. The highest grain yield was recorded from common bean plants inoculated with *Rhizobium* strain HB-429 which was significantly higher than the control (Tarekegn and Serawit, 2017). Inoculation with *Rhizobium laguerreae* strain PEPV40 significantly increases several vegetative parameters such as leaf number, size and weight, as well as chlorophyll and nitrogen contents of spinach (Jimenez-Gomez *et al*., 2018).

Phosphate Solubilizing Microorganisms (PSM)

Phosphorus is a vital nutrient for plants and microorganisms next only to nitrogen. Phosphorus is one of the major essential macronutrients for biological growth and for proper plant development (Dave and Patel, 1999). Organic matter derived from dead and decaying plant debris is rich in organic sources of phosphorus. The deficiency of phosphorus may occur in crop plants growing in soils containing adequate phosphates. This may be partly due to the fact that the plants are able to absorb phosphorus only in available form. Soil phosphates are rendered available either by plant roots or by soil microorganisms through their secretion of organic acids. Therefore, phosphate-solubilizing soil microorganisms play some role in correcting phosphorus deficiency of crop plants. These microorganisms may also release soluble inorganic phosphates into soil through decomposition of phosphate-rich organic compounds.

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Table 1. Response of plants to inoculation of phosphate solubilizing microorganisms.

PSM	Crop	Effect on plant	Reference
B. megaterium and *B. circulans*	Gram	Increase in yield and P uptake	Mane *et al.*, 2000
PSB	black gram	Increase in grain yield	Chendrayan *et al.*, 2003
PSB	soybean	increase in plant height, total dry matter production and seed weight	Govindan and Thirumurugan, 2005
PSB	winter maize	increase in plant height, dry matter, and grain yield	Sutaliya and Singh, 2005
Bacillus laevolacticum B.amyloliquefaciens, Pseudomonas denitrificans, P. rathonis and *Arthrobacter simplex*	wheat and maize	increased plant growth	Egamberdiyeva, 2005
PSB	wheat	enhance the growth and grain yield	Afzal *et al.*, 2005
PSB	*Zea mays*	enhance the growth and grain yield	Yasmin and Bano 2012
PSB	radish	improved vigour index	Lamo *et al.*, 2012
Pantoea agglomerans and *Burkholderia anthina*	mung bean	enhanced shoot and root length, shoot and root dry matter, and P uptake	Walpola, and Yoon, 2013
PSB	Foxtail millet	increased plant height, dry weight of shoot and root over control plants	Rafi and Charyulu, 2016
Phosphate solubilizing bacteria and fungi	*Vigna radiata*	improvement in the growth and yield	Kolekar *et al.*, 2017

Several soil bacteria particularly *Bacillus, Pseudomonas* and fungi possess the ability to solubilize insoluble phosphates into soluble forms. Phosphate-solubilizing bacteria (PSB) and nitrogen-fixing bacteria attracted many workers for their commercial utilization in agriculture. One way to correct the deficiency of phosphorus in plants is to inoculate seeds or soils with phosphate-solubilizing microorganisms (PSMO) along with the use of phosphatic fertilizers (Gaind and Gaur, 1999; Rafi, 2007). Use of PSB as bio-inoculants plays a vital role in maintaining the soil nutrient status and structure and opens up a new horizon for better crop productivity and for greater yield performance (Sonboir and Sarwagi, 1998; Ingle and Padole, 2017). The use of efficient phosphate-solubilizing microorganisms (PSM) opens up a new horizon for better crop productivity and for greater yield performance without affecting the soil health. Increase in the incidence of PSB in natural and alkaline soils is essential to increase and maintain the supply of available phosphorus. Response of different plants to the inoculation of PSMs is shown in Table 1.

Potassium solubilizing bacteria

After nitrogen (N) and phosphorus (P), potassium (K) is the most important plant nutrient and the most abundantly absorbed cation in higher plants. The introduction of high yielding varieties and hybrids during green revolution and with the progressive intensification of agriculture, the soils are getting depleted in potassium reserve at a faster rate. As a consequence, potassium deficiency is becoming one of the major constraints in crop production. Potassium plays an important role in the growth and development of plants. It activates over 80 different enzymes, maintains cell turgor, enhances photosynthesis, reduces respiration, helps in transport of sugars and starches, helps in nitrogen uptake and is essential for protein synthesis. (Archana, 2007; White and Karley, 2010; Almeida *et al.*, 2015; Cecílio Filho *et al.*, 2015; Yang *et al.*, 2015; Gallegos-Cedillo *et al.*, 2016; Hussain *et al.*, 2016). K is the seventh most abundant element in Earth's crust. Total K content in soils ranges between 0.04 and 3% K. Although K is present as an abundant element in soil, only 1 to 2 % of this element is available to plants (Sparks and Huang, 1985).

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The rest are bound with other minerals and therefore are unavailable to plants. There are three forms of potassium found in the soil viz., soil minerals, nonexchangeable and available form. Soil minerals make up more than 90 to 98% of soil potassium. It is tightly bound and most of it is unavailable for plant uptake. The second is non-exchangeable potassium which acts as a reserve to replenish potassium taken up or lost from the soil solution. It makes up approximately 1 to 10% soil potassium. The third type is available potassium which constitutes 1 to 2%. It is found either in the solution or as part of the exchangeable cation on clay mineral. Among three different forms of potassium in soils, the concentrations of soluble K in soils are usually very low but the highest proportion of potassium in soils are in insoluble rocks and minerals (Goldstein, 1994; Archana, 2007).

Many researchers have isolated microorganisms that are capable of transforming unavailable potassium to available forms considered as potassium-solubilizing bacteria (KSB). These microorganisms may be promising tools to increase K availability in soils and are playing pivotal role in plant growth in K deficient soils. Many researchers have established the role of KSB in improvement of crop yield (Javad *et al*., 2013; Zhang and Kong, 2014; Ahmad and Zargar, 2017, Balasubramanian *et al*., 2017). The mechanism of potassium solubilization by KSB has been well reviewed elsewhere (Etesami *et al*., 2017). Inoculation of *Bacillus mucilaginosus* (KSB) recorded increase in the dry matter by 125 per cent and oil content 35.4 per cent of groundnut plant and available P and K is increased from 6.24 and 9.28 mg/kg and 86.57 to 99.60 mg/kg respectively in soil compared to uninoculated control (Sugumaran and Janarthanam, 2007). Plant dry weight and uptake of both K and N by tobacco seedlings increased significantly after being treated with the four KSB strains (Zhang and Kong, 2014). Han *et al*. (2006) evaluated the inoculation effect of *Bacillus megaterium* var. *phosphaticum* (PSB) and *Bacillus mucilaginosus* (KSB) combined together with rock material on pepper and cucumber in nutrient limited soil and reported a consistent increase in mineral availability, uptake and plant growth. Archana (2007) reported that KSB (*Bacillus* species) inoculation increased plant growth, nutrient uptake (K) and yield component of maize plants significantly over control plants. Groundnut plant treated with KSB strains, *Micrococcus varians* and *Corynebacterium kutscheri* showed maximum pods of 16 and seeds 72 per plant as compared to control having pods 7 and seeds 21 Verma *et al*. (2016). Increase in growth vigor, biomass yield and K uptake to different degrees in available K deficient soil in ryegrass upon inoculation with KSB strains was notice by Xiao *et al*., (2017). Studies conducted by Bagyalakshmi *et al*. (2017) on inoculation of indigenous potassium solubilizing bacteria along with different doses of potassium fertilizer established improvement in the productivity and nutrient uptake in tea plantations.

Conclusion

There are several encouraging reports which established the effectiveness of biofertilizers in improving plant growth and crop yields. Yet further extensive investigations on the use of beneficial microorganisms are desirable in different climatic conditions of the world on variety of crops to meet the food needs of the growing population. There are also reports on the beneficial mechanisms of these organisms. Further efforts are to be done to elucidate different mechanisms using r-DNA technology for sustainable agriculture.

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