Mycopath (2013) 11(2): 95-101 **Review Article**

Role of biofertilizers in agriculture: a brief review

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Abstract

Bio-fertilizer contains microorganisms which promote the adequate supply of nutrients to the host plants and ensure their proper development of growth and regulation in their physiology. The production of bacterial bio-fertilizer essentially requires the selection of appropriate strains for a particular crop in a given agro-climate. As agro-climatic conditions and soil characteristics vary widely, a large range of strains of each bio-fertilizer needs to be isolated for each area. Bio-fertilizers are eco-friendly, one of the best modern tools for agriculture and are used to improve the fertility and quality of the soil. It offers an economically attractive and ecologically sound route for augmenting nutrient supply.. Leguminous crops have the ability to fix nitrogen (N) biologically from the atmosphere. This can benefit not only the legumes themselves but also any intercropped or subsequent crops.

Keywords: Agricultural, bio-fertilizers, nitrogen fixation, future directions.

Introduction

The excess uses of chemical fertilizers in agriculture are costly with adverse effects on physico-chemical properties of soils. Therefore, in the recent years several organic fertilizers have been introduced that act as natural stimulators for plant growth and development (Khan *et al*., 2009). The knowledge of such natural stimulator or microbial inoculums has long history started with culture of small scale compost production and passes from generation to generation of farmers (Abdul Halim, 2009). A specific group of this kind of fertilizers includes products based on plant growth-promoting microorganisms named biofertilizer or 'microbial inoculants' that are preparation containing live or latent cells of efficient strains of nitrogen fixing, phosphate solubilizing or cellulytic microorganisms. These are used for application of seed, soil or composting areas with the objective to enhance the numbers of such microorganisms and accelerate certain microbial process to augment the extent of the availability of nutrients in a form which can assimilated by plant (Khosro and Yousef, 2012). Such biofertilizers are important components of integrated nutrients management in soil, while play key role in productivity and sustainability of soil. With every passing days, these biofertilizer replacing chemical fertilizers due to cost effectively, ecofriendly and renewable source of plant nutrients.

Arbuscular mycorrhizal fungi (AMF) (Jeffries *et al*., 2003), plant growth-promoting rhizobacteria (PGPR) (Podile and Kishore, 2006), and nitrogen fixing rhizobia (Franche *et al*., 2009) are three important groups of biocontrol agents extensively utilized globally. However, in this paper we just focused on nitrogen fixers.

Nitrogen fixers

The discovery of nitrogen fixation was attributed to the German scientists Hellriegel and Wilfarth in 1886, who reported that legumes bearing root nodules could use gaseous (molecular) nitrogen. Shortly afterwards, in 1888, Beijerinck, a Dutch microbiologist, succeeded in isolating a bacterial strain from root nodules. This isolate happened to be a *Rhizobium leguminosarum* strain. Stewart (1969) stated that the microbiologists Beijerinck in 1901 and Lipman in 1903 were responsible for isolation of *Azotobacter* spp., while Winodgradsky (1901) isolated the first strain of *Clostridium pasteurianum*. Discovery of nitrogen fixation in blue-green algae (now classified as cyanobacteria) was established much later (Stewart, 1969). Since then, research efforts in these fields have steadily increased resulting in the selection of numerous strains showing several beneficial features (Podile and Kishore, 2006).

Nodule formation

Rhizobial infection occurs via plant root hairs which prior to the infection process, respond to the presence of compatible rhizobia by deformation (shepherd's crooks, cauliflower structures, etc.). At the deformation stage, the plant perceives the rhizobial signal and initiates a developmental program aimed at formation of symbiotically nitrogen-fixing nodules (Dénarié *et* *al.,* 1996). A set of plant genes, initially called nodulins, is specifically activated in response to nodulation factor perception (Geurts and Bisseling, 2002). A nodule meristem is thus formed within the root while the rhizobia enter through a plant-derived infection thread-a tube formed to facilitate rhizobia entry to the deeper layers. The infection threads grow transcellularly and finally, rhizobia wrapped into a plant derived membrane, now called symbiosome membrane, are delivered into plant cells. Nodules are either of an indeterminate type with an apical meristem, or they are determinate, meaning that the peripherally located meristem stops functioning after nodule completion (Foucher and Kondorosi, 2000; Mishra *et al*., 2013).

Nodule physiology

During nodule formation, host tissues develop to form a specialized tissue that maintains an environment in which nitrogen fixation can occur. Functioning of the nodule was reviewed by White *et al*. (2007). In the nodule, specialized organelle-like forms of bacteria called bacteroids are engulfed in plant-derived membranes, forming symbiosomes. The reduction in dinitrogen inside the nodule requires energy provided by the plant. Photosynthate in the form of sucrose is transported to the nodule, whereas dicarboxylic acids further provide the bacteroids with carbon and energy through the symbiosome membrane. For generation of energy through respiration, a high flux but a low internal concentration of oxygen is achieved with the aid of leghemoglobin. Ammonia produced in the bacteroid needs to be transported to the plant through the symbiosome membrane. In addition to ammonia, alanine is transported. An amino acid flux back through the symbiosome membrane has also been proposed to be involved in the transport mechanism (Prell and Poole, 2006). Ammonia is further assimilated into glutamine or asparagine in the plant cytosol. In determinate nodules, these are further converted into ureides in uninfected cells adjacent to the infected ones. In indeterminate nodules, this does not occur, and all plant cells are normally infected. If a root nodule is cut open and the inside is pink/red the nodule is active and fixing lots of nitrogen for the plant. The color is due to the presence of plenty of leghaemoglobin. The redder the nodule, the more active it is. When nodules are young and not yet fixing nitrogen they are white or grey inside. Legume nodules that are no longer fixing nitrogen turn green and may be discarded by the plant. This may be the result of an inefficient *Rhizobium* strain or poor plant nutrition. After harvest legume roots left in the soil decay, releasing organic nitrogen compounds for uptake by the next generation of plants. Farmers take advantage of this natural fertilization by rotating a leguminous crop with a non leguminous one.

Nitrogen fixation and crop productivity

For optimum plant growth, nutrients must be available in sufficient and balanced quantities (Chen, 2006). Following photosynthesis, nitrogen fixation is the second most important process in crop production. Photosynthesis captures sunlight and produces energy, and nitrogen fixation uses nitrogen gas to form ammonium. Nitrogen fixation can provide for free up to 300-400 kg N/ha/yr (Adam, 2002). Atmospheric dinitrogen can fix biologically to ammonia. This ammonia is available to crop plants. The ammonia is converted to nitrate by few microorganisms in soil which is then available to plants.

Biological nitrogen fixation represents annually up to 100 million tons of N for terrestrial ecosystems, and from 30 to 300 million tons for marine ecosystems. In addition, 20 million tons result from chemical fixation due to atmospheric phenomena (Mosier, 2002). The first industrial production of rhizobium inoculant began by the end of the 19th century. However, to sustain production of cereal crops, legumes and other plants of agricultural importance, the supply of nitrogenous chemical fertilizers has been regularly increasing since the Second World War. According to an FAO report, production of N fertilizer for 2007 was 130 million tons of N, and this should further increase in the coming years. Biofertilizers can add 20-200kg N ha (by fixation), liberate growth-promoting substances and increase crop yield by 10-50%. They are cheaper, pollution free, based on renewable energy sources and also improve soil (Saeed *et al*., 2004). Both plant and bacterium can live separately but the association is vary beneficial for them. It was reported that in plants, up to 25% of total nitrogen came from nitrogen fixation. Activity of nitrogen fixing microorganisms depends greatly upon excessive amount of carbon compounds and adequately low level of combined nitrogen (Andrew *et al*., 2007). Carbohydrates come directly from photosynthesis or from decay of organic wastes in soils. Roots of plants release substances into soil, which in certain measure support colonization and nitrogen fixing activity of bacteria in rhizosphere of plants (Nghia and Gyurjan, 1987). The main and direct purposes of applying biofertilizers to soil are: to provide nutrient sources and good soil conditions for the growths of crops when used as a live body; to partially substitute and enhance the function of chemical fertilizer and then subdue the application quantities of fertilizers and still maintain the same crop yields and the capital used for making bio-fertilizers is cheaper than that of chemical fertilizers and to lessen the negative effect aroused from applying chemical fertilizers to soil .On the other hand, the indirect purposes of using bio-fertilizers to soil are: to enhance the growth of root system to increase the water and nutrient absorption abilities of crops, extend the life of root , neutralize and degrade harmful materials accumulated in soil, promote survival efficiency of seedling after transplanting and get shorter time for the flower to come out. Biofertilizers reduced the population of *Meloidogyne incognita* infecting chilli and tomato and *Tylenchulus semipenetrans* on Washington navel orange. Also, Six new commercial Egyptian bio-fertilizers viz.,nitrobien, rizobacterin, cerealin, phosphorine, microbien, blue green algae, and five new commercial Egyptian plant nutrients viz., nuftarein, potassein F, citrein, kotangein and kapronite as for the control of root-knot nematode, *Meloidogyne incognita* on sunflower **(**Youssef and Eissa, 2014).

Common nitrogen fixers

Azotobacter

The genus *Azotobacter* belongs to the ɤ-subclass of the *Proteobacteria* (Becking, 2006) and comprises seven species: *A.chroococcum, A. vinelandii, A. beijerinckii, A. paspali, A. armeniacus, A.nigricans* and *A. salinestri***.** *Azotobacter* spp. are heterotrophic and aerobic dominantly found in soils with property to fix nitrogen non-symbiotically (Doroshenko *et al*., 2007). These free-living, Gram-negative, motile and mesophilic bacteria that are capable of fixing an average 20 kg N/ha/per year. They pleomorphic, oval or spherical form thick-walled cysts and may produce large quantities of capsular slime (Rawia *et al*., 2009). The first representative of the genus *A. chroococcum*, was discovered and described in 1901 by the Dutch microbiologist and botanist Martinus Beijerinck. *A. chroococcum* are mostly found in neutral and alkaline soils (Gandora *et al*., 1998; Martyniuk and Martyniuk, 2003).

There are some species of *Azotobacter* which establish symbiotic relationships with different parts of plants, and may develop special structures as the site of nitrogen fixation (Stephens *et al*., 2000; Gomare *et al*., 2013). All *Azotobacter* species have the capacity to produce oxidases and catalases for the protection of their nitrogenase. Their agronomic importance is due to the capability of synthesizing antibiotics, plant growth promotion substances (Pandey and Kumar, 1990; Pandey *et al*., 1998), vitamins, exopolysaccharides and pigment production (Sabra *et al*., 2001; Jimenez *et al*., 2011), besides their antagonist effect against pathogens (Sudhir *et al*., 1983). They also have the ability to solubilize phosphates in aquaculture systems and vermicompost production (Garg *et al*., 2001; Kumar and Singh, 2001). *A. vinelandii* and *A. chroococcum* produce exopolysaccharides with high potential value related to their wide range of commercial applications (De la Vega *et al*., 1991; Clementi, 1997).

In soils, *Azotobacter* spp. populations are affected by soil physico-chemical (e.g. organic matter, pH, temperature, soil depth, soil moisture) and microbiological (eg. microbial interactions) properties. As far as physico-chemical soil properties are concerned, numerous studies have focused on the nutrients (P, K, Ca) and organic matter content and their positive impact on *Azotobacter* spp. populations in soils (Ridvan, 2009). The population of *Azotobacter* is generally low in the rhizosphere of the crop plants and in uncultivated soils. The occurrence of this organism has been reported from the rhizosphere of a number of crop plants such as rice, maize, sugarcane, bajra, vegetables and plantation crops, (Arun, 2007). It enhanced the productivity of many crops compared with chemical fertilizers, where Das (1991) studied the impact of *Azatobacter* on crop yield compared with chemical fertilizers where increase in yield over yields obtained with chemical fertilizers was 13, 16, 10, 5, 20, 40, 24, 27, 24 and 20% in potato, carrot, rice, maize, cauliflower, tomato, cotton, sugarcane and sorghum, respectively

Rhizobium

Rhizobium belongs to family *Rhizobiaceae* are Gram-negative motile, non-sporulating rods, symbiotic in nature and can fix nitrogen 50-100 kg/ha with legumes only. Successful nodulation of leguminous crops by *Rhizobium* largely depends on the availability of compatible strain for a particular legume. In agricultural settings, perhaps 80% of this biologically fixed N2 comes from symbiosis involving leguminous plants and α-proteobacteria, order Rhizobiales, family Rhizobiaceae, including species of *Rhizobium, Bradyrhizobium, Sinorhizobium, Azorhizobium* and *Mesorhizobium* (Farrand *et al*., 2003).

Rhizobium population in the soil depends on the presence of legume crops in the field **(**Sheraz *et al*., 2010). In absence of legumes, the population generally reported to decreases. Each legume requires a specific species of *Rhozobium* to form effective nodules. Many legumes may be modulated by diverse strains of *Rhizobia*, but growth is enhanced only when nodules are produced by effective strains of *Rhizobia* (Cooper, 2004).

Azospirillum

Members of the genus *Azospirillum* are gram-negative to gram-variable, curved-rod shape, motile, oxidase positive and exhibit acetylene-reduction activity under micro-aerophilic conditions. *Azospirillum* spp. have been identified mainly as rhizosphere bacteria and its colonization of the rhizosphere has been studied extensively along with reporter gene fusion (Burdman *et al.,* 1997; Pereg-Gerk *et al.,* 2000; Steenhoudt and Vanderleyden, 2000). *Azospirillum* plant interactions have been extensively studied since 1970s. It was first isolated and originally named as *Spirillum lipoferum*. This bacterium was later isolated from soil and from dried seaweed in Indonesia and as a phyllosphere bacterium in tropical plants (Becking, 1985). The beneficial effect of *Azospirillum* may derive both from its nitrogen fixation and stimulating effect on root development (Noshin *et al*., 2008). It directly benefits plants improving shoot and root development and increasing the rate of water and mineral uptake by roots (Gonzalez *et al*., 2005) and stimulate plant-growth even in the presence of several stresses such as drought (Creus *et al*., 1996). *Azospirillum* inoculation alleviates low soil moisture effects on wheat plants grown under drought conditions (El-Komy *et al*., 2003). Fulchieri and Frioni (1994) observed that maize inoculated with *Azospirillum* had enhanced dry weight of seed by 59% and the yield.

Azospirillum spp. is not considered to be a classic biocontrol agent of soil-borne plant pathogens. However, there have been reports on moderate capabilities of *A. brasilense* in biocontrol of crown gall-producing *Agrobacterium,* bacterial leaf blight of mulberry, and bacterial leaf and/or vascular tomato diseases (Sudhakar *et al*., 2000; Bashan and de-Bashan, 2002). In addition, *A. brasilense* can restrict the proliferation of other nonpathogenic rhizosphere bacteria (Holguin and Bashan, 1996). These antibacterial activities of *Azospirillum* could be related to its already known ability to produce bacteriocins and siderophores (Shah *et al*., 1992). The mechanisms by which *Azospirillum* spp. can exert a positive effect on plant growth is probably composed of multiple effects including synthesis of phytohormones, N_2-fixation, nitrate reductase activity and enhancing minerals uptake (El-Komy, 2004). *Azospirillum* plant association is accompanied by biochemical changes in roots, which in turn; promote plant growth and tolerance to low soil moisture. The bacteria stimulate plant-growth even in the presence of several stresses such as drought (Noshin *et al*, 2008; Sivasakthivelan and Saranraj, 2013).

N_2-fixing bacteria associated with non-legumes

The list of N_2-fixing bacteria associated with nonlegumes includes species of *Achromobacter*, *Alcaligenes*, *Arthrobacter*, *Acetobacter*, *Azomonas*, *Beijerinckia*, *Bacillus*, *Clostridium*, *Enterobacter*, *Erwinia*, *Derxia*, *Desulfovibrio*, *Corynebacterium*, *campylobacter*, *Herbaspirillum*, *Klebsiella*, *Lignobacter*, *Mycobacterium*, *Rhodospirillum*, *Rhodo-pseudomonas*, *Xanthob-acter*, *Mycobacterium* and *Methylosinus* (Wani, 1990). Although many genera and species of N_2-fixing bacteria (*Azotobacter* and *Azospirillum*) were isolated from the rhizosphere of various cereals, have been widely tested to increase yield of cereals and legumes under field conditions (Simon, 2003).

Conclusion and future prospects

Biofertilizers lead to soil enrichment and are compatible with long-term sustainability. Further they are ecofriendly and pose no danger to the environment can be replaced with chemical fertilizers.

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