Azotobacter chroococcum – A Potential Biofertilizer in Agriculture: An Overview

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Abstract Research on *Azotobacter chroococcum spp*. in crop production has manifested its significance in plant nutrition and its contribution to soil fertility. The possibility of using *Azotobacter chroococcum* in research experiments as microbial inoculant through production of growth substances and their effects on the plant has markedly enhanced crop production in agriculture. Being free living N_2-fixer diazotroph, *Azotobacteria* genus synthesizes auxins, cytokinins, and GA like substances and these growth materials are the primary substances regulating the enhanced growth. It stimulates rhizospheric microbes, protects the plants from phyto-pathogens,

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improves nutrient uptake and ultimately boost up biological nitrogen fixation. These hormonal substances, which originate from the rhizosphere or root surface, affect the growth of the closely associated higher plants. In order to guarantee the high effectiveness of inoculants and microbiological fertilizers it is necessary to find the compatible partners, i.e. a particular plant genotype and a particular *Azotobacter* strain that will form a good association.

Keywords *Azotobacter chroococcum* • Nitrogen fixation • Microbial inoculant • Soil fertility

1 Introduction

Biofertilizers also called as bio-inoculants, the organic preparations containing microorganisms are beneficial to agricultural production in terms of nutrient supply particularly with respect to N and P. When applied as seed treatment or seedling root dip or as soil application, they multiply rapidly and develop a thick population in rhizosphere. Biofertilizers can fix atmospheric N through the process of biological nitrogen fixation (BNF), solubilize plant nutrients like phosphates and stimulate plant growth through synthesis of growth promoting substances. They have C: N ratio of 20:1 indicating the capacity of the biofertilizer to release nutrients. Being eco-friendly, non hazardous and non-toxic products, biofertilizers are nowadays gaining the importance in agriculture (Sharma et al. 2007; Hakeem et al. 2016). They are cheaper and low capital intensive.

Biofertilizers benefiting the crop production include *Azotobacter*, *Azospirillum*, *Blue green algae*, *Azolla, P-solubilizing microorganisms*, *mycorrhizae* and *sinorhizobium* (Selvakumar et al. 2009). *Azotobacter chroococcum* and *Azotobacter agilis* were first of all studied by Beijerinck (1901). The first species of the genus *Azotobacter*, named *Azotobacter chroococcum* family *Azotobacteriaceae*, was isolated from the soil in Holland in 1901. In subsequent years several other types of *Azotobacter* group have been found in the soil and rhizosphere such as *Azotobacter vinelandii*, Lipman (1903); *Azotobacter beijerinckii*, Lipman (1904); *Azotobacter nigricans*, Krassilnikov (1949); *Azotobacter paspali*, Dobereiner (1966), *Azotobacter armenicus*, Thompson and Skerman (1981); *Azotobacter salinestris*, Page and Shivprasad (1991).

2 Taxonomy, Morphology and Distribution of *Azotobacter*

The genus *Azotobacter* includes 6 species, with *A. chroococcum* most commonly inhabiting many soils all over the world (Mahato et al. 2009). Among the saprophytes along with nodular bacteria, genus *Azotobacter* was considered to be the

most extensively studied (Horner et al. 1942). Aerobic bacteria belonging to the genus *Azotobacter* represent a diverse group of free-living diazotrophic (with the ability to use N_2 as the sole nitrogen source) microorganisms commonly inhibiting the soil. The taxonomic classification of *Azotobacter* is shown below.

Domain	:	Bacteria
Kingdom	:	Bacteria
Phylum	:	Proteobacteria
Class	:	Gammproteobacteria
Order	:	Pseudomonas
Family	:	Pseudomonadaceae/Azotobaceriaceae
Genus	:	Azotobacter

Azotobacter represents the main group of heterotrophic, non-symbiotic free living nitrogen-fixing bacteria principally inhabiting the neutral or alkaline soils. These bacteria are Gram negative and vary in shape. They are generally large ovoid pleomorphic cells of 1.5–2.0 um or more in diameter ranging from rods to coccoid cells. The cells can be dispersed or form irregular clusters or occasionally chains of varying lengths in microscopic preparations. In fresh cultures, the cells are mobile due to the numerous flagella present on their body surface but later the cells lose their mobility, become almost spherical and produce a thick layer of mucus, forming the cell capsule. The shape of the cell is affected by the amino acid glycine which is present in the nutrient medium peptone. Their distribution of existence is diverse and occurs either singly, in paired or irregular clumps and sometime in chains of varying length. Fig. 1 shows different stained *Azotobacter* species cells. *Azotobacter* possesses some unique features among the biofertilizers. They possess more than one type of nitrogenase enzymes (Joerger and Bishop 1988). They do not produce endospores but form cysts, oval or spherical bacteria that form thick-walled cysts (means of asexual reproduction under favorable condition) (Salhia 2013). The formation of cysts is induced by changes in the concentration of nutrients in the medium and addition of some organic substances such as ethanol, n-butanol, or β-hydroxybutyrate. The formation of cysts is also induced by chemical factors and is accompanied by metabolic shifts, changes in catabolism, respiration and biosynthesis of macromolecules (Sadoff 1975). The cysts of *Azotobacter* are spherical and consist of the so-called 'central body'a reduced copy of vegetative cells with several vacuoles and the 'two-layer shell'. The inner part of the shell has a fibrous structure and is called intine and outer part has a hexagonal crystalline structure called as exine (Page and Sadoff 1975). The central body can be isolated in a viable state by some chelation agents (Parker and Socolofsky 1968). The main constituents of the outer shell are alkyl resorcinol composed of long aliphatic chains and aromatic rings.

The population of *Azotobacter* is generally low in the rhizosphere of the crop plants in uncultivated soils. Jensen's N-free medium is frequently used for the mass multiplication of *Azotobacter. Azotobacter* grows well at an optimum temperature range between 20 and 30 °C and grows best in neutral to alkaline soil (pH of 6.5–

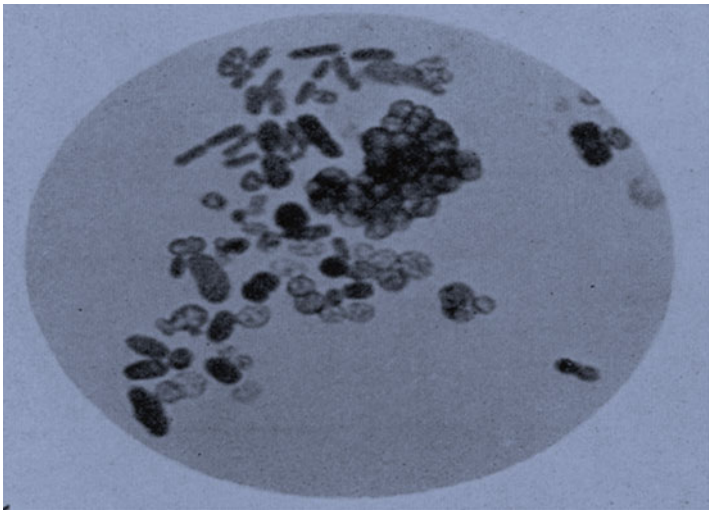

Fig. 1 *Azotobacter* species cells, stained with Heidenhain's iron hematoxylin, ×1000

7.5), but does not thrive when the pH is below 6 and hence not present in acidic soil. This organism has been reported to occur in the rhizosphere of a number of crop plants such as rice, maize, sugarcane, bajra, vegetables and plantation crops (Arun 2007) hence called rhizobacteria and or occurs endophytically (Hecht-Buchholz 1998). They work better in the root region of crop non-symbiotically when sufficient organic matter is present. They are reported to occur also in parenchymatous cells of root cortex and leaf sheath. *Azotobacter* is generally used in any non-legume crop (Singh and Dutta 2006). They can exhibit a variety of characteristics responsible for influencing the overall plant growth (Tippannavar and Reddy 1989). The *Azotobatcteria* also categorised as Plant growth Promoting Rhizobacteria (PGPB) are considered to promote plant growth directly or indirectly. These rhizobacteria derive their food and energy from the organic matter present in the soil and root exudates and fix atmospheric N (Maryenko 1964) depending on the amount of carbohydrates utilized by them. These non-specific associative nitrogen-fixing rhizobacteria are important for ecology and play a great role in soil fertility in agriculture.

3 Mode of Action of *Azotobacter* on Plant Growth

Despite the considerable amount of experimental data available concerning *Azotobacter* stimulation of overall plant development, however the exact mode of action by which *Azotobacter* enhances plant growth is not yet fully understood

(Wani et al. 2013). Three possible mechanisms have been proposed to explain the action: N_2 fixation; delivering combined nitrogen to the plant; the production of phytohormone – like substances that change the plant growth and morphology and bacterial nitrate reduction, thereby increasing nitrogen accumulation in inoculated plants.

3.1 Nitrogen Fixation

Nitrogen fixation is considered as one of the most important biological processes and interesting microbial activity on the surface of earth after photosynthesis as it makes the recycling of nitrogen and gives a fundamental contribution to nitrogen homeostasis in the biosphere. Biological nitrogen fixation plays an important role in maintaining soil fertility (Vance and Graham 1995). *Azotobacteria* is used for studying nitrogen fixation and inoculation of plants due to its rapid growth and high level of nitrogen fixation. They are extremely tolerant to oxygen while fixing nitrogen and this is due to respiration protection of nitrogenase (Robson and Postgate 1980; Hakeem et al. 2016). They have respiratory protection, uptake of hydrogenases and switch on-off mechanisms for protection of nitrogenase enzyme from oxygen (Chhonkar et al. 2009). *Azotobacter chroococcum* is shown to have uptake hydrogenase which metabolises hydrogen (H_2) evolved during nitrogen fixation (Partridge et al. 1980). *Azotobacter* is capable of converting nitrogen to ammonia, which in turn is taken up by the plants (Kamil, et al 2008). Iswaran and Sen (1960a) reported that the presence of optimum levels of calcium nutrient is essential for better growth of *Azotobacter* and its nitrogen fixation. However, the efficiency of *Azotobacter* was found to decrease with increased N level as reported by Soleimanzadeh (2013). *Azotobacter* spp. are non-symbiotic heterotrophic bacteria and capable of fixing about 20 kg N/ha/per year (Kizilkaya 2009) and it may be used in crop production as a substitute for a portion of mineral nitrogen fertilizers (Hajnal et al. 2004). According to Soliman et al. (1995) inoculation with *Azotobacter* replaced up to 50 % of urea-N for wheat grown in a greenhouse trial under aseptic conditions. The isolated culture of *Azotobacter* can fix about 10 mg nitrogen g^{-1} of carbon source under *in-vitro* conditions. The schematic representation of nitrogen fixation involved in nitrogen cycle in the biosphere by diazotrophs (*Azotobacteria*) is shown in Fig. 2.

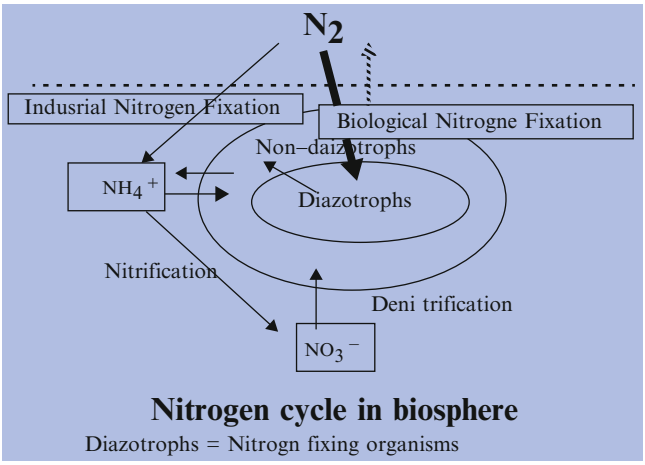

Fig. 2 Nitrogen fixation by diazotrophs (*Azotobacter spp.*)

3.2 *Growth Promoting and Other Substances Produced by Azotobacter*

Although most specifically noted for their nitrogen fixing ability, *Azotobacter* spp. have also been noted for their ability to produce different growth hormones (IAA and other auxins, such as gibberllins and cytokinins) (Barea and Brown 1974), vitamins, antibacterial and antifungal compounds and siderophores (Pandey and Kumar 1989b) which directly or indirectly effect the plant growth and microbial activity. Growth substances or plant hormones are natural substances that are produced by microorganisms and plants alike. They have stimulatory or inhibitory effects on certain physiological and biochemical processes in plants and microorganisms. These hormonal substances which originate from the rhizosphere or root surface affect the growth of the closely associated higher plants. Brakel and Hilger (1965) showed that *Azotobacter* produced indol-3-acetic acid (IAA) when tryptophan was added to the medium while as Hennequin and Blachere (1966) found only small amounts of IAA in old cultures of *Azotobacter* to which no tryptophan was added. Bacteria of the genus *Azotobacter* synthesize auxins, cytokinins, and GA-like substances and these growth materials are the primary substance controlling the enhanced growth of tomato (Azcorn and Barea 1975). *Azotobacter* spp. can also produce antifungal compounds to fight against many plant pathogens (Jen-Hshuan 2006). Many strains of *Azotobacter* have been reported to produces pigments which are involved in the metabolism of microbial organisms. For example, *A. chroococcum* forms a dark-brown water-soluble pigment melanin. This process occurs at high levels of metabolism during the fixation of nitrogen and is thought to protect the nitrogenase system from oxygen (Shivprasad and Page 1989). Other *Azotobacter* species produce pigments from yellow-green to purple colours (Jensen 1954)

Table 1 Effect of inoculation on dehydrogenase activity (µg TPF/10 g of soil)

Variant	Maize hybrids			
	ZP 555 su	620 k	NS 609b	NS 6030
Control	30.25	945.00	877.50	942.00
100 ml *A.chroococcum*	724.50	1244.00	1018.50	943.00
75 ml *A.chroococcum*	1000.75	985.00	914.75	950.00
50 ml *A.chroococcum*	962.75	1063.00	949.00	1055.00

including a green pigment which fluoresces with a yellow-green light and a pigment with blue-white fluorescence. *Azotobacter* acts as indicator hence enhances the microbial activity in soils. Jafari et al. (2012) reported that a more realistic indicator of total microbiological activity of soil is dehydrogenase activity which points to the intensity of oxidation reduction, i.e. the intensity of metabolic activity of microorganisms. In this research, dehydrogenase activity increased in all the variants where *Azotobacter* was applied (Table 1). Mutant *A. vinelandi* is more suitable for the biosynthesis of alginate in view of its latent utilization as a food stabilizer has better qualitative properties (Chen et al. 1985). Alginate the polymers are linear polysaccharides, which are composed of variable amounts of (1–4)-a-D-mannuronic acid and its epimer, a-L-guluronic acid. Several strains of *Azotobacter* are capable of producing amino acids when grown in culture media amended with different carbon and nitrogen sources (Lopez et al. 2005). Substances like amino acid produced by these rhizobacteria are involved in many processes that explain plant-grown promotion. Biochemical analysis of chlorophyll, nitrogen, phosphorous, potassium and protein content was higher in *Azotobacter* inoculated plants as compared to non-inoculated control plants (Naseri et al. 2013).

3.3 Response of Crops to Growth Promoting Substances

Large number of field trials and various experiments carried throughout India and whole world have convincingly established the importance of *Azotobacter* as microbial inoculant. Various crops like wheat, barley, maize, sugar beet, carrot, cabbage, potato were inoculated with *Azotobacterin* during its preparation. In India many crops like wheat, rice, onion, brinjal, tomato and cabbage were tested during experiment to study the effects of *Azotobacter*. Eklund (1970) demonstrated that the presence of *A. chroococcum* in the rhizosphere of tomato and cucumber is correlated with increased germination and growth of seedlings. Elgala et al. (1995) concluded that with microbial inoculation rock phosphate could be used as cheap source of P in alkaline soils and that combined inoculation could reduce the rate of fertilizer required to maintain high productivity. A study conducted by Govedarica et al. (1993) on the production of growth substances by nine *A. chroococcum* strains isolated from a chernozem soil has showed that these strains have the ability to produce auxins, gibberelins, and phenols and in association with the tomato plant, increase

plant length, mass and nitrogen content. Puertas and Gonzales (1999) reported that dry weight of tomato plants inoculated with *A. chroococcum* and grown in phosphate-deficient soil was significantly greater than that of non inoculated plants. Phytohormones (auxin, cytokinin, gibberellin) can stimulate root development. *A. chroococcum* produces an antibiotic which inhibits the growth of several pathogenic fungi in rhizosphere thereby seedling mortality (Subba Rao 2001). Incidence of some diseases of mustard and rapeseeds could be reduced by inoculating with *Azotobacter* (Singh and Dutta 2006) Vijayan et al. (2007) observed that foliar application of *A. chroococcum* to mulberry grown under saline soil conditions showed significant level of improvement in biochemical and morphological parameters of leaf. Under greenhouse conditions inoculation of *A. chroococcum* recorded a significant N and P uptake in both seed and stover in brown sarson (*Brasssica campestris* L.) over the control (Wani 2012). Dual inoculation of *Azotobacter and Azospirillum* showed synergistic effects by improving growth prompting hormones, controlling pathogenesis and growth reducing agents due to producing fungicide antibiotics and compounds (antagonistic effect) and also air molecular N fixing and also producing growth prompting hormones such as oxine, cytokenine and gibberellins and solving mineral compound (Naseri et al. 2013).

4 Interaction of *Azotobacter* with Other Microorganisms

4.1 Interaction with Rhizobium

A synergistic relation of *Azotobacter* with *Rhizobium* interaction as co-inoculants have been observed in a majority of studies conducted under conditions like laboratory, greenhouse or field crops. Combined inoculation of *Azotobacter* and Rhizobium spp. has observed a positive response from crops. By significantly increasing nodulation *Azotobacter* spp. greatly influence Rhizobium activity. Increasing N_2 content within roots and shoots of respiring/metabolizing plant cells improves conditions within the rhizosphere and enhances synergistic interactions between the host and *Azotobacter sp*. in an open field conditions. Associative effect of *A. chroococcum* on Bradyrhizobium strains (BM 42 and BM 43) specific to moong bean (*Vigna radiata*) was also observed (Yadav and Vashishat 1991). The effect was more pronounced when *A. chroococcum* was co-inoculated with both the strains of *Bradyrhizobium*.

4.2 Interaction with Azospirillum

The beneficial effects of *Azotobacter* and *Azospirillum* interaction on plants are mainly attributed to improvements in root development, an increase in the rate of water and mineral uptake by roots, the displacement of fungi and plant pathogenic bacteria and to a lesser extent, biological nitrogen fixation (Okon and Itzigsohn 1995). Associative effect of *Azospirillum lipoferum* and *Azotobacter chroococcum* with Rhizobium spp. improved the growth of chick pea grown on both loamy sand and sandy soils (El-Mokadem et al. 1989). Both *Azotobacter* and *Azospirillum* have been shown to improve growth yields in various soil mineral compositions. This suggests that a mutualistic relationship exists between *Azotobacter* and *Azospirillum* where both interact with the Rhizobium to improve *Cicer arietinum* (chick pea) yields (Parmar and Dadarwal 1997). However, maximum values were obtained with *Azospirillum* application. Similarly, positive reports on application of *Azotobacter* and *Azospirillum* on the yield of mustard (*Brassica juncea*) are available (Tilak and Sharma 2007). Yasari et al. (2009) reported that inoculation of seed with *Azotobacter chroococcum*, *Azospirillium brasilense* and *Azospirillium lipoferum* recorded 1000 seed weight of 4.10 g, pods plant $^{-1}$ of 125.10 and seed yield of 1668 kg ha^{-1} in rapeseed (*Brassica napus. L*) at maturity in a field experiment conducted at Gharakheil Agricultural Research Station in Mazandaran province (Iran) during *rabi* season.

Some of the studies have shown that a relationship exists between chemotactic behaviour and Azotobacter's influence on plant growth such as cotton (*Gossypium hirsutum* L.) and wheat (*Triticum aestivum* L.) (Kumar et al. 2007). In the areas of soil where plant root exudates or secretions such as sugars, glucose, amino acids and organic acids have been deposited, bacteria mobilize towards these exudates through chemotactic attraction. Increased yields and enhanced growth using *A. chroococcum* indicate a positive response attributed to nitrogen fixation, phosphorus mobilization, bacterial production and the release of phytohormones (Kumar et al. 2007).

5 Possibility of Using *Azotobacter* in Crop Production

Azotobacter has beneficial effects on crop growth and yield through biosynthesis of biologically active substances, stimulation of rhizospheric microbes, producing phyopathogenic inhibitors (Lenart 2012). *Azotobacter* makes availability of certain nutrients like carbon, nitrogen, phosphorus and sulphur through accelerating the mineralization of organic residues in soil and avoid uptake of heavy metals (Levai et al. 2008). *Azotobacter* can be an important alternative of chemical fertilizer because it provides nitrogen in the form of ammonia, nitrate and amino acids without situation of over dosage, which might be one of the possible alternatives of

Table 2 Effect Of *Azotobacter* On Crop Yield

S. No	Crop	Increase in yield over yield obtained with chemical fertilizers (%)
1	Wheat	8–10
2	Rice	5
3	Sorghum	15–20
4	Maize	15–20
5	Potato	13
6	Carrot	16
7	Cauliflower	40
8	Tomato	2–24
9	Cotton	7.27
10	Sugarcane	9–24

(Bhattacherjee And Dey 2014)

inorganic nitrogen source (eg. Urea). *Azotobacter* as nitrogen biofertilizer increases the growth and yield of various crops under field conditions (Table 2).

5.1 Effects of Azotobacter on Growth and Yield of Crops

There is increment in dry matter accumulation in Azotobacter inoculated plants; it stimulates development of foliage, roots, branching, flowering and fruiting which is triggered by fixed nitrogen and plant growth regulator like substance produced. It also increases plant tolerance to lack of water under adverse condition (Zena and Peru 1986). The rate of increase in the leaf area determines the photosynthetic capacity of plant, which leads to better assimilation of produce and towards yield. Using *Azotobacter spp*. potato yield has been increased by 33.3 % and 38.3 % (Zena and Peru 1986). Triplett (1996) concluded that the development of the diazotrophic endophytic association in maize appears to be the most likely route to success in the development of a corn plant which does not require nitrogen fertilization for optimum growth and yield. Yield increased ranges from 2 to 45% in vegetables, 9 to 24% in sugarcane, 0 to 31% in maize, sorghum, mustard etc., on *Azotobacter* inoculation (Pandey and Kumar 1989a, b). Tandon (1991) estimated the fertilizer equivalent of important biofertilizers. According to the estimate, fertilizer equivalent of 19–22 kg ha^{-1} for rhizobium 20 kg N ha^{-1} for *Azotobacter* and *Azospirillium*, 20–30 kg N ha^{-1} for blue green algae (BGA) and 3 to 4 kg N ha^{-1} of Azolla. Results of pot experiments a under greenhouse conditions with onion showed that application of *G. fasciculatum* + *A. chrooccocum* + 50 % of the recommended P rate resulted in the greatest root length, plant height, bulb girth, bulb fresh weight, root colonization and P uptake (Mandhare et al. 1998). Laxminarayan (2001) reported that seed inoculation with *Azotobacter* produced higher grain and stover yield compared to

Table 3 Effect of inoculation on the grain yield of maize (t/ha)

Variant	Maize hybrids			
	ZP555 su	620 k	NS 609b	NS 6030
Control	12.27	4.27	8.88	10.59
100 ml *A.chroococcum*	13.32	4.97	8.39	10.90
75 ml *A.chroococcum*	13.24	4.89	8.87	10.75
50 ml *A.chroococcum*	13.31	4.30	8.92	10.96

uninoculated treatments. Singh and Dutta (2006) reported a significant in seed yield (7.86q ha^{-1}) in rapeseed and mustard (var. yella) due to inoculation with Azotobacter. Sharma (2002) reported the effect of biofertilizers and nitrogen on growth and yield of cabbage cv. Pride of India. Biofertilizer application significantly increased the leaf number, weight of non-wrapper leaves per plant, head length and width, gross and net weight of head per plant and yield per hectare over no biofertilizer application. Azotobacter in balanced nutrient condition results in 3.5 % increment in LAI at rosette stage of canola crop and additional application of Azotobacter shot up the yield by 21.17 % over the control (chemical fertilizers) (Yasari and Patwardhan 2007). According to Das and Saha (2007) combined inoculation of Azotobacter, Azospirillium along with diazotrophs increased grain and straw yield of rice by 4.5 and 8.5 kg ha^{-1}, respectively. The dual inoculation of *A. chroococcum* and *P. indica* had beneficiary response on shoot length, root length, fresh shoot and root weight, dry shoot and root weight, and panicle number that affect growth of rice plant (Kamil et al. 2008). Similar result put forwarded by Sandeep et al. (2011) which revealed that there is better growth response of Azotobacter inoculated plants as compared to non-inoculated control plants. Jafari et al. (2011) reported that the use of azotobacter had a positive effect on the grain yield of maize. In the variants where *Azotobacter* was applied, the grain yield increased in three maize hybrids (Table 3). In ZP 555 su, the yield increased by 1000 kg/ha, in NS 6030 by 280 kg/ha and in 620 k by 450 kg/ha. In NS 609b hybrid, the inoculation did not have any effect. An investigation was conducted under field conditions Milosevic et al. (2012), on a chernozem soil to study the effect of wheat seed inoculation (the cultivars Renesansa and Zlatka) with *A. chroococcum*, strain 86 (2–5 × CFU 108 ml^{-1}) reported that inoculation increased the energy of germination by 1 to 9 % and seed viability by 2 to 8 %. The largest increase in 1000 seed weight was obtained in the case of the cultivar Renesansa (16 %). *A. chroococcum* inoculation increased the seed yield of both cultivars and highest yield increase (74 %) was registered in the case of the cultivar Zlatka. According to Salhia (2013) azotobacter inoculants have a significant promoting effect on growth parameters like root, shoot length and dry mass of bamboo and maize seedlings in vitro and in pot experiments. Under green house conditions plant height, leaf number/plant, number of primary and secondary branches/plant, fresh and dry weight of whole plant, number of siliqua/plant, seeds/siliqua of brown sarson increased significantly with *Azotobacter* inoculation than no inoculation with seed and stover yield of 10.107 g pot^{-1} and 22.400 g pot^{-1} respectively

(Wani 2012). Naseri et al. (2013) while studying the effect of *A. chroococcum* and *Azospirillum brasilense* on grain yield, yield components of maize (S.C.704) as a second cropping in western Iran indicated that the dual inoculation with *Azotobacter and Azospirillum* on plant height, number of grain per row, 1000-grain weight, grain yield, biological yield and protein content was significant. Estiyar et al. (2014) reported that, number of branches, pod per plant and 1000 grain weight also increased with *Azotobacter* application.

6 Conclusion

Azotobacter spp. of bacteria, regarded as Plant Growth Promoting Rhizobacteria (PGPR) synthesize growth substances that greatly enhance plant growth and development and inhibit phytopathogenic growth by secreting inhibitors. There is a great significance of *A. chroococcum* in plant nutrition and its contribution to soil fertility. It is thus an important component of integrated nutrient management system due to its significant role in soil fertility. More research is necessary in future to explore the potentiality of *Azotobacter* in soil fertility using modern technology of soil genomics etc. The challenge to the research community will be to develop systems to optimize beneficial plant-endophyte bacterial relationships (Sturz et al. 2000) for long term effective role. In order to guarantee the high effectiveness of inoculants and microbiological fertilizers it is necessary to find compatible partners, i.e. a particular plant genotype and a particular *Azotobacteria* strain that will form a good association especially adapted to local edaphic and climatic conditions.

References

Arun KS (2007) Bio-fertilizers for sustainable agriculture, 6th edn. Agribios Publishers, Jodhpur, pp 76–77

Azcorn R, Barea JM (1975) Synthesis of auxins, gibberellins and cytokinins by *Azotobacter vinelandi* and *Azotobacter beijerinckii* related to effects produced on tomato plants. Plant Soil 43:609–619

Barea JM, Brown ME (1974) Effect on plant growth produced by *A paspali* related to the synthesis of plant growth regulating substance. J Appl Bacteriol 37:583–593

Beijerinck MW (1901) Über ologonitrophile mikroben. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. II Abt. 9: 561–582

Bhardwaj D, Ansari MW, Sahoo RK, Tuteja N (2014) Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. Microb Cell Fact 13:66

Bhattacherjee R, Dey U (2014) A way towards organic farming; A review. Afr J Microbiol Res 8(24):2332–2342

Brakel J, Hilger F (1965) Etude qualitative et quantitative de la synthese de substances de nature auxinique par *Azotobacter chroococcum in vitro*. Bull Inst Agron Stns Rech Gembloux 33:469–487

Chen WP, Chen JY, Chang SC, Su CL (1985) Bacterial alginate produced by a mutant of Azotobacter vinelandii. Appl Environ Microbiol 49:543–546

Chhonkar PK, Pareek RK, Rao DLN, ADiya TK (2009) Soil biology and biochemistry. Fundamentals of soil science, 2nd Edn. Indian Society of Soil Science, Pusa, New Delhi, pp 535–565

Das AC, Saha D (2007) Effect of diazotrophs on mineralization of organic nitrogen in the rhizosphere soils of rice (*Oryza sativa* L.). J Crop Weed 3:69–74

Döberainer J (1966) *Azotobacter paspali* sp. nov., uma bacteria fixadora de nitrogenio na rhizosfera de Paspalum. Pesquisa Agropecuaria Brasileira 1:357–365

Eklund E (1970) Secondary effects of some *Pseudomonads* in the rhizosphere of peat grown cucumber plant. In: Pharis RP, Reid DM (eds) Hormonal regulation of development, vol 3. Springer, New York, p 613

Elgala AM, Ishac YZ, Abdel Monem M, ElGhandour IAI (1995) Effect of single and combined inoculation with Azotobacter and VA micorrhizal fungi on growth and mineral nutrient contents of soil component interactions. In: Huang PM Berthilin J. Bollaj JM, McGill WB, Page AL (eds) Metals other inorganics and microbial activities (vol II). CRC Press, London

El-Mokadem MT, Helemish FA, Abou-Bakr ZYM, Sheteaws A (1989) Associative effect of Azotobacter lipoferum and Azotobacter chroococcum with Rhizobium sp. on mineral composition and growth of chick pea (*Cicer arietinum* L) on sandy soils. Zentralblatt fur Mikrobiologie 144: 255–265

Estiyar HK, Khoei FR, Behrouzyar EK (2014) The effect of nitrogen biofertilizer on yield and yield components of white bean (*Phaseolus vulgaris* cv. Dorsa). Int J Biosci 4(11):217–222

Govedarica M, Miliv V, Gvozdenovi DJ (1993) Efficiency of the association between *Azotobacter chroococcum* and some tomato varieties. Soil Plant 42:113–120

Hajnal T, Jarak M, Milosevic N (2004) Bacterization of maize: yield response of maize to inoculation. Book of abstracts of the 10th international symposium on microbial ecology: isme-10, cancun, mexico: 207

Hakeem KR, Akhtar MS, Abdullah SNA (2016) Plant, soil and microbes – vol 1, Implications in Crop Science Springer International Publishing AG, Gewerbestrasse 11, 6330 Cham, Switzerland. 366 pp

Hecht CB (1998) The apoplast-habitat of endophytic dinitrogen-fixing bacteria and their significance for the nitrogen nutrition of non leguminous plants. J Plant Nutr Soil Sci 161:509–520

Hennequin JR, Blachere H (1966) Recherches sur la synthese de phytohormones et de composes phenoliques par *Azotobacter* et des bacteries de la rhizosphere. Ann Inst Pasteur 3:89–102

Horner CK, Burk D, Allison FE, Sherman MS (1942) Nitrogen fixation by *Azotobacter* as influenced by molybdenum and vanadium. J Agric Res 65:173–193

Iswaran V, Sen A (1960a) Inactivation of Azotobacter by heat. Curr Sci 27(9):341–342

Jafari TH, Latkovic D, Duric S, Mrkovacki N, Najdenovska O (2012) Research. J Agric Sci 44(2)

Jen-Hshuan Chen (2006) The combined use of chemical and organic fertilizers and/or biofertilizer for crop growth and soil fertility. International workshop on sustained management of the soil-rhizosphere system for efficient crop production and fertilizer use 16(20):1–10

Jensen HL (1954) The Azotobacteriaceae. Bacteriol Rev 18(4):195–214

Joerger RD, Bishop PE (1988) Bacterial alternative nitrogen fixing systems. Crit Rev, Microbiol 16: 1–14

Kamil P, Yami KD, Singh A (2008) Plant growth promotional effect of *Azotobacter chroococcum*, *Piriformospora indica* and vermicompost on rice plant. Nepal J Sci Technol 9:85–90

Kizilkaya R (2009) Nitrogen fixation capacity of *Azotobacter* spp. Strains isolated from soils in different ecosystems and relationship between them and the microbiological properties of soils. J Environ Biol 30(1):73–82

Krassilnikov NA (1949) Opred Zlita baktzrij i aktinomyc etov. Mo skva

Kumar R, Bhatia R, Kukreja K, Behl RK, Dudeja SS, Narula N (2007) Establishment of Azotobacter on plant roots: chemotactic response, development and analysis of root exudates of cotton (*Gossypium hirsutum* L.) and wheat (*Triticum aestivum* L.). J Basic Microbiol 47:436–439

Laxminarayana K (2001) Effect of *Azotobacter* and *Azospirillum* on yield performance of maize in hilly regions of Mizoram. Indian J Hill Farm 14(2):134–137

Lenart A (2012) Occurrence characteristics and genetic diversity of *Azotobacter chroococcum* in various soils of Southern Poland. Pol J Environ Stud 21(2):415–424

Levai L, Szilvia V, Nora B, Eva G (2008) Can wood ash and biofertilizer play a role in organic agriculture? Agronomski Glasnic 3:263–271

Lipman JG (1903) Report on the New Jersey Agricultural Experiment Station 24: 217–285

Lipman JG (1904) Report on the New Jersey Agricultural Experiment Station 25: 237–289

Lopez GJ, Pozo RB, Lopez S, Toledo M, Salmeron V (2005) Liberation of amino acids by heterotrophic nitrogen fixing bacteria. Amino Acid 28(4):363–367

Mahato P, Anoop B, Chauhan JS (2009) Effect of *Azotobacter* and Nitrogen onseed germination and early seedling growth in tomato. Researcher 1(4):62–66

Mandhare VK, Patil PL, Gadekar DA (1998) Phosphorus uptake of onion as influenced by *Glomus fasciculatum*, *Azotobacter* and phosphorus levels. Agric Sci Digest 18:228–230

Maryenko VG (1964) Zavisimost lurozaja kukuruzyl balansa azota Aztobacter chroococcum V usbvijah monobakterialznoj kultury (Dependence of maize klyeid and nitrogen balance kon Azotobacter chroococcum in the jkcodnitions of monobacterial cultivatiions). Dokt ISHA 99:399–406

Milosevic N, Tintor B, Protic BC, Cvijanovig R (2012) Effect of inoculation with Azotobacter chroococcum on wheat yield and seed quality. Rom Biotechnol Lett 17(3):7352–7357

Nagananda GS, Das A, Bhattachrya S, Kalpana T (2010) In vitro studies on the effects of biofertilizers (*Azotobacter* and *Rhizobium*) on seed germination and development of *Trigonella foenum-graecum* L. using a novel glass marble containing liquid medium. Int J Bot 6(4):394–403

Naseri R, Moghadam A, Darabi F, Hatami A, Tahmasebei GR (2013) The Effect of deficit irrigation and *Azotobacter Chroococcum* and *Azospirillum brasilense* on grain yield, yield components of maize (S.C.704) as a second cropping in western. Iran Bull Environ Pharmacol Life Sci 2(10):104–112

Okon Y, Itzigsohn R (1995) The development of Azospirillum as a commercial inoculant for improving crop yields. Biotechnol Adv 13:415–424

Page WJ, Sadoff HL (1975) Relationship between calcium and uronic acids in the encystment of *Azotobacter vinelandiil*. J Bacteriol 122(1):145–151

Page WJ, Shivprasad S (1991) *Azotobacter salinestris* sp. nov., a sodium-dependent, microaerophili, and aeroadaptive nitrogen-fixing bacterium. Int J Syst Bacteriol 41: 369–376. Ann Microbiol 51: 145–158

Pandey A, Kumar SJ (1989a) Soil beneficial bacterial and their role in plant growth promotion. Sci Indian Res 48:134–144

Pandey A, Kumar S (1989b) Potential of *Azotobacters* and *Azospirilla* as biofertilizers for upland agriculture: a review. J Sci Ind Res 48:134–144

Parker LT, Socolofsky MD (1968) Central body of the Azotobacter cyst. J Bacteriol 91(1):297–303

Parmar N, Dadarwal KR (1997) Rhizobacteria from rhizosphere and rhizoplane of chick pea (*Cicer arietinum L.*). Indian J Microbiol 37:205–210

Patridge CDP, Walker CC, Yates MG, Postage JR (1980) The relationship between hydrogenase and nitrogenase in *Azotobacter chrocooccum* effect of nitrogen sources on hydrogenase activity. J Gen Microbiol 119:313–319

Puertas A, Gonzales LM (1999) Aislamiento de cepas nativas de *Azotobacter chroococcum* en la provincia Granmay evaluacion de su actividad estimuladora en plantulas de tomate. Cell Mol Life Sci 20:5–7

Robson RL, Postgate JR (1980) Oxygen and hydrgen biological nitrogen fixation. Ann Rev Microbio 34:183–207

Sadoff HL (1975) Encystment and germination in Azotobacter vinelandii. Microbiol Rev 39(4):516–539

Salhia B (2013) The effect of *Azotobacter chrococcum*as nitrogen biofertilizer on the growth and yield of *Cucumis sativus.* The Islamic University Gaza, Deanery of Higher Education Faculty of Science, Master of Biological Sciences, Botany

Sandeep C, Rashmi SN, Sharmila V, Surekha R, Tejaswini R (2011) Growth response of *Amaranthus gangeticus* to *Azotobacter chroococcum* isolated from different agroclimatic zones of Karnataka. J Phytology 3(7):29–34

Sariiv MR, Sariiv Z, Govedarica M (1988) Efficiency of strain combination of different genera of nitrogen fixing bacteria on sunflower genotypes. In: 12th international sunflower conference, Novi Sad, pp 187–191

Selvakumar G, Lenin M, Thamizhiniyan P, Ravimycin T (2009) Response of biofertilizers on the growth and yield of blackgram (*vigna mungo L.*). Recent Res Sci Technol 1(4):169.17

Sharma R. (2002) Ph.D. Thesis, AAU, Jorhat.

Sharma K, Dak G, Agrawal A, Bhatnagar M, Sharma R (2007) Effect of phosphate solubilizing bacteria on the germination of *cicer arietinum* seeds and seedling growth. J Herbal Med Toxicol 1(1):61–63

Shivprasad S, Page WJ (1989) Catechol formation and melanization by Na^+ dependent Azotobacter chroococcum: a protective mechanism for aeroadaptation? Appl Environ Microbiol 55(7):1811–1817

Singh MS, Dutta S (2006) Mustard and rapeseed response to Azotobacter – a review. Agric Rev 27(3):232–234

Soleimanzadeh H, Gooshchi F (2013) Effects of *Azotobacter* and nitrogen chemical fertilizer on yield and yield components of wheat (*Triticum aestivum* L.). World Appl Sci J 21(8):1176–1180

Solimam S, Seeda MA, Ally SSM, Gadalla AM (1995) Nitrogen fixation by wheat plants as affected by nitrogen fertilizer levels and non-symbiotic bacteria. Egypt J Soil Sci 35:401–413

Sturz AV, Christie BR, Nowak J (2000) Bacterial endophytes: potential role in developing sustainable systems of crop production. Crit Rev Plant Sci 19:1–30

Subba Rao NS (2001) An appraisal of biofertilizers in India. In: Kannaiyan S (eds) Biotechnology of biofertilizers. Maximising the use of biological nitrogen fixation in agriculture. Narosa Publising House, New Delhi, p 375

Tandon HLS (1991) Role of sulphur in plant nutrition. Fert News 36(69):79

Thompson JP, Skerman VBD (1981) Validation list no. 6. Int J Syst Bacteriol 31:215–218

Tilak K, Sharma KC (2007) Does *Azotobacter* help in increasing the yield. Indian Farm Digest 9:25–28

Tippanavar CM, Reddy TK (1989) Aztobacter in root stem and leaf tissuesof cells of *Triticum aestivum* and *Triticum durum* L. Curr Sci 58:1342–1343

Triplett GT, Dabney SM, Siefker JH (1996) Tillage systems for cotton on silty upland soils. Agron J 88:507–512

Vance CP, Graham PH (1995) Nitrogen fixation in agriculture: applications and perspectives. In: Tikhonovich IA, Provorov NA, Romanov VI, Newton WE (eds) Nitrogen fixation: fundamentals and applications, current plant science and biotechnology in agriculture 27: 77–86

Vijayan K, Chakraborti SP, Ghosh PD (2007) Foliar application of *Azatobacter chroococcum* increases leaf yield under saline conditions in mulberry (Morus spp.). Sci Hortic 113:307–311

Wani SA (2012) Effect of balanced NPKS, biofertilizer (*Azotobacter*) and vermicompost on the yield and quality of brown sarson (*Brassica rapa* L.), M. Sc thesis, Sher-e-Kashmir University of Agriculture Sciences and Technology, Kashmir, Srinagar

Wani SA, Chand S, Ali T (2013) Potential use of Azotobacter chroococcum in crop production: an overview. Curr Agric Res J 1:35–38

Yadav AS, Vashishat RK (1991) Associative effect of Bradyrhizobium and Azotobacter inoculation on nodulation, nitrogen fixation and yield of Moong bean (*Vigna radiata* L. Wilczek). Indian J Microbiol 31:297–300

Yasari E, Patwardhan AM (2007) Effect of (*Azotobacter* and *Azosprillium*) inoculants and chemical fertilizers on growth and productivity of canola (*Brassica napus* L.). Asian J Plant Sci 6(1):77–82

Yasari ES, Esmaeili MA, Azadgolch MS, Alasthi MR (2009) Enhancement of growth and nutrient uptake of rapeseed (*Brassica napus* L.) by applying mineral nutrients and biofertilizers. Pak J Biol Sci 112:127–133

Zena GG, Peru C (1986) Effect of different rates of *Azotobacter* and frequency of application of Agrispon on yield and quality in the growing of onion (*Allium cepa* L.) in Cajamarca. National University of Cajamarca Faculty of Agriculture Sciences and Forestry