



Azotobacter: A Complete Review

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ABSTRACT

Azotobacter is one of the bio-fertilizer which contains living organisms which when applied on soil surface or seeds help in colonizing the rhizosphere or the interior parts of the plant parts and also help in promoting growth through the increase of the availability of primary nutrients to plants. *Azotobacter* is a free living gram negative bacterium which is oval or spherical in shape. It is an important bio-fertilizer which helps in increasing soil fertility through nitrogen fixation which later helps in increasing crop production through the process of biosynthesis of biologically active substances for plants uptake. It also plays an important role in nutrient cycling and increases nutrient availability so it is more ecofriendly when compared to chemical fertilizer. From research it is found that use of *Azotobacter* also increases the yield of some crops. With the growing importance of conserving natural resources, the use of biofertilizer alone or in combination with some fertilizer or pesticide is found to give good results in some research papers. Therefore, to decrease the rate of exhausting the soil with chemicals it is better to use bio-fertilizers. So, it is important to learn more about its importance and uses about the particular bio-fertilizer before practically using it.

Keywords: *Azotobacter*, biofertilizer, nitrogen fixation, soil fertility

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INTRODUCTION

Bio fertilizers also known as bio-inoculants; which are prepared organically contain microorganisms that are beneficial to agriculture products in supply of nutrients particularly N and P [1]. Bio fertilizers when used as seed treatment [2] or applied in soil [3] they rapidly multiply to develop more population in the rhizosphere [4]. Bio fertilizers are gaining more importance in agriculture nowadays as they are non-hazardous, non-toxic and eco-friendly [5].

Bio fertilizers including *Azotobacter* [6], Blue green algae[7], *Azospirillum*[8], mycorrhizae[9] and p-solubilizing microbes [10] as the advanced tools are being used and impart various benefits to the agriculture sector [11].

Azotobacter spp. are free-living, gram negative, oval or spherical in shape, aerobic soil dwelling bacteria [12,13]. *Azotobacter* was first discovered by a Dutch microbiologist and botanist Beijerinck in 1901 [14]. The genus *Azotobacter* has 7 different species which includes *Azotobacter croococcum*, *A. armeniacus*, *A. beijerinckii*, *A. paspali*, *A. salinestrus*, *A. nigricans* and *A. vinelandii* [15,16]. Their size ranges from 1-2 μm wide and 2-10 μm long [17].

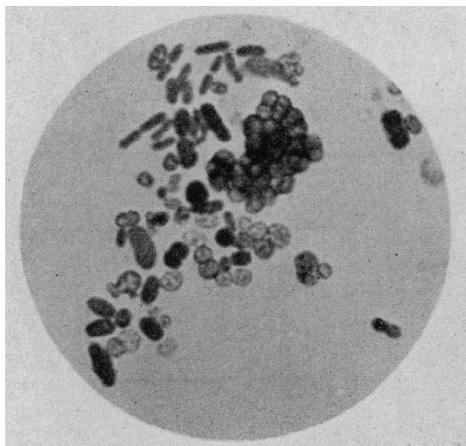


Figure 1: *Azotobacter* species cells (Beijerinck, 1901)

The atmospheric nitrogen gas is utilized by these bacteria for synthesis of their cell protein [18]. This cell protein will be mineralized after the *Azotobacter* cell is dead which will contribute the available nitrogen of the crop plants [19]. It is found that the *Azotobacter* spp. are sensitive to high temperature above 35°C, acidic pH and high salts [20]. This bacterium has beneficial effects on growth and yield of crop by stimulating rhizospheric microbes [21, 22], bio-synthesizing the active substance and producing phytopathogenic inhibitors [23, 24].

***Azotobacter* AS A BIO INOCULANT AND BIO FERTILIZER**

The use of *Azotobacter* as bio fertilizer was introduced by Gerlach and Voel (1902) for supplying N to soil which is biologically fixed N₂ as one of the activities for this microbe [25]. This bacterium is playing a multifaceted role in stimulation of growth to plants which is not only the fixing of atmospheric N₂ but also helps in possessing other activities of growth promotion such as phosphate solubilisation, PGRs production like auxins, cytokinins, gibberellins, amino acids and vitamins [26, 27, 28, 29, 30]. A report from Apte and Shende [28] shows that this bacterium possesses a high range of N₂ fixation in the amount of 2-15mg N fixed per gram of glucose consumed and also has a high acetylene reduction assay (ARA). *Azotobacter chroococcum* helps in reducing the infection of nematodes by 48% which is followed by *Azospirillum* (4%) and *Pseudomonas* (11%) [31].

***Azotobacter* IN SOIL FERTILITY**

As chemical fertilizers are quite expensive and give high cost of production which also have adverse effects on microbial population as well as soil health [23], in such a situation, bio fertilizer becomes the best alternative for maintaining soil fertility [32, 33]. Bio fertilizers being environmentally friendly and economic [34], they are found to be very useful for better crop production and yield [35].

Azotobacter spp. in soils has so many benefits on growth of plants, helps in improving germination of seeds [36, 37] and also has positive response on Crop Growth Rate (CGR) [38], also the abundant presence of these bacteria has positive relation to many of the soil physico-chemicals (e.g. organic matter, pH, soil moisture and temperature of the soil) and microbiological properties [39]. According to the soil profile depth, the abundance also varies [40].

Nitrogen fixation

Nitrogen fixation turns out to be the most important microbial activity [39] and biological processes [41] happening on the earth surface right after photosynthesis. The role of biological nitrogen fixation is very important in maintaining fertility of the soil [42]. *Azotobacter* can be used for the study of nitrogen fixation as well as plant inoculation because of its rapid growth and having high levels of nitrogen fixation [43, 44]. *Azotobacter* has the capability for conversing nitrogen into ammonia which later on taken up by the plants [45]. As *Azotobacter* spp. being non-symbiotic [46] and heterotrophic bacteria, they have the capability of fixing 20 kg N per hectare per year which can be used for crop production [47]. Bacterization helps in improving growth of plants [48] and increases soil nitrogen by utilizing carbon through nitrogen fixation for its metabolism [49, 50].

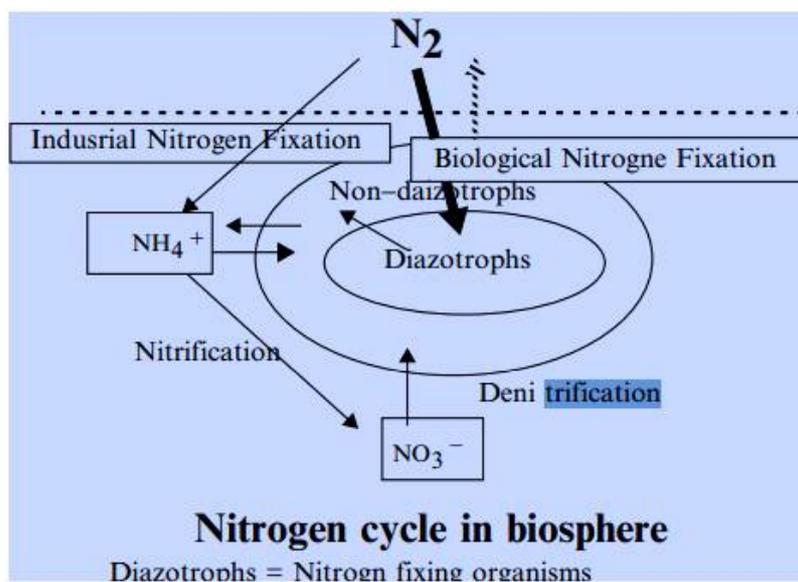


Figure 2: Nitrogen Fixation by *Azotobacter* spp. [4].

Azotobacter IN CROP PRODUCTION

Azotobacter spp. have so many beneficial effects on growth of crops through the process of biosynthesis of biologically active substances [51] producing phytopathogenic inhibitors and stimulation of rhizospheric microbes [52]. *Azotobacter* also makes the availability of some nutrients like carbon, phosphorus, nitrogen and sulphur through the process of accelerating the mineralized organic residues in soil [4] while avoiding the uptake of different heavy metals [53]. *Azotobacter* has become an important alternative for chemical fertilizers as it can provide nitrogen in ammonium form, amino acids and nitrate without leaving the situation over dosage [54]. *Azotobacter* as a nitrogen bio fertilizer helps in improving the growth and yield of different crops in field conditions [55] **Table 1**.

Azotobacter IN GROWTH AND YIELD OF CROPS

Azotobacter helps in stimulating the development of branching, flowering rooting foliage formation and fruiting which is initiated by plant growth regulator and fixed nitrogen [56]. This bacterium also helps plants in increasing tolerance to lack of water supply under adverse climatic conditions [57]. A report says that the yield of potato has been greatly increased after using *Azotobacter* spp. by 33.3% [57]. There is also a report that shows a significant increase in yield of mustard (var. Yella) and Rapeseed (7.86q ha⁻¹) after inoculation with *Azotobacter* [58]. From a report of Das and Saha (2007) [59], the combined inoculation of *Azotobacter* with *Azospirillum* as well as *diazotrophs* helps in increasing the straw and grain yield of rice by 4.5 to 8.5 kg ha⁻¹. Plants inoculated with *Azotobacter* have better crop yield as compared to those with non-inoculated plants and have a positive response on grain yield of maize [60]. The grain yield increased while using *Azotobacter* in three different maize hybrids is shown in **Table 2**.

Table 1: *Azotobacter* effects on crop yield

Sl. No.	Crop	Yield increased over yield obtained from chemical fertilizers (%)
1	Rice	5
2	Sorghum	15-20
3	Wheat	8-10
4	Maize	15-20
5	Potato	13
6	Tomato	2-24
7	Carrot	16
8	Cauliflower	40
9	Cotton	7.27
10	Sugarcane	9-24

Source: Bhattacharjee and Dey [54]

Table 2: Effect of *Azotobacter* spp. in maize yield

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

Source: [87]

Azotobacter IN NUTRIENT CYCLING

Azotobacter allows certain nutrients available like carbon, sulphur, phosphorus and nitrogen through the mineralization process of organic residues in soil while avoiding the uptake of different heavy metals [53]. Nowadays *Azotobacter* has become one of the most important alternatives for chemical fertilizers as it can provide nitrogen into ammonia, amino acids and nitrate without overdosing the plants [54]. The bacterium helps in sustaining the growth of plants and yield even when the soil content is low in phosphate and also helps in taking up the macro and micro nutrients for better utilizing the plant root exudates [61].

Azotobacter IN SEED INOCULATION AND NUTRIENT UPTAKE

When seeds are inoculated with *Azotobacter*, the bacterium helps in the uptake of macronutrients like N and P along with some micronutrients like Fe and Zn [62, 63]. These bacteria strains are also used for

improving the nutrition of rice, wheat and maize [64,65]. The yield of crops is profoundly increased as the *Azotobacter* helps in supplying nitrogen in standing crops [66]. *Azotobacter* inoculated seeds are found to be increased in protein and carbohydrate content of two varieties of corn (Inra260 and Inra210) in an experiment done in a greenhouse [67]. The combined application of manure with *Azotobacter* gives increase in biomass of maize crop [68].

EFFECTS OF *Azotobacter* COMBINED WITH CHEMICAL FERTILIZERS

Azotobacter when applied in combination with 50% chemical fertilizers i.e., N and P, has some effects on growth of plants, number of branches, height, dry weight and freshness of safflower as compared to chemical fertilizers only [69]. Same goes for the organic fertilizers as well when applied with *Azotobacter* biphosphate along with half dose of chemical fertilizers helps in increasing the economic yield of safflower [71]. With increased N levels, the efficiency of *Azotobacter* is found to be decreasing [70]. The best combination turns out to be with NH₄Cl @ 0.1 g/L while the combination with copper and *Azotobacter* are found to be toxic even when applied in low concentration [72, 76].

EFFECTS OF *Azotobacter* COMBINED WITH PESTICIDES

The herbicide 2, 4-D along with its products p-chlorophenol and p-chlorophenoxy-acetic acid are utilized by *Azotobacter croococcum* in the form of carbon, which later on stimulate nitrogenase enzyme [73]. Result found from a report that the insecticide carbofuran helps in stimulating the activity of nitrogenase enzyme [74]. A report says that the herbicide simazine has no effect on growth of *Azotobacter croococcum*, neither growth nor sterilized or dialyzed soil medium [75]. With the presence of simazine, the *Azotobacter* can be grown with cells having higher ATP content.

STRESS TOLERANCE CHARACTERISTICS OF *Azotobacter*

Most of the important pollutants through irrigation in agricultural soils are the heavy metals [77]. The consecutive accumulation of these heavy metals lead to retardation of plant growth later on affects the yield [78]. Exopolysaccharides of *Azotobacter* have a great role in immobilizing heavy metals [79]. High absorptive nature of EPS removes heavy metals from the soil [80, 81]. The EPS of *Azotobacter* can directly uptake and bind the heavy metals like Cr and Cd in contaminated soils [82, 83]. Macronutrients and micronutrients can also be supplied through the decomposition of EPS from *Azotobacter* [84]. The EPS based *Azotobacter* helps in increasing the aqueous dispersion of some poorly soluble compounds by changing the affinity and magnitude between hydrocarbons and microbial soils [85, 86]. The microbial activity can differentiate the biological process between compacted soil and non-compacted soils.

CONCLUSION

Azotobacter spp. are gram negative and capable of fixing 20 kg N per hectare per year. This bacterium is regarded as PGPR which helps in synthesizing growth substances and takes a great role in enhancing growth, development, and inhibiting phytopathogenic growth as they secrete inhibitors. From more research, *Azotobacter* spp. helps in increasing soil fertility, germination rate and has a positive response on Crop growth rate which results in more yield and healthy growth. *Azotobacter* spp. can also be used combining with chemical fertilizers as well as pesticides which help in increasing the economic yield. More stress can be tolerated by this bacterium as they can also produce EPS. More research is required so as to get more good qualities of the *Azotobacter* spp.

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