

## Azotobacter: A Plant Growth Promoting Rhizobacteria a Biofertilizer for Wheat Crop

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**ABSTRACT:** In the present context, the best alternative of chemical fertilizer is necessary because of its adverse effects on the soil health. There are several alternatives available to enhance the soil fertility one of them is *Azotobacter*. It is a free-living N<sub>2</sub>-fixer diazotroph that has several beneficial effects on the crop growth and yield. It helps in synthesis of growth regulating substances like Auxins, Cytokinins and Giberellic Acid (GA). In addition, it stimulates rhizospheric microbes, protects the crops from major phytopathogens, improves nutrient uptake and ultimately boost up biological nitrogen fixation. The abundance of these bacteria in soil is related to many factors, mostly soil pH and fertility.

**Keywords:** Wheat; PGPR's; *Azotobacter*; Biological nitrogen fixation and Soil fertility.

**INTRODUCTION:** Wheat is the major grain that sustains humanity. Wheat grows in temperate climate and it is staple food for 35% of world's population. On other hand, it provides more calories and protein in the diet than any other crop. Wheat (*Triticum* spp., most commonly *T. aestivum*) is a cereal grain (botanically, a type of fruit called a caryopsis) originally from the Levant region but now cultivated worldwide<sup>1</sup>. In 2016, world production of wheat was 749 million tonnes, making, it the second most-produced cereal after maize (1.03 billion tonnes), with more than rice (499 million tonnes) (*United Nations, Food and Agriculture Organization. 2016*). Since 1960, world production of wheat and other grain crops has tripled and is expected to grow further through the middle of the 21<sup>st</sup> Century.<sup>2</sup>

This grain is grown on more land area than any other commercial food (220.4 million hectares, 2015-16) Food and Agriculture Organization Corporate Statistical Database (*FAOSTAT, 2015*). World trade in wheat is greater than for all other crops combined. Globally, wheat is the leading source of vegetal protein in human food, having a protein content of about 13%, which is relatively high as compared to other major cereals and staple foods.

When eaten as the whole grain, wheat is a source of multiple nutrients and dietary fiber, and is associated

with lower risk of several diseases, including coronary heart disease, stroke, cancer and diabetes.<sup>3</sup>

**Nutrition:** In 100 grams, wheat provides 327 calories and is a rich source (20% or more of the Daily Value, DV) of multiple essential nutrients, such as protein, dietary fibre, manganese, phosphorus and niacin. Several B vitamins and other dietary minerals are in significant content. Wheat seeds contain 13% water, 71% carbohydrates, and 1.5% fat. Its 13% protein content is comprised mostly of gluten as 75-80% of total wheat protein which upon digestion contributes amino acids for human nutrition.<sup>4</sup>

**Production and consumption:** In 2016, global wheat production was 749 million tonnes, Food and Agriculture Organization (FAO 2016). Wheat is the primary food staple in North Africa and the Middle East, and is growing in uses in Asia. Unlike rice, wheat production is more widespread globally, though 47% of the world total in 2014 was produced by just four countries – China, India, Russia and the United States, Food and Agriculture Organization Corporate Statistical Database (*FAOSTAT 2014*).

During the past century, industrialization of agriculture has provoked a significant and essential productivity increase, which has led to a greater amount of food available to the general population. Along with this abundance the appearance of serious environmen-

tal and social problems came with the package: problems that must be faced and solved in the not too distant future. Nowadays, it is urgent to maintain that high productivity, but it is becoming urgent to alter as little as possible the environment. Clearly we must then head for a more environmentally sustainable agriculture while maintaining ecosystems and biodiversity. One potential way to decrease negative environmental impact resulting from continued use of chemical fertilizers, herbicides and pesticides is the use of plant growth-promoting rhizobacteria (PGPR). This term was first defined by Kloepper and Schorth (1978)<sup>5</sup> to describe soil bacteria that colonize the rhizosphere of plants, growing in, on or around plant tissues that stimulate plant growth by several mechanisms.<sup>7</sup>

**Table 1: Top Wheat producers in 2018-19.**

Country	Millions of tonnes
China	126.2
India	95.8
Russia	59.7
United States	55.1
France	39.0
Canada	29.3
Germany	27.8
Pakistan	26.0
Australia	25.3
Ukraine	24.1
<b>World</b>	<b>720</b>

Source: UN Food and Agriculture Organization

**Rhizosphere:** The rhizosphere is coined more than hundred years ago by Hiltner (1904).<sup>8</sup> It is a nutrient-rich habitat for microorganisms, where severe, intense interactions take place between the plant, soil, and microfauna.<sup>9</sup> They may have positive, negative or no visible effect on plant growth. Plant growth and productivity is highly affected by these interactions. Different type of microorganisms such as bacteria, fungi, protozoa, algae coexist among them. Bacteria contribute most to plant health by releasing many organic exudates, thus creating a selectively sensitive environment where diversity is less. Out of them plant growth promoting bacteria (PGPR) are most abundant among all others in the rhizosphere.<sup>10</sup>

**Role of PGPR:** PGPR are free living bacteria that resides in soil. They either directly or indirectly assist rooting<sup>11</sup>. They play different roles in the soil which proves beneficial for plant health and productivity. They colonize the rhizosphere and protect plant from its pathogens, produce secondary metabolites such as antibiotics that suppress harmful rhizobacteria, pro-

duce siderophores, and phytohormones, can fix atmospheric nitrogen, and help in providing nutrition uptake by solubilizing phosphate and produce biologically active substances which influence the plant growth and development.<sup>12</sup>

**Occurrence and forms of PGPR:** The mechanism by which PGPR exerts their beneficial effect on plants can be very diverse. They can establish themselves on root's surface or inside the roots. PGPR can be classified into extracellular plant growth promoting rhizobacteria (ePGPR) that may exist in the rhizosphere, on the rhizoplane or in the spaces between the cells of root cortex. The bacterial genera such as *Agrobacterium*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Flavobacterium*, *Pseudomonas* and *Serratia* belong to ePGPR. The other category is intracellular plant growth promoting rhizobacteria (iPGPR) that locates generally inside the specialized nodular structures of root cells *Rhizobium* spp.<sup>13</sup>

**Azotobacter:** *Azotobacter* spp. are Gram negative, free-living, aerobic soil dwelling<sup>14</sup>, oval or spherical bacteria that form thick-walled cysts (means of asexual reproduction under unfavorable condition), can grow well on N-free medium.<sup>15</sup> There are around six species in the genus *Azotobacter*<sup>16</sup> some of which are motile by means of peritrichous flagella, others are not. They are typically polymorphic and their size ranges from 2-10  $\mu\text{m}$  long and 1-2  $\mu\text{m}$  wide<sup>15</sup>. The *Azotobacter* genus was discovered in 1901 by Dutch microbiologist and botanist (founder of environmental microbiology). *A. chroococcum* is the first aerobic free-living nitrogen fixer.<sup>17</sup>

These bacteria utilize atmospheric nitrogen gas for their cell protein synthesis. This cell protein is then mineralized in soil after the death of *Azotobacter* cells thereby contributing towards the nitrogen availability of the crop plants. *Azotobacter* spp. is sensitive to acidic pH, high salts, and temperature.<sup>18</sup> *Azotobacter* has beneficial effects on crop growth and yield through, biosynthesis of biologically active substances, stimulation of rhizospheric microbes, producing phytopathogenic inhibitors.<sup>19-20</sup>

Besides being quite expensive and making high cost of production, chemical fertilizers have adverse effect on soil health and microbial population. In such situation, biofertilizers can be the best alternative for enhancing soil fertility. Being economic and environmental friendly, biofertilizers can be used in crop production for better yield. Similarly, microbial products are considered safer, self-replicating, target specific, which is regarded as major component of inte-

grated nutrient management from soil sustainability perspective.<sup>21</sup>

#### **Role of *Azotobacter* in soil:**

**Azotobacter in soil:** The presence of *Azotobacter* sp. in soils has beneficial effects on plants, but the abundance of these bacteria is related to many factors, soil physico-chemical (e.g. organic matter, pH, temperature, soil moisture) and microbiological properties.<sup>22</sup> Its abundance varies as per the depth of the soil profile.<sup>23</sup> Azotobacteria are much more abundant in the rhizosphere of plants than in the surrounding soil and that this abundance depends on the crop species.<sup>24</sup>

**Nitrogen fixation:** Nitrogen is the component of protein and nucleic acids and chlorophyll. Thus, nitrogen supply to the plant will influence the amount of protein, amino acids, protoplasm and chlorophyll formed. Therefore, adequate supply of nitrogen is necessary to achieve high yield potential in crop.

The atmosphere comprises of ~78% nitrogen as an inert, in unavailable form. Above every hectare of ground there are ~80000 tones of this unavailable nitrogen. In order to be converted to available form it needs to be fixed through either the industrial process or through Biological Nitrogen Fixation (BNF). Without these nitrogen-fixers, life on this planet may be difficult.

Nitrogen (N) deficiency is frequently a major limiting factor for crops production<sup>25</sup>. Nitrogen is an essential plant nutrient, widely applied as N-fertilizer to improve yield of agriculturally important crops. An interesting alternative to avoid or reduce the use of N-fertilizers could be the exploitation of Plant Growth-Promoting Bacteria (PGPB) capable of enhancing growth and yield of many plant species, several of agronomic and ecological significance. *Azotobacter* spp. are non-symbiotic heterotrophic bacteria capable of fixing an average 20 kg N/ha/per year.<sup>22</sup>

Bacterization helps to improve plant growth and to increase soil nitrogen through nitrogen fixation by utilizing carbon for its metabolism.<sup>26</sup>

**Seed inoculation with *Azotobacter* and nutrient uptake:** Seed Inoculated with *Azotobacter* helps in uptake of N, P along with micronutrients like Fe and Zn, in wheat, these strains can potentially be used to improve wheat nutrition<sup>27</sup>. Seed inoculation of *Azotobacter* profoundly contribute to increase yield by supplying nitrogen to the crops. Inoculation of seeds with *Azotobacter chroococcum* increased carbohydrate and protein content of two corn varieties (Inra210 and Inra260) in greenhouse experiment.<sup>28</sup>

There is increment in Maize biomass with the application of manure and *Azotobacter*.<sup>29</sup> In nitrogen-deficient sand, seed inoculation increased plant length, dry weight, and nitrogen content in addition to a significant increase in soil nitrogen.<sup>30</sup>

Seeds of wheat (*Triticum aestivum*) were inoculated with 11 bacterial strains of *A. chroococcum*, Research result showed that all *A. chroococcum* strains had positive effect on the yield and N concentrations of wheat.<sup>31</sup>

**Role in growth substances production and promotion:** Besides, nitrogen fixation, *Azotobacter* produces, Thiomin, Riboflavin, Nicotin, Indol Acetic Acid and Gibberellins. When *Azotobacter* is applied to seeds, seed germination is improved to a considerable extent. (Brake J and Hilger F 1965)<sup>32</sup> showed that *Azotobacter* produced Indol-3-Acetic Acid (IAA) when tryptophan was added to the medium. (Hennequin JR, Blachere H 1996)<sup>33</sup> 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 wheat.<sup>34</sup> These hormonal substances, which originate from the rhizosphere or root surface, affect the growth of the closely associated plants. (Eklund.1990)<sup>35</sup> demonstrated that the presence of *Azotobacter chroococcum* in the rhizosphere of tomato and cucumber is correlated with increased germination and growth of seedlings. Phytohormones (auxin, gibberellin and cytokinins) can stimulate root development.

High Gibberillic acid production was detected in *Azotobacter* (71.42 %) isolates. Higher phosphate solubilization was detected in the isolates of *Azotobacter* (74.28 %) followed by *Pseudomonas* (63.00 %). Gibberellins applied in small quantities to the soil or rosettes on the leaves and shafts of certain plant produces an increase in height. In grains such as wheat and corn, they also cause an increase in length of the leaves. In some cases they also increase the fresh weight and dry weight. However it doesn't produce any effect on the growth of the roots.<sup>31</sup>

These responses suggest that *Azotobacter* probably influences the development of plants by producing growth-regulating substances. Therefore, *Azotobacter* spp. is often regarded as a member of "Plant Growth Promoting Rhizobacteria (PGPR)".<sup>36</sup>

**Dry matter accumulation:** 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.<sup>37</sup> 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. Better crop growth response ultimately results in better dry matter accumulation.<sup>38</sup>

**Biochemical effects:** Several strains of *Azotobacter* are capable of producing amino acids when grown in culture media amended with different carbon and nitrogen sources.<sup>39</sup> Substance like amino acid produced by these rhizobacteria are involved in many processes that explain plant-growth promotion. Biochemical analysis of chlorophyll, nitrogen, phosphorous, potassium and protein content was higher in *Azotobacter* inoculated plants as compared to non-inoculated control plants.<sup>40</sup>

**Anti-pathogenic response:** *Azotobacter* spp. are capable to produce siderophore, they bind to the available form of iron  $Fe^{3+}$  in the rhizosphere, thus making it unavailable to the phytopathogens and protecting the plant health; similarly Hydrogen Cyanide (HCN) production was higher in traits of *Azotobacter* (77.00 %). *Azotobacter* secretes an antibiotic with a structure similar to anisomycin, which is a documented fungicidal antibiotic. *Azotobacter*, in sufficient numbers, will out-compete pathogens for food. Some of the pathogens that have been controlled by *Azotobacter* in the soil and on the leaf include: *Alternaria*, *Fusarium*, *Collectotrichum*, *Rhizoctonia*, *Microfomina*, *Diplodia*, *Batryiodiplodia*, *Cephalosporium*, *Curvularia*, *Helminthosporium* and *Aspergillus* etc.<sup>36</sup>

**Effects of chemical fertilizer inoculation with *Azotobacter*:** Combined application of bio-fertilizer with 50% of chemical fertilizers (N and P) has significant effect in plant growth, plant height, number of branches, fresh and dry weight of saf flower in comparison with chemical fertilizers alone. Similarly, application of *Azotobacter* biophosphate and organic fertilizers, with half dose of chemical fertilizers increase the economic yield of wheat.<sup>41</sup> Efficiency of *Azotobacter* found decreased with increased N level<sup>42</sup>. The best combination was recorded with  $NH_4Cl$  at 0.1 g/L whereas, action of copper in *Azotobacter* found toxic even in very low concentration. The population of *Azotobacter* may suffer due to high amount of nitrates and the acidic environment created because of chemical fertilizer.<sup>43</sup>

**Effect of pesticides in *Azotobacter*:** Balajee & Mahadevan, (1990)<sup>44</sup> reported that, the effect of herbicide 2, 4-D and its products; p-chlorophenoxy-acetic acid and p-chlorophenol were utilized by *A. chroococcum* as carbon source, which ultimately stimulate nitrogenase enzyme. Similar result found by (Kanungo *et al.* 1995)<sup>45</sup> which revealed insecticide carbofuran also stimulates nitrogenase enzyme activity. Martinez *et al.* 1991)<sup>46</sup> found that, herbicide simazine have no effect on *A. chroococcum* growth either on standard medium or on dialysed soil and sterilized soil medium. The presence of 50-300 mg of simazine per ml of culture or in one gram of soil did have a stimulating effect on *Azotobacter*. When *Azotobacter* is grown in presence of simazine, the cells have a higher ATP content than the control. Whereas organophosphorous insecticides profenofos and chloropyrifos had adverse effect on the number of aerobic nitrogen fixers and decreased nitrogen fixation.<sup>47</sup>

***Azotobacter* in nutrient cycling:** *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. *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 inorganic nitrogen source (eg. Urea). It also helps to sustain the plant growth and yield even in case of low phosphate content soil, as well as helps in uptake of macro and certain micro nutrients which facilitates better utilization of plant root exudates itself.<sup>48</sup>

**CONCLUSION:** *Azotobacter* spp. are free living, non-symbiotic, heterotrophic bacteria capable of fixing an average of 20 kg N/ha per year. These bacteria are regarded as Plant Growth Promoting Rhizobacteria (PGPR) which synthesize growth substance that enhances plant growth and development and inhibit major phytopathogenic growth by secreting inhibitors. It also helps in nutrient uptake and produces some biochemical substances such as protein, amino acids etc. *Azotobacter* improves seed germination and has beneficiary response on Crop Growth Rate (CGR). It helps to increase nutrient availability and to restore soil fertility for better crop response. It is an important component of integrated nutrient management system due to its significant role in soil sustainability. More research is necessary in future to explore the potentiality of *Azotobacter* in soil fertility.

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