### **Review Article**



# Utilization of *Azospirillum* as a Biofertilizer – An Overview

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### ABSTRACT

A soil, *Azospirillum* is the foremost common plant growth-promoting rhizobacteria (PGPR) that are generally related with grasses, rice, wheat and sugarcane. *Azospirillum* could be an advantageous bacterium and is an important biofertilizer utilized in cultivation of rice. The Indole acidic acid (IAA) is one of the most significant physiologically active phytohormones. IAA delivered rhizobacteria are important constituents of biofertilizer and broadly utilized to extend crop development and consequent yield. The plant development controllers like auxins, gibberellin, ethylene, siderophores, HCN and anti-microbials are created by *Azospirillum sp*. hence it was utilized as a biofertilizer. The application of these bacterial species as biofertilizers might be the substitute source of synthetic fertilizers since these bacterial species (*Azotobactor, Azospirillum*) have incredible potential to fix atmospheric nitrogen as well as to solubilize the phosphorus within the soil. It fixes atmospheric N2, improves take-up of supplements additionally produces plant growth promoting substances.

Keywords: Azospirillum sp, Indole acetic acid, Nitrogen fixation, Bacterial biofertilizer.

#### **INTRODUCTION**

zospirillum is a great plant growth-promoting rhizobacteria (PGPR)<sup>1-2</sup>. Biofertilizers remain on behind the soil environment exceedingly included numerous sorts of micro and macro supplements by the process of nitrogen fixation, phosphate solubilization or mineralization, that are adaptable substances for plant growth and arrangement of antibiotics and biodegradation of natural matter within the soil<sup>3</sup>. As long as superior supplement takes up and increased resistance towards drought and moisture stress. Azospirillum under stress circumstance great plant development by fixing atmospheric Nitrogen and by the generation of growth promoting substances and influence root improvement, causing superior take up of supplements from the arrive, and inhibit pathogenic fungi and bacteria in the rhizosphere<sup>4</sup>. Azospirillum inoculation may well obviously increment the development in terms of height; number of leaf and plant; length and breadth of leaf; and new and dry weight/plant of rice plant<sup>5</sup>. In common, microbes commonly utilized as biofertilizers might be Azotobacter, Azospirillum, Rhizobium as nitrogen fixing soil bacteria<sup>6</sup>. Azospirillum is well-known to fix a significant amount of atmospheric nitrogen and arrangements to the crop, improve the fertilizer utilized great organization, soil fertility assessment and ensures fragmentary saving of nitrogenous fertilizer.

The capability of the *Azospirillum* to extend within the rhizosphere of crop recommends its capacity to get way better the supplement accessibility to the plants and be able to improvement the costly inorganic and organic fertilizers. Indeed in spite of the fact that decreased in rice production and yield in the 2015/16 season, compared

among the past one, individually 14.3 and 0.6%<sup>7</sup>. The present research was planned to separate and characterization of *Azospirillum* sp. from paddy field soil samples. Biofertilizer are carrier based inoculants that contain valuable or useful microorganisms in a suitable state with seed or soil relevance. These are generally ought to improve soil fertility and to encourage plant development by desirable biological activity. The useful microorganisms favored for the great biofertilizer preparation, which helps to increase greatest plant development and effectiveness.

Table 1:	Types	of Biof	ertilizer
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Biofertilizer	Microorganisms
Nitrogen fixing	Rhizobium, Azotobacter, Azospirillum, Radyrhizobium
Phosphorus Solubilizing (PSB)	Bacillus, Pseudomonas, Aspergillus.
Plant Growth Promoting	Pseudomonas
Phosphorus solubilizing (Fungi)	Mycorrhiza
Algae	Cvanobacteria and Azolla sp

Biofertilizers form an imperative portion of Integrated Plant Nutrient Supply System (IPNS). In inoculants innovation, the survival of microorganisms for the length of capacity and other characteristics viz., biotic factors, nutrients composition of culture media and location of sampling which were used for study includes dehydrates and drying efficiency<sup>8</sup> and the temperature resistivity of inoculums<sup>9</sup> which is affect their own are one of the main problem in inoculant technology. So, the study stated that to expend the shelf life of inoculants or decision substitute



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formulations for carrier based inoculants are important, Liquid biofertilizers are special liquid formulation contain the favored microorganisms and their nutrients, or substance that promote formation of inactive spores or cysts for longer shelf life and resistance to unfavorable conditions.

### **AZOSPIRILLUM**

*Azospirillum* is a species of bacteria which is utilized as one of the leading fundamental biofertilizer in nature. Which is involved in the fixation process of atmospheric nitrogen in to the world's most portion staple food crops like rice, maize, sorghum, wheat and millets. The genus of *Azospirillum* are narrowly found in soils and its inoculation of cereal and feed crops resulted in yield extended in different field experiments<sup>10</sup>, during the formation of essential plant development promoting substances.

Azospirillum could be a smaller scale aerophilic, gram negative, rod shaped, plant growth promoting bacteria, which is well develop in Nitrogen free semi-solid malate medium. Azospirillum could be a symbiotic bacterium. These are the foremost critical biofertilizer in the cultivating of rice. Azospirillum bacteria present in the root zones of rice and are able to fix more atmospheric nitrogen which is locked in by the plants. The organism multiply under both anaerobic and oxygen consuming condition, in spite of the fact that it favor micro aerophilic condition for development, a part from nitrogen fixation, and the main important work of Azospirillum is growth promoting substance production (IAA), illness resistance and drought tolerance are some of the extra benefits suitable to Azospirillum inoculation. In this survey, we discussed about the biofertilizer arrangement by using Azospirillum as an inoculants.

#### Isolation methods of Azospirillum

Azospirillum sp., was separated from the rhizospheric soil by the following laboratory techniques. First of all 1 g of rhizosphere soil was collected and the soil sample was permitted to serial dilution by utilizing sterile refined water up to10<sup>8</sup> dilutions. The diluted sample 1 ml has been taken by the range between  $10^6 - 10^8$ . Then the 0.1 ml sample was shifted to test tube with nitrogen free bromothymol blue by semi solid media condition further tube were incubated for 24 hours in 32°C of temperature. The different colors colonies (pink, yellow and white) of Azospirillum was separated from the plates and inoculated in to the fresh salt agar medium consisting plates by streaking. The plates were incubated in the same temperature and duration. If the colonies growth was satisfactory then the samples was harvested and stored for further use.

# Mechanism of *Azospirillum* SP for Promoting Plant Growth

The primary component was carried out to check the capability of *Azospirillum* sp how that empower the plant development by the process of biological nitrogen

fixation<sup>11</sup>. The reason of that mechanisms are: (1) The inoculation prepare essentially increments the whole nitrogen substance in shoots and grains; (2) The inoculation commonly decreases the specified dosages of nitrogen fertilization for numerous plant species; (3) The inoculation contributes to create the N adjust of plants<sup>12</sup>. Oppositely, Bashan and Holguin<sup>13</sup> appeared that the association of nitrogen fixation by Azospirillum come to less than 20% of the overall Nitrogen improve within the plant. This conclusion showed agronomic result than fundamentally were predictable for this bacterial genus<sup>14</sup>. A decade presently, modern considers appeared adjacent is not unmistakable evidence that nitrogen fixation by Azospirillum or other free-living nitrogen fixers plays a vital part in agriculture15. Advance mechanisms of plant growth promoting exercises are taken by Azospirillum like phytohormones or siderophores generation, phosphate solubilization16, biocontrol of phytopathogens<sup>17</sup> and security of plants close to stress like soil saltiness or harmful compounds were the primary to propose that Azospirillum sp. might upgrade plant development by auxins and especially indole-3-acetic corrosive (IAA) production, and taking after studies appear the capability of this class to create various other phytohormones and plant development controllers. In spite of the fact that numerous alluring mechanisms are carried and plant growth was estimated and confirmed by Azospirillum sp., also their single cell mechanism did not affect the total output Azospirillum sp. The mode of activity of Azospirillum sp., was well clarified by the "additive hypothesis" which permits enhancement the plant growth promoting impacts reasonable to inoculation. This theory was optional more than 20 years back and consider a few components instead of one mechanism taking part within the successful organization of Azospirillum with plants. Nowadays, behind more than eight decades of considers, we know that these rhizobacteria have been related with the gathering of auxins<sup>18</sup>, gibberellins<sup>19</sup>, ethylene<sup>20</sup>, and other plant development controllers, such as abscisic acid<sup>21</sup>, nitric oxide<sup>22</sup> and polyamines like spermidine, and the diaminecadaverine<sup>23</sup>. Numerous numbers of organically dynamic plant controllers made by Azospirillum sp.<sup>24</sup>.

#### Nitrogen Fixation of Azospirillum

Azospirillum can subsist generously or in beneficial interaction and in additionally case entraps atmospheric nitrogen and changes over the un responsive nitrogen to NH3, A assortment that's readily expend by plants, this movement is term as organic nitrogen fixation (BNF) and is catalyze by the oxygen helpless enzyme nitrogenase show interior the bacteria by the taking after reaction;

N2 + 8H + 8e+ 16 ATP Nitrogenase 2NH3 + H2 + 16 ADP + 16 Pi ------ (1)

The capability of an endophytic to settle atmospheric nitrogen contained by a have has been demonstrated utilizing not at all like approaches: acetylene reduction measure, 15N isotope weakening<sup>25</sup>. *Azospirillum sp* is the



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foremost well-organized N fixers within the field whereas all the compulsory circumstance for organic nitrogen fixation are show<sup>26</sup>. They up to 18 percent of the plant nitrogen were ensuing from nitrogen fixation<sup>27</sup>. All natural sort Azospirillum organisms were available for the nitrogen fixtration by non-living and associated forms with plants<sup>28</sup>. It coupled with rice, *Azospirillum lipoferum* N-4 contributed as respects 66% of the total nitrogen availability in plants were confirmed with N isotopes<sup>29</sup>. Mutated Azospirillum lipoferum with mesophilic lac-z marking did not show significant variations in nitrogen filtration at the range of 45°C temperature out of 40 thermo tolerant mutants. Among the mutants, only 14 mutants were grow efficiently and fix the nitrogen when the temperate over 40°C<sup>30</sup>. The impact of inoculation of Azospirillum are the early development, high yield and N fixation of rice. Microscopic organisms of the class Azospirillum energize plant development specifically more over by amalgamate phyto-hormones or by promoting nourishment by the process of natural nitrogen fixation<sup>31</sup>.

#### Plant Growth Substances Production by Azospirillum

Hartmann et al.<sup>32</sup> found that clear mutants of A. brasilense applied higher amounts of IAA display upto 16 ug per one ml of culture. Vaccinated A. brasilense by mutation may deliver results such length, weight and numbers of root<sup>33</sup>. Horemans et al.<sup>34</sup> explained that the enlargement of in vitro may depends on cytokinin in Azospirillum<sup>35</sup>. The changes of root length and hairs may vary when Azospirillum inoculated in the sorghum experiments. Also the huge changes were found in the IAA, IBA when A. brasilense was incorporated with plants<sup>36</sup>. The co-culture of A. brasilense and Arthrobacter giacomelloi may produce the large quantity of gibberellin and cytokine than the monocultures and it has revealed that the interaction between rhizosphere may significantly induced the auxiliary metabolism in plants<sup>37</sup>. Making of total quantity of IAA and Gibberellin at sensibly more seasoned stages of the bacterial development would simply that this may well be appropriate to the field rhizosphere interaction of Azospirillum with plants where the microorganisms are rarely at the logarithmic stage however influence plant broadening. This may other than clarify why very old total societies actuated positive plant reactions behind inoculation<sup>38</sup>. For distant better much higher stronger an improved a higher estimation of the part of hormones in all inclusive and IAA in specific in plant promotion, adjacent may be a require for a mutant completely missing in IAA production. This mutant is still out of stock. The same is genuine for other phytohormones. Addition of IAA and auxins may External IAA and manufactured auxins can be included within the up directive of expression of *A. brasilense*<sup>39</sup>. IAA made by microscopic organisms of the class Azospirillum sp. can promote plant development by motivating root arrangement. Local Azospirillum sp. isolated from Iranian soils have been evaluated. The roots of wheat seedlings react emphatically to the a few bacteria inoculations by an increment in root length, dry weight and by the

sidelongroot hairs. Perrig et al.<sup>40</sup> detailed stated that plant growth promoting compounds made by two strains of *Azospirillum* phytohormones. Indole-3 acidic acid (IAA), Gibberellic acid (GA3), Abscisic Acid (ABA) and development controllers' putrescine, spermine, spermidine and cadaverine were found in culture supernatant of together strains.

#### **Role of Biofertilizer in Agriculture**

The biofertilizer play a vital part in progressing the richness of the soil<sup>41-42</sup>. In collection, their reason in soil progresses the structure of the soil minimize the sole utilize of chemical fertilizers. Beneath low and arrive circumstance, the work of BGA +Azospirillum demonstrated exceptionally much useful in progressing LAI.Grain yield and collect direct moreover create with misuse of biofertilizer. Azotobacter + Rhizobium + VAM infused paddy crops gave the most elevated increment in straw and grain vield plants with shake phosphate as a P fertilizer. Azolla is low-cost, economical, neighborly, which give yield in terms of carbon and nitrogen enhancement of soil. Something else A few commercially reachable biofertilizer are in addition utilized for the crop yield. Raj<sup>43</sup> recorded that microorganisms (B. subtils, Thiobacillus thioxidans and Saccharomyces sp.) can be utilized as biofertilizers for solubilization of settled micronutrients like zinc. Soybean plants, like different other vegetables can fix atmospheric nitrogen symbiotically and almost 80 to 90%nitrogen require might be provided by soybean through beneficial interaction<sup>44</sup>. Development, abdicate and quality parameters of guaranteed plant recognizably expanded with biofertilizer containing bacterial nitrogen fixer, phosphate and potassium solubilizing bacteria and microbial strains of a few microscopic organisms<sup>45</sup>.

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