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The Potentiality of Selected Strain of PGPR: *Azotobacter*, for Sustainable Agriculture in India

Ruchi Singh¹, Jaspal Singh², Ravi Deval¹, Sudhir Upadhyay³ and Dinesh Kumar^{1*}

¹Department of Biotechnology, Invertis University, Bareilly, U.P. 243123 INDIA

²Department of Environmental Science, Bareilly College, Bareilly U.P. 243005 INDIA

³Department of Environmental Science, Purvanchal University, Jaunpur, U.P., INDIA

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ABSTRACT

In modern agriculture, large numbers of pesticides are being used to inhibit pests to get maximum crop production. The rapidly growing industrialization along with an increasing population has resulted in the accumulation of a wide variety of chemicals. One promising treatment method is to exploit the ability of microorganisms to remove pollutants from contaminated sites. Plant growth promoting rhizobacteria (PGPR) are a group of free-living bacteria that can be found in the rhizosphere and contribute to enhance the growth and yield of crop plants. In the last few decades, many microorganisms show positive effect on plant development. *Azotobacter* has been used as a potential nitrogenous fertilizer to increase crop growth. *Azotobacter* has the capacity of fixing atmospheric nitrogen, it include the production of plant hormones like auxins, gibberellins and cytokinins, nitrogen fixation, These qualities makes *Azotobacter* the most successful and widespread group among the PGPR, as well as they produce substances that change the plant growth and morphology. Co-inoculants of *Azotobacter* with *Rhizobium* has been reduced water stress and dual inoculation of *Azospirillum* and *Azotobacter* significantly increased the concentrations of indole-3-acetic acid (IAA), Potassium, Magnesium, Nitrogen and total soluble sugars (TSS). *Azotobacter* increases the tolerance capacity of plants. *Azotobacter* inoculum with high yielding EPS (exopolysaccharides) and high nitrogen fixing ability can be utilized to satisfy the future demand of augmented crop production attributed to increase plant growth promoting agents.

1) INTRODUCTION

Agricultural sustainability, food security and energy renewability depends on a healthy and fertile soil. However, rapid acceleration of desertification and land degradation by numerous anthropogenic activities leads to an estimated loss of 24 billion tons of fertile soil from the world's crop lands [1].

Plant growth promoting rhizobacteria (PGPR) are a group of free-living bacteria that can be found in the rhizosphere and contribute to enhance the growth and yield of crop plants. The rhizosphere is the zone that are surrounding by the plant roots in which complex relations occur among the plant, the soil microorganisms and the soil itself. In the last few decades, many microorganisms show positive effect on plant development. Besides the well-known plant growth promoting rhizobacteria, present in the rhizosphere can stimulate the plant growth or reduce the damage that are caused by soil-borne plant pathogens, a process is known as bio-control activity [2]. Bacteria identified as PGPR have diverse taxonomy and include strains of the genera *Azospirillum*, *Azotobacter*, *Bacillus*, *Enterobacter*, *Gordonia*, *Klebsiella*, *Paenibacillus*, *Pseudomonas*, *Serratia*, among others [3]. *Azotobacter* has been used as a potential nitrogenous fertilizer to increase crop growth. The *Azotobacter* genus was discovered in 1901 by Dutch microbiologist and botanist Beijerinck (founder of environmental microbiology). *A. chroococcum* is the first aerobic free-living nitrogen fixer [4].

Azotobacter is an aerobic, free living nitrogen fixer. They multiply rapidly and develop a thick population in rhizosphere, when applied as seed treatment or seedling root-dip or as soil application [5].

For sustainable agriculture, the best alternative of chemical fertilizer is necessary because of its adverse effect on the soil health. Many alternatives available to enhance the soil fertility for better crop production, one promising treatment method is to exploit the ability of microorganisms to remove pollutants from contaminated soils, one of them is *Azotobacter*. It is an important component of integrated nutrient management system due to its significant role in soil sustainability [6].

Taxonomy, Morphology and Distribution of *Azotobacter*

The genus *Azotobacter* belongs to the subclass of the Proteobacteria and comprises seven

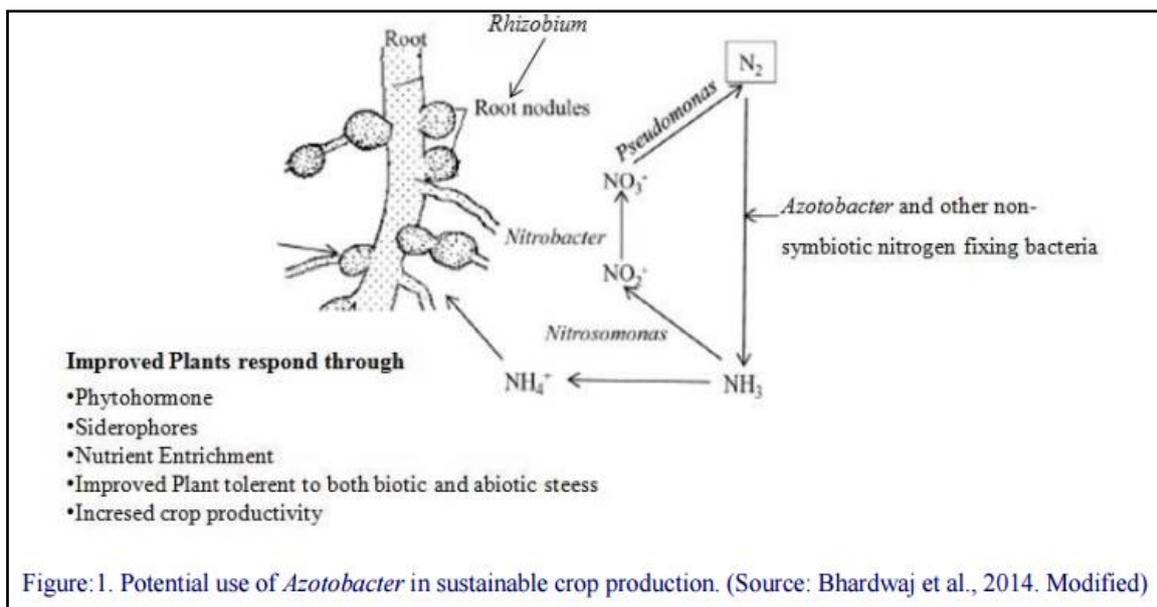
Species: *A. chroococcum*, *A. vinelandii*, *A. beijerinckii*, *A. paspali*, *A. armeniacus*, *A. nigricans* and *A. salinestri* [7, 8]

The taxonomic classification of *Azotobacter* is shown below:

Domain: Bacteria
Kingdom: Bacteria
Phylum: Proteobacteria
Class: Gammaproteobacteria
Order: Pseudomonas

* Corresponding Author: Dr. Dinesh Kumar

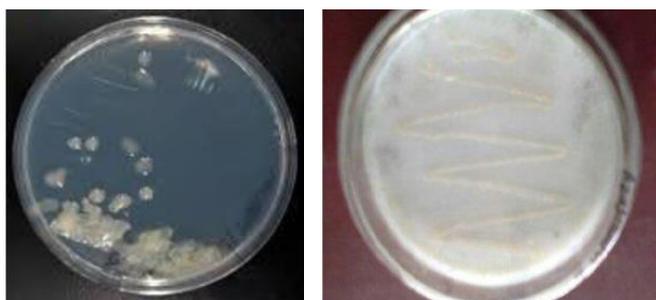
Email address: Dinesh.p@invretis.org



Family: Pseudomonadaceae/Azotobacteriaceae

Genus: *Azotobacter*

Azotobacter species are gram negative, free living aerobic bacteria [9]. The shape of *Azotobacter* is oval or spherical that form thick-walled cysts (means of asexual reproduction under favorable condition) [10]. The population of *Azotobacter* is generally low in the rhizosphere of the crop plants in uncultivated soils. Jensen's N-free medium is used for rapid 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 6.5-7.5), but does not develop when the pH is below 6 and hence not present in acidic soil. This rhizobacteria has been reported to present in the rhizosphere of a number of crop plants such as rice, maize, sugarcane, bajra, vegetables and plantation crops [11]. *Azotobacter* is generally used in any non-legume crop [12].



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2. Mode of Action

Azotobacter has the capacity of fixing atmospheric nitrogen. *Azotobacter* include the production of plant hormones like auxins, gibberellins and cytokinins, These qualities makes *Azotobacter* the most successful and widespread group among the PGPR, as well as they produce substances that change the plant growth and morphology.

(a) Nitrogen fixation

Azotobacter is used for 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 [13, 14].

Azotobacter species are non-symbiotic heterotrophic bacteria capable of fixing an average 20 kg N/ha/per year [15]. Bacterization helps to improve plant growth and to increase soil nitrogen through nitrogen fixation by utilizing carbon for its metabolism [16].

(b) Role of *Azotobacter* in growth substances production and promotion

Azotobacter directly or indirectly affects the plant growth and microbial activity by producing different growth hormone (IAA and other auxin, such as gibberellins and cytokinins) [17]. Growth substances are natural substances that are produced by microorganisms. *Azotobacter* is the genus of great interest in agricultural application due to their free nitrogen fixing ability. Here, emphasis is given on the role of exo-polysaccharide in sustainable agriculture system and also to the survival in its own environments [18]. Substances like amino acid produced by *Azotobacter* 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 [19]. Many strains of *Azotobacter* also exhibited fungi static properties against plant pathogens such as *Fusarium*, *Alternaria* and *Helminthosporium*. 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, [20].

(c) Response of Crops to Growth Promoting Substances

Large number of field trials and various experiments carried throughout India and whole world has convincingly established the importance of *Azotobacter* as microbial inoculant. In India various crops like barley, maize, sugarbeet, carrot, cabbage, potato, wheat, rice, onion, brinjal, tomato and cabbage were inoculated with *Azotobacter*, it increased germination and growth of seedlings [21]. Thus *Azotobacter* produced three growth-promoting substances which were gibberellin-like in character in that they induced shoot elongation of dwarf mutant plants whose growth responses are

thought to be specific to the gibberellins [22]. *Azotobacter* produces indole acetic acid (IAA), gibberlic acid (GA) which are important plant growth hormones and these hormones will help in seed germination and plant growth considerably [23]. Pesticide tolerant *Azotobacter* species was isolated from paddy soils and are known to produce IAA in media supplemented with 5% pesticide.[24] 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 Nitrogen fixing and also producing growth prompting hormones such as auxin, cytokinin and gibberellins and solving mineral compound [25].

3. Relation to other PGPRs

(a) Interaction with Rhizobium

Water stress condition has reduced nodule number and nodule dry weight. It is observed in many studies that a synergistic relation of *Azotobacter* with Rhizobium interaction as co-inoculants of *Azotobacter* with Rhizobium has been reduced water stress. Co-inoculants increased most of growth parameters, water and nutrient uptake under the deficit irrigation because alleviated effect of shortage of water [26].

(b) 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 [27].

Some of the studies have shown that a relationship of *Azospirillum* and *Azotobacter* counts in dual inoculations. These decreases may be due to the antagonistic activity. Single and dual inoculations stimulated plant growth, significantly increased the concentrations of indole-3-acetic acid (IAA), Potassium, Magnesium, Nitrogen and total soluble sugars (TSS).The significance of IAA, nitrogen fixation and antibacterial substances, produced by such agronomical beneficial bacteria [28].

4. Possibility of using *Azotobacter* in crop Production

Azotobacter has been used as a potential nitrogenous fertilizer to increase crop growth and has the capacity of fixing atmospheric nitrogen, thus *Azotobacter* is a best alternative of chemical fertilizer.

S .No.	Crops	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	7.24
10.	Sugarcane	9-24

Bhattacharjee and Dey 2014. [29]

(a) Stress tolerance characteristics

In the scarcity of water, under adverse condition *Azotobacter* increase the tolerance capacity of plants. It produced growth

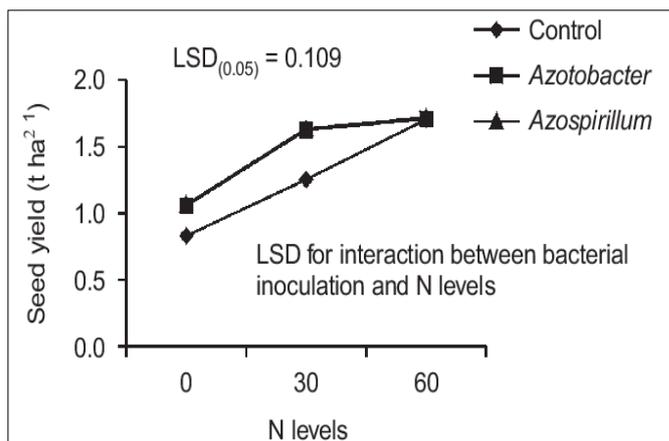
regulator that stimulates development of foliage, roots, branching, flowering and fruiting which triggered by fixed nitrogen [30]. One of the species of *Azotobacter vinelandii* has a potential of salinity stress tolerance in rice. *A. vinelandii* sustains growth and improve compatible solutes that results crop improvement.

5. Effects of *Azotobacter* on growth and yield of crops

Azotobacter is urea adaptive bacteria. It is concluded that when *Azotobacter* alone and combination of different conc. of urea is applied to rice seeds, the co-inoculation of chemical and biofertilizers improves the growth of plants [31]. Seedling of tomato were treated with dual inoculation of *Azotobacter* and *Azospirillum* it showed high performance in whole plant (Plant height, no. of leaves, no. of leaves per plants, no. of fruits, yield per plants, chlorophyll and protein content). It all treatments co-inoculation shows maximum yield when compared with single inoculation and control [32].

The effect of vermicompost and biofertilizers (*Azotobacter*) on the performance of cabbage among the various levels of bio-fertilizers inoculation, *Azotobacter* resulted in maximum number of leaves/head, while the length of head and head yield/plant were maximum with *Azotobacter*/ha. [33].

Table: Effect of *Azotobacter* inoculation on yield of some



important crop plants

Crop	Average yield in control (metric cwt/ha) after incubation	Yield Increase
Barley	21.0	9.1
Maize	36.2	8.1
Oats	17.2	12.0
Potatoes	178.0	8.0
Spring wheat	15.0	8.3
Sugar beet	283.1	7.2
Winter Wheat	21.3	9.8

Source -: Microorganism and Plant Growth (Manisha Garg)

Azotobacter have shown that the yields of some important crop plants can be substantially increased by *Azotobacter* inoculation

6. CONCLUSION

Azotobacter could be one of the bio-fertilizer options for sustainable and environmental eco-friendly. *Azotobacter* may

be further enhanced with the optimization and acclimatization according to the prevailing soil conditions. In future, they are expected to replace the chemical fertilizers, pesticides and artificial growth regulators which have numerous side-effects to sustainable agriculture. *Azotobacters* are unique biofertilizers to maintain the N level in agriculture soil. Co-inoculants increased most of growth parameters, water and nutrient uptake under the deficit irrigation. *Azotobacter* has a potential to stress tolerance that results crop improvement. *Azotobacter* shows high performance in whole plant like Plant height, no. of leaves, no. of leaves per plants, no. of fruits, yield per plants, chlorophyll and protein content. Hence, understanding and manipulating this feature may be of great agro-ecological interest for future crop improvement.

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