



## ***Azospirillum* AS BIOFERTILIZER AND BANGLADESH PERSPECTIVE**

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**Abstract.** *Azospirillum* has potential use as biofertilizer in agriculture. A review of *Azospirillum* as biofertilizer and Bangladesh perspective has been discussed. Habitat and distribution of *Azospirillum* have been reviewed. The species of *Azospirillum*, their isolation, cultivation and preservation have been reviewed. General characteristics and identification of *Azospirillum* have also been discussed. In this paper, all the possible mode of action of *Azospirillum* as biofertilizer has also critically reviewed and the crops affected has also been discussed. Studies on *Azospirillum* carried out in Bangladesh still now, as biofertilizer also reviewed. Commercial use of *Azospirillum* has also been discussed in this paper.

**Keyword:** *Azospirillum*, Biofertilizer, Bangladesh, Isolation, Mode of action

### **Introduction**

Bangladesh, one of the most densely populated countries, mostly depends on agriculture for living.

The population explosion has created a tremendous pressure on agriculture.

The use of agricultural land for various industrial purposes reduces the area for production.

Most of agricultural lands are deprived of some of the essential nutrients for growth and development of crop plants.

One of the major essential elements and the most common limiting factor for growth of plants is nitrogen.

Nitrogen is required in large quantities for plants to grow, since it is the basic constituent of proteins, and nucleic acids. In soils used for intensive cropping, soil fertility is maintained by addition of nitrogenous fertilizer.

Manufacture of nitrogenous fertilizer is an energy intensive process. It requires two tones of fuel oil for every tones of nitrogen fertilizer produced and manufacturing of 1 kg nitrogenous fertilizer requires six times more energy than needed to produce 1 kg P or K fertilizer [RASUL, 1999].

In addition, during the manufacturing of nitrogen fertilizer, CO<sub>2</sub> gas is produced that is involved in

greenhouse effect. Excessive use of fertilizer in agriculture leads to the formation of nitrous oxide (N<sub>2</sub>O) and nitrate (NO<sub>3</sub><sup>+</sup>).

Nitrous oxide (N<sub>2</sub>O) is a greenhouse gas and nitrates (NO<sub>3</sub><sup>+</sup>) pollute the ground water, and thus are hazardous to human health and environment leading to severe eutrophication, ethemoglobinemia of infant and animal and the formation of nitrosamine which is involved in carcinogenesis.

Therefore, it is not realistic to advise farmers to apply high doses of expensive fertilizer that they could hardly afford.

In developing countries like Bangladesh, non-availability and high price of fertilizer-N due to constraints on technological development of fertilizer industries, inadequate power supply, non-availability of raw-materials, poor storage, improper distribution and extension services limit its use on large scale.

In the area where the fertilizers are available on affordable prices, there is also a need to use fertilizer more efficiently because of higher environmental cost linked to its heavy use. Moreover, the efficiency of added urea-N is very low, often only 30-40% and, in some cases, even lowers [De DATTA 1978; CHOUDHURY and KHANIF 2001, 2004].

Rice requires 20-40 kg soil N,



during 3–5 month, for every tone of grain produced. According to an estimate, total global fertilizer–N consumption during 1990 was about 77 million tones.

Out of this, 34% nitrogen was used for rice (9.276 million tones) and wheat (16.082 million tones) [RASUL, 1999].

Although Bangladesh is now trying to achieve self–sufficiency in cereal food production, it will not be able to ensure food security for an ever–increasing population, as the requirement will double in the next 25 years while the natural resource base will shrink.

To keep pace with population growth and the shrinking land resource base, total production of food crops will have to be increased by 60–70% within that period [SATTAR *et al.*, 2008].

Increase in total yield through horizontal expansion (i.e. using more land for production) is not possible, so vertical increase (yield/unit area) is the only option.

In this regard the Bangladesh Rice Research Institute (BRRI) and Bangladesh Institute of Nuclear Agriculture (BINA) are developing modern rice varieties that have high yield potential. But these high–yielding varieties need high amounts of N for biomass production.

Due to problems associated with chemical fertilizer use, worldwide attention has been developed to use biological approaches for increasing crop production. Biological approaches are usually less expensive, harmless and in the reach of all the countries.

The utilization of biological nitrogen fixation (BNF) technology can also decrease the use of urea–N, prevent the depletion of soil organic matter and reduce environmental pollution to a considerable extent.

Application of biofertilizers can not only reduce chemical fertilizer consumption by 20 to 50 per cent but also can simultaneously increase the yield of crop by 10 to 20 per cent [SAIKIA and BORAH, 2007]. For the cultivation of leguminous and other crops nitrogen fixing bacteria *Rhizobium*, *Azospirillum* and *Azotobacter*

are being used as biofertilizer in several countries of the world. In Bangladesh, works on *Rhizobium* have received much dimension and this organism is now being used as biofertilizer for the cultivation of a number of leguminous crops.

The importance of other nitrogen–fixing bacteria should also be considered for other crops especially for grain crops. *Azospirillum* is a plant growth promoting soil bacterium capable of producing associative symbiosis in the roots of various plants including grain crops such as rice and wheat. Inoculation of plants with *Azospirillum* has been found to cause significant increases in growth and yield of different crops including rice and wheat. Yield response to bacterization with *Azospirillum* inoculants was almost equivalent to that attainable due to application of 15–20 kg N/ha. A yield increase in rice due to inoculation of *Azospirillum* is reported to be in the 5–60% range [KUMAR and BALASUBRAMANIAN, 1986].

Inconsistency of crop responses of *Azospirillum* has been more common than *Rhizobium* and responses depended on crops, their varieties, location, season, crop management practices, bacterial strains, level of soil fertility and native microbial population.

This organism has wide adaptability to different environmental conditions.

The use of beneficial microorganisms associated with roots may accelerate the restorations of disturbed areas [CARRILLO–GARCIA–A *et al.*, 2000].

Under such stress condition *Azospirillum* might be naturally adapted to attain remarkable efficiency in fixing atmospheric nitrogen and in enhancing plant growth by this and by some other ways like production of growth promoting substances and influencing root development, causing increased uptake of nutrients from the land, and inhibiting pathogenic fungi and bacteria in the rhizosphere.

In the present paper, habitats, distribution, isolation, cultivation, preservation, characteristics, identification, nitrogen fixation, effects on plant, crops affected *etc.* have been critically reviewed.



### **Azospirillum and Biofertilizer**

The bacteria belonging to the genus *Azospirillum* (K-subclass of proteobacteria) are known for many years as plant growth promoting rhizobacteria (PGPR) [OKON, 1994].

They were isolated from the rhizosphere of many grasses and cereals [DEBOREINER and DAY, 1976].

Both in greenhouse and in field trials, *Azospirillum* was shown to have beneficial effects on plant growth and crop yields [BODDEY *et al.* 1986].

At present, seventeen species have been described: *Azospirillum lipoferum* [BEIJERINCK 1925; TARRAND *et al.*, 1978], *Azospirillum brasilense* [TARRAND *et al.*, 1978], *Azospirillum amazonense* [MAGALHÃES *et al.*, 1984], *Azospirillum halopraeferens* [REINHOLD *et al.*, 1987], *Azospirillum irakense* [KHAMMAS *et al.*, 1989], *Azospirillum canadense* [MEHNAZ *et al.* 2007a], *Azospirillum doebereineriae* [ECKERT *et al.*, 2001], *Azospirillum largimobile* [LONGTONGLANG *et al.*, 2002], *Azospirillum melinis* [PENG *et al.*, 2006], *Azospirillum oryzae* [XIE and YOKOTA, 2005], *Azospirillum picis* [LIN *et al.*, 2009], *Azospirillum rugosum* [YOUNG *et al.*, 2008], *Azospirillum thiophilum* [LAVRINENKO *et al.*, 2010] and *Azospirillum zeae* [MEHNAZ *et al.*, 2007b], *Azospirillum formosense* [LIN *et al.* 2012], *Azospirillum fermentarium* [LIN *et al.*, 2013], *Azospirillum humicireducens* [ZHOU *et al.*, 2013].

### **Habitat**

Free-living nitrogen-fixing bacterium, *Azospirillum brasilense*, was found to adhere to root hairs of tomato, pepper, cotton, sorghum, bajra and rage plants. In this case the bacteria bound to root hair and to the epidermal cells of the elongation zone. The bacteria appear to form clusters held together by fibrils [TEJERA *et al.*, 2005; BASHAN, 1999].

It is isolated from various parts of plants such as roots, stems and leaves and rhizosphere soils [PATIL, 2005; KHAN and HOSSAIN, 1990]. It also found in soil.

### **Distribution**

*Azospirillum* appear to have a worldwide distribution [LADHA and WATANBE, 1987] and occur in large number (upto  $10^7$ /g) in rhizosphere soils in association

with the roots of a variety of C3 and C4 plants. *Azospirillum* occurs in about 90% of tropical soils and in about 60% of soils in the temperate zone. *Azospirillum* has been isolated from the roots of wild and cultivated grasses, cereals and legumes from tropical, subtropical and temperate soils world wide [PATIL, 2005; TORTORA, 2007; PELCZAR, 2007]. *Azospirillum* is a great root colonizer and is not plant-specific bacterium [IDRESS *et al.*, 2010].

But *Azospirillum brasilense* is attributed to have affiliation with plants with photosynthesis of type C3 (wheat), while *Azospirillum lipoferum* is considered to have affiliation with plants of type C4 (maize) [SWEDRZYNSKA and SAWICKA, 2001].

This organism has been found to occur in soil and in root of different plants in various environments including desert showing wide adaptability to different environmental conditions. *Azospirillum* has also been found to occur in the saline non-rhizosphere soil [RAHMAN *et al.*, 2007] and in the coastal region of Bangladesh [JOLLY *et al.* 2010; KHAN *et al.*, 2003]. In Brazil, this rhizosphere bacterium is also capable of unlimited persistence in soils, whereas in Israel, and in some American and Canadian soils, it survived poorly [BASHAN and LEVANONY 1990], showing variations in persistence. Various strains of *Azospirillum* spp. were isolated from the bulk and rhizospheric soils of leguminous and non-leguminous plants distributed in a unique Mediterranean-type climate, Al Jabal Al Akhdr eco-region, in eastern Libya [ATTITALLA *et al.*, 2010].

### **Isolation**

Isolation of microorganisms, screening for desirable characters and selection of efficient strains are important steps to optimize high crop yields and improve the sustainability of the ecosystem [HOLGUIN and BASHAN 1996].

Different species of *Azospirillum* were isolated from different samples.

For example, recently identified species *Azospirillum formosense*, *Azospirillum fermentarium*, *Azospirillum humicireducens* were isolated from agricultural soil in Yunlin County, Taiwan; a fermentative tank in Taiwan; microbial



fuel cell (MFC) [LIN *et al.*, 2012, LIN *et al.*, 2013, ZHOU *et al.* 2013]. Various media were used for

isolation of *Azospirillum* (Table 1).

Table 1.

Different media used for isolation of *Azospirillum*

Species	Media	Reference
<i>A. brasilense</i> , <i>A. lipoferum</i> <i>A. melinis</i> , <i>A. halopraeferens</i>	Semi-solid N-free bromothymol blue (Nfb)	Dobereiner, 1980; Dobereiner, 1992; Peng <i>et al.</i> , 2006; Reinhold <i>et al.</i> , 1987
<i>A. brasilense</i> , <i>A. lipoferum</i> , <i>A. melinis</i> , <i>A. amazonense</i>	Semi-solid LGI medium	Dobereiner, 1992; Peng <i>et al.</i> , 2006
<i>Azospirillum</i> spp., <i>A. halopraeferens</i>	Semi-solid modified Nfb medium	Dobereiner, 1992; Reinhold <i>et al.</i> , (1987)
<i>A. brasilense</i> , <i>A. lipoferum</i>	Ashby's medium	Shilyaeva and Yakouleva, 1988
<i>Azospirillum</i> spp.	Potato agar	Dobereiner, 1992
<i>Azospirillum</i> spp.	OAB medium	Bashan <i>et al.</i> , 1993
<i>Azospirillum</i> spp.	Congo red-NFb	Bashan <i>et al.</i> , 1993
<i>Azospirillum</i> spp.	BL and BLCR Media	Bashan <i>et al.</i> , 1993
<i>A. canadense</i>	M medium	Xie & Yokota, 2005
<i>A. doebereineriae</i>	DYGS medium	Rodrigues Neto <i>et al.</i> , 1986
<i>Azospirillum oryzae</i>	M medium	Xie and Yokota, 2005
<i>A. picis</i>	Tryptone soya agar Brain-heart infusion	Lin <i>et al.</i> , 2009
<i>A. rugosum</i>	(BHI) agar and tryptone soy agar	Young <i>et al.</i> , 2008
<i>Azospirillum zeae</i>	M medium	Mehnaz <i>et al.</i> , 2007

### Cultivation and preservation of cultures

For rapid multiplication, the *Azospirillum* spp. can be grown in liquid media based on semi-solid N-free bromothymol blue (Nfb), semi-solid LGI medium, semi-solid modified Nfb medium, potato agar (omitting the agar) to which a combined nitrogen source has been added (7 gm/L NH<sub>4</sub>Cl or 0.4 gm/L of yeast extract). In such media with rapid stirring or shaking, after 24 hours cell concentrations of 10<sup>6</sup> to 10<sup>8</sup> per mL are reached.

Changes in the pH of the medium have to be minimized however. [RAHMAN, 2002].

Routine preservation of *Azospirillum* spp. is possible on potato agar within tightly closed tubes or under mineral oils.

However, several replicate tubes should always be maintained, because once such cultures are open they frequently died. *A. amazonense* must be

stored on sucrose medium because elevation above pH 6.5 kills the bacteria within a few days.

Also, *A. lipoferum* is relatively sensitive to alkaline media.

Storage of centrifuged cells in liquid N<sub>2</sub> preserves the cells for large culture collections.

Although previous information indicated that *Azospirillum* do not survive lyophilization well, it has now been found that viability of lyophilized cells is maintained for several years if cultures are grown to late log phase in Vincent described medium [VINCENT, 1970].

### General Characteristics and identification

According to Bergey's manual of systematic bacteriology general characteristics of the genus *Azospirillum* are as follows: cells are plump, slightly curved and straight rods, about 1.0 µm in diameter and 2.1–3.8 µm in length often with pointed ends.



Intracellular granules of poly- $\beta$ -hydroxy butyrate are present which can be stored for use latter or during long-term survival [BASHAN and LEVANONY, 1990].

The main differential characteristics of *Azospirillum* spp. when compared with other microaerobic diazotrophs are their vibroid cell shape, very characteristic corkscrew-boring or vibroid movement, the veil like pellicle in semisolid media due to aerotactic attraction and their capacity to associate with plant roots.

$N_2$ -fixers, exhibiting  $N_2$ -dependent growth under microaerobic conditions.

Grow well under an air atmosphere in the presence of a source of fixed nitrogen such as an ammonium salt.

Posses mainly a respiratory type of metabolism with oxygen or nitrate as the terminal electron acceptor, but weak fermentative ability may also occur.

Under severe oxygen limitation nitrate is dissimilated to nitrite or to nitrous oxide and nitrogen gas.

Optimum temperature 35–37°C.

Doubling time 1hr in ammonia containing medium, 5.5 to 7hr. on malate containing semi-solid medium.

Optimum pH values lie between 6.0 and 7.0. Gram negative to gram variable, Oxidase and catalase positive and some strains require biotin.

Chemoorganotrophic; some strains are facultative hydrogen auxotrophs.

Grow well on the salt of organic acids such as malate, succinate, lactate or pyruvate. Fructose and certain other sugars can also be used as carbon source. Disaccharides are not used.

Occur free living in the soil or associated with the roots of cereal crops, grasses and tuber plants.

Root nodules are not induced.

The mol % G+C of the DNA is 69–71 (Tm) detected by thermal denaturation methods [KRIEG and DOBEREINER, 1984].

There is no evidence of their being pathogenic [BASHAN, 1999]. The minimum requirement of  $pO_2$  is 0.003 atm. [RASUL, 1999]. *Azospirillum* show phase variation with genomic architecture changes [VIAL *et al.*, 2006]. They form cysts like structures which are called C forms.

The cysts are non motile, ovoid and large cells, which are devoid of flagella and contain PHB granules [RASUL, 1999] which improves survival under stress condition [BASHAN and LEVANONY, 1990].

They also produce siderophores that are hydroxamate and phenolate compounds and have high affinity for  $Fe^{3+}$  [RASUL, 1999].

After 72 h incubation at 37°C in Nfb medium, *Azospirillum* form a white, dense, undulating, diffuse pellicle, appearing 1 to 4 mm below the surface.

Light-pink and colorless colonies were observed after 48 h in streaked on plates of RC medium. After 72 h, the light-pink colonies became scarlet.

Small scarlet colonies indicated the presence of *Azospirillum* spp. among the contaminants. Colonies of *Azospirillum* spp. could be readily distinguished from colonies of other diazotrophs by scarlet coloration in culture media in which Congo red was included [RODRIGUEZ CACERES, 1982].

Colonies on potato agar are typically light or dark pink often wrinkled and nonslimy. *Azospirillum* species show single flagellum when grown in MPSS broth while lateral flagella when grown on MPSS agar at 30°C.

They also form wrinkled, dark pink colonies when grown on MPSS agar [INDRESS *et al.*, 2010].

#### **Mode of actions of *Azospirillum* as biofertilizer on plant**

The members of the genus *Azospirillum* have been widely discussed as a biofertilizer. The inoculation with *Azospirillum* has shown 60–70% experimental success with 5–30% increase in yield [RASUL, 1990].

It has been reported that the growth promotion depends on the presence of live bacteria and the morphological changes in the root system are directly related to bacterial concentration of *Azospirillum*: higher than optimal levels had inhibitory effects, while low bacterial doses had no effect.

The optimal bacterial concentration that produces a promoting effect on corn has been reported to be  $10^7$  cfu per plant [PUENTE *et al.*, 2009].



*Azospirillum* serves as a model for elucidating the mode of action of beneficial plant–rhizobacterial interaction [LALDHA and WATANBE, 1987].

It was suggested that the net beneficial effect to the plant upon *Azospirillum* inoculation is the result of all the above–mentioned mechanisms operating either simultaneously or sequentially.

Moreover, soil parameters, bacterial community interactions, plant growth phase, and growth phase of the bacterial inoculums may influence the participation of one or several of these mechanisms [HOLGUIN *et al.*, 1999].

*Azospirillum* spp. has diverse effects on plants such as rice, maize, wheat and legumes, which follows:

**Nitrogen fixation:** Nitrogen fixation was the first mechanism proposed to explain improved plant growth following inoculation with *Azospirillum*.

This was mainly because of an increase in the number of nitrogenous compounds and the nitrogenase activity in inoculated plants [BASHAN and HOLGUIN, 1997].

Contribution of nitrogen fixation by *Azospirillum* spp. is ranged from 5–18% of total plant increase [RASUL, 1990].

Inoculation of wheat and maize has indicated that 5–10% and up to 18% of the plant N was derived from N<sub>2</sub> fixation.

Furthermore, of the entire N fixed by the bacteria, less than 5% was incorporated into the host plants [BASHAN and LEVANONY, 1990].

In numerous studies, *Azospirillum* inoculation has been reported to reduce the use of chemical fertilizers in particular nitrogen by 20%–50% [IDRESS *et al.*, 2010].

**Hormone production:** Bashan and Holguin stated that hormonal effects are the main mechanism in plant growth promotion by *Azospirillum* [BASHAN and HOLGUIN 1997]. Many *Azospirillum* strains produce several plant hormones including indole–3–acetic acid (IAA) (in large quantity) and indolelactic acid, indole–3–butyric acid (IBA), indole–3–ethanol, indole–3–methanol, unidentified indole compounds, several gibberellins, abscisic acid (ABA), and cytokinins (detected in

low quantity) [BASHAN and LEVANONY, 1990; CASSAN, 2009] which were determined by paper chromatography, thin layer chromatography (TLC), column chromatography, high performance liquid chromatography (HPLC) and bioassay [RASUL, 1990]. The production of these hormones depends on the growth phase of *Azospirillum* [BASHAN and LEVANONY, 1990].

These hormones increase absorption of water and nutrients, root hair density, stem diameter, number of ear and tillers in wheat and rice, stimulates root development [PATIL, 2005].

**Increased germination:** Cassan and collab. proposed that plant growth regulator compounds produced by *Azospirillum* are responsible of the response in the early developmental stages such as germination since they are the first contact between the bacterial formulation and the seed [CASSAN *et al.* 2009].

**Facilitating up take of the water and minerals:** Enhancement in uptake of NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>, PO<sub>4</sub><sup>2-</sup>, K<sup>+</sup>, Rb<sup>+</sup>, and Fe<sup>2+</sup> by *Azospirillum* was proposed to cause an increase in foliar dry matter and accumulation of minerals in stems and leaves.

During the plant reproductive period, these minerals could have been transferred to the panicles and spikes and finally resulted in a higher yield.

Increased mineral uptake by plants has been suggested to be due to a general increase in the volume of the root system and not to any specific enhancement of the normal ion uptake mechanism.

It has been further suggested that *Azospirillum* inoculation may promote availability of ions in the soil by helping the plant scavenge limiting nutrients; this may explain accumulation of N compounds in the plant without any apparent N<sub>2</sub> fixation.

The plant may take up N more efficiently from the limited supply in the soil, resulting in a lower requirement of N fertilization to attain a certain yield.

Supporting evidence for increased mineral uptake by inoculated roots is provided by enhancement in proton efflux



activity of wheat roots inoculated with *Azospirillum* [BASHAN and LEVANONY, 1991].

It is well known that proton efflux activity is directly related to the balance of ions in plant roots.

*Azospirillum halopraeferens* has phosphatase activity which is responsible for solubilization of inorganic phosphates facilitating phosphate uptake [RASUL, 1999].

In addition to improved mineral uptake, *Azospirillum* inoculation improved water status in stressed sorghum plants.

Inoculated plants were less water stressed, having more water in their foliage, higher leaf water potential, and lower canopy temperature than noninoculated plants.

Total extraction of soil moisture by *Azospirillum*-inoculated plants was greater and water was extracted from deeper layers in the soil profile.

Therefore, sorghum yield increase in inoculated plants was attributed primarily to improved utilization of soil moisture [BASHAN and LEVANONY, 1990].

**Helping in the production of vitamins:** *Azospirillum* plays a vital role in producing pantothenic acid, thiamine, riboflavin and niacin. The production of vitamins by *Azospirillum brasilense* and their liberation were significantly affected by the presence of different carbon sources and the age of the culture [BASHAN and HOLGUIN, 1997; TILAK *et al.*, 2010].

#### ***Azospirillum* as biocontrol agent:**

The interesting indirect effects of colonizing bacteria may be by removing deleterious organisms and chemicals from the environment.

It produces chemicals that suppress the growth of phytopathogens in the rhizosphere.

*Azospirillum* produces siderophores which have high affinity for  $Fe^{3+}$ .

The iron-siderophore complex is formed because of this affinity which creates an iron deficient atmosphere and inhibits the growth of pathogenic fungi and bacteria in the rhizosphere.

The mechanism has been proved by using partially purified siderophores and synthetic non-chelating agent

ethylenediamine di-o-hydroxy phenylacetic acid [RASUL, 1999].

They also have fungistatic activity against a wide range of pathogenic fungi [TILAK *et al.*, 2010].

In an experiment, *P. syringae* pv. Tomato, the causal agent of bacterial speck of tomato and *A. brasilense* were co-inoculated onto tomato plant and sprayed with malic acid. *Pseudomonas* is unable to metabolize organic acids while *Azospirillum* does it efficiently.

This resulted in decreasing the population of *Pseudomonas*, eliminating disease development and improving the plant growth [PATIL, 2005].

*Azospirillum* sp. B510 inoculation enhanced disease resistance to virulent rice blast fungus and the bacterial pathogen *Xanthomonas oryzae*.

*Azospirillum lipoferum* M produced catechol-type siderophores under iron-starved conditions that exhibited antimicrobial activity against various bacterial and fungal isolates.

The effect of *Azospirillum brasilense* on crown gall formation in dicotyledonous plants was studied after inoculating them with virulent strains of *Agrobacterium tumefaciens* and gall formation was inhibited.

When *Azospirillum brasilense* Cd was mixed in a culture with the mangrove rhizosphere bacterium *Staphylococcus* sp., the population of the latter was significantly reduced [BASHAN and HOLGUIN, 1997].

**Strengthens the effect of organic fertilizer:** Application different organics with *Azospirillum* favorably influence the soil physical, chemical and biological environment such as bulk density, water holding capacity, organic carbon, available nitrogen, beneficial bacterial and fungal population over the inorganic alone applied plot.

Among the different organic N sources the application 75 per cent Vermicompost with *azospirillum* was found to be superior in improving soil health over the other treatments [KANNAN *et al.*, 2005].



**Bioremediation:** *Azospirillum lipoferum* was capable of reducing 4–chloronitrobenzene, an aromatic compound used in the manufacturing of pesticides, dyes, explosives, and industrial solvents and an environmental pollutant [BASHAN and HOLGUIN, 1997; JENA *et al.*, 1992; GALLORI *et al.*, 1991].

*Azospirillum*–inoculated plants accumulated less Arsenic than did the surface–sterilized uninoculated plants.

This study shows that *A. brasilense* Sp245 in association with wheat changes the speciation, bioavailability, and plant uptake of Arsenic [LYUBUN *et al.*, 2006].

#### **Crops affected**

Host range of *Azospirillum* has not been fully known. In addition to the claims of *Azospirillum* specificity for certain cereal species, it has been shown that *Azospirillum* is a general root colonizer and can colonize crop plants, weeds and annual and perennial plants.

Two strains of *Azospirillum lipoferum* enhanced the growth of sunflower significantly and *Azospirillum brasilense* improved the development of oak seedling.

*Azospirillum* proved good inoculants for four tropical plants i.e. *Delonix regia*, *Jecopina corsinea*, *Lagerstroemia indica* and *Lawsonia inermis*. Inoculation of mulberry by *Azospirillum brasilense* was beneficial under low level of fertilizer application.

The inoculation of cactus with *Azospirillum brasilense* enhanced the seed germination and growth of seedlings and delayed the spine senescence.

In addition to that *Azospirillum* sp. promotes the growth of tomato, egg plant, pepper, cotton and mustard and colonizes 64 plant species including 48 weeds [RASUL, 1999]. *Azospirillum* also affects *Oryza sativa* [CHENG–HUI *et al.*, 2005], miscanthus [ECKERT, 2001], *Zea mays*, sugarcane, barley, bajra, ragi, sorghum, turmeric, all fruits, vegetables, horticulture crops, *etc.*

#### **Recent use of *Azospirillum***

In Brazil 100% of the soybean production use PGPR and not the fertilizers to obtain 100% of the N necessary for the plant nutrition.

Though *Azospirillum* was tested, isolated and described many years ago, very few attempts were taken to develop a commercial product using this bacterium.

In United States, *A. brasilense* was used to develop Azo–Green™ by a company called Genesis Turfs Forages, was recommended to be applied on the seeds for improving germination, root system, drought resistance, and plant health.

In Italy, Germany, and Belgium another product named Zea–Nit™ containing a mixture of *A. brasilense* (strain Cd) and *A. lipoferum* (strain Br17) was produced by Heligenetics.

It was recommended the reduction of 30–40% of the N fertilization of the plants.

In France another AzoGreen™ was used in a maize application in the Agbasar Station, at Northeast of Tongo, Africa. Uruguay also developed a product called Graminante™.

It was commercialized as a powder mixed with calcium carbonate [REIS *et al.*, 2011]. In Mongolia, it is found that *Azospirillum* along with oligochitosan have synergy effect on the growth and yield of tomato growing in *Fusarium* contaminated soil.

In Philippines, biofertilizer with a brand name **Bio N** containing *Azospirillum* as its major component with soil and charcoal as its carrier was developed by National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Banos [FNCA biofertilizer newsletter, 2014].

#### ***Azospirillum* in Bangladesh**

In Bangladesh, Bangladesh Institute of Nuclear Agriculture, Bangladesh Agricultural University, National Institute of Biotechnology, Bangladesh Agricultural Research Institute and Bangabandhu Sheikh Mujibur Rahman Agricultural University are organizations involved in biofertilizer activities.

Among them, Environmental biotechnology division of National Institute of Biotechnology (NIB) is involved in the development of rice biofertilizer with *Azospirillum*.



Soil and rice root samples have been collected and from different localities of the country to isolate *Azospirillum* strains.

The strains were identified and nitrogen fixing potentiality has been studied. Rice seeds are inoculated by the selected strains to observe their effect on germination and plant growth [FNCA biofertilizer newsletter, 2012]

Recently works on *Azospirillum* are gradually receiving enormous importance particularly for the development of inoculants for grain crops.

Heluin and collab. isolated 17 strains of *Azospirillum* from Brahmaputra flood plain soil of Bangladesh and reported its nitrogen fixing potential in association with rice cv. Nizirshail [HELUIN et al., 1989]

Khan and Hossain reported the distribution and nitrogen fixing potential of *Azospirillum* in the rice fields of Chittagong University Campus [KHAN and HOSSAIN 1990]

Rahman and collab. conducted a field experiment to evaluate the effect of some isolates of *Azospirillum brasilense* and *Azospirillum lipoferum* on growth and yield of lentil (*Lens esculenta*) [RAHMAN et al., 2007]. Khan and Akond isolated five strains of *Azospirillum brasilense* from the roots of rice plants cultivated in Savar, Dhaka and observed the effects of temperature, pH, NaCl and Zn on the nitrogen-fixing potential of the strains [KHAN and AKOND 1996]

Molla and collab. studied the potential enhancement of root growth and nodulation in vegetable soybean (AGS190) with application of *Azospirillum brasilense* (Sp7) and *A. lipoferum* (CCM3863) co-inoculated with two *Bradyrhizobium japonicum* strains (TAL102 and UPMR48) [MOLLA et al., 2001].

Significant root growth stimulation and nodulation were observed in *Azospirillum* as well as during its co-inoculation with *Bradyrhizobium*.

Nuruzzaman and collab. carried out an experiment at the Farm of the Department of Crop Botany, Bangladesh Agricultural University, Mymensingh from March to July, 2001 to investigate the

effect of biofertilizers on morpho-physiological characters of okra [NURUZZAMAN et al., 2003]

### ***Azospirillum* as commercial biofertilizer**

The bottom line of every inoculation technology is its successful application under agricultural and industrial conditions.

The inoculum formulation and application technology are crucial for the development of commercial *Azospirillum* inoculants.

A technique that works mainly under research-laboratory conditions is unlikely to gain success under commercial and competitive markets.

Surprisingly, relatively few studies have addressed the application of *Azospirillum* technology, which has been slow to make a significant impact on the inoculation market.

After introducing the concept of synthetic inoculants made of alginate into *Azospirillum* technology, several commercially oriented studies addressed its application.

An optimized process for manufacturing a crop inoculant was developed with an *Azospirillum lipoferum* strain.

This process involves the entrapment of living cells in alginate beads and air dehydration.

This latter unfortunately eliminated the vast majority of the original cells, but the remaining cells were sufficient to serve as inoculants.

The highest survival rate was obtained by addition of skim milk and controlled dehydration in air of the alginate beads.

Finally, a powdered inoculant was obtained. It was easy to store and handle and can be used in the field as a micro-granule or as a seed coating.

The biodegradability insures that there is no environmental pollution [BASHAN and HOLGUIN, 1997]

*Azospirillum* inoculant is available for a variety of crops in Europe and Africa [DOBBELAERE et al., 2001]



*Azospirillum* is being commercially used as biofertilizers.

Different private companies are recently offering these biofertilizers. Few of Indian companies include BIO PROMOTOR AZOSPIRILLUM, BioN-Plus, PEAK AZOLAM, Azosfer etc.

### Conclusions

In Bangladesh population explosion has created a tremendous pressure on agriculture.

The production of crops is insufficient hampering Bangladesh to ensure food security.

The only possible way to increase food production is the vertical increase (yield/unit area) by using high yielding modern crop varieties requiring high amounts of N for biomass production.

N-deficiency of most of the rice soils of Bangladesh and other problems restrict chemical fertilizer use in proper amounts, hampering crop production.

Among growth promoting rhizobacteria, *Azospirillum* is known to be a very active nitrogen fixer under laboratory as well as soil conditions providing fast growth, better health of the plant and higher yield.

Inoculation of plants with *Azospirillum* has been found to cause significant increases in growth and yield which is equivalent to that is attainable by application of 15–20 kg N/ha.

Like other countries such as Europe, Africa, India, China, Indonesia, Japan, Malaysia, The Philippines, Thailand, Vietnam, in Bangladesh *Azospirillum* can be used as substitute and/ or supplement of N-fertilizer.

Like these countries biofertilizer industry can be developed in our country which will generate employment, alleviate poverty, and improve the socio-economic condition of the people having a profound impact on national economy.

Different biological nitrogen fixation (BNF) systems including the use of plant growth promoting bacteria are use on a limited scale in Bangladesh agriculture.

Before large-scale extension of biological nitrogen fixation (BNF) systems at the farm level, further research is

needed to determine their N supplement potentials.

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