Biofertilizers for sustainable agriculture: *Azotobacter, Azospirillium*

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Azotobacter

- *Azotobacter* belongs to the family *Azotobacteraceae*.
- This family includes various gram negative, aerobic, heterotrophic, catalase positive, free-living diazotrophic bacteria.
- The first species of the genus *Azotobacter*, named *Azotobacter chroococcum*, was isolated from the soil in Holland in 1901 by Beijerinck.
- Along with *Rhizobium, Azotobacter* is the most extensively studied genus among the saprophytes.
- Winogradsky (1932) discovered that *Azotobacter* released ammonia into the soil. This started work on harnessing of *Azotobacter* for the benefit of plant and to improve soil fertility.

At present six species of *Azotobacter* are known –

1. *A. chroococcum*
2. *A. vinelandii*
3. *A. nigricans*
4. *A. paspali*
5. *A. armenicus*
6. *A. salinestris*

Azotobacter are much more abundant in the rhizosphere of plants than in the surrounding soil and that this abundance depends on the crop species.

A. chroococcum is the most commonly found species in soils.

Identifying characteristics of the genus *Azotobacter*

1. Large ovoid cells 1.5-2.0 μm or more in diameter.
2. Pleomorphic, ranging from rods to coccoid cells.
3. All the species of this genus form cysts.
4. Motile by peritrichous flagella or non-motile.
5. Aerobic
6. Production of water-soluble and water insoluble pigments.
7. Nitrogen fixers, generally fix nonsymbiotically at least 10 mg of atmospheric nitrogen/g of carbohydrate consumed.
8. Catalase positive.

IMAGE : *Azotobacter* cyst.

Bio-fertilizers

Bio-fertilizers are not fertilizers, which directly give nutrition to crop plants. These are cultures of microorganisms like bacteria, fungi, packed in a carrier material. Thus, the critical input in Biofertilizers is the microorganisms. They help the plants indirectly through better Nitrogen (N) fixation or improving the nutrient availability in the soil.

Mode of action of *Azotobacter*

The beneficial influence of *Azotobacter* on plant growth is attributed to a number of factors. These being –

Direct mechanism of plant growth improvement

- Biological nitrogen fixation under free-living conditions
- Productions of phytohormones like indole 3-acetic acid, gibberrillin-like substances and cytokinins
- Solubilization of insoluble phosphates

Indirect mechanism of plant growth improvement by biocontrol

Antagonism against phytopathogens by

- Production of siderophores
- Production of antifungal compounds
- Induction of defense enzymes

Commercial Manufacture of *Azotobacter*

The manufacturing process in short involves

- Selection of suitable strain of the organism for which market demand is identified.
- Mass multiplication.
- Mixing of the culture with carrier material and packing.

The steps involved are

- Culture selection and maintenance.
- Purchase of desired strains from the identified authentic sources like Agricultural Universities, IARI, some ICAR institutions, Regional biofertilizer labs of MOA, etc. There are international sources of supply also like NifTAL, IRRI etc. These sources maintain pure mother cultures.
- They have to be further sub-cultured and maintained purely for mass production by adopting standard techniques under the supervision of trained microbiologist.

Culture augmentation

Culture has to be mass multiplied in two levels

a.at primary level using **shakers in flasks**
b.Secondary stage multiplication in **fermenters**

The important factor in this is the preparation of growing medium in which the culture is mass multiplied. There are standard media on which information is available from published sources like Norris and Date, Fred *et al.*, ISI approved etc.

After the media is formulated and sterilized in fermenter, it is inoculated using the shorter cultures multiplied in the flasks at definite ratios usually 5%. The bacteria growing medium is called broth and it is continuously aerated by passing sterile air from compressors.

After about 3-4 days fermentation period, the broth will be ready for packing in a carrier material. At various stages the quality is tested by drawing samples.

Carrier sterilization

- While the broth is getting ready in the fermenter, the carrier material, which is usually the carbon source for the cultures to survive, is sterilized in autoclaves and kept ready for mixing with the broth.
- The carrier is either sterilized in bulk or it is packed and then the packets are sterilized.
- Peat imported from countries like U.S., Australia is reported to be the best source of carrier material. However, as it is costly lignite, charcoal : soil mix are used extensively in India.
- The pH of the carrier material is adjusted to 7 for better results.
- Moisture is generally maintained at 10%.

Mixing and packing

There are 2-3 alternatives depending upon the sophistication and automation of the unit.

Under **non sterile system**, the broth is harvested from the fermenter into sterilized carrier - the mixing is done manually under aseptic condition and packed in polythene bags of desired quantity.

In a **slightly upgraded method**, the broth and sterilized carrier are mixed mechanically in a blender and the material is packed using semiautomatic packing and sealing machine. In a slightly modified method some units are packing by delivering desired quantities of carrier and broth simultaneously from separate pipe conveyance system into the polythene bags.

Under a completely **sterile system** the carrier is taken in autoclavable polypropylene bags and pre sealed - into which the broth from fermenter is directly injected with the help of dispenser. The injection hole is immediately sealed. The packets are kept in incubation room for about a week before transferring to store room.

Sterile system of packing using auto syringe and dispenser is recommended to be the best method and all new units should follow and adopt this system.

Indian standard specification for *Azotobacter*

S.N.	Parameters	*Azotobacter* Inoculant IS – 9138-2002
1.	Base	Carrier based
2.	Viable cells	10^7cells/g of carrier within 15 days manufacture.
3.	Cell no at the time of expiry	10^6 cells/g within 15 days before expiry date
4.	Shelf life or expiry period	6 months from the date of manufacture
5.	Permissible contamination	No contamination at 10^5 dilution
6.	PH	6.0-7.5
7.	Moisture %	35-40%
8.	Strain	*A. chroococcum* mentioned
9.	Carrier	Should pass through 100 micron IS sieve
10.	Efficiency test	Minimum amount of N-fixation not less than 10 mg/g of sucrose utilized.

Methods of Application

Application of bio-fertilizer require technical methods, thus it should be shown to farmers.

There are three methods of application

1. **Seed treatment:** Normally, 500 g of bio-fertilizers is required for seeds to be applied in a hectare of field. Bio-fertilizer is mixed with water and adhesive material so that seed coat is not broken, is dried for half an hour before sowing.
2. **Seedling treatment:** Bio-fertilizers are being used for seedling treatment. This is mainly done in transplanted crops. A slurry of *Azotobacter* biofertilizer is prepared. Seedlings are dipped in this slurry for about 15 minutes, allowed to dry and then transplanted.
3. **Soil application :** About 2 kg of bio-fertilizers are mixed with 40-50 kg of decomposed FYM and are broad cast at the time of sowing/prior to sowing.

Azospirillium

INTRODUCTION

- Azospirillum species are Free living in soil & plant-associated diazotrophs
- Association with the roots of cereal crops, grasses and tuber plants.
- subclass of Alphaproteobacteria.

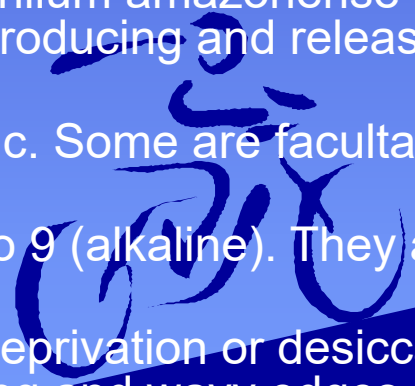
"**Azo**" comes from French word , Means "**Nitrogen**"

Spirillum - shape of bacteria.

- Azospirillum was First isolated by **Beijerinck (1922)** in Brazil from the roots of Paspalum and named it as Azotobacter paspali and later named as Spirillum lipoferum.
- **Dobereiner and Day (1976)** reported the nitrogen fixing potential of some age grasses due to the activity of S. lipoferum in their roots.
- Taxonomy was re-examined and **Tarrand et al. (1978)** designated this organism as **Azospirillum.**
- An aerobic or micro aerophilic , Gram negative rod.
- Non sporeformer and inhibiting the plant roots both externally and internally.
- Being a micro aerophilic organism, it can be isolated on a semi solid malate medium by enrichment procedures.
- It is used as **BIOFERTILIZER.**
- Azospirillum fixes nitrogen from 10 to 40 kg/ha.

Azospirillium

- These bacteria are spiral or slightly curved rod-shaped nonspore-forming cells with polyhydroxybutyrate granules that can form cysts.
- The Gram staining is negative, and the cells are very motile with a single polar flagellum and several lateral flagella, shorter in length.
- The DNA G+C content varies between 64 and 71 mol %.
- The genus mainly includes nitrogen-fixing rhizobacteria. They can be found in soil and freshwater but are most abundant in the rhizospheres and roots of crop plants like corn, rice, wheat, sugarcane etc.
- These bacteria enhance plant growth by forming beneficial associations with them. Hence they are called plant growth-promoting rhizobacteria (PGPR).
- Azospirillum lipoferum, Azospirillum brasilense and Azospirillum amazonense efficiently fix nitrogen in the rice rhizosphere. They also stimulate plant growth by producing and releasing various plant hormones.
- Azospirillum species are gram-negative and microaerophilic. Some are facultative anaerobes, capable of surviving without oxygen.
- They can tolerate a wide range of soil pH, from 5 (acidic) to 9 (alkaline). They are curved rods and motile. The cells usually remain dispersed.
- They can produce spherical cysts in response to nutrient deprivation or desiccation. Their colonies have a circular, flat morphology with prominent red colouring and wavy edges.



Applications of *Azospirillium*

- They are used as biofertilizers and seed inoculants for non-leguminous plants for the following reasons:
- They can convert atmospheric nitrogen into ammonia.
- They have a crucial role in maintaining the usable form of N_2 , a vital macronutrient in soil.
- They can help to mineralise other nutrients like phosphorus, carbon, sulphur, overall increasing soil fertility.
- They increase uptake of macronutrients like N, P and some micronutrients like Zn, Fe.
- They release vital phytohormones and other biologically active substances to promote growth of crop plants.
- They also stimulate flowering, rooting, foliage formation, seed vigour.
- These microorganisms help in biological remediation of heavy metals and other soil pollutants.
- Azospirillum spp. can produce antioxidants to mitigate oxidative stress of plants.
- These bacteria induce plant defence mechanisms to fight various biotic and abiotic stresses.

Difference Between Azotobacter and Azospirillum

Criteria	Azotobacter	Azospirillum
Definition	Azotobacter spp. are aerobic, non-symbiotic diazotrophs	Azospirillum spp. are microaerophilic, associative diazotrophs
Habitat	Free living, widely found in soil, water and sediments	Surface colonising rhizobacteria, commonly found in the rhizosphere or roots of crops like rice, wheat, corn etc.
Biological interaction	Non-symbiotic	Symbiotic or associative
Oxygen tolerance	Aerobic	Microaerophilic, facultative anaerobe
pH tolerance	Neutral to alkaline, optimal growth & N2 fixation at pH- 7-7.5	Acidic to alkaline, optimal growth & N2 fixation at pH-7
Growth temperature	Mesophilic bacteria, grows between 25 °C to 30 °C, optimal growth temperature- 30 °C	Grows between 5 °C to 42 °C, optimal growth temperature- 37 °C

… Difference Between Azotobacter and Azospirillum

Criteria	Azotobacter	Azospirillum
Shape	Oval or spherical shape	Curved rod shape
Colony characteristics	Convex, circular, mucoid colonies with dark brown colour	Flat, circular colonies with red colour
Family	Pseudomonadaceae	Azospirillaceae
Functions	• Mainly function as free living nitrogen fixer • Carry out phosphorus solubilisation in soil • Improve soil fertility • Used in biofertilizers and • Used for production of biopolymers • Can induce root growth by secreting certain plant metabolites and hormones	• Function as associative nitrogen fixer • Promote plant growth by producing phytohormones and plant metabolites- polyamine, and trehalose • Produce antioxidants to relieve oxidative stress of plants • Carry out degradation of soil pollutant • Used in biofertilizers for non-legume crops
Examples	Azotobacter chroococcum, Azotobacter vinelandii, Azotobacter beijerinckii	Azospirillum lipoferum, Azospirillum brasilense, Azospirillum amazonense