

# Azotobacter Biofertilizer for North-East Region of India



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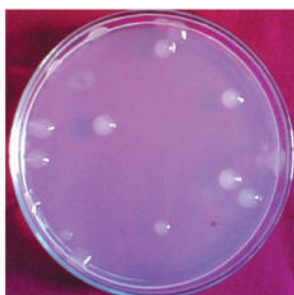
## Background

The importance of *Azotobacter* as potential inoculant has been convincingly established since its discovery in 1901. In addition to N<sub>2</sub>-fixation, *Azotobacter* has emerged as a potential contributor in plant growth by its ability to produce phytohormones, antibiotic substances, siderophores and enhance mineral nutrition. Genus *Azotobacter* belonging to family Pseudomonadaceae of the subgroup γ-proteobacteria, comprises of aerobic, motile, free living N<sub>2</sub>-fixing bacteria which are widespread throughout the world. Its occurrence is also governed by a number of factors such as pH, organic matter and mineral content of soil. Although exotic strains of the microbial agents can perform better, molecular technique-based evidence suggests that genotypes of beneficial bacteria are endemic to a biogeographical region and may prove to be highly efficient.

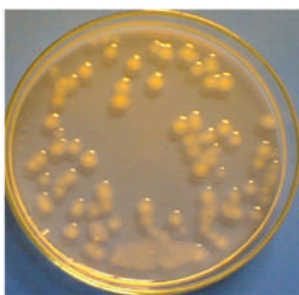
ICAR All India Network Project on Soil Biodiversity - Biofertilizers (AINP-SBB) has developed *Azotobacter* biofertilizer technology for North East Region of India. Potential strains were isolated after intensive screening of native isolates. These strains are being used for mass production of *Azotobacter* biofertilizers. The performance of the biofertilizer was evaluated on wheat and vegetables commonly grown in NEH region. *Azotobacter* biofertilizer enhanced crops yield by 20-25% and saved inorganic nitrogenous fertilizer significantly. Technology has been transferred to 3 industries for mass production to meet the farmers demand. During last 3 years, about 740 farmers have been benefitted and 15 state agricultural and KVK officers were trained to intensify use of this technology. State agriculture department has adopted this technology and documented in the package of practices.

## *Azotobacter* in North Eastern Region of India

AINP on Soil Biodiversity-Biofertilizers at Assam Agricultural University has been engaged in screening and evaluation of efficient *Azotobacter* from the pristine ecosystems of the region. Eight potential *Azotobacter* strains were isolated from rice, toria and wheat rhizosphere and has been utilized for preparation of Biofertilizers. Based on PGPR activities, potential strain *Azotobacter* sp. (MW301147) identified for biofertilizer production for North Eastern Region.



Pure *Azotobacter* colonies in Burks media



Exopolysaccharide secretion by *Azotobacter* in Burks media



Black to brown pigmentation by *Azotobacter* in Burks media

## Nitrogenase activity of isolated culture of *Azotobacter*

<i>Azotobacter</i> Isolate	AZT1	AZT2	AZT3	AZT4	AZT52	AZT6	AZT7	AZT8
N <sub>2</sub> -ase Activity ( $\eta$ moles of C <sub>2</sub> H <sub>4</sub> mg <sup>-1</sup> protein h <sup>-1</sup> )	0.825	0.762	0.622	0.730	0.916	0.870	0.695	0.573

## Procedure of *Azotobacter* biofertilizer

**Step I:** Mass multiplication of *Azotobacter* in Burk's broth by inoculating the mother culture @1.0 % (mL). Allowed the *Azotobacter* to grow for 5-7 days in incubator shaker/BOD incubator at 30±1°C till the broth turns dense and opaque.

**Step II:** The carrier material (compost+charcoal,50:50) is powdered to a fine powder so as to pass through 212 micron IS sieve. The mixed carrier material is sterilized in an autoclave to eliminate the contaminants for three consecutive days at 24h of interval.

**Step III:** The sterilized carrier material is spread in a clean, dry, sterile metallic or plastic tray. The full grown (population approx 10<sup>9</sup> cell/mL) *Azotobacter* culture is added to the sterilized carrier and mixed well by manual (by wearing sterile gloves). The culture suspension is added to a level of 40 – 50% water holding capacity of the carrier materials. Curing is done by spreading the inoculant on a clean floor/polythene sheet/ trays with polythene covering for 2 -3 hours at room temperature before packaging. Packaging of *Azotobacter* biofertilizer is carried out in polythene bags (thickness 50-75 micron) and sealed with electric sealer.

**Step IV:** Each packet is marked with the name of the manufacturer, name of the product, strain number, the crop to which recommended, method of inoculation, date of manufacture, batch number, date of expiry, price, full address of the manufacturer and storage instructions etc.

**Step V:** The packet is stored in a cool place away from the heat or direct sunlight. The population of inoculant in the carrier inoculant packet is determined at 15 days interval till its application. There should be more than 10<sup>9</sup> cells / g of inoculant at the time of preparation and 10<sup>7</sup> cells/g on dry weight basis before expiry date.



Mother Cultures



Growth of *Azotobacter* in broth



Mixed with sterilized carrier materials



Packaging and Sealing

## Benefits of *Azotobacter* inoculants

- ✓ Fixes 20-25 kg of N/ha/season in soil
- ✓ Increases grain yield by 20-30%
- ✓ Increases vegetative growth and leaf yield by 10-30%
- ✓ Secretes growth promoting and antibiotic like substances
- ✓ Can supplement more than 25% of nitrogenous fertilizer requirement by the crop.

## Strategies for application of *Azotobacter* biofertilizers

These biofertilizers can be applied to different crops by three different ways.

- ✓ Seed treatment
- ✓ Root dip treatment
- ✓ Soil treatment

In non-leguminous crop such as wheat, toria, vegetables, etc. *Azotobacter* can effectively be used as seed treatment. In this method 400-500gm of *Azotobacter* biofertilizer is suspended in 500mL of water to make slurry, which is then mixed with 10-12kg of seeds, until the seeds get uniformly coated. The treated seeds are dried in shade for 1-2 hour and sown immediately.

In case of transplanted vegetables, slurry of *Azotobacter* biofertilizer is prepared and the roots of seedlings are dipped for 1 hour for root coating, and then transplanted in the main field.

For soil treatment depending upon the crops the required quantity of biofertilizers is mixed with compost and broadcast evenly in the field.

In case of integrated nutrient management (INM), to supplement phosphatic and potassic fertilizers, phosphate solubilizing bacteria and potash solubilizing bacteria can be used along with *Azotobacter*.

Enriched compost can be prepared by addition of *Azotobacter*, PSB and KSB @1.0% and rock phosphate @1.0% (as P) in 80-90days immature aerobic compost following 25-30 days incubation.



Seed coating of *Azotobacter* biofertilizer in wheat

## Effect of *Azotobacter* biofertilizer on different crops

Crop	Application and crop response	
Wheat	1. <i>Azotobacter</i> and PSB @50g/kg seeds with NPK@ :60:35:32 kg/ha Wheat yield:9.50q/ha 2. <i>Azotobacter</i> and PSB @50g/kg seeds with NPK@ :80:46:42 kg/ha Wheat yield:8.25q/ha Yield advantage due to <i>Azotobacter</i> +PSB is 15.15% amid reduction of 25% RDF	
Toria	<i>Azotobacter</i> + PSB: Application : Seeds treated with <i>Azotobacter</i> +PSB @ 40g/kg seed. Yield with treated : 7.35q/ha Yield in control : 5.01q/ha	
Organic okra	Yield: 1. <i>Azotobacter</i> +PSB+RP(313kg/ha)+ Vermicompost (@5 t/ha)=110.70 q/ha 2. <i>Azotobacter</i> +PSB+RP(313kg/ha)+ Vermicompost (@5t/ha)=103.05q/ha	
Organic cabbage	1. Application of Biofertilizer ( <i>Azotobacter</i> +PSB) & RP (135kg/ha) in Vermicompost (5t/ha) recorded the highest yield of 17.94t/ha, 2. Application of ( <i>Azotobacter</i> +PSB) & RP (135kg/ha) in FYM (10t/ha) produces 17.72 t/ha	
Organic carrot	1. Enriched compost (5t/ha) with <i>Azotobacter</i> , PSB and RP(135kg/ha) recorded highest yield of 21.44 t/ha. Application of ( <i>Azotobacter</i> +PSB) & RP (135kg/ha) with normal compost (5t/ha) produces 20.97 t/ha	

## Trainings / package of practices/recommendations

Farmers benefitted (2017-2020)

About 740 farmers of NEH regions and 15 state agriculture officers were trained.

Technology transfer

1. Technology transferred to State Agriculture Department, Assam.

Supply of biofertilizer strains to industries

1. Green Tech Ecosolutions Pvt Ltd, Manipur, Imphal, PIN: 795146  
2. Green Agri Biotech, Moran, Abhaypur, P.O. Moran, Dibrugarh, 785670, Assam  
3. Director of Agriculture, Mizoram Government, Aizwal: 796001

Revenue earned (2017-2020)

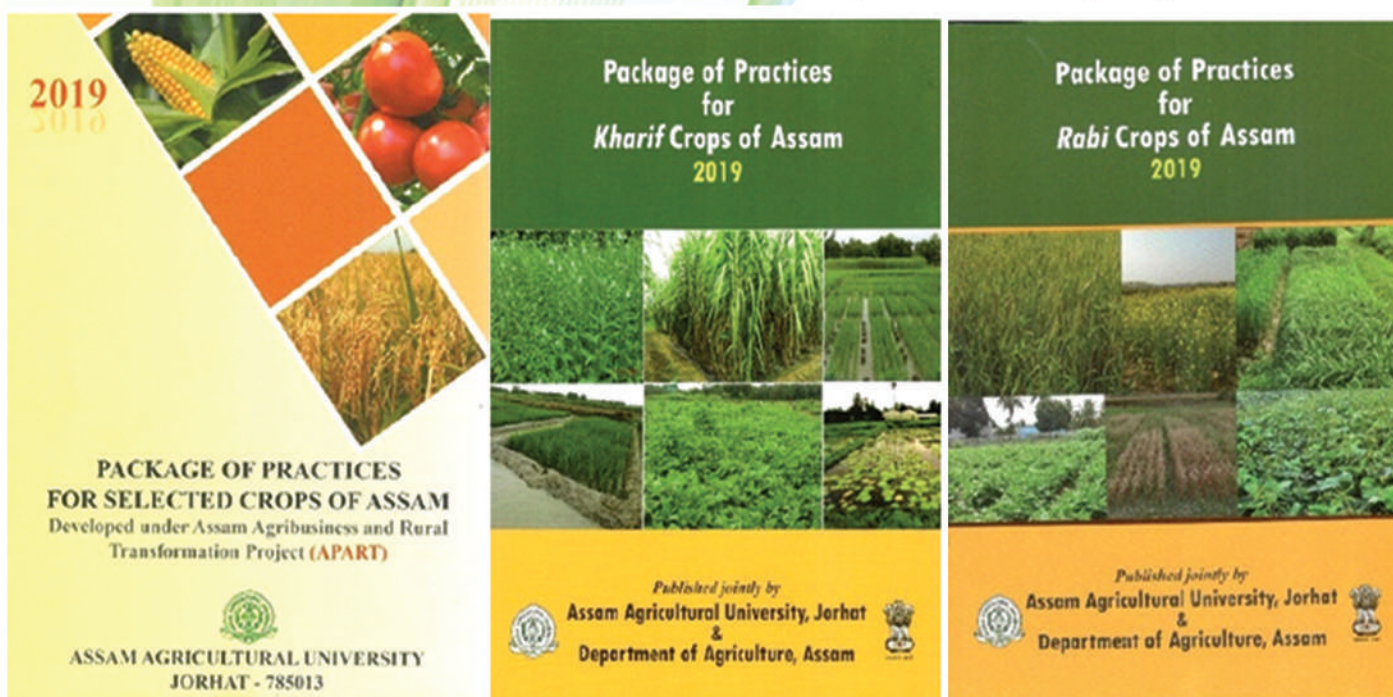
Rs.590730.00 (This includes biofertilizer strains and biofertilizer packets of *Azospirillum*, *Azotobacter*, *Rhizobium* and Phosphate solubilizing bacteria)

Package of practices

Package of practices developed and recommended to farmers of NEH regions. Published by AAU, Jorhat & State Agriculture Department, Assam as follows:

- Package of practices for Rabi crops of Assam, 2019
- Package of practices for selected crops of Assam, 2019

### Assam Agricultural University and State Agriculture Department - Package of practices



The information given in the document is based on the experiments carried out at the AINP centre- Department of Soil Science, Assam Agricultural University (AAU), Jorhat, Assam. For training, demonstration and other enquiries please contact Principal Investigator, AINP on Soil Biodiversity-Biofertilizers, AAU, Jorhat-13, Assam.

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