

Azospirillum Biofertilizer Technology for North-East Region of India



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Background

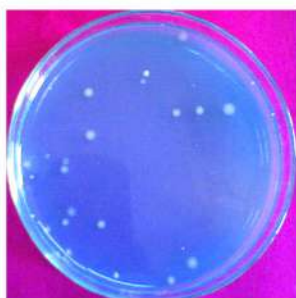
Azospirillum is one of the most widely studied and commercially used plant growth-promoting rhizobacteria (PGPR) in crop production. It is found in association with many plants worldwide in microaerophilic and free-living habitats. *Azospirillum* is known for their plant growth promoting (PGP) activities such as nitrogen fixation, production of the phytohormones like indole 3-acetic acid (IAA), cytokinin, abscisic acid (ABA), ethylene, gibberellic acid and zeatin, plant growth regulatory substances such as polyamines, osmotic stress response in plants and siderophore production. Besides their versatile PGP activities, these bacteria adapt to most rhizospheric environment and show higher competitiveness. Being microaerophilic in nature, these bacteria perform better in medium heavy to heavy textured soils. ICAR- All India Network Project on Soil Biodiversity Biofertilizers (AINP-SBB) has developed *Azospirillum* biofertilizer technology for North East Region of India. A potential *Azospirillum* sp isolated after intensive screening of native isolates. The *Azospirillum* strain (MW301143) being used for mass production of biofertilizers. The performance of the biofertilizer was evaluated on rice and vegetables commonly grown in NEH regions. The *Azospirillum* biofertilizer enhanced crops yield by 13-20% and saved chemical fertilizer significantly (about 20%). A revenue of Rs 5.9 lakhs generated from commercialization. Technology has been transferred to 3 industries for mass production to meet the farmers increasing demand. During last 3 years, about 640 farmers were benefitted and 12 state agricultural officers were trained. State agriculture department of Assam has adopted this technology and published the recommendations as package of practices in 2019.

Isolation of *Azospirillum* sp

AINP on Soil Biodiversity-Biofertilizers at Assam Agricultural University has been engaged in screening and evaluation of efficient *Azospirillum* from the pristine ecosystems of the region. Eighteen *Azospirillum* strains were isolated from rice rhizosphere and potential strain is utilized for preparation of biofertilizers.



Pellete Formation in semisolid NFB media



Pure colonies in solid NFB media



Antibiotic resistance against common antibiotics

Azospirillum biofertilizer production protocol

Step I: Mass multiplication of *Azospirillum* in nitrogen free bromothymol blue (NFB) broth is done by inoculating the mother culture @1.0 % (v/v). Allowed the *Azospirillum* to grow for 5-7 days in an incubator shaker/BOD incubator at $30 \pm 1^\circ\text{C}$ till the broth turns into blue color.

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 on a clean, dry, sterile metallic or plastic tray. The full grown (population approx 10^9 cell/mL) *Azospirillum* culture is added to the sterilized carrier and mixed well manually (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 *Azospirillum* 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 10^9 cells/g of inoculant at the time of preparation and 10^7 cells/g on dry weight basis before expiry date.

Benefits of *Azospirillum* inoculants

- ✓ Fixes 15-25 kg of N/ha/season in soil
- ✓ Increases grain yield by 10-20%
- ✓ 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.



Mother Culture



Growth of *Azospirillum* in broth



Mixed with sterilized carrier materials



Packaging and Sealing

Strategies for application of *Azospirillum* biofertilizers

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

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

In non-leguminous crop such as wheat, seed sown upland paddy etc. *Azospirillum* can effectively be used as seed treatment. In this method 400gm of *Azospirillum* biofertilizer is suspended in 500mL of water to make slurry and mixed with 10-12kg of seed until seeds are uniformly coated. The treated seeds are dried in shade and sown immediately.

In transplanted rice, *Azospirillum* is generally used as root dip treatment. In this method a small size bed is prepared in a corner of the paddy field. To transplant one bigha of land, 500gm *Azospirillum* biofertilizer is mixed with 10-15kg compost and just sufficient quantity of water in the bed. Uprooted rice seedlings are dipped in the bed for 8-12 hours before transplanting.

Transplanted vegetables such as tomato, brinjal, chilli, cauliflower etc are also inoculated through root dip treatment. Required quantity of *Azospirillum* is mixed with water and the roots of the seedlings are dipped in for 20-30 minutes before transplanting.



Seedling root dip treatment of paddy

For soil treatment depending upon the crops the required quantity of biofertilizers is mixed with compost and broadcast evenly in the field. For sugarcane, the biofertilizer is applied in furrows near the root zone and in potatoes it should be applied 20 days after planting or at the time of earthing up operation.

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

Effect of *Azospirillum* biofertilizer on different crops

Crop	Application and crop response	
Winter rice	<i>Azospirillum</i> + PSB <i>Azospirillum</i> and PSB @3.5kg/ha with NPK@ : 30:10:40 kg/ha Rice yield:3.99t/ha Rice Yield with RD of NPK(60:20:40 kg/ha):4.07t/ha. Application of <i>Azospirillum</i> and PSB could save 30 and 10kg of nitrogenous and phosphatic fertilizers	
Winter rice	<i>Azospirillum</i> + compost: Application : 1t/ha enriched compost (primed with 1.0% each of <i>Azospirillum</i> and PSB& rock phosphate(as P)) + 50% RDF (NPK at 30:10:40) Yield with microbial enriched compost : 4.28t/ha Yield with RDF : 3.79t/ha	
Rice (organic)	Application: Compost 5.0t/ha+biofertilizers (<i>Azospirillum</i> , and PSB) as seedling root dip. Yield with microbial enriched compost+ Biofertilizers :3.35 t/ha Yield with normal compost :2.82t/ha	
Jute	NPK@15:13:25(kg/ha) + biofertilizer (<i>Azospirillum</i> + PSB, 3.5kg each) as seed coating recommended as INM for Jute. INM yield: 27.60qt fibre yield/ha RD of NPK(30:25:25kg/ha): 26.60qt fibre/ha This technology could produce highest (2760 kg /ha) fibre yield, fibre fineness (2.8, tex) and fibre strength (26.7 tex/g) which was comparable with the 100% chemical fertilizers (2660 kg /ha).	
Okra	Biofertilizers (7.5g each/100g seed)+Rock Phosphate (313kg/ha) + FYM @5 t/ha + Vermicompost @1 t/ha Yield with biofertilizer :111.03 q/ha Yield with recommended NPK@60:30:120: 175.67qt/ha (Yield with biofertilizer and organic is less in organic cultivation compared to sole NPK fertilizers)	

Trainings / package of practices/stakeholders (2017-20)

Beneficiaries

Farmers benefitted
State agriculture officers

640 farmers trained on biofertilizer use
12 agriculture officers trained

Stakeholders

Technology transferred /
Supply of Biofertilizer strains to industries

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

Revenue earned
(2017-2020)

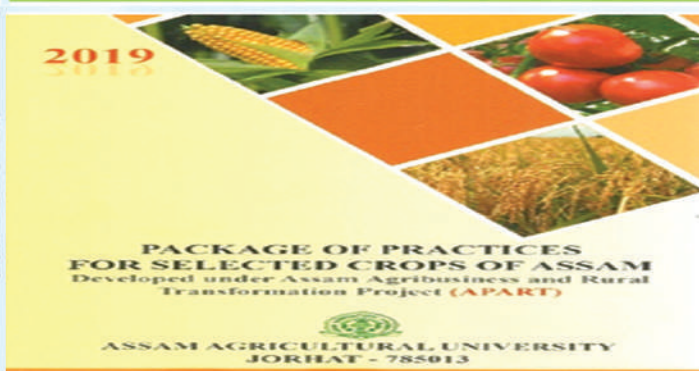
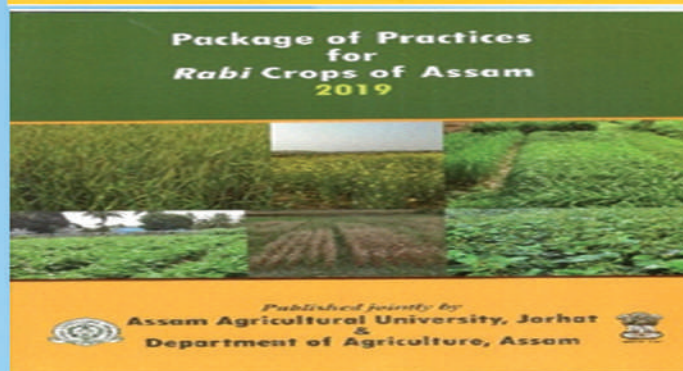
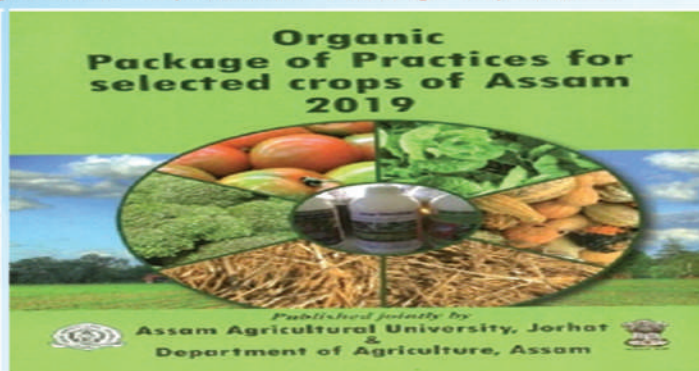
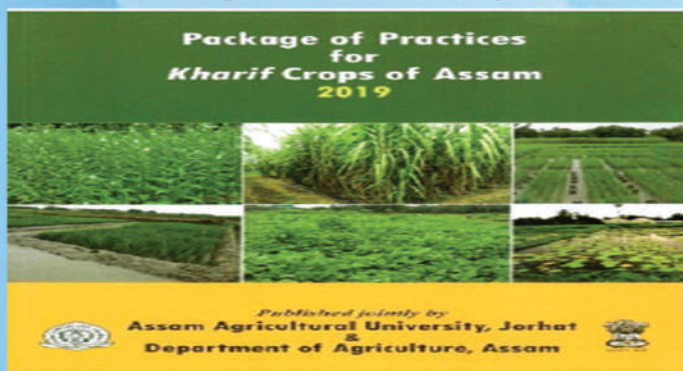
Rs.5.90730 lakhs
(Includes commercialization of strains and biofertilizer packets)

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 kharif crops of Assam, 2019
- Package of practices for Rabi crops of Assam, 2019
- Organic 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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