

# PHOSPHORUS SOLUBILIZING BIOFERTILIZER TECHNOLOGY FOR MAJOR CROPS OF MAHARASHTRA

2022



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# Background

The phosphates in soil remain in bound state with other compounds like aluminum and iron as aluminum/iron phosphate. The phosphate solubilizing microbes are used for making this biofertilizer. These microorganisms possess the ability to breakdown insoluble phosphates in soil into soluble forms by secreting organic acids such as formic, acetic, lactic and fumaric acids etc. These acids lower the  $P^H$  and bring out dissolution of bound forms of phosphate. Example-several soil bacteria particularly belonging to the genera *Pseudomonas* (*Pseudomonas striata*) and *Bacillus* (*Bacillus megaterium* and *Bacillus subtilis*) and fungi belonging to the genera, *Penicillium spp.* and *Aspergillus awamori* possess the ability to degrade insoluble phosphates present in soil, making easier for plants to uptake through roots. Bacterial cultures are termed as PSB (*Phosphate solubilizing Bacteria*) and fungal cultures are termed as PSF (*Phosphate Solubilizing Fungi*).

Increase in yield due to phosphate solubilizing bacteria could be obtained in crops like cotton, sorghum, maize, soybean, green gram, pigeon pea, sugarcane, wheat, chickpea, safflower, fruits and vegetables. The main input of inorganic P in agricultural soil is applying phosphorus fertilizers. Nearly, 70 to 90 % of phosphorus fertilizers applied to soils is fixed by cations such as  $Ca^{2+}$  in calcareous or normal soils to form a complex calcium phosphate and with  $Al^{3+}$  and  $Fe^{3+}$  in acidic soils to form aluminum phosphate and ferrous phosphate. These are insoluble forms and consequently unavailable. These accumulated phosphates in agricultural soils are adequate to maintain maximum crop yields if it could be mobilized, converted into soluble P forms using PSM.

Phosphate solubilizing biofertilizer technology developed under ICAR-All India Network Project on Soil Biodiversity-Biofertilizers (AINPSBB) at Vasantnaik Naik Marathwada Krishi Vidyapeeth, Parbhani. The technical bulletin highlights production of phosphates solubilizing biofertilizers, quality and field evaluation. The PSB biofertilizer improves crop yield by 3-20%. The product is commercially produced and marketed to farmers. During 2017-2022 revenue generated was Rs.49.94 lakhs. The technology benefited to 9500 farmers. The technology has been transferred to two industry (Microbial Solutions Pvt.Ltd. Hingoli and Atmanirbhar Baliraja Agri Business Pvt.Ltd., Jintur ) and marketed through KVKs.

## Isolation of Phosphorus Solubilizing Bacteria (PSB)

1. 1 g rhizosphere soils suspended in 9 ml sterile water and mix vigorously.
2. Serial dilution of the sample and dilutions are restricted to  $10^{-4}$  or  $10^{-5}$
3. Sterilized media is poured in petri plates and allowed for solidification.
4. Using sterile pipettes or micro tip 0.1 ml of each dilution is transferred aseptically to the petri plates containing solid media and spread uniformly with spreader.
5. Petri plates are incubated in BOD incubator at  $30 \pm 1^\circ\text{C}$  for 3-5 days.



### Media for growth of Phosphorus Solubilizing Bacteria (Modified Pikovskaya's medium)

Composition	Quantity (g/L)
Glucose	10.0
ZnO/ZnCO <sub>3</sub> /Zn <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub>	1.00
(NH <sub>4</sub> )SO <sub>4</sub>	0.50
KCL	0.20
MgSO <sub>4</sub>	0.10
FeSO <sub>4</sub>	0.005
MnSO <sub>4</sub>	0.005
Yeast extract	0.50
pH	7.00
Agar	15-20





## Large Scale Production of Liquid *Phosphorus Solubilizing* Biofertilizers

Liquid phosphorus solubilizing biofertilizer production technology includes isolation or procurement of efficient bacterial strains for required purpose, selection of suitable effective strain, preparation of mother culture, broth preparation, addition of cell protectants and their mixing, followed by packaging, storage and dispatch. The phosphorus solubilizing strains most adopted and found suitable in Marathwada region are produced at biofertilizer production unit, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani.

### Preparation of liquid *phosphorus Solubilizing* biofertilizers

#### 1. Preparation of mother or starter cultures

Starter cultures of selected strains are obtained after ascertaining their performance in laboratory, pot culture experiments and at field levels. The pure culture of efficient strain of particular microorganism is grown on respective agar medium on slant and maintained in the laboratory. A loopful of inoculum from the slant is transferred in a 250 ml capacity conical flask containing liquid medium. The conical flask was kept on rotary shaker for at least 72 hrs depending whether they are fast growing or slow growing. The content of these flasks usually attains a load of  $10^{14}$  cells per ml called mother culture or starter culture. This mother culture is further multiplied in larger flasks called as broth preparation.

#### 2. Preparation of broth cultures

Prepare liquid medium for phosphorus solubilizers. Distribute equal quantity in big conical flasks (1000 ml). Sterilize it in autoclave for half an hour at 15 lbs pressure. After sterilization each flask containing suitable broth is inoculated with the mother culture in 1:5 proportions aseptically under laminar flow. Keep the flasks on rotary shaker for 72 hours or in sterile fermenter until the viable count per ml reaches to  $10^{14}$  cells. The broths become thicker in consistency. This broth culture with population of at least  $10^{12}$  cells per ml should be stored at suitable temperature and condition.



#### 3. Preparation of liquid phosphorus solubilizing biofertilizer with cell protectant

To prepare the liquid phosphorus solubilizing biofertilizers from the above prepared broth, all the ingredients required should be mixed thoroughly under laminar flow to avoid the contamination.

### Ingredients

Sr.No.	Component
A)	Liquid broth
B)	Cell protectants
C)	Glycerol

\* Mix A+B+C as per above and make the volume 100 liters with sterile water

#### 4. Filling and packaging of bottles

After preparation of liquid phosphorus solubilizing biofertilizer as mentioned above the requisite quantity as per need should be filled in the sterile auto-lock high-density polyethylene (HDPE) plastic bottles under laminar flow to avoid contamination.



#### Dosage of liquid phosphorus solubilizing biofertilizer in different crops

Recommended liquid phosphorus solubilizing biofertilizers, their application methods and quantity to be used for different crops is given follows:

Crop	Recommended Biofertilizer	Application method	Quantity to be used
Sorghum, Pearl millet, Maize, Paddy, Sunflower, Safflower	<i>Bacillus megaterium</i>	Seed treatment	100 ml 10 kg <sup>-1</sup> seed
Cotton	<i>Bacillus megaterium</i>	Seed treatment	10 ml kg <sup>-1</sup> seed
Sugarcane, Potato, Ginger	<i>Bacillus megaterium</i>	Planting material	500 ml in 500 lit. of water
Vegetable crops	<i>Bacillus megaterium</i>	Seedling deeping	100 ml in 25 lit. of water
Fruit crops	<i>Bacillus megaterium</i>	Through drip irrigation	2.5 L. ha <sup>-1</sup>

## Effect of various phosphorus solubilizing biofertilizers on yield of various crops

Crops	Treatments	Yield (q ha <sup>-1</sup> )		Percent increase in yield
		Uninoculated	Inoculated	
Cotton	RDF+ Seed treatment with <i>Bacillus megaterium</i>	16.81	19.00	11.52
Wheat	RDF+ Seed treatment with <i>Bacillus megaterium</i>	15.39	19.45	20.00
Soybean	RDF+ Seed treatment with <i>Bacillus megaterium</i>	19.41	20.01	3.00
Pigeon Pea	RDF+ Seed treatment with <i>Bacillus megaterium</i>	13.39	16.72	19.92
Safflower	RDF+ Seed treatment with <i>Bacillus megaterium</i>	16.96	18.78	9.69
Groundnut	RDF+ Seed treatment with <i>Bacillus megaterium</i>	23.50	24.96	5.84
Guava	RDF + Application of <i>Bacillus megaterium</i> through drip	174.00	213.00	18.30

### Evaluation of biofertilizer : Front line demonstration

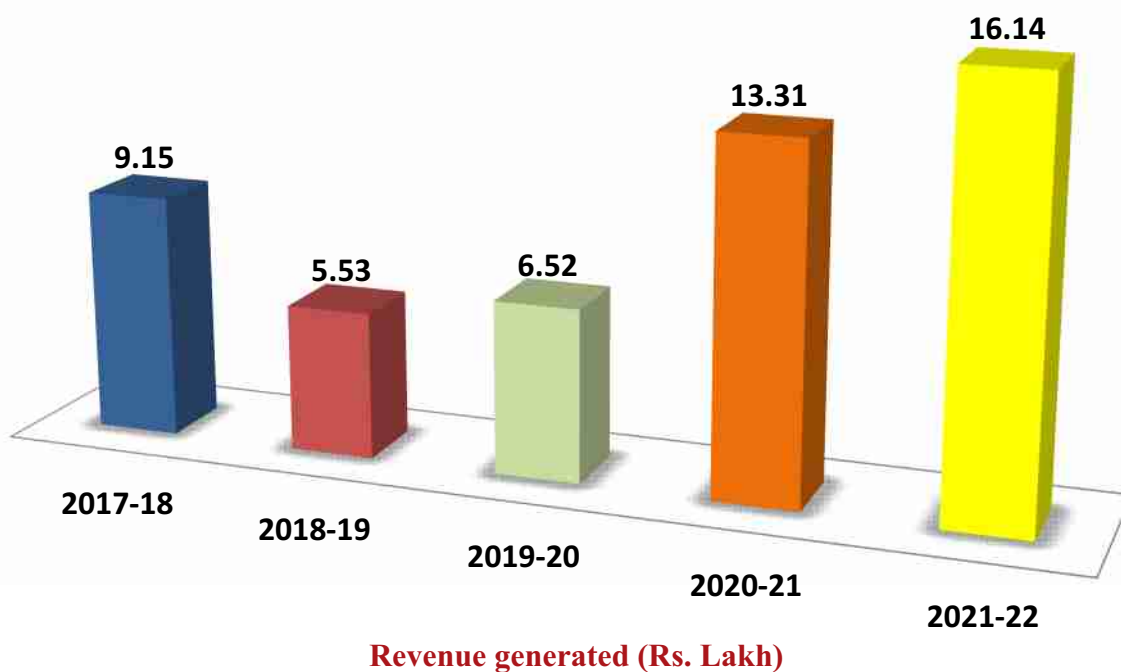




## Evaluation of biofertilizer : Front line demonstration



## Commercialization and revenue generated through Liquid biofertilizer



## Technology Transferred

Industry	M/s Microbial Solution Pvt. Ltd. Hingoli (Maharashtra)	Atmanirbhar Baliraja Agri Business Pvt. Ltd. Jintur (Maharashtra)
	 <p><b>Certificate</b></p> <p>This is to certify that we have received Liquid Biofertilizer Technology for (Rhizobium spp., Azotobacter, PSB, Sulphur Oxidizing Bacteria, Zinc Solubilizers) from the ICAR All India Network Project on Soil Biodiversity Biofertilizers, Department of Soil Science and Agricultural Chemistry, Vasanttrao Naik Marathwada Krishi Vidhyapeeth, Parbhani, Maharashtra on 18/5/2018. The technology is used for commercial production of biofertilizer.</p> <p>Microbial Solution Pvt. Ltd. Hingoli, Maharashtra</p> <p>Principal Investigator ICAR - All India Network Project on Soil Biodiversity Biofertilizers, Department of Soil Science and Agricultural Chemistry, Vasanttrao Naik Marathwada Krishi Vidhyapeeth, Parbhani, Maharashtra</p> <p>Dated: 18th July 2018</p>	 <p><b>CERTIFICATE</b></p> <p>This is to certify that we have received Liquid Biofertilizer Technology for (Rhizobium Spp., Azotobacter, PSB, Sulphur Oxidizing Bacteria, Zinc Solubilizers) from the ICAR - All India Network Project on Soil Biodiversity Biofertilizers, Department of Soil Science and Agricultural Chemistry, Vasanttrao Naik Marathwada Krishi Vidhyapeeth, Parbhani, Maharashtra on 20.12.2021. This technology is used for Commercial Production of Biofertilizer.</p> <p>Atmanirbhar Baliraja Agri Business Pvt. Ltd.</p> <p>Principal Investigator ICAR - All India Network Project on Soil Biodiversity Biofertilizers, Deptt. of Soil Science and Agril. Chemistry, Vasanttrao Naik Marathwada Krishi Vidhyapeeth, Parbhani</p> <p>Dated: 20 Dec 2021</p>

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Plate : Phosphorus solubilizing biofertilizer for sale

The information given in the document is based on the experiments carried out at the ICAR - AINP-SBB Centre, Department of Soil Science, VNMKV Parbhani, Maharashtra. For training, demonstration and other enquiries please contact the department.