Booklet on

BIOFERTILIZER

(PHOSPHOBACTERIA)

Shri AMM Murugappa Chettiar Research Centre
Taramani, Chennai –600113.

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Title : **BIOFERTILIZER (PHOSPHOBACTERIA)**

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Contents

Biofertilizer

There are two types of supplies for agriculture, specifically fertilizer and pesticide. It can be said that fertilizer is food, and pesticide is medicine for plants in conventional agriculture. On the other hand, biofertilizer and/or biopesticide are referred to each of them respectively in sustainable or environmentally friendly system. Biofertilizers are low cost, renewable sources of plant nutrients which supplement chemical fertilizers. These are nothing but selected strains of beneficial soil microorganisms cultured in the laboratory and packed in a suitable carrier. They can be used either for seed treatment or soil application. Biofertilizers generate plant nutrients like nitrogen and phosphorous through their activities in the soil or rhizosphere and make available to plants in a gradual manner. Biofertilizers are gaining momentum recently due to the increasing emphasis on maintenance of soil health, minimize environmental pollution and cut down on the use of chemicals in agriculture. In rainfed agriculture, these inputs gain added importance in view of their low cost, as most of the farmers are small and marginal and cannot afford to buy expensive chemical fertilizers. Biofertilizers are also ideal input for reducing the cost of cultivation and for practising organic farming.

Biofertilizer is still an unclear term. It can be easily found that biofertilizers are identified as plant extract, composted urban wastes, and various microbial mixtures with unidentified constituents, and chemical fertilizer formulations supplemented with organic compounds. However biofertilizer is most commonly referred to the use of soil microorganisms to increase the availability and uptake of mineral nutrients for plants. So it is necessary to define the term “Biofertilizer”. There is a proposal that “biofertilizer” be defined as a substance which contains living microorganisms which colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrient and/or growth stimulus to the target crop, when applied to seed, plant surfaces, or soil.

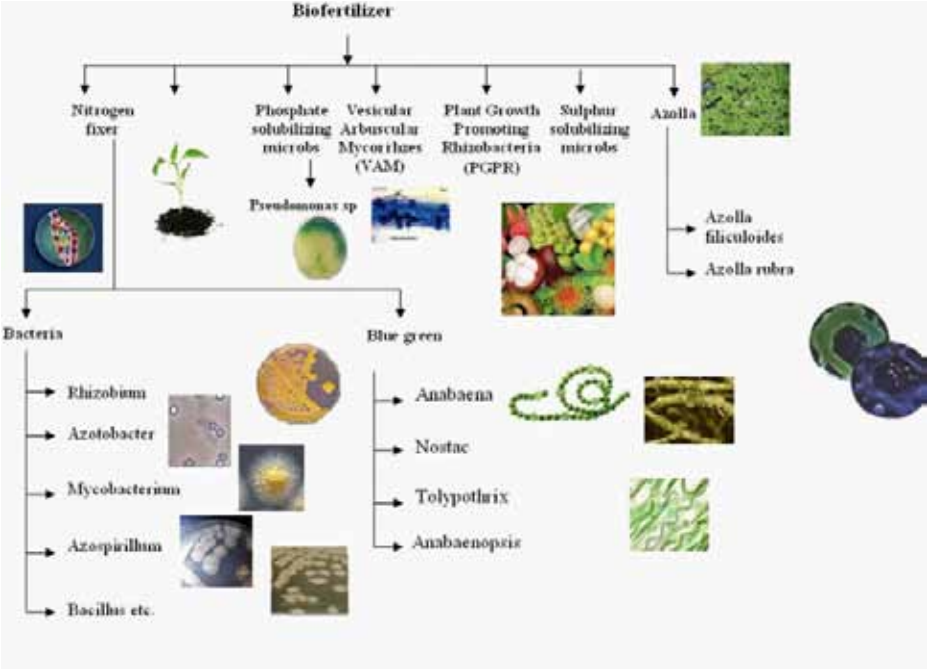
Whether the existence of a microorganism increases the growth of plants by making nutrients more available or replacing soil nutrients or increasing plant access to nutrient, as long as the nutrient status of the plant has been enhanced by the microorganisms, the substance that was applied to the plant or soil containing the microorganisms, can be characterized as a biofertilizer. This definition separates biofertilizer from organic fertilizer containing organic matter.

Microbial Functions Newly suggested as Biofertilizer

Numerous species of soil bacteria which flourish in the rhizosphere of plants, but which may grow in, on, or around plant tissues, stimulate plant growth. These bacteria are collectively known as plant growth promoting rhizobacteria (PGPR). Some PGPR appear to promote growth by acting as both biofertilizer and biopesticides. The search for PGPR and investigation of their modes of actions are increasing at a rapid pace as efforts are made to exploit them commercially as biofertilizers. Modes of PGPR action include fixing N2, increasing the availability of nutrients in the rhizosphere, positively influencing root growth and morphology, and promoting other beneficial plant-microbe symbiosis. The combination of these modes of actions in PGPR is also addressed, as well as the challenges facing the more widespread utilization of PGPR as biofertilizers.

MCRC have been interested in microorganisms mainly nitrogen fixers, phosphate solubilizers, cellulose degraders and mycorrhizae as main sources for biofertilizer and production of these strains in low cost medium. There are several limitations to the use of biofertilizer for agricultural system. Primarily, efficacy is not reliable for most biofertilizers. This is because the mechanism of action of the biofertilizer in promoting growth is not well understood. However, research into biofertilizer is increasing, attempting to deal with these issues. Research needs also to be conducted to determine if and how variations in soil type, management practices, and weather affect biofertilizer efficacy. Furthermore, there is a block in biofertilizer development. It is difficult to test inoculant in field

as routine experiments.



Types of Biofertilizers

The following types of biofertilizers are available to the farmers in India.

- Nitrogen fixing biofertilizers eg. *Rhizobium, Bradyrhizobium, Azospirillum and Azotobacter.*
- Phosphorous solubilising biofertilizers (PSB) eg. *Bacillus,Pseudomonas and Aspergillus*
- Phosphate mobilizing biofertilizer eg. *Mycorrhiza*
- Plant growth promoting biofertilizers eg. *Pseudomonas sp.*

How biofertilizers work?

- Biofertilizers fix atmospheric nitrogen in the soil and root nodules of legume crops and make it available to the plant.
- They solubilise the insoluble forms of phosphates like tricalcium, iron and aluminium phosphates into available forms.
- They scavenge phosphate from soil layers.
- They produce hormones and anti metabolites which promote root growth.
- They decompose organic matter and help in mineralization in soil.
- When applied to seed or soil, biofertilizers increase the availability of nutrients and improve the yield by 10 to 25% without adversely affecting the soil and environment.

Phosphate Solubilizing Biofertilizers (PSB)

Phosphate Solubilizers

Most soils are deficient in soluble forms of phosphorus (P), one of the major essential macronutrients required for plant growth. Phosphorus, besides to nitrogen is one of the most important elements in crop production. It makes up to about 0.2% of plant dry weight. It has a defined role in plant metabolism such as cell division, development, photosynthesis, breakdown of sugar, nuclear transport within the plant, transfer of genetic characteristics from one generation to another and regulation of metabolic pathways. The plants obtain their phosphate requirements from the soil pool. It occurs in soil as inorganic phosphate, produced by weathering by parent rock or as organic phosphate derived from decayed plant, animal or microorganisms.

The phosphate available for plant growth depends not only on the total amount of phosphorous in the environment but also on its solubility, which in turn is dictated by chemical reactions and biological interaction in the soil. The makeup of a soil (soil texture) and its acidity (pH) determine the extent to which nutrients are available to plants. The diverse soil

phosphate forms can be generally categorized as soil solution phosphate, insoluble organic and insoluble inorganic phosphate. Many phosphate-solubilizing bacteria (PSB) belonging to the *Pseudomonas, Bacillus, Rhizobium, Agrobacterium, Burkholderia, Achromobacter, Micrococcus, Aerobacter, Enterobacter, Flavobacterium,* and *Erwinia genera* have been isolated from soil samples.

ISOLATION OF *PHOSPHO BACTERIA* FROM RHIZOID SOIL

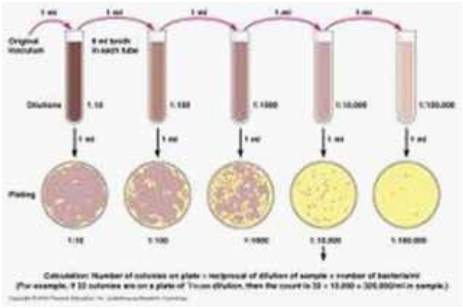
1. Sample collection:

Soil samples collected from different agricultural land and screened.

2. Serial dilution method:

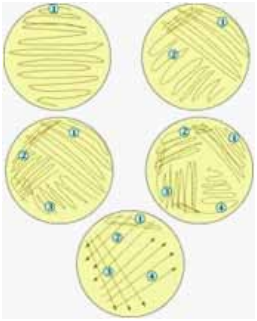
10g of soil sample was dissolved in 100ml of distilled sterilized water and mix the sample well and considered the dilution as 10-1. Then serially diluted the soil sample in sterilized distilled water up to 10-7 dilution (Each tubes containing 9ml of sterilized distilled water).Then 10 -5, 10-6, 10 -7 dilutions taken for spread plate technique.

3. Spread plate technique:



Sterilized Nutrient Agar prepared and poured into petridishes. After solidification of the medium, 0.1 ml of sample was poured into agar medium plate. By using L- rod, spread the sample evenly over the agar surface and then incubated at 37°C for 24 hours.

Nutrient Agar Medium :



Beef extract	-	3g
Peptone	-	5g
Nacl	-	5g
Agar	-	15g
Distilled water	-	1000ml
pH	-	6.8

Pikovskaya's Media Composition : (Experimental media for phosphate solubilizing organisms)

Ingredients		**g/l**
Yeast ex tract	-	0.5
Dextrose	-	10
Calcium phosphate	-	5
Ammonium phosphate	-	0.5
Potassium chloride	-	0.2
Magnesium sulphate	-	0.1
Manganese sulphate	-	0.0001
Ferrous sulphate	-	0.001
Agar	-	15
pH	-	6.8

This medium is used to identify the phosphate solubilizing microorganisms. In that halo zone formation considered as positive result.

(a) King's B Broth (Commercial medium for *Pseudomonas* sp.)

Peptone	-	20g
Di-potassium sulphate	-	1.5g
Magnesium Dichloride	-	1.5g
Glycerol	-	10 ml
pH	-	7.2±0.2

The culture was inoculated into King's B broth and incubated at room temperature for 24 hours.

LOW COST MEDIUM PREPARATION

Formulation - 1 (MCRC-DST CORE)

- Alternative for Kings B Broth (*Pseudomonas* sp.)

Fish extract	- 10ml
Algal water	- 25ml
Aloe vera extract	- 5ml
Tap water	- 100ml
pH	- 7.2±0.2

The broth prepared was inoculated with *P.fluorescens* and was incubated at 27ºC for 24hrs on rotary shaker.

Formulation - 2 (MCRC-DST CORE)

- Alternative for Nutrient Broth *(Bacillus megaterium)*

Fish extract	- 4ml
NaCl	- 0.5g
Yeast extract	- 0.15g
Tap water	- 100ml
pH	- 7.2±0.2

Uses of PSB :

PSB can be used for all crops including paddy, millets, oilseeds, pulses and vegetables. Methods recommended for application are :

- Seed treatment
- Seedling dipping
- Soil application

Seed treatment :

Dosage : 10 kg of normal size seeds such as moong, urd, arhar, cowpea, lentil and berseem may be treated with 200 g of PSB inoculant by slurry method. Large size seeds such as groundnut, chickpea, soybean and pea, etc., require 400 to 500 g of inoculant for 10 to 12 kg of seeds. In case, the seeds are to be treated with fungicides, insecticides and bio agents, apply PSB at the last. Apply PSB 24 hr after treating with other chemicals.

Dosage : 10 kg medium size seeds such as groundnut, wheat, cotton, maize etc., may be treated with 200 g of inoculant, whereas 100 g per acre inoculant is enough for treatment of small size seeds.

Seedling dipping : This method is useful where the transplantation of seedlings is required. It is ideal for vegetable crops.

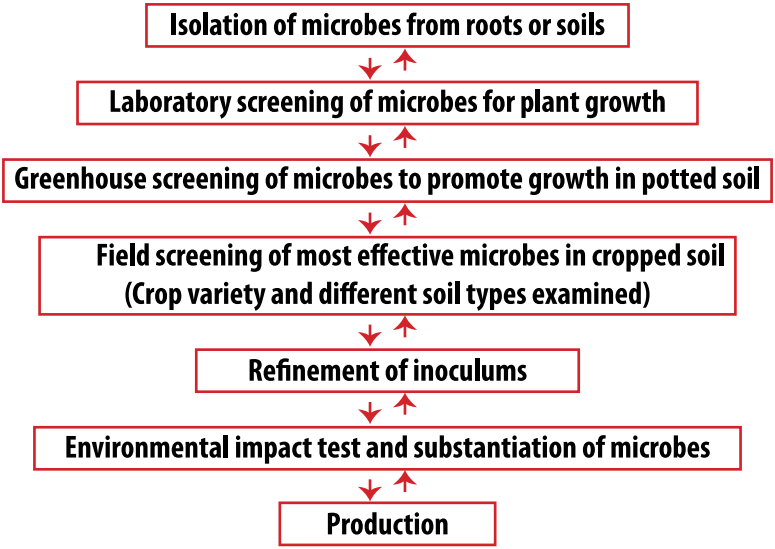
- Prepare the inoculant suspension in water in the ratio of 1:10.
- Dip the roots of seedlings in suspension and keep them immersed for about 5 minutes
- Take out the seedlings from the suspension and transplant as early as possible.

pseudomonas fluorescens

Dosage : Suspension of one kg in 10 to 15 litre of water for treating of seedlings for one acre.

Soil application : Mix 3-5 kg inoculant with 50 kg finely powdered FYM. Broad cast this mixture at the time of last ploughing.

Note : In case of PSB, best results are obtained when applied with well decomposed organic manure.



Carrier Materials

Biofertilizers are usually prepared as carrier-based inoculants containing effective microorganisms. Assimilation of microorganisms in carrier material enables easy-handling, long-term storage and high effectiveness of biofertilizers. Among various types of biofertilizers, bacterial inoculant is one major group which includes nitrogen-fixing rhizobacteria, plant growth-promoting rhizobacteria, phosphate-solubilizing bacteria, and so on. Basically, the carrier-based inoculant of these bacteria can be prepared by a common procedure.

The most common way of inoculation is “seed inoculation”, in which the inoculant (bacteria-carrier mixture) is mixed with water to make slurry-form, and then mixed with seeds. In this case,the carrier must be in the form of fine powder. To achieve the tight coating of inoculant on seed surface, use of adhesive, such as gum arabic, methylethylcellulose, sucrose solutions and vegetable oils is recommended. Any locally available

sticky material, which is non-toxic to bacteria and seeds, can be used as adhesive. Seed inoculation may not always be successful, i.e. the inoculation results in low nodule occupancy of the inoculated rhizobial strain, or low establishment of the inoculated rhizobacterial strain. This might be due to low population and/or low survival of the inoculated bacterial strain on the seed surface and in the soil. In such instance, "soil inoculation" can be adopted, whereby a large population of a bacterial strain can be introduced into the soil. For soil inoculation in general, granular inoculant is placed into the furrow under or alongside the seed. This enhances the chance for the inoculated strain to be in contact with plant roots. Different types of materials are used as carrier for seed or soil inoculation. For preparation of seed inoculant, the carrier material is milled to fine powder with particle size of 10 - 40 μm.

The properties of a good carrier material for seed inoculation are:

1) Non-toxic to inoculant bacterial strain
2) Good moisture absorption capacity
3) Easy to process and free of lump-forming materials
4) Easy to sterilize by autoclaving or gamma-irradiation
5) Available in adequate amounts
6) Inexpensive
7) Good adhesion to seeds
8) Good pH buffering capacity and
9) Non-toxic to plant (Somasegaran and Hoben, Springer, 1994)

For soil inoculation, carrier material in granular form (0.5 – 1.5 mm) is generally used. Granular forms of peat, perlite, talcum powder, charcoal or soil aggregates are suitable for soil inoculation. Other essential criteria for carrier selection relating to survival of the inoculant bacteria should be considered. (1) Survival of the inoculant bacteria on seed. Seeds are not always sown immediately after coating with the inoculant bacteria.

The bacteria have to survive on seed surface against drying condition until placed into soil. (2) Survival of the inoculant bacteria during the storage period. (3) Survival of the inoculant bacteria in soil. After being introduced into the soil, the inoculant bacteria have to compete with native soil microorganisms for the nutrient and habitable niche, and have to survive against grazing protozoa. Such carrier materials that offer the available nutrient and/or habitable micro-pore to the inoculants bacteria will be desirable. In this sense, materials with micro-porous structure, such as soil aggregate and charcoal, will be good carrier for soil inoculants.

Sterilization

AUTOCLAVE- FOR STERLIZATION

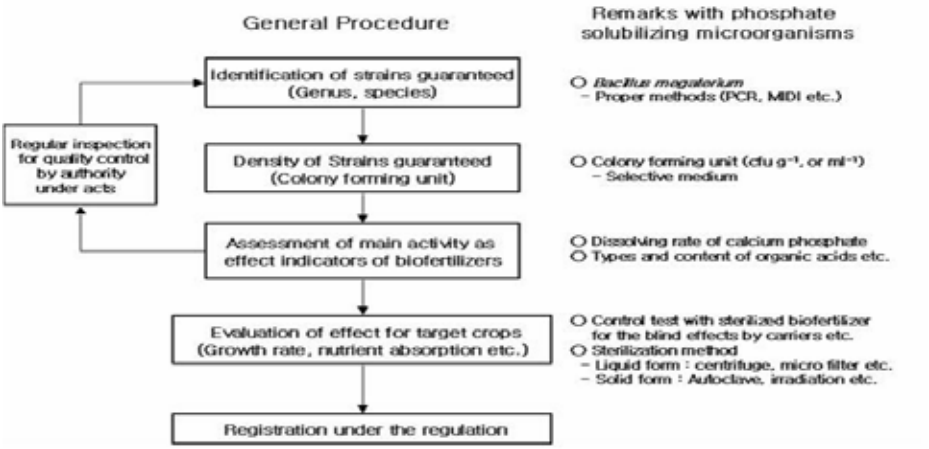
Sterilization of carrier material is essential to keep high number of inoculant bacteria on carrier for long storage period. Gamma-irradiation is the most suitable way of carrier sterilization, because the sterilization process makes almost no change in physical and chemical properties of the material. In brief, carrier material is packed in thin-walled polyethylene bags, and then gamma-irradiated at 50 kGy (5 Mrads). Another way of carrier sterilization is autoclaving. Carrier material is packed in partially opened, thin-walled polypropylene bags and autoclaved for 60 min at 121 ºC. It should be noted that during autoclaving, some materials change their properties and produce toxic substance to some bacterial strains.

Guidelines on Buying and Storage of Biofertilizers :

1. Ensure biofertilizer package before buying. Make sure to buy biofertilizer for the specific crops (e.g. corn).
2. Make sure that the biofertilizer is fresh. Check the expiration date.
3. Keep the package in a cool place until ready to use. Storage in refrigerator is good. Cool temperature will not harm the bacteria but high temperatures can be damaging to the biofertilizer microorganisms.
4. It is best to inoculate seeds prior to planting. Bacteria die quickly on drying seeds.
5. Chemicals on seeds and applied to the soil (e.g. insecticides, fungicides) may be toxic to the bacteria.
6. Store the inoculated seeds in a cool protected place until planting. Keep them out of direct sunlight and protect them from excessive drying.
7. Leftover inoculants may be kept safely in the package provided it is closed tightly to prevent excessive drying. Leftover inoculant stored in a refrigerator at 4oC or lower will remain effective for several months.

Cautions and Limitations of Biofertilizer

- Never mix with chemical nitrogen fertilizers
- Never apply with fungicides, plant ash etc. at the same time
- Never directly expose to sunlight
- Do not keep used solution overnight
- Store at room temperature, not below 0°C and over 35°C



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