

Biofertilizers for sustainable agriculture: *Rhizobium*

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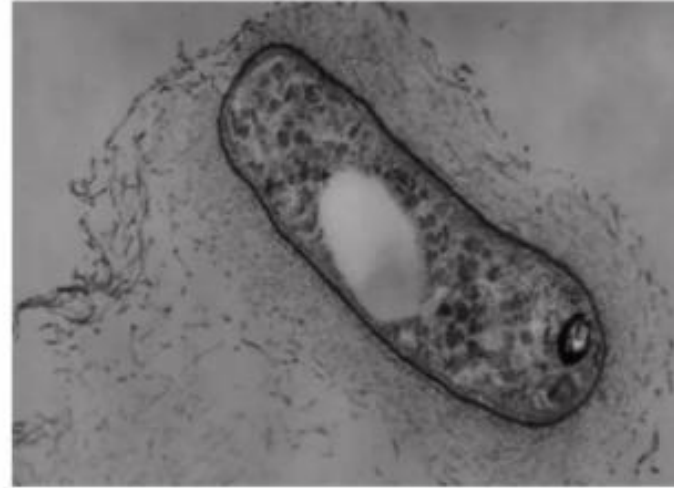
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INTRODUCTION

- It is soil bacteria.
- Rhizobium basically found in soil
- Most bacteria in soil are about one micron in length or diameter (there are a thousand microns in a millimetre).
- *Rhizobium*, has the ability to fix atmospheric nitrogen in symbiotic association with host legumes.
- Bacteria in environments that have high levels of nutrients may be larger than those in nutrient poor conditions.
- It converts atmospheric nitrogen into ammonium,
- Recommended for :
Pulses: chickpea, pea, lentil, black gram, green gram, cowpea, pigeon pea. **Oil seeds:** soybean, groundnut.

MORPHOLOGY:

- It belongs to rhizobiaceae family,
- Rhizobium are symbiotic diazotrophs
- It form a endosymbiotic association with legumes.
- Rhizobium is a Gram negative Soil Bacteria.
- They are non sporing bacteria
- Rod shaped cells.



CLASSIFICATION

Kingdom: Bacteria

Phylum: Proteobacteria

Class: Alphaproteobacteria

Order: Rhizobiales

Family: Rhizobiaceae

Species: *Rhizobium leguminosarum*, *R. lentil*.



Rhizobium can be classified on the basis of the types of the plant they are associated with and also the rate of growth.

Few species of *Rhizobium* bacteria include:

- *Rhizobium leguminosarum*
- *Rhizobium alarii*
- *Rhizobium lantis*
- *Rhizobium japonicum*
- *Rhizobium trifolii*
- *Rhizobium phaseolii*
- *Rhizobium smilacinae*

MORPHOLOGY



- They appear as elongated rods when viewed under the microscope
- Like a number of other bacteria, *Rhizobium leguminosarum* do not form spores in their life cycle.
- They possess several flagella on their polar end.
- They are aerobic as such, they need oxygen for respiratory purposes.
- Various strains of the bacteria have granules
- They can tolerate higher temperatures of about 38⁰ C.

STEPS OF MASS PRODUCTION



1. Inoculum preparation
2. Processing of carrier material
3. Selection of ideal carrier material.
4. Preparation of carrier material
5. Mixing the carrier and the broth culture and packing
6. Preparation of Inoculants packet

1. Inoculum preparation



- Prepare appropriate media for specific to the bacterial inoculant in 250 ml, 500 ml, 3 litre and 5 litre conical flasks and sterilize.
- The media in 250 ml flask is inoculated with efficient bacterial strain under aseptic condition
- Keep the flask under room temperature in rotary shaker (200 rpm) for 5- 7 days.
- Observe the flask for growth of the culture and estimate the population, which serves as the starter culture.
- Using the starter culture (at log phase) inoculate the larger flasks (500 ml, 3 litre and 5 litre) containing the media, after obtaining growth in each flask

2. Processing of carrier material



The use of ideal carrier material is necessary in the production of good quality biofertilizer

1. Peat soil
2. Lignite
3. Vermiculite
4. Charcoal
5. Press mud
6. Farmyard manure and soil mixture can be used as carrier materials.

The neutralized peat soil/lignite are found to be better carrier materials for biofertilizer production.

3. Selection of ideal carrier material.

1. Cheaper in cost
2. Should be locally available
3. High organic matter content
4. No toxic chemicals
5. Water holding capacity of more than 50%
6. Easy to process, friability and vulnerability



4. Preparation of carrier material



- The carrier material (**peat or lignite**) is powdered to a fine powder so as to pass through 212 micron IS sieve.
- The pH of the carrier material is neutralized with the help of calcium carbonate, since the peat soil / lignite are acidic in nature (pH of 4 - 5).
- The neutralized carrier material is sterilized in an autoclave to eliminate the microorganisms

5. Mixing the carrier and the broth culture



Inoculant packets are prepared by mixing the broth culture obtained from fermenter with sterile carrier material.

6.Preparation of Inoculants packet



- The neutralized, sterilized carrier material is spread in a clean, dry, sterile metallic or plastic tray.
- The bacterial culture drawn from the fermenter is added to the sterilized carrier and mixed well by manual (by wearing sterile gloves) or by mechanical mixer.
- The culture suspension is to be added to a level of 40- 50% water holding capacity depending upon the quantity of carrier material
- The inoculant packet of 200 g quantities in polythene bags, sealed with electric sealer and allowed for curing for 2-3 days at room temperature.
- Curing can be done by spreading the inoculant on a clean floor/polythene sheet/ by keeping in open shallow tubs/ trays with polythene covering for 2-3days at room temperature before packaging.

Specification of the polythene bags



- The polythene bags should be of low density grade.
- The thickness of the bag should be around 50 - 75 micron.
- **Each packet should be 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 to farmers

Storage of biofertilizer packet



- The packet should be stored in a cool place away from the heat or direct sunlight.
- The packets may be stored at room temperature or in cold storage conditions in lots in plastic crates or polythene / gunny bags.
- The population of inoculant in the carrier inoculant packet may be determined at 15 days interval.
- 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

MASS PRODUCTION



Application of Rhizobium



1

Seed treatment or seed inoculation

2

Seedling root dip

3

Main field application