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Sundarakkottai, Mannargudi-614 016.
Thiruvarur (Dt.), Tamil Nadu, India.

ENVIRONMENTAL AND AGRICULTURAL MICROBIOLOGY

SEMESTER –II

CODE: P16MB22

NITROGEN FIXATION AND BIOFERTILIZER

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BIOFERTILIZERS

- A **biofertilizer** (also **bio-fertilizer**) is a substance which contains living micro-organisms which, when applied to seeds, plant surfaces, or soil, colonize the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant.

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- Nutrient inputs of biological origin for plant growth
- Preparation containing living or dormant cells of efficient Nitrogen fixing,Phosphate solubilising and Cellulolytic microorganisms
- Otherwise termed as' Bioinoculants' and Microbial inoculants -suggested By Subba Rao 1982

Types of biofertilizers

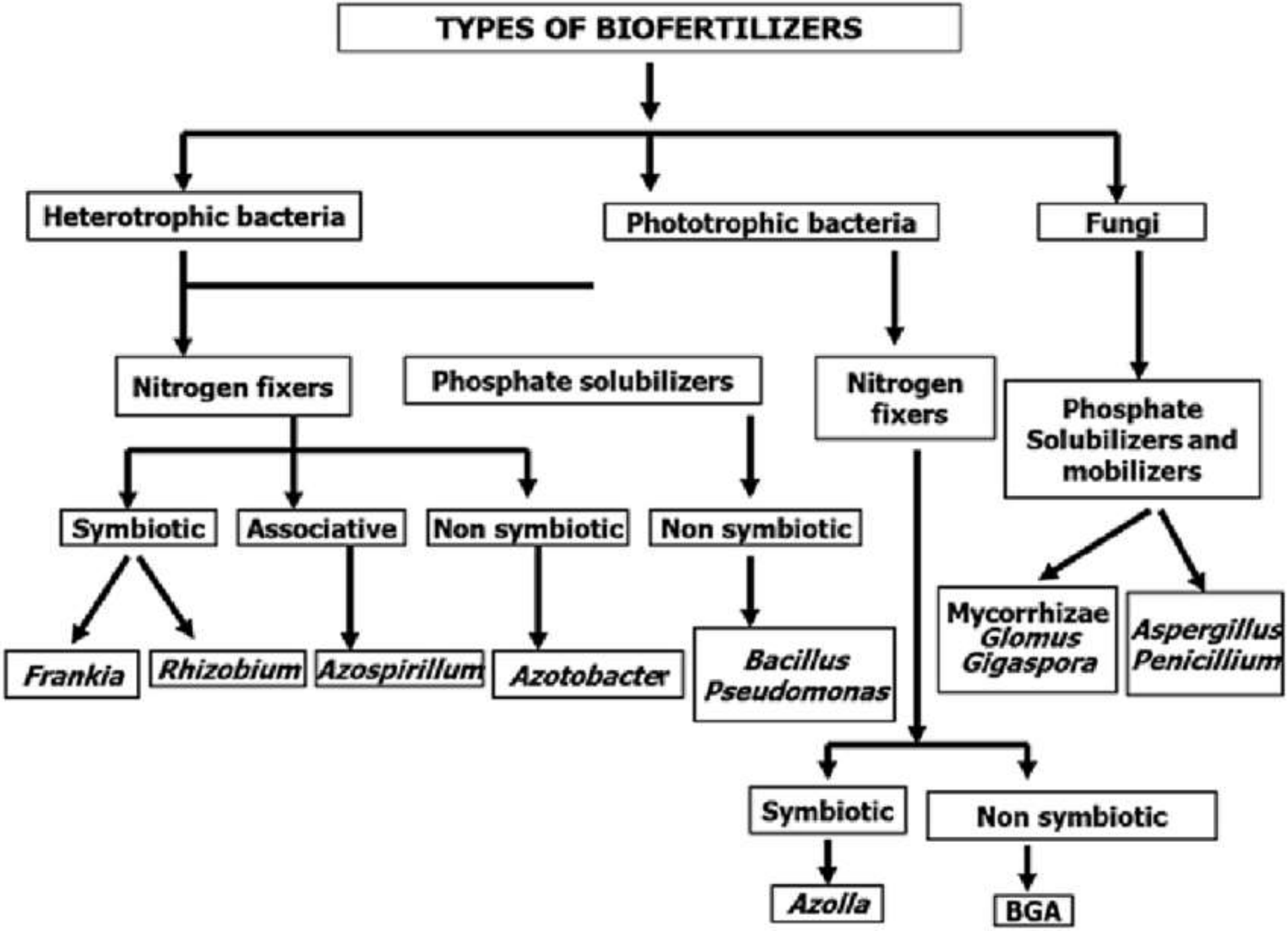
- Symbiotic fertilizers:*Rhizobium*
- Non symbiotic(free living):

Azotobacter,Azospirillum(Associative)

,Frankia

- Algal biofertilizer:BGA association with Azolla
- Phosphate solubilizing microbes
- Mycorrhizal biofertilizers

Azolla>Rhizobium>Cyanobacteria>Azospirillum>Azotobacter>PSM


TYPES OF BIOFERTILIZERS
Heterotrophic bacteria
Phototrophic bacteria
Fungi
Nitrogen fixers
Phosphate solubilizers
Nitrogen fixers
Phosphate Solubilizers and mobilizers
Symbiotic
Associative
Non symbiotic
Non symbiotic
Mycorrhizae
Glomus
Gigaspora
Aspergillus
Penicillium
Frankia
Rhizobium
Azospirillum
Azotobacter
Bacillus
Pseudomonas
Symbiotic
Non symbiotic
Azolla
BGA

ROLE OF BIOFERTILIZERS:-

*Makes availability of nutrients.
*Make the root rhizosphere more lively.
*Growth Promoting Substances are produced.
*More root proliferation.
*Better germination.
*Improve quality and quantity of produce.
*Improve fertilizer use efficiency.
*More biotic and abiotic stress tolerance.
*Improve soil health.
*Residual Effect.
*Make the system more sustainable.

Symbiotic nitrogen fertilizer

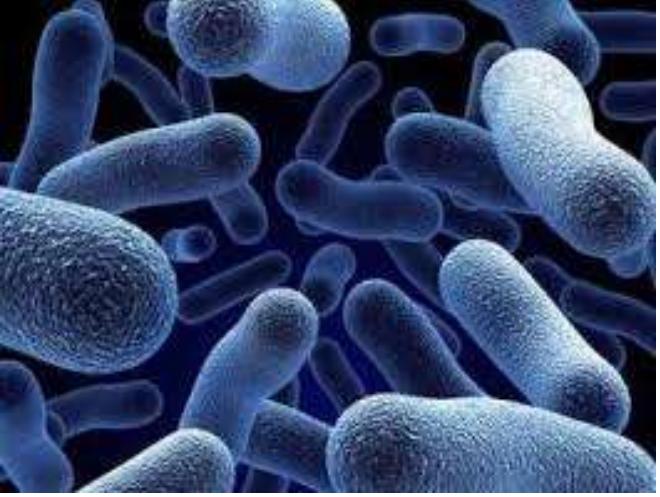
- *Rhizobium*- Gram Negative, fast growing ,Whitish, transparent colonies formed in YEMA,lives in soil,fix the atmospheric nitrogen in root nodules of leguminous plants.
- Carrier: Peat,Lignite
- It has been estimated that 40-250 kg N/ha/annum

Recommended for:Legumes like ground nut,Green Gram,Bengal gram, Soy bean,Cowpea and fodder legumes.

Non Symbiotic nitrogen fertilizers

- *Azosprillium*: Isolated by Beijernick Gram negative,Isolated from soil and plant roots ,motile,PHB granules,Okon's medium is used. Increase yield 15-30%. *A.lipoferum,A.brasilense* are most commonly used as biofertilizers
- Carrier: Farmyard manure

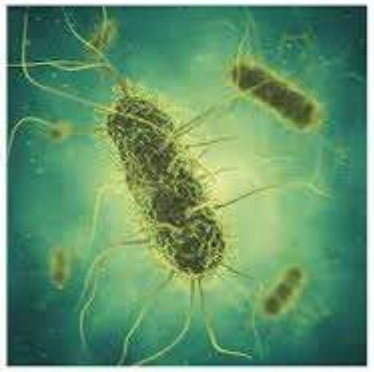
Recommendedfor:Rice,wheat,maize,Sorghum.Sugarcane



Azotobacter

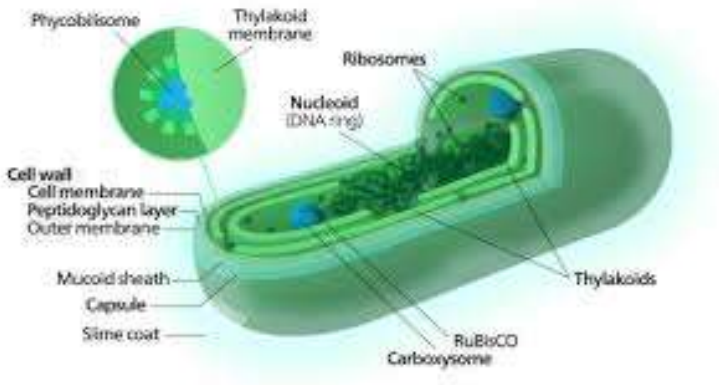
Free living,nitrogen fixing,

- Azotobacteriaceae family
- Gram negative,aerobic,rod shaped,Jensen medium is used for cultivation
- It produces IAA,gibberllic acid,vitamin
- Important species are: *A.beijernicka,A.agilis,A.paspali,* *A.vinelandii* etc.,
- Recommended for:Rice,wheat,millets, Cotton etc



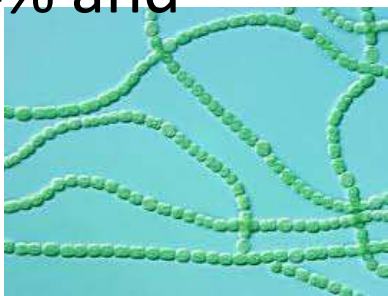
Azolla

- Free floating,freshwater fern lives in endophytic cyanobacterium *Anabaena azollae,fix* atmopheric nitrogen.first isolated in vietnam.Now it is popular in China,India,Bangaladesh,Phillipines and Indonesia.
- Six species are *A.pinnata,A.rupra,A.mexicana,A.microphylla, A.filiculoides,A.caroliniana*
- Mass cultivation:Microplots in soil
- Recommended for:Rice



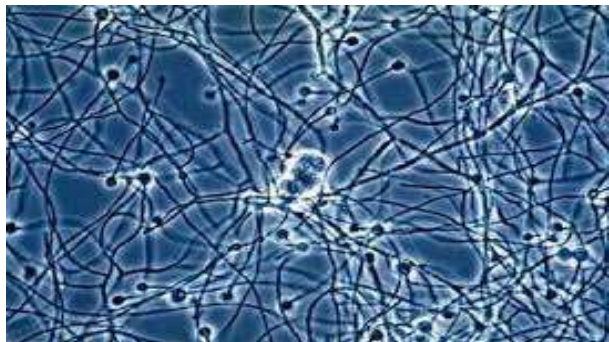
Cyanobacteria

- In water logging conditions, Cyanobcateria multiply, fix atmospheric nitrogen and released into surrounding medium in the form of aminoacids,proteins,and other growth promoting substances. *Ex:Anabaena,Nostoc,Plectonema, Cylindrospermum*
- Isolation:Fogg's medium. Mass cultivation:cemented tank,shallow metal trough,Polythene lined pit,Field method
- Recommended for :Rice increase crop yield 34% and saves nitrogen fertiliser by 30%



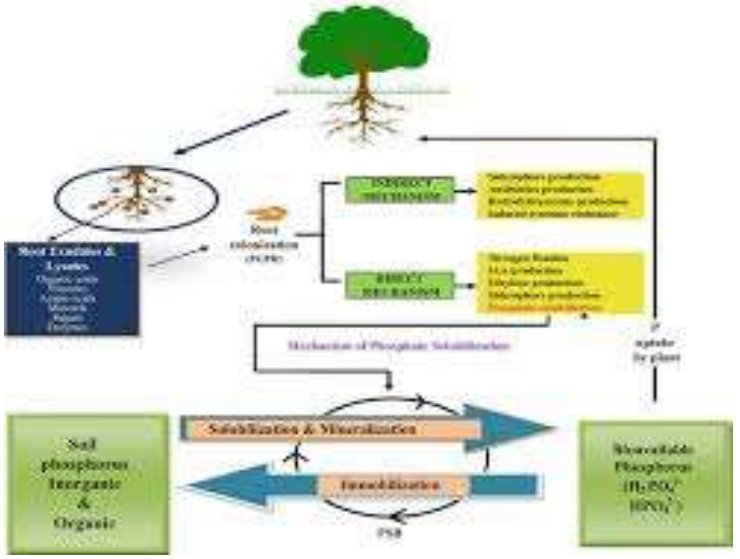
Frankia

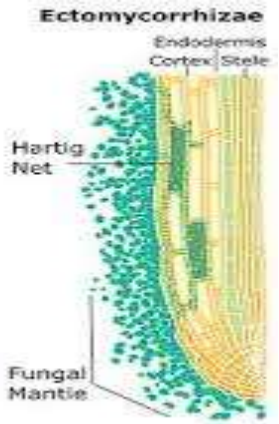
- Member of Actinomycetes,form symbiotic structure with roots of non leguminous plants called actinorrhizae.BAP medium is used,Grow slowly,very long log phase
- *Alnus,Hippophae,Allocasuarina, Causuarina, Gymnostoma*
- Recommended for:Increase nitrogen content 61-157kg/ha/annum



Phosphorous Solublizing Microorganisms(PSM)

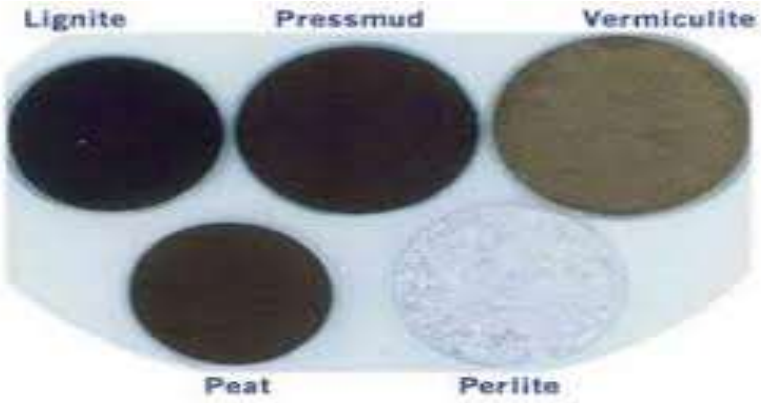
- *Pseudomonas,Bacillus,Micrococcus,Flavobacterium,Aspergillus,Penicillum,Fusarium, Sclerotium*
- PSM save 30-50 kg/ha of super phosphate and increase crop yield up to 200-500kg/ha
- Medium: Pikovskaya
- Carrier: Wood,Charcoal,
- Recommended for:All crops





Mycorrhizae

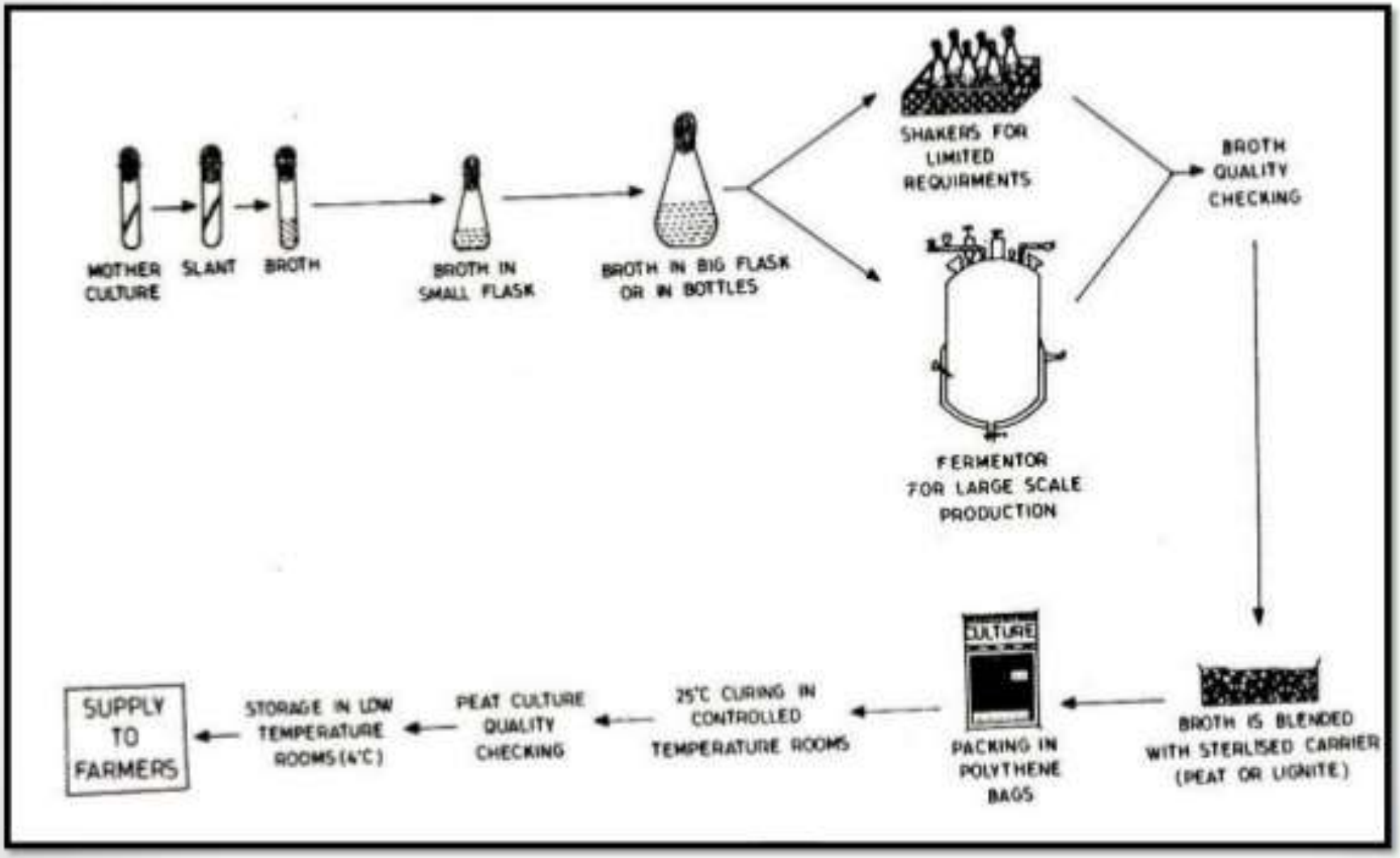
- Fungus root -Discovered by Frank. Mutualistic symbiosis between plant root and fungus. Phosphorus mobilising fungi,penetrate into root cortex,forms external hyphae,vesicles,arbuscles
- Types of Mycorrhizae:Ecto,Endo,,VAM, Ericoid,Orchid, Arbutoid,Monotrophid
- Require living host for multiplication(Onion,Maize,Sorghum)
- Benefits:increase longevity of feeder roots,uptake minerals,increase resistance in plants,nutrient transfer mainly phosphorus,zinc,sulfur



- Inert material,locally available,water holding capacity,high organic content,easily handled, packed and transaported
- Ex: Peat,Lignite, FYM, Charcoal powder
- Specifications of polythene Bags

Name of the manufacturer, Product,Strain no. Batch no, ISI, Date of Expiry,Price,Method of inoculation,Recommended crop, Storage instructions

Production

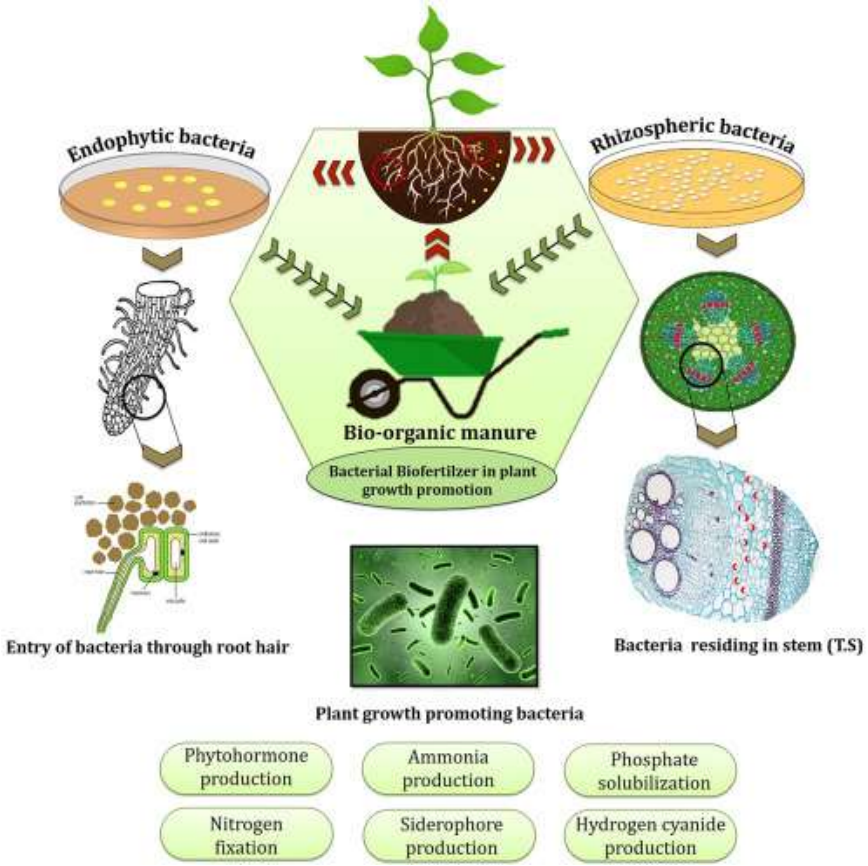

MOTHER CULTURE
SLANT
BROTH
BROTH IN SMALL FLASK
BROTH IN BIG FLASK OR IN BOTTLES
SHAKERS FOR LIMITED REQUIRMENTS
FERMENTOR FOR LARGE SCALE PRODUCTION
BROTH QUALITY CHECKING
BROTH IS BLENDED WITH STERLISED CARRIER (PEAT OR LIGNITE)
CULTURE
PACKING IN POLYTHENE BAGS
25°C CURING IN CONTROLLED TEMPERATURE ROOMS
PEAT CULTURE QUALITY CHECKING
STORAGE IN LOW TEMPERATURE ROOMS (4°C)
SUPPLY TO FARMERS

Application of biofertilizer

- Seed treatment: *Rhizobium, Azotobacter,PSM,Azospirillum*
- Seedling treatment:*Azospirillum*
- Soil treatment : PSM.Mix with 400-600 kgs of cow dung along with ½ bag of rock phosphate with 2kg of PSM

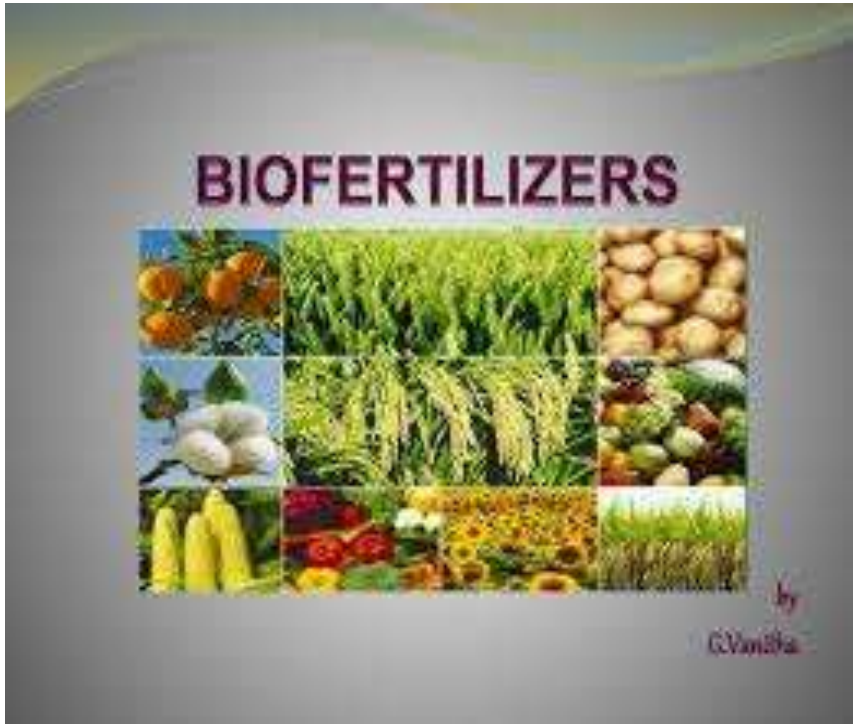
Advantages of biofetilizer:

- Increase crop yield
- Replace chemical fertilizers such as nitrogen and phosphoros by 25%
- Stimulate plant growth,Secrete growth promoting substances
- Cost effective ,ecofriendly
- Free from pollution hazards and increase fertility



Disadvantages

- Require special care for long term storage
- Too hot or too dry loss the viability
- Must be used before expiry date



Biology of Nitrogen fixation

- Biological nitrogen fixation (BNF), discovered by Beijerinck in 1901 , is carried out by a specialized group of prokaryotes.
- These organisms utilize the enzyme nitrogenase to catalyze the conversion of atmospheric nitrogen (N_2) to ammonia (NH_3).
- Because it is the principal source of the nitrogen in the soil, plants need to grow, nitrogen fixation is one of the most important biochemical processes on Earth.
- Nitrogen within living organisms is eventually decomposed and converted to atmospheric nitrogen (N_2).

Application of Biofertilizers in Agriculture

- . They supplement chemical fertilizers for meeting the integrated nutrient demand of the crops.
- Increased mineral and water uptake, root development, vegetative growth and nitrogen fixation.
- Improved physico-chemical properties of soil
- Increase organic matter and fertility of soil, secrete antibiotics which act as pesticides

Genes and Regulation of Rhizobium

- The ***nif* genes** are genes encoding enzymes involved in the fixation of atmospheric nitrogen fixation into a form of nitrogen available to living organisms.
- The primary enzyme encoded by the *nif* genes is the nitrogenase complex which is in charge of converting atmospheric nitrogen (N_2) to other nitrogen forms such as ammonia which the organism can use for various purposes.
- Besides the nitrogenase enzyme, the *nif* genes also encode a number of regulatory proteins involved in nitrogen fixation.
- The *nif* genes are found in both free-iving nitrogen fixing and in symbiotic bacteria associated with various plants.
- The expression of the *nif* genes is induced as a response to low concentrations of fixed nitrogen and oxygen concentrations. The first Rhizobium genes for nitrogen fixation (nif) and for nodulation (nod) were cloned in the early 1980s b yGary Ruvkun and SharonR.Long in fredrick M.Ausubel's laboratory

Contd…

- In most bacteria, regulation of *nif* genes transcription is done by the nitrogen sensitive NifA protein. When there isn't enough fixed nitrogen available for the organism's use, NtrC triggers NifA expression, and NifA activates the rest of the *nif* genes.
- If there is a sufficient amount of reduced nitrogen or oxygen is present, another protein is activated: NifL. NifL inhibits NifA activity resulting in the inhibition of nitrogenase formation. NifL is regulated by the products of *glnD* and *glnK*.
- The *nif* genes can be found on bacterial chromosomes, but in symbiotic bacteria they are often found on plasmids with other genes related to nitrogen fixation

NODULIN Genes

- Some specific polypeptide called nodulins are secreted by the chromosomal DNA in the root nodules of the plant. Nealy 20 nodulins are secreted inside the nodule. Basically all the nodulins are divided into three groups I,II,III
- Group I:Nodulins 23,24,26 and36 include structural polypeptides of nodules
- Group II: Enzymes associated with uride metabolism
- Group III : Regulate the reduction of nitrogen


Thank You
Use Biofertilizers
For Healthy and living soil