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Biofertilizers (Microbial Inoculants)

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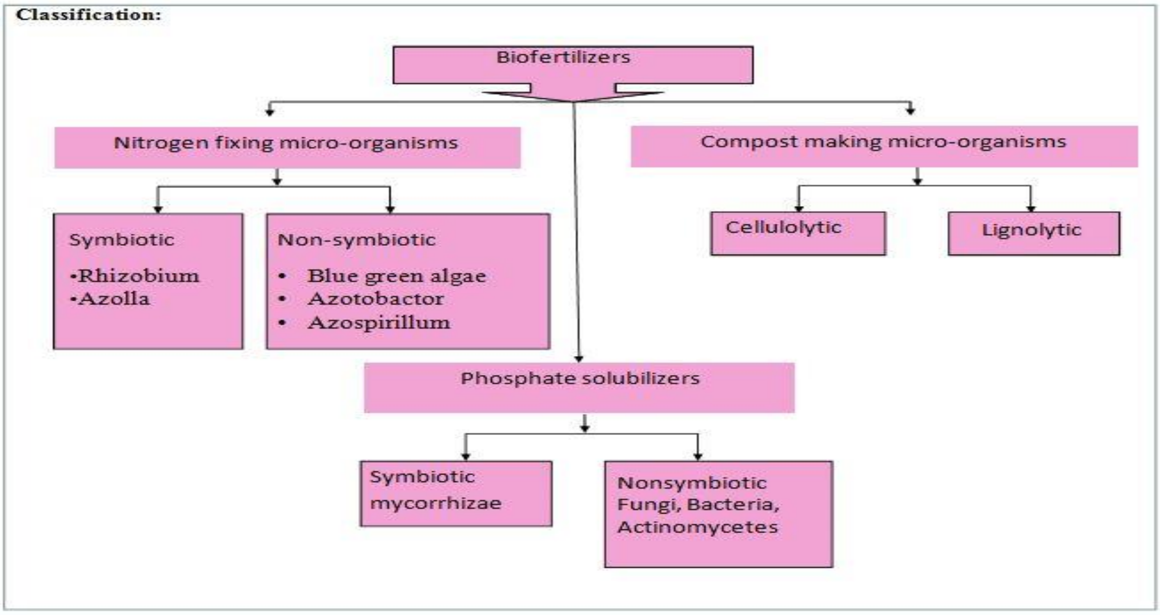
Abstract

Bio-fertilizers are living microbial inoculants of bacteria, algae, fungi alone or in combination and they augment the availability of nutrients to the plants. Bio-fertilizers in agriculture assumes special significance, particularly in the present context of increased cost of chemical fertilizer and their hazardous effects on soil health. Increase in human population raises a big threat to the food security of each people as land for agriculture is limited and even getting reduced with time. Therefore, it is essential that agricultural productivity should be enhanced significantly within the next few decades to meet the large demand of food by emerging population. The present review elucidates various mechanisms that have been exerted by bio-fertilizers in order to promote plant growth and also provides protection against different plant pathogens.

Introduction

Bio-fertilizer is a broad used for products containing living or dormant micro- rganisms such as bacteria , fungi, actinomycetes and algae alone or in combination, which on application help in fixing atmospheric N or solubilise/ mobilize soil nutrients in addition to secreting growth- promoting substances. They are also known as bio-inoculants or microbial cultures strictly speaking, although widely used , the term bio-fertilizer is a misnomer. Unlike fertilizer, these are used to provide nutrients present in them except in the case of azolla used as green manure. Chemical fertilizers Which are now being used extensively since the green revolution have depleted soil health by making the soil ecology non-inhabitable for soil micro flora and micro fauna which are largely responsible nutrients to plants. Bio fertilizers are the products containing one or more species of micro-organisms which have the ability to mobilize nutritionally important elements from non usable to usable from through biological process such as nitrogen fixation, phosphate solubilisation, excretion of plant growth promoting substances or cellulose and bio-degradation in soil, compost and other environments . The role of bio-fertilizer in agriculture assumes special significance, particularly in the present context of increased cost of chemical fertilizer and their hazardous effect on soil health.



N-fixing biofertilizers:

- Rhizobium
- Azotobacter
- Azospirillum
- Clostridium
- Acetobacter
- BGA or cyanobacteria and the fern Azolla (which works in symbiosis with BGA).

Rhizobium:

Bacteria of the genus Rhizobium are able to establish symbiotic relationships with many leguminous plants, as a result of which the nitrogen gas (N2) of the air is “fixed” or converted to ammonium ions that can be utilized by plants. These bacteria survive in the soil as spores. Where a root of a compatible species grows close to the spore, recognition occurs and symbiosis begins. The root hair curls and an infection thread appears from the spore and enters the root cells. The root responds by multiplying cells and these form the nodules on the roots that contain the bacteria. The root nodules act as the site of N fixation. The optimal temperature for their growth is 25–30 °C and the optimal pH is 6–7. Inoculation with

Rhizobium is recommended for legumes (pulses, oilseeds and forages). On average, yield response to Rhizobium inoculation varies from 10 to 60 percent depending on the soil–climate situation and efficiency of the strain.

The *Rhizobium* species that can form nodules and fix N with specific leguminous plants are:

Rhizobium sp	**Crops**
R. leguminosarum	Peas *(Pisum)*, lathyrus, vicia, lentil *(Lens)*
R. Tripoli	Berseem *(Trifolium)*
R. phaseoli	Kidney bean *(Phaseolus)*
R. lupine	*Lupinus. Ornithopus*
R. japonicum	Soybean *(Glycine)*
R. meliloti	*Melilotus.* Lucerne *(Medicago)*, Fenugreek *(Trigonella)*
Rhizobium ciceri	Chickpea
Rhizobium etli	Beans

Azotobacter:

Azotobacter is a non-symbiotic, aerobic, free-living, N-fixing soil bacterium. It is generally found in arable soils but its population rarely exceeds 102–103/g soil. Its six species are: *Azotobacter armeniacus, A. beijerinckii, A. chroococcum, A. nigricans, A. paspali* and *A. vinelandi*. Unlike Rhizobium, inoculation with Azotobacter can be done for a wide variety of crops. Grain yields obtained from plots untreated with fertilizer N but inoculated with N-fixing bacteria are similar to yields obtained from the application of 20–35 kg N/ha.

Azotobacter also synthesizes growth-promoting substances, produces group B vitamins such as nicotinic acid and pantothenic acid, biotin and heteroauxins, gibberellins and cytokinin-like substances, and improves seed germination of several crops. Both carrier-based and liquid-based Azotobacter biofertilizers are available. It is recommended as a biofertilizers for cereals and horticultural crops including flowers and vegetables. Its application is usually done through seed treatment, seedling treatment or soil application

Azospirillum:

Azospirillum, a spiral-shaped N-fixing bacteria, is widely distributed in soils and grass roots. Major species of Azospirillum are Azospirillum brasilense and Azospirillum lipoferum. It can fix 20–50 kg N/ha in association with roots. It also produces hormones such as indole acetic acid (IAA), gibberellic acid (GA), cytokinins and vitamins.

Acetobacter:

Acetobacter is a rod-shaped, aerobic, N-fixing bacteria. Acetobacter diazotrophicus is an N-fixing bacteria found in the roots, stems and leaves of sugar cane with the potential to fix up to 200 kg N/ha. It is capable of growth at pH 3. It can also solubilize insoluble forms of P. Inoculation with Acetobacter is recommended for sugar cane.

Blue green algae:

BGA are photosynthetic, unicellular, aerobic, N-fixing algae. They are also known as cyanobacteria and are used primarily as a biofertilizer in flooded-rice culture. More than 100 species of BGA are known to fix N. Commonly occurring BGA are Nostoc, Anabaena, Aulosira, Tolypothrix and Calothrix. These are used as biofertilizer for wetland rice (paddy) and can provide 25–30 kg N/ha in one crop season, or up to 50 kg N/ha/year. The BGA also secrete hormones, such as IAA and GA, and improve soil structure by producing polysaccharides, which help in the binding of soil particles (resulting in better soil aggregation). BGA are also used as a soil conditioner and, through mat formation, they protect the soil against erosion.

Soil pH is the most important factor in determining BGA growth and N fixation. The optimal temperature for BGA is about 30–35 °C. The optimal pH for BGA growth in culture media ranges from 7.5 to 10, and its lower limit is about 6.5–7. Under natural conditions, BGA growth is better in neutral to alkaline soils. BGA need all the plant nutrients for their growth and N fixation. N fertilizers generally inhibit BGA growth and N fixation. Adequate available P should be present in the floodwater as P enhances BGA growth and N fixation. Consequently, P deficiency causes drastic reduction in BGA growth and, hence, in N fixation. Mo is another essential nutrient for the growth and performance of BGA.

Azolla is another N-fixing biofertilizer of specific interest in rice cultivation. Azolla itself is a fern. N fixation is carried out by the cyanobacterium *Anabaena azollae* in the leaf cavities of Azolla. The most common species of Azolla are:

***Azolla pinnata*:**

This is the most important species. It is widespread in the Eastern Hemisphere, tropical Africa, Southeast Asia, etc. Of its two forms, *Azolla pinnata* var. *pinnata* and *Azolla pinnata* var. *imbricata*, *pinnata* is more common. Its favourable temperature is 20–30 °C.

***Azolla caroliniana*:** A multitolerant species of *Azolla*, it is pest resistant, shade tolerant and thrives under a wide temperature range.

***Azolla filiculoides*:** It is cold tolerant (-5 ºC), and heat sensitive (exceeding 30 °C).

***Azolla microphylla*:** It is heat tolerant but cold sensitive.

***Azolla nilotica*:** Reported to occur in the Nile River in Africa.

On average, dry *Azolla* contains 2.08 percent N, 0.61 percent P2O5, 2.05 percent K2O, and has a C:N ratio of 14:1. It is known to accumulate significant amounts of K. *Azolla* can accumulate 30–40 kg K2O/ha from irrigation water in the paddy- field. The N-enriched *Azolla* biomass is incorporated into the soil, thus providing the N fixed by the cyanobacteria and all other nutrients absorbed by the fern from the soil and irrigation water. Thus, it is more of a green manure than a conventional biofertilizer. One crop of *Azolla* can provide 20–40 kg N/ha to the rice crop in about 20–25 days.

P-solubilizing/mobilizing biofertilizers:

These include phosphate- solubilizing bacteria (PSB) and phosphate-solubilizing micro-organisms (PSMs), e.g. Bacillus, Pseudomonas and Aspergillus. Mycorrhizae are nutrient-mobilizing fungi, also known as vesicular-arbuscular mycorrhizae.

Composting accelerators:

(i) Cellulolytic (Trichoderma); and

(ii) Lignolytic (Humicola).

Plant-growth-promoting rhizobacteria (PGPR): Species of Pseudomonas. These do not provide plant nutrients but they enhance plant growth and performance.

Phosphate-solubilizing biofertilizers:

There has been much research conducted on the use of organisms to increase P availability in soils by "unlocking" P present in otherwise sparingly soluble forms. These microbes help in the solubilization of P from PR and other sparingly- soluble forms of soil P by secreting organic acids, and in the process decreasing their particle size, reducing it to nearly amorphous forms. The earliest known commercial P-solubilizing biofertilizer, Phospho-bacterin, contained Bacillus megatherium var. phosphaticum. Phosphate-solubilizing organisms include:

Bacteria:

Bacillus megatherium var. phosphaticum, Bacillus polymyxa, Bacillus subtilis, Pseudomonas striata, Agrobacterium sp. Acetobacter diazotrophicus, etc.

Fungi: Aspergillus awamori, Penicillium digitatum, and Penicillium belaji;

Yeast: Saccharomyces sp., *etc*.

Actinomycetes: Streptomyces sp., Nocardia sp.

Phosphate -mobilizing biofertilizers:

The most prominent among nutrient mobilizers in the soil are the soil fungi mycorrhizae. These form symbiotic relationships with the roots of host plants. These are of two types:

Ectomycorrhizae:

These form a compact sheath of hyphae over the surface of roots of a limited number of plants such as Pinus and Eucalyptus.

Endomycorrhizae:

These penetrate the roots and grow between the cortical cells. They produce storage "vesicles" ("saclike" structures) between the cells and multibranched "arbuscules" within the cells. Hence, the name vesicular- arbuscular mycorrhizae (VAM). They also produce thin hyphae that grow out up to 2 cm from the root surface.

VAM

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Responses to field inoculation with VAM are rare except in crops such as onions that have no root hairs to facilitate P uptake and require a rapid supply of P. Responses to soil inoculation do not occur where there is ample P in the soil. Because mycorrhizae cannot be cultured in the same way as rhizobia, commercial inoculation is not possible at this stage. Where inoculation is required, soil from infected plants is used. Application of organic manures stimulates VAM.

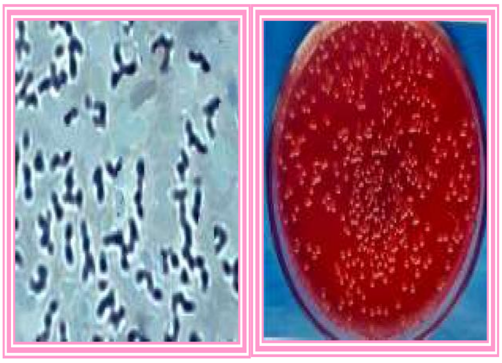
The relationship between mycorrhizae and plant roots is useful in improving the capability of plants for soil exploration and nutrient uptake. VAM have been associated with increased plant growth and with enhanced accumulation of plant nutrients, mainly P, Zn, Cu and S, primarily through greater soil exploration by the mycorrhizal hyphae. Out of their special structures, the arbuscules help in the transfer of nutrients from the fungus to the root system and the vesicles store P as phospholipids. Thus, the exploratory capacity of the root system is improved far beyond the zones of nutrient-depleted soil that may surround the root.

Types of Bio-fertilizer:

AZOTOBACTOR

RHIZOBIUM

VA-MYCHORRHIZA



AZOSPIRILLUM

List of commonly produced bio-fertilizers in India

Name	Crops suited	Benefits usually seen	Remarks
Rhizobium strains	Legumes like pulses, groundnut, soybean	10-35% yield increase, 50-200 kg N/ha.	Fodders give better results. Leaves residual N in the soil.
Azotobacter	Soil treatment for non-legume crops including dry land crops	10-15% yield increase- adds 20-25 kg N/ha	Also controls certain diseases.
Azospirillum	Non-legumes like maize, barley, oats, sorghum, millet, Sugarcane, rice etc	10-20% yield increase	Fodders give higher/enriches fodder response. Produces growth promoting substances. It can be applied to legumes as co-inoculant
Phosphate Solubilizers (there are 2 bacterial and 2 fungal species in this group)	Soil application for all crops	5-30% yield increase	Can be mixed with rock phosphate.
Blue-green algae and Azolla	Rice/wet lands	20 -30 kg N/ha, Azolla can give biomass up to 40-50 tonnes and fix	Reduces soil alkalinity, can be used for fishes as feed. They have growth

		30-100 kg N/ha	promoting hormonal effects.
Microhizae (VAM)	Many trees, some crops, and some ornamental plants	30-50% yield increase, enhances uptake of P. Zn, S and Water.	Usually inoculated to seedlings.

Application of biofertilizers to crops:

Seed treatment

Each packet (200g) of inoculants is mixed with 200 ml of rice gruel or jaggery solution. The seeds required for one hectare are mixed in the slurry so as to have uniform coating of the inoculants over the seeds and then shade dried for 30 minutes. The treated seeds should be used within 24 hours. One packet of inoculant is sufficient to treat to 10 kg seeds. Rhizobium, Azospirillum, Azotobacter and Phosphobacteria are applied as seed treatment.

Seedling root dip

This method is used for transplanted crops. Five packets (1.0 kg) of the inoculants are required for one ha and mixed with 40 litres of water. The root portion of the seedlings is dipped in the solutions for 5 to 10 minutes and then transplanted. Azospirillum is used for seedling root dip particularly for rice.

Soil treatment

4 kg each of the recommended biofertilizers are mixed in 200 kg of compost and kept overnight. This mixture is incorporated in the soil at the time of sowing or planting.

Use of VAM Biofertilizer

- The inoculum should be applied 2-3 cm below the soil at the time of sowing.
- The seeds are sown or cuttings planted just above the VAM inoculums so that the roots may come in contact with the inoculums and cause infection.

- Bulk inoculum of 100 gm is sufficient for one meter square area.
- Seedlings raised in the polythene bags need 5-10 g of bulk inoculums for each bag.
- At the time of planting of saplings, VAM inoculums is to be applied at the rate of 20g /seedling in each spot.
- In the existing tree, inoculums of 200 g is required for each tree.

Use of Blue Green Algae (BGA)

- Algal culture is applied as dried flakes at 10 kg/ha over the standing water in field rice.
- This is done two days after transplanting in loamy soils and six days after planting in clayey soils.
- The field is kept water logged for few days immediately after algal application.
- The biofertilizer is to be applied for 3-4 consecutive seasons in the same field.

Use of Azolla

- **Green manure:** Azolla is applied @ 0.6-1.0 kg/m^2 (6.25-10.0 t/ha) and incorporated before transplanting of rice.
- **Dual crop:** Azolla is applied @ of 100 g/m^2 (1.25t/ha), one to three days after transplanting of rice and allowed to multiply for 25-30 days. Azolla fronds can be incorporated into the soil at the time of first weeding.

Conclusion

Bio fertilizer help in increasing crop productivity. Bio fertilizer replace 25-30% of chemical fertilizers. Bio fertilizers can be applied through seed treatment, seedling inoculation, set inoculation, seed pelleting. Carrier based bio fertilizer commercialized on large scale.