# MICROORGANISMS AND CARRIER MOLECULES USED IN BIOFERTILIZER FORMULATIONS

**ABSTRACT**

Rhizobacteria with plant growth promoting potential are used widely for agricultural use. They have been used as supplements to chemical fertilizers. Cyanobacteria, nitrogen fixing bacteria, phosphate mineralizing bacteria, and potassium solubilizing microorganisms have been used as biofertilizers for different crops. The survival of these rhizobacteria in soil is affected by number of abiotic as well as biotic factors that limits the growth of rhizobacteria. To enhance the survival rate of rhizobacteria in soil and to enhance their viability and efficiency, the bacteria is mixed with carrier material or additive to increase their activity. Carrier molecule enhances the adhesion of bacteria to the plant roots. The processing of bacteria with carrier molecule ensures easy handling and processing or agricultural use. Addition of additive molecule to the bacterial culture provides excellent survival rate and slow cell release at different soil pH. Different additive molecules used as carrier molecules are discussed in this article.

**Keywords:** Biofertilizers, inoculants, carrier molecule, additive, growth, development

## 1. Introduction

Biofertilizers are "Preparations containing live microbes that augment the plant growth by enhancing nutrient availability in soil and protecting the crop from various biotic and abiotic factors (Kaur and Kaur, 2020). Biofertilizers holds great potential in sustainable agriculture. They can be used as a replacement to chemical fertilizers in organic farming and forms an important part of integrated nutrient management system. Microorganisms including phosphate and potassium solubilizing fungi/bacteria, nitrogen-fixers, cyanobacteria and other microorganisms with plant growth promoting traits can be utilized as biofertilizers (Rani et al., 2020). One of the most attractive features of biofertilizers is their environment friendly and cost-effective nature.

Different micro inoculums are used as biofertilizer for good practices of agriculture for increasing crop yield. These microorganisms themselves contain various properties which are vital for increasing the product value. The microbial formulations are used to stimulate certain processes which can convert insoluble nutrients to soluble forms that can be used by plants. The microbial inoculum is mixed with carrier molecule that helps the microbial culture to bind to it and maintain its viability for longer period. A carrier is basically a formulation carrying microorganisms, peat, vermiculite, lignin, etc. (Kaur and Kaur, 2018). Different microorganisms that can be used as biofertilizer are discussed below-

## 1.1Cyanobacteria

Cyanobacteria (blue green algae) are abundantly found in marine environment and have a great potential to be used for sustainable agriculture (Pisciotta et al., 2012). It is well known to maintain the nitrogen level in soil, improve its water holding capacity and aeration (Paumann et al., 2005). *Nostoc* and *Anaebena* are two most common cyanobacteria used as biofertilizer (Table 1). The striking feature of cyanobacteria that makes it suitable for use as biofertilizer is that it does not require any kind of substructure or host for its cultivation, growth, and production of molecules responsible for plant growth promoting. *Azolla-Anaebena* that is known for its symbiotic association also serves to increase crop yield. Cyanobacterial biofertilizers can be directly supplied into the soil or can be applied to the seeds. Due to the exopolysaccharide secreting potential, the cyanobacteria can be used for the reclamation of infertile soils (Paul and Nair, 2008). They also have roles in reducing global warming and maintaining bio-geochemical cycles (Fig 1).

**Table 1.** Effect of cyanobacteria on different crop

| **Bacterial Species** | **Crop Effect** | **Effect on the crops** | **Reference** |
|---|---|---|---|
| *NostocPunctiforme* PCC 73102 | Rice (*Oryza sativa* L.) | Yield of plant was increased | Álvarezet al., (2020) |
| Cyanobacteria | Lettuce (*Lactuca sativa L)* | Leaf length, fresh and dry weight of the plant was increased | Menamo &Wolde (2013) |
| Cyanobacterium *Anabaena* | Wheat | Phosphorous uptake was enhanced | Swarnalakshmi et al., (2013) |

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| Nostoc | Maize | Enhances structure of soil, soil fertility, and growth of maize | Maqubela et al., (2009) |

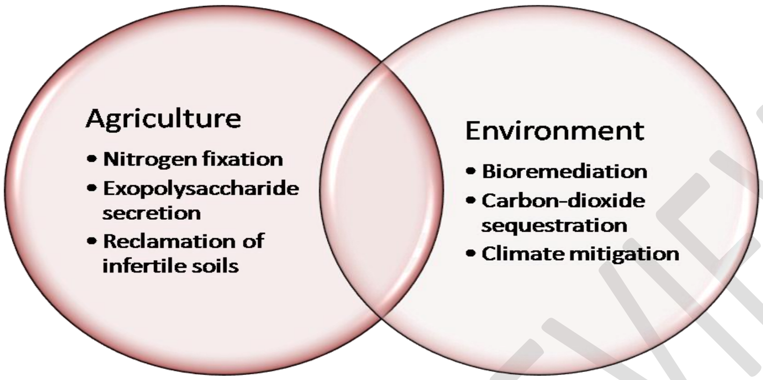


**Fig.1**. Different roles of cyanobacteria (Pisciotta et al., 2012)

## 1.2. **Nitrogen fixing bacteria**

Nitrogen is the macro nutrient essential for the growth and development of non-legume plants and cereals. Therefore, nitrogen must be supplied through fertilizers. However, injudicious use of synthetic nitrogenous fertilizers contributes to contamination of soil and groundwater, leading to human health hazards, and threatens agricultural sustainability (Kaur and Kaur, 2018). Rhizobium containing biofertilizer enhance the productivity of leguminous plants through supply of nitrogen via symbiotic associations. These bacteria induce nodule formation in leguminous plants. Various other bacteria belonging to the genus *Azotobacter*, *Azoarcus*, *Burkholderia*, *Enterobacter*, *Gluconacetobacter*, *Azospirillum*, *Pseudomonas* have potential to fix nitrogen non-symbiotically in wheat, rice, sunflower, maize and other non-legume crops (Benidire et al., 2017) (Table 2,3).

**Table 2.** Effect of nitrogen fixing bacteria on different crop

| **Bacterial species** | **Crop** | **Effect on the crops** | **Reference** |
|---|---|---|---|
| *Azospirillum lipoferum* or *Azotobacter* | Maize | It helps in maintaining the negative effects on salt stress on maize | Latefet al., (2020) |

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| *chroococcum* | | plants. Improves physiological activities under saline conditions for maize growth. | |
| *Azospirillum brasilense* | Maize | Enhanced growth of maize. Nitrogen use efficiency of maize was also increased. | Zeffa et al., (2019) |
| *Rhizobium leguminosarum* | Lettuce (Lactuca sativa) and Carrots (Daucus carota) | Dry matter weight was increased. Increases uptake of N and P in the edible parts of both plants. | Flores-Félix et al., (2013) |
| *Rhizobium Leguminosarum* (RhOF34, RhOF125 and RhOF15) | Broad bean, Fava bean, (VICIA FABA) | Improved growth of the plants. Mineral and nitrogen uptake was improved. | Benidire, (2017) |
| *Pseudomonas stutzeri* A1501 | Rice | It helps in the growth of rice in the presence of salt concentration and heavy metals. | Han, (2015) |

## 1.3Actinomycetes

Actinomycetes comprise a major group of microorganisms which are found in rhizosphere and inside the plant roots as endophytes and are documented for their role to improve plant growth. They are well known for their potential to recycle nutrient by degradation of chitin, cellulose, starch, lipids and complex carbohydrates and converting them into simple sugars. Actinomycetes are the key component of agricultural ecosystems and are important in promotion of plant growth by various means (Fig. 2). These microorganisms around the plant root surfaces perform an important role of breakdown of organic matter and making it available for the plant uptake. These microorganisms also show their potential role in solubilization of phosphate, production of siderophores, hydrogen cyanide, auxin, ammonia and lytic enzymes (Sousa et al., 2016). Their morphology renders them more efficient for solubilizing phosphorus by increasing surface to volume ratio. Actinomycetes are known to secrete various enzymes and low molecular weight organic acids that help in solubilization of phosphate and potash rocks. Some species of actinomycetes such as *Streptomyces venezuelae, S. alboniger, S. ambofaciens* and *S. lienomycini* have been known to secrete phytate degrading enzymes phytase that help in solubilization of organic phosphorus pool in the soil and making it available to the plants. The potential of actinomycetes to produce indole acetic acid also makes them important candidate for use as biofertilizers (Rani et al., 2018).

**Table 3.** Effect of nitrogen fixing bacteria on different crop

| **Bacteria species** | **Crop** | **Effects on the crop** | **Reference** |
|---|---|---|---|
| Bacillus spp. | Crop plants (sugar cane, wheat, rice, corn, maize) | Helped managing the abiotic stresses (water, salinity and heavy metal). | Nanjundappa (2019) |
| *Micrococcus sp.* TISTR2221 | Maize grain (*Z. mays L.*) | Significantly promoted shoot length, root length, and dry biomass of Maize. | Sangthong et al., (2016) |
| *Flavobacterium johnsoniae* strain GSE09 | Black Pepper (Piper nigrum) | Exhibited biofilm formation and produced indolic compounds and showed biocontrol activity against *Phytophthora capsici.* | Sanget al., (2012) |
| *Aspergillus niger F7* | Crop plants (sugar cane, wheat, rice, corn, maize) | Maintained available phosphate level for crops. | Srividya et al., (2009) |
| *Penicillium funiculosum* LHL06 | Soybean (Glycine max L.) | Improved soybean crop growth by reducing toxic effects on metals. Gibberellin production was observed during copper stress. | Khan & Lee (2013) |

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| *Pseudomonas fluorescens* | Crop plants (sugar cane, wheat, rice, corn, maize) | Known to produce antibiotics, volatiles, siderophores, and growth-promoting substances | Khan & Lee (2013) |
| *Pseudomonas fluorescence (SS5)* | Tomato | Plant growth promotion reported due to increase in root, shoot weight, length and fruit yield per plant. | Ahirwar et al., (2015) |



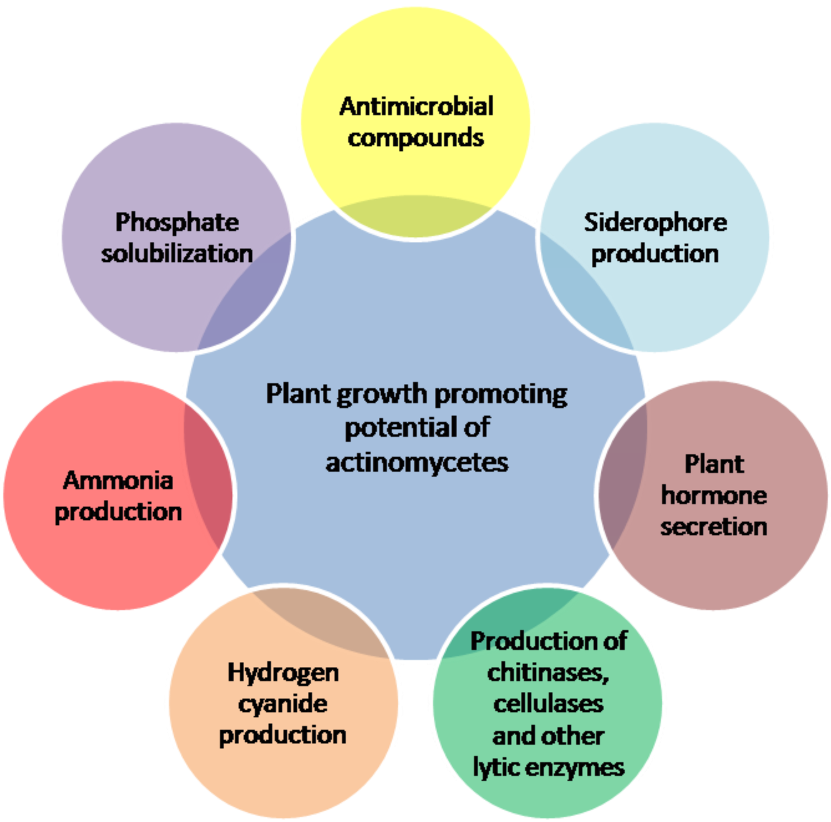


**Fig- 2**. Different bioactive produced by actinomycetes (Sousa et al., 2016)

## 2. Carrier and Additives for Biofertilizer

Plant growth promoting microorganisms are served as bioinoculants for agricultural use by incorporating them in carrier material for easy handling, high effectiveness and high shelf life. Various different types of carrier materials are used for biofertilizer development (Fig. 3). The carrier material should have certain properties such as:

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
5. Available in adequate amounts
6. Inexpensive
7. Good adhesion properties
8. non-toxic to plant

For soil inoculation, carrier material like peat, charcoal, perlite, clay of size 0.5-1.5mm is generally used. For seed inoculation, the carrier material should be milled to fine powder with particle size of 10 -40 µm.

**2.1 Clay Minerals as Additives**

There are some wide ranges of carrier material used in bio immobilized system which provides various properties such as bulking capacity, stability and protection against physical stress. Torelease microorganism slowly into the soil and to protect the microbial cells against dessication, the combinations of soil microorganism inoculants and clay minerals is used (Bashan et al., 2002). This combination had provided a great effect on the significantly increasing microorganism survival by reducing UV effects compared to normal cells (He et al., 2015). For example, when *Pseudomonas* microencapsulated in a mixture of alginate and starch was combined, under saline conditions there was increase in soluble protein content, carotenoid concentrations and cotton biomass, chlorophylls (He et al., 2017). Clay minerals such as pyrophyllite, bentonoite and kaolin in combination with alginate, is used for immobilization of *Pantoea agglomerans*, *Trichoderma harzianum* and other plant growth promoting bacteria (Zohar-Perez et al., 2003).

**2.2 Skim Milk**

Skimmed milk is used in bio formulations which enhances cell viability during storage conditions (Yu et al., 2001). Encapsulation of *Azospirillum* and *Pseudomonas* with skim milk powder significantly increases the cell number and viability (Bashan et al., 2002). The cell release rate is also found to higher in cells encapsulated with skim milk. The recovery rate of 100% in *P. fluorescens* was observed in alginate- skim milk encapsulated beads. For encapsulation of rhizobacteria, skim milk can be used in combination of clay mineral for strategic increase in cell count and cell viability.

### 2.3 Starch

Starch is used as carrier or additive for use in encapsulation of rhizobacteria. Starch is reported to improve the survival of bacterial cells by reducing the stress on microbial cells (Bashan et al., 2002). The microbial cells adhere to the starch molecules which protects it from physical stress. The cell adhesion to starch is based on the effect of starch and stress conditions on the microbial cells.

### 2.4 Chitin/Chitosan as Additives

Chitin or its polymer chitosan, is a bioactive oligosaccharide polymer which is used as filler material or a cell protectant in biofertilizers. Chitin is known or its antibacterial activity, non-toxic and biodegradability potential (Muxika et al., 2017). Reports have shown that the encapsulation of *Bacillus* in a matrix containing chitin, improves the multiplication of bacterial cells and enhances its antifungal potential (Manjula et al., 2001). Also, for the biocontrol activity, chitin is an excellent chelating agent against pathogens (Berger et al., 2014) and it also enhances stress tolerance, antioxidant activity, and osmoregulator potential in plants (Dar et al., 2015). Being a coating material, it plays an important role in lowering the formulation cost and providing the strength to plants. Chitosan is an excellent carrier which can maintain the viability of beneficial microorganisms for plants (Chanratana et al., 2018). Chitosan has also been widely used for seed coating. Seed of soyabean, maize, wheat, rice and peanut were found to have better germination rates when coated with chitin (Crini et al., 2019).

### 2.5 Humic Acid Additives

To promote the populations of specific microorganism, humic acid and its derivatives are used in biofertilizers (Pukalchik et al., 2019). Formulation of microbial inoculants immobilized with humic acid additives has shown excellent survival, releases cell slowly at various pH, and provide uniform growth to bacteria. To enhance root colonization by fungi, humic acid is usually added in biofertilizers (Gryndler et al., 2005).

## 3. Conclusion

The development of microbial bioformulations for agricultural use has leads us towards the sustainable option against chemical pesticides and fertilizers. Towards this approach, microbial inoculants with plant growth promoting activities are being used. However, their direct application in soil decreases their viability and plant growth promoting potential. Therefore, they are applied in combination with carrier molecules or additives that help the bacteria binds to it and enhances its survival rate in the soil. Different carrier molecules are used which have been discussed. Currently, the carrier based biofertilizers are being replaced by encapsulated biofertilizers. These additives can also be used as polymer matrix for encapsulation.

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