

USAGE OF INDIGENOUS MICROBIAL STRAINS IN THE DEVELOPMENT OF LIQUID BIOFERTILIZER

Manish Kumar Singh

Research scholar Sai Nath University

Dr. Dinesh Kumar

Professor Sai Nath University

ABSTRACT

A problem for agrotechnology is to increase crop output while simultaneously feeding a growing population without compromising the quality of the surrounding environment. In spite of the fact that it helped achieve food self-sufficiency and security, the green revolution was responsible for a number of unintended consequences. The cultivation of crops evolved into a highly capital- and energy-intensive activity that also contributed to the degradation of soil and water. These kinds of outcomes brought up the issue of sustainability, which refers to keeping output at a higher level without reducing the quality of the environment, as well as making use of the natural resources that are available right now—primarily land, which is a valuable natural resource—without diminishing their capacity for use in the future. A management strategy is required due to the growing use of chemical fertilizers, the high prices associated with them, their low efficiency, the loss of soil structure, and the pollution concerns they cause. The amount of fertilizer that is produced in our nation falls short of the amount that is necessary. Alternative sources of nutrients, like as biofertilizers and organic waste, will need to be sought for in order to fill this void in the supply of nutrients. They have the potential to be utilized as a replacement supply in order to fulfill the nutrient requirements of crops and to fill in any gaps that may appear in the foreseeable future.

Keywords: Biofertilizer, Soil fertility, Crop productivity

INTRODUCTION

The high expense of nitrogen fertilizers in recent years, along with increased environmental awareness over the usage of chemical fertilizers, has been a catalyst for the quest for an alternative that can increase agricultural production per unit area. In light of the potentially damaging and dangerous consequences of chemical fertilizers, biofertilizers present a potentially safer option to chemical inputs. Furthermore, they will assist in the process of lowering the rate at which ecological disturbance occurs. A product that contains living microorganisms and, when applied to seeds, plants, or soil, colonizes the rhizosphere or the inside of the plants and promotes plant growth by boosting the availability of nutrients to the host plant is referred to as a biofertilizer. Under optimal conditions, the fixation of biofertilizers can contribute up to 30–300 kg of nitrogen per hectare, and they can also solubilize immobilized 30–50 kg of phosphorus–2–5 per hectare. They do this by releasing chemicals and vitamins that encourage growth and by contributing to the continued fertility of the soil. The occurrence of pathogens that cause plant illnesses can be reduced by using biofertilizers, which results in an increase of 10–35% in crop field. The majority of the nitrogen that is fixed on a global scale is done so through biological processes. The symbiosis between legumes and rhizobia is the most promising since it provides around 80–90% of the total amount of nitrogen that legumes require. The ensuing cereal crop benefits

from the symbiotic relationship's residual nitrogen. When used for an extended period of time, biofertilizers improve the fertility level of the soil while also being cost effective and environmentally benign. According to Gaur (2010), they are the most cost-effective source of plant nutrients, as well as highly major suppliers of micronutrients and organic matter, secretors of growth hormones, and contributors to mitigating the adverse effects of the use of artificial fertilizers.

History of biofertilizer

The yield of crops is the primary focus of agricultural activities. In order to achieve higher levels of productivity, chemical fertilizers are utilized. It has a detrimental impact on the soil's health because it alters the chemical makeup, microbial flora, and ecology of the soil, all of which can have an effect on the soil's biodiversity. In the early nineteenth century, chemical fertilizer manufacturers began developing synthetic fertilizers and pesticides consisting of phosphorous (P), potassium (K), and nitrogen (N) to enhance crop productivity while also protecting plants from a variety of illnesses. These were done in an effort to combat the widespread use of natural fertilizers and pesticides. According to studies, the application of chemicals over extended periods of time and in a continuous manner led to the acidity of the soil and a decrease in the soil's quality, both of which had a negative impact on human health and contributed to environmental instability (Geisseler and Scow, 2014). As a result of this, there is a growing need to have alternative sustainable farming practices in order to boost crop output. By preserving the variety of ecosystems and enhancing the health of the soil, microorganisms play a significant part in this method of ensuring the continuation of sustainable agricultural practices. Plant growth-promoting bacteria, also referred to as PGPB for short, are bacteria that have characteristics that are beneficial for the plant in terms of growth and protection against disease. When the discovery of *Beauveria bassiana* infection in silkworm was made by Bassi in 1835, bioformulations were already common agricultural practice. Because of this revelation, the important role that bacteria play in illness prevention was uncovered. According to Sayyed et al. (2003), the discovery of the Bt (*Bacillus thuringiensis*) toxin provided additional support for the argument that researchers should think more about microbes as an alternative for pesticides. After some time, it was discovered that the majority of the bacteria had the ability to promote plant development and operate as biocontrol agents. According to Glick and Bashan (1997), a number of studies indicated that the successful application of diverse bioformulations in controlling the disease and improving plant growth was possible. In the latter part of the eighteenth century, the process of commercializing PGPR began, and the substance quickly acquired popularity as a result of its successful application as bioinoculants. The use of PGPB in environmentally friendly farming practices is becoming increasingly important. The mechanism of action of these microbial inoculants might vary and is highly dependent on the host as well as the area. On the basis of the characteristics expressed by these organisms, a large number of biofertilizers with a wide variety of different formulations have been developed. Recent research in the field of agriculture has shown that the activities of microbiomes in soil and sustainable agricultural practices are intricately linked to one another (Fig. 1; Tables 1 and 2).

Table.1 Major inoculation groups with inoculant and host plants

Cross inoculation Group	<i>Rhizobium</i> species	Host Legume
Pea group	<i>R. leguminosarum</i>	Pea, sweet pea

Alfalfa group	<i>R. meliloti</i>	Sweet clover
Clover group	<i>R. trifoli</i>	Clover / berseem
Bean group	<i>R. phaseoli</i>	All beans
Soybean group	<i>Bradyrhizobium japonicum</i>	Lupins
Cowpea group	<i>Rhizobium</i> sp.	Cowpea, arhar, urd, moong and groundnut

Table.2 Effect of *azotobacter* on crop yield (Dudeja *et al.*, 1981)

Crop	Increase in yield over yields obtained with chemical fertilizers (%)	Crop	Increase in yield over yields obtained with bio fertilizers (%)
Wheat	8-10	Potato	16
Rice	5	Carrot	40
Maize	15-20	Cauliflower	2-24
Sorghum	15-20	Tomato	7-27
Other	13	Cotton	9-24

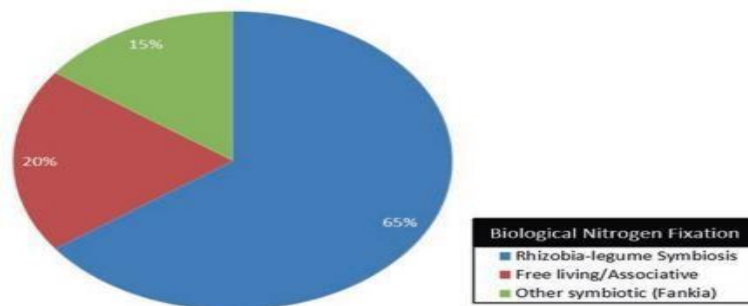


Fig.1 Biological nitrogen fixation

Symbiotic N₂ fixers

In a symbiotic relationship with Rhizobium, which can be found in the root or stem nodules of leguminous plants, these plants are able to fix nitrogen from the atmosphere. Rhizobium is a type of bacterium that fixes nitrogen into the soil. These gram-negative bacteria are rod-shaped, motile, and feature one polar or subpolar flagellum. The establishment of a successful Rhizobium legume symbiosis is contingent upon the entry of a micro symbiont, known as Rhizobia, into a macro symbiont, known as legume. This entry takes place in stages. Infection and the development of nodules both appear to be host-specific events. According to Allito *et al.* (2015), the genesis of this symbiotic relationship needs a complicated interaction between the host and

the symbiont. This interaction ultimately leads to the production of nodules in which Rhizobia colonize as intracellular symbionts. Rhizobium, Bradyrhizobium, Sinorhizobium, Azorhizobium and Mesorhizobium are collectively referred as Rhizobia. The presence of a symbiotic relationship between a Rhizobium strain and its host may be established when a host legume lectin (glycoprotein) binds to a specific carbohydrate receptor on the Rhizobium cell surface. Lectins have recognition functions. The only two people who can become bound together are those who are compatible. When binding takes place, the root hair at the tip of the root bends, allowing bacteria to enter and develop in the form of an infection tube. Rhizobia only proliferate in tetraploid cells because normal diploid cells are eradicated in the process. Following the production of growth hormones, the root epidermal cells will begin the process of mitosis, or cell division. In order to generate a nodule, the cells that are next to diploid cells in the surrounding area are stimulated to divide quickly. The quickly dividing bacteria populate malformed cells known as bacteroides that are more than ten times as large as rhizobia. The peribacteroid membrane surrounds the bacteroids, whether they are present singly or in groups. Because it contains leghaemoglobin, the tissue that contains the bacteroids appears red. As a result of the degradation of leghaemoglobin into green bile pigments, the nodules turn green as a normal part of the aging process. The death of the nodules triggers the release of stationary phase rhizobia, which then have the ability to proliferate by utilising the byproducts of nodule breakdown as a food source. Nitrogenase is an enzyme that is present in all nitrogen-fixing microorganisms. This enzyme has the ability to convert nitrogen into ammonia with the assistance of energy in the form of ATP. The nitrogenase enzyme consists of two different components, one of which contains Mo-Fe protein and the other consists of Fe protein. Azorhizobium is a species of stem nodule-forming symbiotic bacteria that belongs to the Rhizobium genus. It is responsible for the formation of stem nodules and the fixation of atmospheric nitrogen. In addition to this, they create a sizeable quantity of indole acetic acid (IAA), which stimulates the growth of plants. Not only is Bradyrhizobium an effective nitrogen fixer, but when it is inoculated in mucuna seeds, it also increases the amount of total organic carbon, nitrogen, phosphate, and potassium that is present in the soil. As a result, it encourages the growth of plants, increases the population of microbes in the soil, increases the biomass of plants, and decreases the number of weeds.

Free living N₂ fixers

Fixers of nitrogen who can live independently or in associations The bacteria Azotobacter is capable of fixing nitrogen in the environment. This bacteria is extremely aerobic and heterotrophic, and it thrives in pH ranges ranging from neutral to alkaline. The genus Azomonas and the species Azotobacter are both included in the family Azotobacteriaceae as members. Azotobacter vinelandii, Azotobacter beijerinckii, Azotobacter insignis, and Azotobacter macrocytogenes are some of the other species that have been described. A. chroococcum is the species that is most common in the soil in this area. In the rhizosphere of a wide variety of crop plants, including rice, maize, sugarcane, bajra, vegetables, and plantation crops, the presence of Azotobacter has been documented. Azotobacter possesses a number of characteristics that are exclusive to it, including the production of cysts that hold a novel liquid, the presence of more than one type of nitrogenase, and an exceptional tolerance to oxygen. In order to flourish, Azotobacter requires a pH that is neutral to slightly alkaline. Azotobacter is the free-living nitrogen-fixing organism that has been studied and isolated the most frequently. It is an important component of the natural flora and the fertility of the soil. It is believed that Azotobacter makes a significant contribution to the nitrogen content of soil. This organism possesses a number of beneficial properties, including increased nitrogen fixation, ammonia excretion, production of vitamins and growth promoters, production of siderophores, and production of antifungal

antibiotics. It has been discovered that *Azotobacter* secretes compounds that increase root growth and the uptake of plant nutrients. Additionally, these molecules limit the growth of specific root diseases.

Azolla as a biofertiliser

Azolla is a free-floating cyanobacterium that floats in water and fixes atmospheric nitrogen in combination with nitrogen-fixing blue-green algae called *Anabaena azollae*. Azolla, which contains between 0.2 and 0.4 percent of nitrogen when it is wet but between 0.4 and 0.5 percent when it is dry, can be a highly helpful source of organic manure and nitrogen for rice farmers. Before rice is planted in a field, azolla can be worked in as a biofertilizer to improve the soil quality. *Azolla pinnata* is the species of Azolla that is most commonly used in India, and it is able to be vegetatively propagated on a large scale for commercial purposes. *Azolla caroliniana*, *Azolla microphylla*, *Azolla filiculoides*, and *Azolla mexicana* are some of the other species of Azolla that may be found in India. These species were brought there because of their high biomass output. The rice plants are able to make effective use of the nitrogen that is provided by azolla, which decomposes easily in the soil. In addition to that, it helps to deliver a sufficient quantity of micronutrients like phosphorus, potassium, zinc, iron, and molybdenum.

Effect of biofertilizer in photosynthesis

According to Long et al. (2006), higher levels of photosynthesis result in improved plant development. This is because around 90 percent of plant biomass is obtained from the assimilation of CO₂ through photosynthesis. It was found that certain test strains of Rhizobia significantly increased the surface areas of plant leaves, the net photosynthetic rate of plants, stomatal conductance, as well as the efficiency with which water was used. This suggests that rhizobial inoculation of rice can increase the photosynthetic capacity of the plant as well. According to Heidari and Golpayegani (2012)'s research, the combination of three bacterial biofertilizers—*Pseudomonades*, *Bacillus lentus*, and—was more effective than using any of them alone.

A. *brasilense* increased the expression of antioxidant enzymes as well as increased chlorophyll content in leaves under stress.

Therefore, the application of biofertilizer to a plant can stimulate the plant's photosynthetic activity, which allows the plant to maintain healthy growth despite adverse environmental conditions.

Effect of biofertilizer in amino acid synthesis

The term "rhizosphere" refers to a community of bacteria in the rhizosphere that has the capacity to colonize the surrounding root environment. Root exudates are the name given to the compounds that are secreted by roots into the surrounding soil. Different microbes can be chemically drawn to these chemicals because of their attractive chemical properties. Exudation of a variety of chemical compounds changes the physicochemical properties of the soil and significantly influences the structure of the soil microbial community in the area immediately surrounding the root surface. Therefore, the types of amino acids that plants produce, in addition to the composition of the root exudates that they emit, are dependent on the species of plants and the microorganisms that are linked with the plant.

Effect of biofertilizer on bioremediation of metals

Pollutants such heavy metals, toxic waste, organic contaminants, and many more have been released into the environment as a result of extensive agricultural activities, rapid increases in industry and urbanization, and other factors that have led to various environmental concerns. Zinc (Zn), arsenic (As), chromium (Cr), cadmium (Cd), mercury (Hg), copper (Cu), nickel (Ni), and lead (Pb) are all examples of potentially hazardous heavy metals that can exist in a variety of valence states. Although plants require metals as micronutrients, an accumulation of heavy metals is detrimental to the majority of plant species. However, a decline in soil fertility and an effect on the microbial community can be caused by excessive concentrations of heavy metals in the soil. Numerous studies have been conducted to investigate the function that PGPR plays in the bioremediation of metal toxicity, and the findings have shown that a wide variety of microorganisms play an essential part in the removal of the harmful effects of heavy metals. *Achromobacter xylosoxidans*, *A. chroococcum*, *B. subtilis*, *B. megaterium*, *Bradyrhizobium*, *Pseudomonas* sp., *Brevibacillus* sp., *Kluyvera ascorbata*, *Mesorhizobium*, *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Ralstonia metallidurans*, *Rhizobium*, *Sinorhizobium* sp., *Variovoxparadoxus*, *Ochrobactrum* sp., *Psycrobacter* sp., and *Xanthomonas* sp. are a few PGPR among the wide range of PGPR that play an important role in bioremediation of heavy metal toxicity. The creation of 1-aminocyclopropane-1-carboxylate (ACC) deaminase is the most important of the many different defense mechanisms that are activated by PGPR. This enzyme lowers the amount of the stress-inducing hormone ethylene that accumulates in plant tissues. The generation of microbial siderophores is found to be an effective strategy by PGPR to lower the toxicity of metals.

Effect of biofertilizer in remediation of pesticides

Because it is simple for it to enter the tissues of living species and lead to bioaccumulation, the use of pesticides in excessive amounts and for an extended period of time has the potential to be harmful to the environment. It also poses a potential threat to the plant kingdom as well as to people. It has been shown that bacteria such as *Azospirillum*, *Azotobacter*, *Bacillus*, *Enterobacter*, *Gordonia*, *Klebsiella*, *Paenibacillus*, *Pseudomonas*, and *Serratia*, among others, have the ability to reduce the toxicity of pesticides. Actinomycetes, in addition to these strains, offer a sizeable potential for the biotransformation and biodegradation of pesticides. Enzymatic degradation is the primary process that microorganisms use to break down pesticides. Enzymatic degradation is the most important. In addition, it has been found that certain enzymes that catalyze a wide variety of reactions, such as hydrolysis, oxidation, addition of amino group to a nitro group, dehalogenation, reduction of nitro group to amino group, replacement of sulfur with an oxygen, ring cleavage, and metabolisms of side chains, can reduce the toxicity that is caused by pesticides. These enzymes can also be found in foods.

Effect of biofertilizers on plant parasitic nematodes

According to El-Haddad et al. (2011), certain bacterial biofertilizers, such as bacteria that fix nitrogen, such as *Paenibacillus polymyxa* (four strains), bacteria that solubilize phosphate, such as *Bacillus subtilis*, and *Bacillus subtilis*,

B. It was discovered that all of the applied microbial biofertilizers showed significant nematicidal activity on tomato plants that were infested with the root-knot nematode M. incognita in potted sandy soil. B. megaterium (three strains) and the potassium-solubilizing bacteria, Bacillus circulans (three strains), were inoculated individually on tomato plants. It was found that inoculating nematode-infested chili (Capsicum annum) with a biological nitrogen fixer (Azospirillum and Azotobacter) boosted the plant's

growth, yield, and quality.

Effect of biofertilizer on ecosystem

The effects of biofertilizers on non-target members of the soil rhizosphere and food web have been studied to a significant extent, and the majority of these studies have suggested that there will be measurable changes as a result of the introduction of bio inoculants to the rhizosphere. However, the magnitude of these changes and their significance on the ecological functions have not yet been reported. According to some reports, the degree to which biofertilizers have an influence on the organisms that live in the soil is dependent on a number of circumstances. These elements include the features of the soil, the technique for applying biofertilizers, the various environmental conditions, and so on. Before releasing biofertilizers into the ecosystem, it is necessary to conduct multi-dimensional analyses of the efficacy, diversity, and risk assessment studies of biofertilizers. These analyses must be conducted using methods that have a better resolution in addition to standard techniques.

Effect of biofertilizer in reclamation of degraded land

The growth of an active indigenous microbial community is essential to the successful reclamation of mine spoil dumps (Juwarkar and Singh, 2007; Kumar et al., 2013). This community is responsible for the formation of the soil structure, the growth of plants, and the production of plant nutrients through a variety of biogeochemical cycles (Juwarkar and Singh, 2007; Kumar et al., 2013). The mining process turns the soil in the mining areas into a very acidic substance, which is detrimental to the growth of plants. According to Diacono and Montemurro (2010), the pH of these soils can be raised by applying organic amendments, which not only raise the pH of the soil but also improve its quality, water-holding capacity, and give a delayed release of fertilizer. As a result, biofertilizers have the potential to partially restore the soil quality of degraded land.

Effect of biofertilizers in crop production

The addition of biofertilizers to the soil plays a significant role in enhancing the fertility of the soil, as well as the yield-attributing characteristics and, as a consequence, the final yield. Because biofertilizers improve the overall health of plants and soil while also boosting crop yields in a manner that is environmentally responsible, they are increasingly being used in agricultural production. Rice farming makes effective use of the nitrogen-rich azolla biofertilizer due to the rapid rate at which it decomposes in soil and the immediate accessibility of the nitrogen it contains to rice plants. The application of Rhizobium biofertilizers in pulse crops grown in climates with moderate temperatures considerably improved the agronomic yield attributes, while the application of Azospirillum in agricultural crops improved the leaf area index, harvest index, and yield attributes.

Mode of application of formulated biofertilizer

There are many methods for applying formulated biofertilizer into soil, and some of these methods are as follows: (a) seed inoculation with powder formulations; (b) dry biofertilizers mixed with the seeds in the seed hopper; (c) biofertilizer and adhesive are applied as slurry to seeds and coated with ground material like lime; (d) slurry method (the biofertilizer is suspended in water then added to the seeds and mixed); (e) (Bashan,1998) suggests the following practices: (h) seed treatment or seed inoculation; (i) soil application; and (j) seedling root dip..

An exciting development in biofertilizer technology: liquid biofertilizers

(Hegde, 2008) A liquid biofertilizer is a suspension that contains the microorganisms that are sought as well as any particular cell protectants or compounds that accelerate the creation of dormant spores or cysts in order to give the product a longer shelf life and greater tolerance to unfavorable circumstances. According to Herrmann and Lesueur (2013), liquid biofertilizers have become increasingly popular due to the ease with which they may be handled and applied, either on seeds or in soil. In general, liquid biofertilizers circumvent the effect of high temperature, maintain high cfu more than 10^9 ml⁻¹ up to 12 months, and better survive on seeds and soil. In addition, liquid biofertilizers are easy to use, handle, and store by farmers; the dosage is ten times less than that of powder form; it can be packed in different volumes; and it saves carrier materials (Ve). These are the advantages that liquid biofertilizers have over powder. In addition, liquid formulations are compatible with the gear typically used on big farms, such as seed augers and air seeders. However, due to certain constraints, its application is now impossible in the vast majority of underdeveloped countries. Because they lack carrier protection, biofertilizers that are derived from broth cultures lose their viability on seed very soon. However, the inclusion of other components such as sucrose, glycerol, and other such substances may boost the microbes' ability to live in liquid conditions.

CONCLUSION

A more widespread use of biofertilizers will involve addressing a few concerns, giving them greater attention, and taking the necessary steps to fix those issues before they can be used more widely. The investigation of the efficacy of a given strain in relation to a certain crop, as well as the soil and climate elements involved. Along with expanding the scope of the extension wing, it is essential to invest more resources in research and development. A standardized approach to the application of biofertilizers to a given crop and soil. In order to expand the use of biofertilizers beyond the scope of research conducted in a laboratory or greenhouse and into large-scale commercial use, a variety of innovative new ways will need to be developed for the cultivation, storage, transportation, formulation, and application of these bacteria. In order to assure the success of plant-microorganism symbiosis and investigate its potential benefits, a quality control system for the production of inoculants and their use in the field should be established. It is necessary to enact the "Biofertilizer Act" and impose stringent regulations for quality control in both the market and the application process. Finding a better carrier material in order to extend the viability of strains and increase their shelf life.

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