

Lecture 11. Biofertilizers: Application of Microorganisms to Improve Plant Nutrition and Increase Yields

Introduction

Modern agriculture requires effective and environmentally sustainable approaches to maintaining soil fertility and increasing crop productivity. For many decades, mineral fertilizers have been the primary source of nitrogen, phosphorus, potassium, and other nutrients required for plant growth. Their application has significantly increased agricultural productivity; however, excessive and inefficient fertilizer use can lead to nutrient losses, contamination of surface and groundwater, changes in soil properties, increased production costs, and negative effects on soil microbial communities. For this reason, increasing attention is being paid to biological approaches that can improve nutrient availability while maintaining soil health. One of these approaches is the application of biofertilizers. Biofertilizers are preparations containing living beneficial microorganisms that colonize the rhizosphere, root surface, or internal plant tissues and enhance plant nutrition through natural biological processes. Unlike conventional mineral fertilizers, biofertilizers generally do not directly supply large quantities of nutrients. Instead, microorganisms improve the availability and utilization of nutrients already present in the soil or atmosphere. Their activities include biological nitrogen fixation, phosphate and potassium solubilization, improvement of micronutrient availability, stimulation of root development, and formation of symbiotic relationships with plants. Biofertilizers therefore represent an important component of sustainable agriculture and modern plant biotechnology.

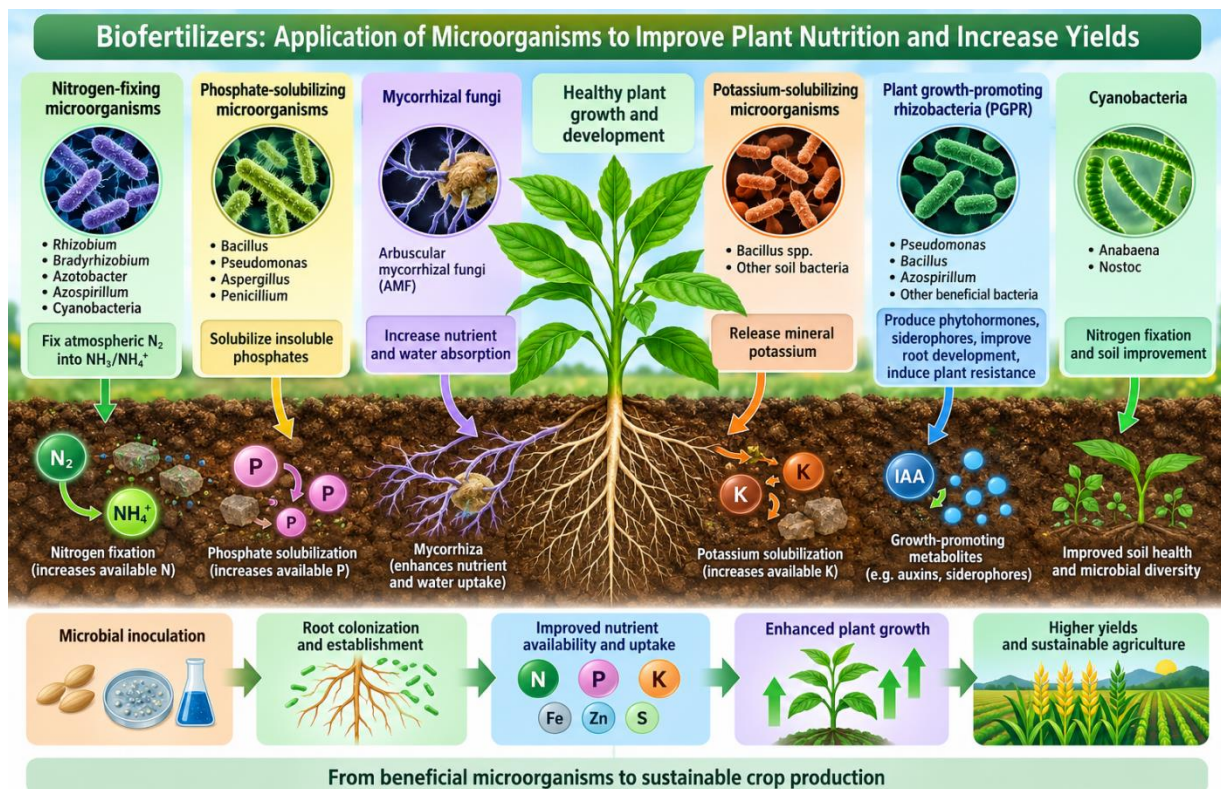


Figure 1. Major Mechanisms of Biofertilizer-Mediated Plant Growth Promotion and Sustainable Crop Production

Plant–Soil–Microorganism Interactions

Plants exist in continuous interaction with microorganisms inhabiting the surrounding soil. The soil region directly influenced by plant roots is known as the rhizosphere, and it contains diverse populations of bacteria, fungi, archaea, and other microorganisms. Plant roots release a wide variety of organic substances, including sugars, amino acids, organic acids, vitamins, phenolic compounds, and secondary metabolites. These substances, collectively known as root exudates, provide nutrients and chemical signals for microorganisms and strongly influence the composition of the rhizosphere microbiome. Beneficial microorganisms use these compounds and, in return, can improve the nutritional status of the plant. Some microorganisms convert atmospheric nitrogen into biologically available nitrogen compounds, while others release phosphorus and potassium from poorly soluble soil minerals. Mycorrhizal fungi extend their hyphae beyond the root surface and significantly increase the volume of soil accessible to the plant. Other beneficial microorganisms produce metabolites that influence root development and improve the plant's ability to absorb water and nutrients. Thus, the relationship can be represented as: plant roots → root exudates → microbial colonization → nutrient transformation → improved nutrient availability → enhanced plant growth and productivity.

Major Groups of Biofertilizers

Biofertilizers can be classified according to the microorganisms they contain and the biological functions they perform. Nitrogen-fixing biofertilizers include symbiotic bacteria such as *Rhizobium* and *Bradyrhizobium*, associative bacteria such as *Azospirillum*, and free-living microorganisms such as *Azotobacter*. Phosphate-solubilizing microorganisms include various species of *Bacillus*, *Pseudomonas*, *Aspergillus*, and *Penicillium*. Mycorrhizal biofertilizers contain beneficial fungi that establish symbiotic relationships with plant roots and improve the absorption of phosphorus and other nutrients. Potassium-solubilizing microorganisms contribute to the release of potassium from soil minerals. Plant growth-promoting rhizobacteria, commonly referred to as PGPR, can influence plants through several mechanisms simultaneously, including nutrient mobilization, production of growth-promoting compounds, and interactions with other members of the rhizosphere microbiome. Cyanobacteria such as *Anabaena* and *Nostoc* can also contribute to soil fertility, particularly in flooded agricultural systems. In practice, the boundaries between these functional groups are not absolute because a single microbial strain may simultaneously fix nitrogen, solubilize phosphorus, produce biologically active compounds, and contribute to plant stress tolerance.

Biological Nitrogen Fixation

Nitrogen is one of the most important macronutrients required for plant growth because it is an essential component of amino acids, proteins, nucleic acids, chlorophyll, enzymes, and many other cellular molecules. Although molecular nitrogen constitutes a large proportion of the atmosphere, plants cannot directly utilize atmospheric N_2 . Certain prokaryotic microorganisms possess the biological machinery required to convert atmospheric nitrogen into biologically available forms. This process is known as biological nitrogen fixation and is primarily associated with the nitrogenase enzyme complex. In simplified form, the process can be represented as $atmospheric\ N_2 \rightarrow NH_3/NH_4^+ \rightarrow organic\ nitrogen\ compounds \rightarrow plant\ proteins\ and\ other\ nitrogen-containing\ molecules$. Nitrogen-fixing microorganisms may live freely in soil, associate with plant roots, or establish specialized symbiotic relationships with plants. Biological nitrogen fixation is particularly

important because it introduces biologically available nitrogen into agricultural ecosystems through natural microbial processes and can complement mineral nitrogen fertilization.

Rhizobium–Legume Symbiosis

One of the most extensively studied examples of biological nitrogen fixation is the symbiotic relationship between leguminous plants and bacteria belonging to genera such as *Rhizobium* and *Bradyrhizobium*. Leguminous crops include soybean, pea, bean, lentil, chickpea, clover, and alfalfa. The establishment of symbiosis begins with molecular communication between the plant root and compatible bacteria in the rhizosphere. Root-derived compounds stimulate bacterial responses, and bacterial signaling molecules subsequently influence plant root cells. Following successful recognition, bacteria interact with root hairs, enter plant tissues, and contribute to the formation of specialized structures called root nodules. Within these nodules, bacteria differentiate into nitrogen-fixing forms and convert atmospheric nitrogen into compounds that can ultimately be incorporated into plant metabolism. The plant provides the bacteria with carbon compounds and energy generated through photosynthesis, while the bacteria provide the plant with biologically available nitrogen. The relationship can therefore be summarized as plant signals → bacterial recognition → root colonization → nodule formation → biological nitrogen fixation → improved nitrogen nutrition of the plant.

Free-Living and Associative Nitrogen-Fixing Bacteria

Not all nitrogen-fixing microorganisms require specialized root nodules. *Azotobacter* represents an important group of free-living aerobic soil bacteria capable of biological nitrogen fixation. These microorganisms occur in the rhizosphere of many plant species and may contribute to plant development through nitrogen fixation and the production of biologically active metabolites. Another important group is represented by bacteria of the genus *Azospirillum*, which frequently associate with the roots of cereals and grasses such as wheat, maize, and rice. Rather than forming specialized nodules, these bacteria colonize regions near or on plant roots and influence plant development through multiple mechanisms. Their effects may include contributions to nitrogen metabolism, modification of root architecture, and production of compounds associated with plant growth regulation. A more developed root system provides a larger surface area for nutrient and water absorption and may therefore contribute significantly to crop productivity.

Phosphate-Solubilizing Microorganisms

Phosphorus is another essential macronutrient required for plant growth and development. It is involved in ATP-mediated energy transfer, nucleic acid synthesis, membrane formation, photosynthesis, respiration, root development, flowering, and seed formation. Although agricultural soils may contain considerable quantities of phosphorus, much of it can occur in forms that are poorly available to plant roots. Phosphate-solubilizing microorganisms can increase phosphorus availability through biological and chemical processes in the rhizosphere. Important representatives include bacteria belonging to *Bacillus* and *Pseudomonas* and fungi such as *Aspergillus* and *Penicillium*. These microorganisms can produce organic acids and other metabolites that modify the chemical environment surrounding soil particles and facilitate the release of phosphate from poorly soluble mineral compounds. The process can be summarized as insoluble soil phosphorus → microbial activity and organic acid production → phosphate solubilization → increased soluble

phosphate → root absorption → improved plant growth. Because phosphorus availability frequently limits agricultural productivity, phosphate-solubilizing microorganisms are important candidates for biofertilizer development.

Mycorrhizal Fungi

Mycorrhiza represents a mutualistic association between fungi and plant roots. Arbuscular mycorrhizal fungi are particularly important in agriculture because they establish associations with a wide range of crop species. After colonizing the root, fungal hyphae extend from the root into the surrounding soil. These microscopic structures can explore soil volumes that are difficult for plant roots to access directly, thereby increasing the effective absorption area of the root system. Mycorrhizal fungi are particularly important for phosphorus acquisition because phosphorus has relatively limited mobility in many soils. They can also influence the uptake of nitrogen and micronutrients and contribute to plant water relations. The plant supplies the fungus with carbon compounds derived from photosynthesis, while the fungus assists the plant in obtaining mineral nutrients and water. The relationship can be represented as soil nutrients and water → fungal hyphae → mycorrhizal structures → plant roots → plant growth, while photosynthetically derived carbon flows from the plant toward the fungal partner. This exchange demonstrates the importance of biological cooperation in plant nutrition.

Plant Growth-Promoting Rhizobacteria

Plant Growth-Promoting Rhizobacteria, or PGPR, are beneficial bacteria capable of colonizing the rhizosphere or plant roots and positively influencing plant growth. Important PGPR include species of *Bacillus*, *Pseudomonas*, *Azospirillum*, and several other bacterial genera. Their effects may result from several direct and indirect mechanisms operating simultaneously. Direct mechanisms include biological nitrogen fixation, phosphate solubilization, mobilization of mineral nutrients, production of metabolites associated with plant growth regulation, and modification of root architecture. Indirect mechanisms can involve competition with potentially harmful microorganisms for nutrients and ecological niches and stimulation of plant defense responses. The ability of PGPR to perform multiple functions makes them particularly interesting for modern agricultural biotechnology. Rather than considering individual microorganisms only as nutrient suppliers, modern research increasingly views PGPR as components of complex plant-associated microbial communities capable of influencing plant nutrition, development, and environmental adaptation.

Microbial Influence on Root Development

The efficiency of plant nutrient uptake is strongly dependent on root architecture. A larger and more highly branched root system can explore a greater volume of soil and therefore obtain more water and nutrients. Certain beneficial microorganisms produce compounds or influence signaling pathways associated with plant hormones. One important compound is indole-3-acetic acid, or IAA, which is associated with auxin-mediated regulation of plant development. Microbial influences on auxin signaling may affect root elongation, lateral root formation, and root-hair development. These changes can increase the surface area available for nutrient absorption. The overall process can therefore be represented as beneficial microorganism → growth-related metabolites and signaling → modification of root architecture → increased soil exploration → improved water and

nutrient absorption → enhanced plant development. This mechanism illustrates how biofertilizers can stimulate plant growth without necessarily providing nutrients directly.

Siderophores and Micronutrient Availability

Plants require micronutrients in relatively small quantities, but these elements remain essential for normal metabolism. Iron, for example, participates in electron transport, photosynthesis, respiration, and numerous enzymatic reactions. In many soils, iron availability is limited because it occurs in poorly soluble forms. Some rhizosphere microorganisms produce low-molecular-weight compounds known as siderophores, which have a high affinity for iron. These compounds bind iron in the environment and facilitate its acquisition by microorganisms. Siderophore-mediated processes can modify iron dynamics within the rhizosphere and influence interactions among plants and microorganisms. Competition for iron may also restrict the development of some competing microorganisms. Consequently, siderophore-producing bacteria can contribute to the ecological stability of the rhizosphere and may indirectly support plant nutrition and health.

Potassium-Solubilizing Microorganisms

Potassium is an essential macronutrient involved in enzyme activation, osmotic regulation, stomatal function, maintenance of cellular water balance, transport processes, and plant responses to environmental stress. Although soils may contain large quantities of potassium, much of it can be incorporated into mineral structures and therefore may not be immediately available to plant roots. Certain microorganisms contribute to mineral weathering through the production of organic acids and other metabolites. These processes can promote the release of potassium ions from minerals into the soil solution. The general pathway can be represented as mineral-bound potassium → microbial metabolism and mineral weathering → release of K^+ → root absorption → improved plant nutrition. Potassium-solubilizing microorganisms are therefore being investigated as components of microbial fertilizers, especially for agricultural soils where the availability of potassium limits crop productivity.

Cyanobacteria as Biofertilizers

Cyanobacteria are photosynthetic prokaryotic microorganisms that can contribute to soil fertility and nutrient cycling. Certain cyanobacteria, including representatives of *Anabaena* and *Nostoc*, are capable of biological nitrogen fixation. Their application has been particularly important in flooded agricultural ecosystems such as rice fields, where environmental conditions support their growth and biological activity. Cyanobacteria can contribute biologically fixed nitrogen, organic matter, and other metabolites to agricultural soils. Their growth can also influence microbial activity and soil biological processes. Consequently, cyanobacteria represent an important example of how naturally occurring microorganisms can be utilized to support plant nutrition and agricultural productivity.

Production and Formulation of Biofertilizers

The successful agricultural application of beneficial microorganisms requires more than simply identifying a microorganism with desirable properties. Selected strains must be produced under controlled conditions and formulated so that they remain viable during storage, transportation, and application. Biofertilizers may be produced as liquid

formulations, powders, granules, carrier-based preparations, seed coatings, root inoculants, or soil inoculants. Carrier materials can provide physical protection, moisture retention, and suitable conditions for microbial survival. An effective formulation should maintain an adequate population of viable microorganisms throughout its shelf life and enable rapid establishment after application. Production therefore involves strain selection, microbial cultivation, formulation development, quality control, packaging, storage, and evaluation under greenhouse and field conditions. Microbial identity, purity, viability, concentration, stability, and functional activity are important quality parameters for commercial biofertilizer products.

Methods of Biofertilizer Application

The method of biofertilizer application depends on the crop species, microbial strain, formulation, soil conditions, and agricultural production system. Seed inoculation is one of the most widely used approaches and involves applying microbial preparations to the surface of seeds before planting. After germination, microorganisms can colonize the developing root system. Root treatment is frequently used for transplanted crops and involves treating seedlings or their roots with microbial preparations before transplantation. Soil application introduces microbial inoculants directly into the root zone, while liquid formulations may be applied through irrigation or other agricultural systems. Successful inoculation requires appropriate environmental conditions because microorganisms must survive, establish themselves in the rhizosphere, interact with the host plant, and compete with native microbial populations before they can produce a significant biological effect.

Factors Affecting Biofertilizer Efficiency

Biofertilizer performance under field conditions can vary considerably because microbial activity is strongly influenced by environmental and biological factors. Soil pH affects nutrient solubility and microbial growth, while temperature and soil moisture influence microbial metabolism and survival. Salinity, organic matter content, soil texture, nutrient availability, and agricultural management practices can also affect microbial establishment. The genetic characteristics of both the microorganism and plant are important because beneficial interactions may be strain-specific or crop-specific. Introduced microorganisms must also compete with native microbial communities already adapted to local soil conditions. Storage temperature, formulation quality, shelf life, inoculum concentration, and application method further influence the final effectiveness of a product. Therefore, promising laboratory results do not automatically guarantee successful field performance. Biofertilizer development requires laboratory experiments, greenhouse studies, field trials, and evaluation under different environmental conditions.

Advantages and Limitations of Biofertilizers

Biofertilizers have considerable potential for improving agricultural sustainability because they utilize natural biological processes to increase nutrient availability and nutrient-use efficiency. They may contribute to biological nitrogen fixation, phosphorus and potassium mobilization, improved micronutrient dynamics, development of larger root systems, and maintenance of biologically active soils. They can complement mineral fertilizers and contribute to integrated nutrient-management strategies. However, biofertilizers also have limitations. Microbial survival can decrease during storage or after introduction into unfavorable soils, and field performance may vary according to

temperature, moisture, soil chemistry, crop genotype, and native microbial communities. Some products may have relatively short shelf lives or require specific storage conditions. Consequently, biofertilizers should not automatically be considered complete substitutes for conventional fertilizers. A more effective strategy is often integrated nutrient management, in which microbial inoculants, organic amendments, crop rotation, and appropriately optimized mineral fertilizers are combined according to soil characteristics and crop requirements.

Biofertilizers and the Plant Microbiome

Advances in molecular biology have transformed the understanding of plant–microorganism interactions. Plants are now recognized as hosts for complex microbial communities collectively described as the plant microbiome. These microorganisms occur in the rhizosphere, on root surfaces, inside plant tissues, and on above-ground organs. Modern techniques such as 16S rRNA gene sequencing, ITS sequencing, metagenomics, metatranscriptomics, metabolomics, and bioinformatics make it possible to characterize these microbial communities and investigate their functions. Instead of searching exclusively for individual beneficial strains, researchers can now investigate networks of microorganisms associated with healthy and productive plants. This has led to growing interest in microbial consortia containing several complementary microorganisms. For example, one strain may fix atmospheric nitrogen, another may solubilize phosphorus, another may improve micronutrient availability, and another may stimulate root development. Such combinations could potentially provide more stable biological functions than individual strains, although their effectiveness must be carefully evaluated under field conditions.

Role of Biofertilizers in Sustainable Agriculture

The application of biofertilizers fits within the broader concept of sustainable agriculture, which aims to maintain high productivity while protecting soil, water, biodiversity, and other natural resources. Conventional nutrient management is often based primarily on supplying nutrients directly through fertilizers. Biological nutrient management considers the entire soil–plant–microorganism system. In this approach, soil microorganisms participate actively in nutrient cycling and can improve the efficiency with which plants utilize available resources. The concept can be summarized as soil + plant + beneficial microbiome + organic matter + optimized mineral nutrition → efficient nutrient cycling → improved plant growth → sustainable crop production. Biofertilizers can therefore be integrated with crop rotation, organic fertilizers, reduced tillage, precision agriculture, and other sustainable agricultural practices.

Future Perspectives

The future development of biofertilizers is closely associated with microbial genomics, plant microbiome research, biotechnology, precision agriculture, and systems biology. Genome sequencing can help identify genes associated with nitrogen fixation, nutrient solubilization, root colonization, stress tolerance, and production of beneficial metabolites. Metagenomic studies can reveal previously unknown microorganisms and functional interactions within agricultural soils. Another promising direction is the development of synthetic microbial communities in which microorganisms with complementary biological functions are combined into carefully designed consortia. Such systems may integrate nitrogen fixation + phosphorus solubilization + potassium mobilization + micronutrient acquisition + stimulation of root development + environmental

stress tolerance. Future biofertilizer technologies are also expected to become increasingly crop-specific and soil-specific. Integration with soil sensors, remote sensing, precision fertilization, and digital agriculture could allow microbial products to be applied under environmental conditions most favorable for their establishment and activity.

Conclusion

Biofertilizers are an important biotechnology for improving plant nutrition and supporting sustainable agricultural production. They contain beneficial microorganisms capable of influencing nutrient availability, root development, plant–soil interactions, and nutrient cycling. Important groups include nitrogen-fixing bacteria such as *Rhizobium*, *Bradyrhizobium*, *Azotobacter*, and *Azospirillum*; phosphate-solubilizing microorganisms such as *Bacillus*, *Pseudomonas*, *Aspergillus*, and *Penicillium*; arbuscular mycorrhizal fungi; potassium-solubilizing microorganisms; cyanobacteria; and multifunctional plant growth-promoting rhizobacteria. Their major mechanisms include biological nitrogen fixation, phosphorus and potassium mobilization, improvement of micronutrient availability, modification of root architecture, and establishment of beneficial symbiotic relationships. The effectiveness of biofertilizers depends on the microbial strain, crop genotype, soil properties, climate, formulation, storage, and method of application. Therefore, their greatest potential lies in scientifically designed integrated nutrient-management systems where biological fertilizers complement other sustainable agricultural technologies. In the future, microbiome engineering, microbial genomics, synthetic microbial consortia, and precision agriculture are expected to increase the reliability and effectiveness of biofertilizers and strengthen their role in environmentally sustainable crop production.