


## Review

# Agricultural Sustainability: Microbial Biofertilizers in Rhizosphere Management

Oluwaseun Adeyinka Fasusi <sup>1</sup>, Cristina Cruz <sup>2</sup> and Olubukola Oluranti Babalola <sup>1,\*</sup> 

<sup>1</sup> Food Security and Safety Niche, Faculty of Natural and Agricultural Sciences, North-West University, Private Mail Bag X2046, Mmabatho 2735, South Africa

<sup>2</sup> Department of Plant Biology, Faculdade de Ciências da Universidade de Lisboa, 1749-016 Lisboa, Portugal

\* Correspondence: olubukola.babalola@nwu.ac.za; Tel.: +27-183892568

**Abstract:** The world's human population continues to increase, posing a significant challenge in ensuring food security, as soil nutrients and fertility are limited and decreasing with time. Thus, there is a need to increase agricultural productivity to meet the food demands of the growing population. A high level of dependence on chemical fertilizers as a means of increasing food production has damaged the ecological balance and human health and is becoming too expensive for many farmers to afford. The exploitation of beneficial soil microorganisms as a substitute for chemical fertilizers in the production of food is one potential solution to this conundrum. Microorganisms, such as plant growth-promoting rhizobacteria and mycorrhizal fungi, have demonstrated their ability in the formulation of biofertilizers in the agricultural sector, providing plants with nutrients required to enhance their growth, increase yield, manage abiotic and biotic stress, and prevent phytopathogens attack. Recently, beneficial soil microbes have been reported to produce some volatile organic compounds, which are beneficial to plants, and the amendment of these microbes with locally available organic materials and nanoparticles is currently used to formulate biofertilizers to increase plant productivity. This review focuses on the important role performed by beneficial soil microorganisms as a cost-effective, nontoxic, and eco-friendly approach in the management of the rhizosphere to promote plant growth and yield.

**Keywords:** beneficial microorganisms; biofertilizers; crop production; soil fertility; sustainable agriculture



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## 1. Introduction

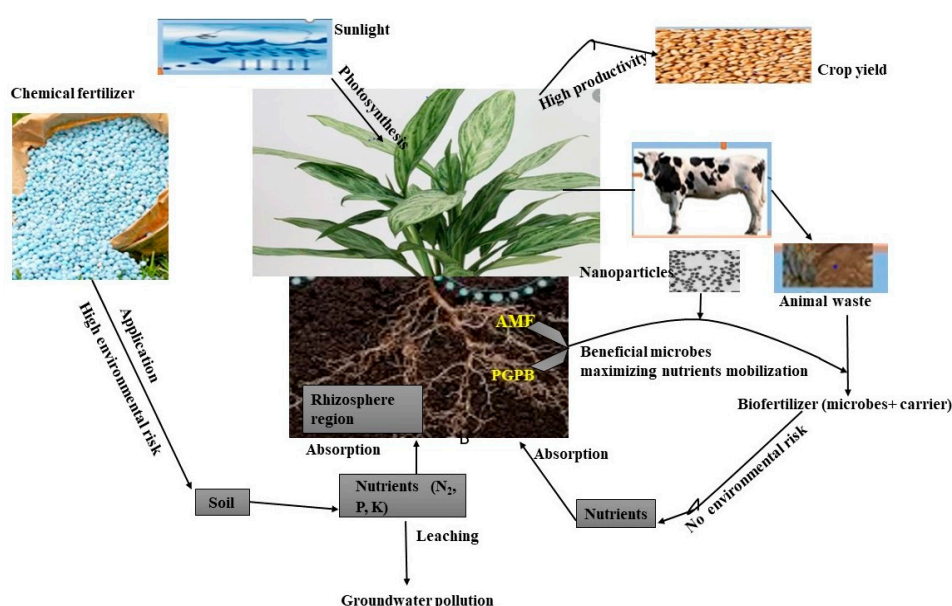
According to the Food and Agriculture Organization (FAO) of the United Nations, the population of the world is expected to increase to more than nine billion by 2050, a third more people to feed than today. It is, therefore, necessary to dramatically increase agricultural production by managing the rhizosphere in a relatively short period [1] to ensure food security. Some factors are necessary to meet this goal, including the right environmental conditions and availability of fertile soil [2] conditions that are becoming rarer with time. From the middle of the 20th century until date, chemical fertilizers have helped in feeding the world's population. This has been done through the provision of the required nutrients, such as phosphorus (P), nitrogen (N), and potassium (K), to plants. About 53 billion tons of NPK fertilizers are used yearly to supplement the number of nutrients needed for plant growth and yield performance [3]. Unfortunately, only a small percentage of these nutrients are used by plants, while a greater percentage is precipitated by metal cations present in the soil. Moreover, the extensive and inappropriate use of chemical fertilizers results in environmental issues that are a major concern to farmers, furthering the argument for the introduction of agricultural practices that do not harm the environment [4]. Scientists everywhere have begun to direct their interests towards ensuring agrarian sustainability using beneficial soil microorganisms instead of chemical fertilizers and pesticides [5].

Rhizosphere management can be defined as the process of improving the nutrient efficiency in the soil to enhance the nutrient needed for plant growth and improve plant yield [6]. Beneficial soil microorganisms enhance the management of the rhizosphere through different mechanisms that are multidimensional. These include the following: production of siderophore, nitrogen fixation, lytic acid production, production of hydrogen cyanide, phosphate solubilization, and production of indole acetic acid [7,8]. The mechanisms of action of these beneficial microorganisms play a crucial role in improving soil fertility, plant growth, and yield.

Many strains of beneficial soil microorganisms have been isolated for their potentials in the management of the rhizosphere to enhance plant yield [9] and are currently used in biotechnology as tools to improve food security and agricultural sustainability. Currently, mycorrhiza fungi and plant growth-promoting rhizobacteria (PGPR) are perceived by soil researchers as microorganisms that play vital roles in ensuring nutrients availability in the soil to enhance plant growth and increase yield. The application of biofertilizers is gaining more awareness since it is an environmentally friendly and cost-effective means of enhancing crop productivity and soil fertility [10]. Microbial biofertilizers consist of viable cells of beneficial microorganisms, with plant growth-promoting potentials that interact with the rhizosphere or endosphere of plants by improving soil fertility and stimulating nutrient uptake to increase yield [11]. The application of biofertilizers reduces the high cost of purchasing chemical fertilizers and addresses the world's demand for green technology for crop production [12]. Thus, this review focuses on the management of the rhizosphere to improve plant growth and yield through the application of PGPR and mycorrhizal fungi in the formulation of cost-effective and ecologically friendly microbial biofertilizers.

## 2. Rhizosphere, as the Zone of Interaction between Soil, Plant, and Microorganisms

The rhizosphere is the region of the soil that includes the area immediately around plant roots and a large number of microorganisms [13]. The rhizosphere is a region with a high turnover of nutrients and a high microbial density where biotic and abiotic factors are under the strict control of each other [14]. Examples of microorganisms that can be found in the rhizosphere include PGPR and mycorrhizal fungi. The microbial diversity of the rhizosphere is determined by the diversity and quantity of organic nutrients exuded, root system architecture, root branching order, root chemistry, and is used by the biotic community, including plants themselves (Figure 1) [15–17].



**Figure 1.** Overview of rhizosphere as the bottleneck in controlling nutrients uptake by plants through the application of chemical and biofertilizer; Arbuscular mycorrhiza fungi (AMF), Plant growth-promoting rhizobacteria (PGPR).

Microorganisms displaying plant growth-promoting characteristics are recruited by the plant to inhabit the rhizosphere. The microbial composition of the rhizosphere is optimized through the production and exudation of specific compounds, such as pyrone and sesquiterpenes, that prevent the growth of specific microbes [18]. However, root exudates represent about 20% of the carbon fixed by the plant through photosynthesis and represent an important source of carbon for the microbial community [19], especially in soils with different compositions of organic matter [20].

Bacteria that live in the rhizosphere, generally referred to as rhizobacteria, develop specific communication pathways with the plant and may influence plant physiology [21], including the type and amount of root exudates produced by the plant. Examples of the outputs of these interactions are biotic and abiotic plant stress tolerance induced by microorganisms [22].

### 3. Plant Growth-Promoting Rhizobacteria

PGPR live in close association with plants and plant tissues (bacterial endophytes) and may stimulate plant nutrient uptake, modulate the level of phytohormones in plant tissues, and/or increase plant biotic or abiotic stress tolerance [16]. Even phylogenetically distinct bacteria may exhibit similar mechanisms in promoting plant productivity [8,10]. Various examples of PGPR with their mechanisms of action are presented in Table 1. Some of the best-known PGPR belong to the genera *Rhizobium*, *Bradyrhizobium*, *Bacillus*, *Azospirillum*, *Azotobacter*, *Acinetobacter*, and *Pseudomonas*.

### 4. Mycorrhizal Fungi

The term mycorrhiza describes a symbiotic association between plant roots and certain fungi. In the mycorrhiza association, the fungi colonize the plant root either intracellularly or extracellularly, depending on the type of plant and fungus involved in this association. In a simple view, the relationship that occurs between a host plant and fungus may be described as mutual in the sense that the fungus is supplied with carbohydrates needed for its metabolic activities by the host plant, and, in exchange, the host plant is supplied with nutrients and water needed for its growth by the fungus. Thus, the association between the fungus and the host plant is a mutually beneficial symbiotic association [23]. Mycorrhizal fungi play a key role in enhancing the uptake of water and nutrients, such as phosphorus from the soil, which is needed for plant growth and productivity. Similarly, the inorganic phosphate transporter (Pi) in mycorrhiza *Glomus versiformis* hyphae was reported to enhance the absorption of phosphate from the soil to the host plant [24]. Mycorrhizal fungi may also facilitate the detoxification of both organic and inorganic soil pollutants that may harm plant productivity.

Mycorrhizal fungi are classified into two major types: endomycorrhiza is common to more than 86% of plant species, where the hyphae penetrate plant root cortical cells forming intracellular arbuscules; and ectomycorrhiza characteristic of trees and shrubs, where hyphae do not penetrate plant root cells [25]. The establishment of the mycorrhiza is mediated through the partner's recognition and the "Common Symbiotic Pathway" [26]. Kang et al. [27] found that a cysteine-rich mycorrhizal protein (MISSP7) plays a crucial role in facilitating the mutual interaction between mycorrhizal fungi and host plants.

### 5. Categories of Microorganisms Used in the Production of Biofertilizers

#### 5.1. Nitrogen-Fixing Microbes

Microorganisms that belong to the family Rhizobiaceae, which are made up of different genera, such as *Rhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, and *Sinorhizobium*, are known to be the best symbiotic nitrogen fixers and live in the plant root nodules (Figure 2). *Rhizobium*, in the root nodule, fixes atmospheric nitrogen in leguminous plants. Nitrogen is used by the plant to synthesize vitamins, amino acids, nucleic acids, and other nitrogenous compounds. All nitrogen-fixing microorganisms use the same enzyme—nitrogenase [28]. The role played by *Rhizobia* in nitrogen fixation makes leguminous plants less dependent on the

application of chemical nitrogen fertilizers and is the key success for the crop rotation strategy for sustainable agriculture.

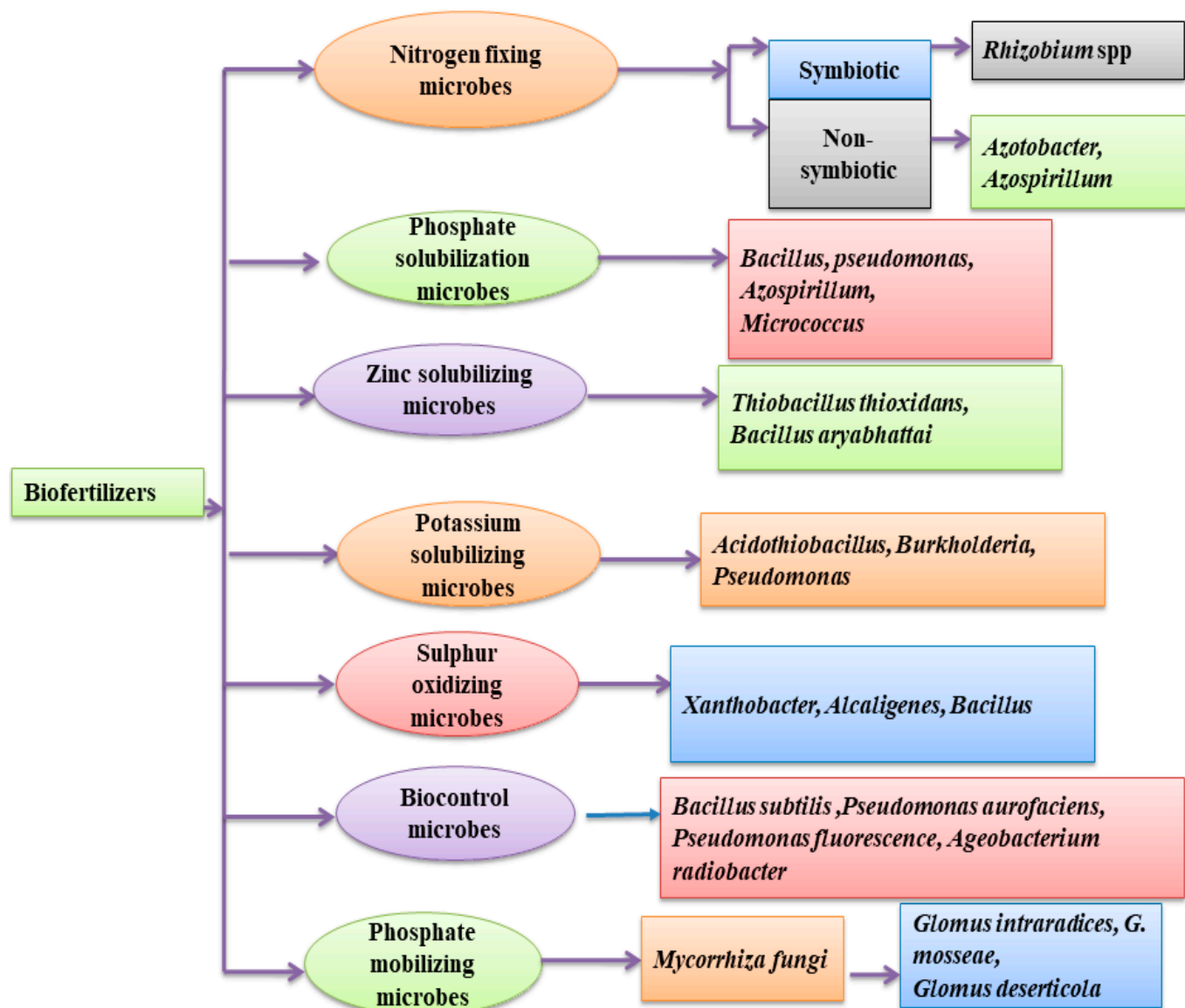


Figure 2. Schematic diagram of categories of microorganisms used as biofertilizers.

Nodule formation is enhanced by the low availability of nitrogen, but microorganisms that produce an enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, have the potential to degrade 1-aminocyclopropane-1-carboxylate before its conversion to ethylene [29,30] and may also enhance the formation of a nodule. Such formation is part of a common strategy developed by leguminous plants and Rhizobiaceae bacteria to decrease the concentration of oxygen to which the nitrogenase is exposed due to the inhibitory effect of oxygen on nitrogenase activity. However, there are other nitrogen-fixing microorganisms, such as those of the *Acetobacter* genus, able to fix nitrogen even under aerobic conditions.

Certain strains of *Azotobacter* (*Azotobacteriaceae* family) have the potential to colonize the roots of sugarcane, coffee, cotton, wheat, rice, and vegetables [29,31]. Co-inoculation of wheat plants with specific strains of *Azotobacter* and *Pseudomonas* increases grain yield, protein content, and harvest index when compared to uninoculated plants, which allowed a decrease in the application of chemical fertilizer in the field by 25–50% [32]. *Azotobacter* is an example of a nitrogen-fixing bacteria genus, able to fix nitrogen under aerobic

conditions and can act as a biocontrol agent. *Azotobacter indicum* have been reported by Mahanty et al. [12] to have fungicide properties.

Several species of *Azospirillum* belonging to the family Rhodospirillaceae (*A. zeae*, *A. thiophilum*, *A. rugosum*, *A. picis*, *A. oryzae*, *A. canadense*, *A. mazonense*, and *A. melinis*) have been found associated with grass rhizosphere [8] while fixing nitrogen. Plant inoculation with *Azospirillum* strains promotes plant growth and yield by causing changes in the cell wall elasticity or the morphology of the root, or both through the production of phytohormones (auxin) [33].

**Table 1.** Rhizobacteria used in the production of biofertilizer, biocontrol traits, and their effect on plant productivity

Microbial Strains	Plant Growth-Promoting Traits	Biocontrol Traits	Effect on Plant Productivity	References
<i>Bradyrhizobium</i> sp.	Production of siderophore, production of indole acetic acid, nitrogen fixation, and phosphate solubilization	Production of antibiotics, secretion of an enzyme that can degrade the cell wall of plant-pathogen, production of hydrogen cyanide and, production of siderophore	Increases growth parameters and seed yield in mungbeans plant	[34,35]
<i>Rhizobium meliloti</i>	Production of siderophore and nitrogen fixation	Production of antibiotics against phytopathogens and production of chitinases	Increases peanuts growth, yield attributes, quality of pods, and efficiency in the use of nitrogen	[36,37]
<i>R. leguminosarum</i>	Phosphate solubilization	Production of antibiotics, secretion of an enzyme that can degrade the cell wall of plant pathogens and enhances the production of phytoalexins in plant	Increases growth of soybean and yield performance under drought stress	[38]
<i>Bacillus</i> spp.	Production of phytohormone, such as auxin, phosphate solubilization	Formation of endospore and biochemical compound against phytopathogens, induces systemic resistance and competition in plant	Increases strawberry fresh and dry weight parameters, increases yield over the control plant	[39,40]
<i>Chryseobacterium</i> sp.	Production of siderophore, phosphate solubilization	Production of proteases	Increases grain yield, shoot mass, and nodule mass in chickpea	[10,41,42]
<i>Herbaspirillum</i> spp.	Synthesis of indole acetic acid, nitrogen fixation	Production of siderophore	Enhances mineral uptake in maize plant and increases yield	[43–45]
<i>Paenibacillus glucanolyticus</i>	Synthesis of indole acetic acid	Production of chitinases and glucanases	Increases tissue dry weight and nutrient uptake in black pepper	[46,47]
<i>Streptomyces</i> spp.	Production of siderophore and synthesis of indole acetic acid	Production of glucanases	Increases tomato growth parameter and modulates metabolic activity	[48]
<i>Burkholderia</i> spp.	Solubilization of phosphate	Production of antibiotic pyrrolnitrin	Increases fenugreek growth and yield performance	[49,50]
<i>Athrobacter</i>	Solubilization of phosphate	Production of chitinases	Increases broccoli growth and yield	[51,52]
<i>Phyllobacterium</i>	Production of siderophore	NA	Increases grain yield in sorghum	[53,54]
<i>Acinetobacter</i> spp.	Production of ACC deaminase, Indole acetic acid synthesis, and phosphate solubilization	Production of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase	Promotes wheat growth in a greenhouse experiment	[55–57]
<i>Acidithiobacillus ferrooxidans</i>	Potassium solubilization	NA	Increases pumpkin growth parameters, yield, and oil composition	[46,58]
<i>Enterobacter cloacae</i>	Nitrogen fixation, phosphate solubilization, siderophore production	Production of the lytic enzyme for chitinolytic activity, production of ACC deaminase	Enhances potato growth and promotes yield performance	[59,60]
<i>Erwinia</i>	Phosphate solubilization	Ethylene synthesis	Promotes growth and yield parameters in wheat	[61,62]
<i>Pseudomonas</i> spp.	Production of ACC deaminase phosphate solubilization, ammonia production, production of IAA	Production of hydrogen cyanide, siderophore production, production of cell wall degrading enzymes, such as chitinase and laminarinase, production of ACC deaminase, quorum sensing, and quenching	Enhances growth and yields in tomato plants	[10,63]

## 5.2. Phosphorus Solubilizing Microbes

Phosphorus is a macronutrient, and its low availability severely limits plant development and productivity. In the majority of situations, the presence of phosphorus available in the soil is at high concentrations as phosphate, which may be in an organic or inorganic form. Only a small fraction of inorganic phosphate is available to the biosphere in the soil solution; the majority of inorganic phosphate is immobilized in insoluble salts. Phosphorus solubilization involves local acidification or alkalization and has been observed in some



species of *Pseudomonas*, *Cyanobacteria*, and *Bacillus* isolated from the rhizosphere of plants (Table 1).

Organic phosphate is the largest pool of soil phosphate, but organic phosphate compounds tend to be complex (nucleic acids, phospholipids, etc.) and have to be transformed by microorganisms before they can be absorbed by plants [64]. Hence, the phosphorus mineralization process in the soil involves the production of enzymes, such as phosphatases and phytases [65]. Phosphate solubilizing and mineralizing characteristics are found in some species of *Pseudomonas*, *Cyanobacteria*, and *Bacillus* (Table 1).

### 5.3. Potassium Solubilizing Microbes

Potassium is an essential macronutrient regulating many enzyme activities, including that of amylases (enzymes involved in starch degradation), which are involved in the coordination of root shoot ratio [30]. An insufficient supply of potassium leads to poor development of the plant root, an increase in the susceptibility of the plant to pathogens, and a reduction in plant growth and yield.

A large number of potassium solubilizing microorganisms live in the soil and have been reported in different studies [66]. These include some bacteria, such as *Bacillus mucilaginosus*, *Azotobacter chroococcum*, and *Rhizobium* spp., which have been reported for potassium solubilization, resulting in increased maize, chili, cotton, pepper, sorghum, and wheat productivity [67]. Recently, the inoculation of wheat grown with a potassium solubilizing strain of *Bacillus edaphicus* was reported to have shown a great increase in roots and shoots growth when compared to uninoculated plants [46].

The production of organic acids is among the mechanisms used by potassium solubilizing microorganisms, and this makes its usage in agriculture the best strategy for promoting plant productivity and enhancing soil fertility. The inoculation of plants with potassium solubilizing microorganisms increases the uptake of potassium, plant yield, and growth.

### 5.4. Phosphorus Mobilizing Microbes

Endomycorrhizal fungi are very important actors for improving phosphorus bioavailability (Figure 1), and some genera (*Scutellospora*, *Glomus*, *Acaulospora*, and *Gigaspora*) are already in use as biofertilizers. Since the fungal hyphae can penetrate the soil pores, sites where the root system cannot reach, the mycorrhizal plant root can efficiently explore a bigger soil volume than non-mycorrhizal plants [68]. Recently, the use of *Glomus mosseae* as a biofertilizer was reported [69] to increase the shoot length, root dry weight, and root length in wheat, and, at the same time, mycorrhizal hyphae also contribute to an increase in the soil structure [70]. The application of mycorrhizal fungi in agriculture (Table 2) is low cost and eco-friendly and increases plant yield when compared to the cost of purchase of chemical fertilizers without any negative effect on the environment [71]. However, the beneficial role played by arbuscular mycorrhizal fungi to plants is negatively affected by tillage and the application of chemical fertilizers or pesticides (fungicides in particular). The application of arbuscular mycorrhizal fungi towards ensuring sustainable agriculture is gaining more awareness each day with their role in improving plant health, productivity, and soil fertility.

### 5.5. Sulphur Oxidizing Microbes

Sulphur is an essential macronutrient needed by plants in high concentration. It is part of some amino acids, such as cysteine, cystine, and methionine, and is among the components that regulate enzyme activity in plants, such as superoxide dismutase, ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase, and glutathione reductase. A deficiency in sulphur in plants results in low nitrogen metabolism, which causes chlorosis, low lipid percentage, and low plant growth and yield [72]. In the soil, sulphur exists in two forms: organic and inorganic, although the inorganic form of sulphur is primarily absorbed by plants (i.e.,  $\text{SO}_4^{2-}$ ). Conversion of organic into inorganic

sulphur forms may be carried out by sulphur-oxidizing microbes belonging to the genera *Xanthobacter*, *Alcaligenes*, *Bacillus*, and *Pseudomonas*. Some plant growth-promoting activities of sulphur-oxidizing microorganisms have been reported [46]. Pourbabae et al. [73] reported the positive effect of *Thiobacillus* spp. on maize plants by increasing plant height, yield, and nitrogen uptake. Similarly, the positive effect of sulphur-oxidizing microorganisms on garlic plants was reported by Hejazirad et al. [74] to increase plant height, fresh and dry leaf mass, as well as bulb weight and diameter. Recently, the application of sulphur-oxidizing microorganisms has been recommended in the formulation of biofertilizer for onion, oats, ginger, grape, garlic, and cauliflower under alkaline soil conditions [75,76].

#### 5.6. Zinc Solubilizing Microbes

Zinc is an essential micronutrient. The results of zinc deficiency in plants are reduction in leaf size, chlorosis, increase in plant susceptibility to heat, light stress, and pathogenic attack [77]. The application of Zn fertilizers has been suggested to pose a threat to the environment [78]. Thus, the application of zinc solubilizing microorganisms as an alternative to Zn supply is gaining traction. Several strains of Zn solubilizing microorganisms have been applied in the production of biofertilizers. These include *Pseudomonas* spp., *Rhizobium* spp., *Bacillus aryabhattai*, *Thiobacillus thiooxidans*, and *Azospirillum* spp. [79]. Solubilization of Zn by microorganisms depends on both soil pH and capacity of cation exchange. Application of *Bacillus* spp. AZ6, as a Zn solubilizing biofertilizer on maize, was reported by Hussain et al. [80] to have a positive impact on total maize biomass and increase plant physiology, chlorophyll content by 90%, and yield when compared to uninoculated plants. Similarly, the application of *Bacillus aryabhattai* increased Zn uptake in maize, resulting in better growth and mitigation of yield loss in maize when compared to uninoculated plants [81]. Moreover, the effect of zinc solubilizing bacteria *Rhizobium*, *Azospirillum*, and *Pseudomonas* was reported to increase the zinc content in wheat when evaluated at different growing stages [82]. The effect of inoculation of these microorganisms enhances uptake of nutrients and production of the wheat plants with better quality. Additionally, the effect of zinc solubilizing bacterium *Bacillus megaterium* was recently reported by Bhatt and Maheshwari [83] to increase growth parameters resulting in maximum zinc content in *Capsicum annuum* L. fruit.

**Table 2.** Contribution of arbuscular mycorrhizal fungi to plant growth promotion and soil nutrients

Mycorrhizal Fungi	Plants	Effect on Plant	Effect on Soil	References
<i>Glomus versiforme</i> <i>Glomus mosseae</i>	Tomato	Promotes growth and yield under water stress and more efficient conditions	Increases phosphorus concentration in the soil	[84]
<i>Glomus etunicatum</i>	Maize	Improves chlorophyll content and nutrient uptake in maize	Increases soil quality	[85]
<i>Acaulospora lacunosa</i>	Strawberry	Enhances nutrient uptake in strawberry	Increases soil nutrient for horticultural crops productivity	[86]
<i>Rhizophagus irregularis</i>	Wheat	Improves tolerance to stress, enhances plant growth, and increases seed yield	Increases soil nutrient needed for wheat production	[87]
<i>R. irregularis</i>	Maize	Enhances tolerance to salt stress, improves growth parameters	Reduces the concentration of salt in the soil for better plant development	[88]
<i>G. mosseae</i> and <i>G. geosporus</i>	Strawberry	Enhances growth and improves its tolerance to water stress	Increases soil nutrient to enhance its colonization on the plant root system	[89]
<i>Rhizophagus irregularis</i>	Tomato	Protects plants against pathogens ( <i>Sclerotinia sclerotiorum</i> ) and improves nutrient uptake in plants	Increases soil micronutrient, triggers the defense of the plant against pathogens	[90]
<i>Glomus deserticola</i>	Snapdragon	Increases the total dry matter, chlorophyll content and improves Snapdragon tolerance to water stress	Increases soil nutrients needed for plant growth promotion	[91]
<i>Glomus</i> spp. and <i>Mortierella</i> spp.	Seashore mallow	Increases shoot and root weight under salt stress	Increases soil nutrient and enhances its absorption by plants	[92]
<i>Glomus versiforme</i>	<i>Mentha arvensis</i> L.	Increases dry weight and improves nutrient uptake in salt stress conditions	Increases soil nutrient and enhances its absorption by the plant to enhance its tolerance to salinity	[93]

## 6. Beneficial Role of Biofertilizers on Plant Yield, Photosynthesis and Soil Nutrient

Biofertilizers are products composed of viable strains of microorganisms used to enhance plant growth without causing harm to human health or the environment [94]. Examples of microorganisms used in the production of biofertilizers that can increase plant growth and yield are nitrogen-fixing microbes, phosphorus solubilizing microbes, sulphur solubilizing microbes, mycorrhizal fungi, and potassium solubilizing microorganisms [95].

Recent research has revealed the effect of *Rhizobium leguminosarum*, *Rhizobium* spp. IRBG 74, and *Bradyrhizobium* spp. IRBG 271 increases plant biomass, yield, and chlorophyll content in plants compared with uninoculated plants. The highest increase was recorded with the IRBG strains, which showed a 14% increase when compared to uninoculated plants [96]. Similarly, certain strains of *Rhizobia* can increase the surface area, photosynthetic rate, water uptake capacity, yield, and stomatal conductance of inoculated plants [97].

Furthermore, inoculation of a consortium of bacteria, namely, *Pseudomonas*, *Bacillus lentus*, and *Azospirillum brasilense*, was reported to increase chlorophyll content in plants and the expression of antioxidant enzymes under stress conditions [98]. Khalid et al. [99] found that the application of biofertilizers on spinach increases growth, chlorophyll content, antioxidant activity, yield, and phenolic compounds. The total phenolic compounds were reported to be 58% higher when compared to uninoculated spinach. Similarly, Arora et al. [100] reported an increase in growth, yield, phenolic compounds, anthocyanins, and carotenoid content of lettuce when inoculated with *Azotobacter chroococcum* and *Piriformospora indica*. Kapoor and Singh [101] examined the biosynthesis of antioxidants by arbuscular mycorrhizal fungi. Likewise, Hassen et al. [102] reported an 80% increase in the yield of soybean when inoculated with nitrogen-fixing *Rhizobium* and *Bradyrhizobium*. Production of secondary metabolites, such as tannins, ortho-dihydroxy, and flavonoids, had also been reported in *Begonia malabarica* and *Calamus thwaitesii*, after being inoculated with *Glomus mosseae*, *Trichoderma viride*, and *Bacillus coagulans* [103].

The effect of biofertilizer in increasing plant growth and increasing plant yield for more food production has also been attached to its application. This is evident in research conducted by Dicko et al. [104], who reported that biofertilizer made from plant growth-promoting Actinomycetes (*Actinomycetes* sp. H7, O19, and AHB12) improved maize yield. Data obtained for the study revealed that the highest yield performance was recorded in biofertilizer made from a combination of O19 and AHB12, with a yield increase to 311.5 g for 1000 seeds compared to 178.28 g for the control plant. Recently, the effect of biofertilizer made from a plant growth-promoting *Bacillus pumilus* strain TUAT-1 was evaluated on two forage rice genotypes. The result obtained revealed that biofertilizer made from the *Bacillus* species increased rice productivity when compared to uninoculated [105]. Additionally, the application of biofertilizer in increasing maize growth and yield performance was reported by Fathi [106]. In the study, biofertilizer formulated using phosphate solubilizing bacteria was reported to enhance maize growth and yield when compared to uninoculated control.

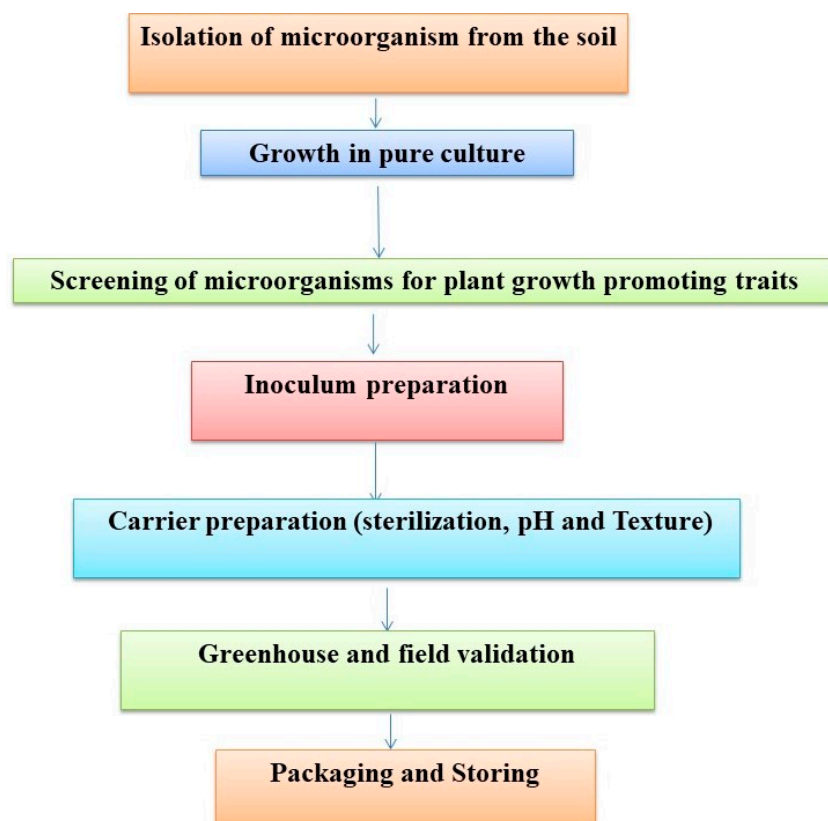
More importantly, soil nutrients are reduced as a result of different activities that occur in the soil, which include runoff, bush burning, and leaching of agricultural soil. Nutrients in the soil migrate to the water body through runoff caused by rainfall, where it causes eutrophication and contamination of the water body [107]. This causes a major threat to the natural environment. Thus, the application of nutrient-rich biofertilizer made from plant growth-promoting microorganisms that have the potentials, such as nitrogen fixation, potassium solubilization, and phosphate solubilization, are essential in the recovery of soil nutrients to enhance plant growth and yield performance [108].

## 7. Formulation of Biofertilizers for the Management of the Rhizosphere

Formulation is a crucial step in the production of a biofertilizer since it has to maintain the viability of the microorganism used while maintaining its activity at low levels [109]. The formulation process involves preparation of inoculum, inclusion of additives, selection of the best carrier, sterilization of carrier material, scaling up, good quality control measures, and adequate packaging with the best delivery method (Figure 3). Formulation of microbial



biofertilizer is regarded as a mixture that comprises one or more viable (active) strains of microorganisms aimed at improving plant metabolic activities at the site of application and is regarded as an alternative approach to chemical fertilizers [79]. Formulation aims to provide long shelf life to microorganisms. The carrier material serves as a support for the proliferation of microorganisms and ensures that the microorganism establishes itself with the plant. Additives protect the formulation from any unfavorable environmental conditions and improve the properties of the formulation [110]. Scaling up provides optimum growth conditions for the proliferation of the microbe used in the formulation process [111].



**Figure 3.** Simplified flow chart for the production of biofertilizer.

A good biofertilizer formulation must not be toxic or pollutant, should be economically viable (preferentially made from inexpensive materials), must permit nutrient uptake by the plant, increase plant yield, have a long shelf life, and remain viable under unfavorable conditions [30,112].

Additionally, the important issue to tackle in the production of biofertilizers for widespread use is the production of large quantities of pure inocula that have a high level of infectivity. Major aspects of the inoculation technology of plant growth-promoting microorganisms are the use of a good strain of microorganisms with plant growth-promoting functional genes for the preparation of inoculum, selection of an appropriate carrier, and the use of an appropriate method of delivery.

Furthermore, the selection of a viable strain of microorganism is important in this step (Table 2); once this is done, the production process, using standardized industrial methods, can follow. The cost of production of commercial biofertilizers is a significant constraint, and there are different carriers used in the production of biofertilizers (Table 3). Thus, the need to use some organic matter that is cheap and readily available arises; materials that have been used successfully include whey, water sludge, animal waste, and compost [113]. An alternative approach to reduce the cost of producing biofertilizer is to use residues from agro-industries that are enriched with rock phosphate. During the process of composting,

microorganisms that can produce organic acid, improve phosphate solubilization activity are added to the carrier to ensure that the nutrients are made available to the plant [114].

Currently, inoculation of crops with mycorrhizal fungi as biofertilizers is becoming more common because of the reduction in the population of indigenous mycorrhiza fungi in the soil through the application of chemical fertilizer [115]. However, in selecting appropriate mycorrhizal fungi, it is essential to select high-quality mycorrhiza fungi, which will be able to colonize plant roots, act in the presence of bacteria, and have a long shelf life in the field and greenhouse [19]. The easiest way of propagating arbuscular mycorrhizal fungi is through the propagation of a viable spore using sterile soil and a suitable host plant. This requires the cultivation of inoculated host plants in sterile soil and arbuscular mycorrhizal fungi spores being allowed to develop and propagate within the host plant [116]. This method of producing inoculum is referred to as soil-based inoculum, which is the most common method used in the multiplication of arbuscular mycorrhiza spore.

The successful use of arbuscular mycorrhizal fungi depends on the strain utilized, the host plant, and the substrate used for propagation. More importantly, host plants that are commonly used in the propagation of arbuscular mycorrhizal fungi are sorghum and maize because of their high infectivity by mycorrhizal fungi. Roots and soil containing mycorrhizal fungi are harvested at the growing cycle, dried, and used as an inoculum.

Recently, new technology has been introduced in the formulation of biofertilizer, which involves the amendment of plant growth-promoting microbes with nanoparticles [117]. This technique involves the use of nanoparticles made from organic or inorganic material with at least 100 nm in size. In agriculture, this technique is referred to as the agro-nanotechnology approach. Plant growth-promoting microbes are integrated into the nanostructure to enhance yield performance in plants [118]. The formulation of nano-biofertilizer has efficiently enhanced agricultural productivity by increasing high retention in soil moisture content and increasing essential nutrient due to the direct and indirect effects of nanomaterial coating on plant growth-promoting microorganisms, and its application has been reported to increase yield performance in cereal and leguminous plants by stimulating the germination potency in plants [119].

**Table 3.** Classification of carrier materials for the production of biofertilizer.

Categories of Carrier Material	Carrier Materials	References
Natural materials	Peat, lignite, coal, clay, and organic soil	[120]
Inert materials	Talc, vermiculite, perlite kaolin, bentonite, silicate, rock phosphate, calcium sulfate, and zeolite	[121,122]
Synthetic polymers	Polyacrylamide, polystyrene, and polyurethane	[123]
Natural polymers	Xanthan gum, carrageenan, agar agar, and agarose	[124]
Organic materials	Charcoal, biochar, composts, farmyard manure, sawdust, maize straw, vermicompost, cow dung, corn cob, and wheat husk	[125–128]
Agro-industry by-product	Sludge ash, jaggery	[120,129]

## 8. Forms and Applications of Biofertilizer Formulations

There are two forms of biofertilizer formulations; liquid and solid. The liquid formulations consist of a culture of microorganisms along with some compounds, such as water, oil, and other substances that help in increasing the adhesion and dispersion ability in the formulated product [130]. The major advantage of liquid formulations is that they can be easily processed, and the cost of production is not high [131]. The solid formulation, which

is also referred to as carrier-based formulation. It is a biofertilizer formulation that is based on the presence of either an organic or inorganic carrier, which is prepared as granules or powder [132]. The most important component in the formulation of a solid biofertilizer, which could be in the form of powder or granular product, is the organic or inorganic carrier.

The application of biofertilizer depends on the type of bioformulation to be applied. There are several methods through which biofertilizers can be applied as follows: seed inoculation, root dipping, and soil application with either dry or liquid biofertilizers [12].

In seed inoculation, carrier biofertilizers are mixed with water to make a slurry. Sterile seeds are mixed inside the slurry to give a uniform mixture of inoculants coating the seed, and then the mixture is air-dried before sowing [133]. The root dipping method of biofertilizer application is used for transplanted crops. The biofertilizer is mixed with water, and the root of the plant is dipped inside the mixture for a while before it is transplanted. In soil application, biofertilizer is spread on the soil at a particular time when the farmer is ready to plant the seed and can also be applied as a foliar spray [133].

### 9. Effect of Biofertilizers on the Production of Volatile Organic Compounds (VOCs) and Amino Acids

The production of volatile organic compounds (VOCs) is among the metabolic activities possessed by beneficial microorganisms used as a signal of interaction between plants and microorganisms to promote plant growth and yield [134]. Many plant growth-promoting microorganisms and plants produce VOCs, which include acetone, 3-butanediol, terpenes, jasmonates, and isoprene, and are classified as natural compounds that can promote plant productivity. VOCs produced by PGPR play a crucial role in influencing systemic resistance in plants and inhibiting the effect of phytopathogens on plant productivity [135]. Bailly and Weisskopf [136] examined the impact of several genera of bacteria in producing VOCs with their positive effect in promoting plant productivity; these include *Bacillus*, *Pseudomonas*, *Arthrobacter*, and *Serratia*. Recently, *Bacillus* spp. has been reported to produce two VOCs, namely, acetoin and 2, 3 butandiol, that can inhibit fungal pathogens and enhance plant growth [137]. Similarly, Park et al. [138] analyzed the production of 1-3-tetradecadien-1-ol, 2-butanone, and 2-methyl-n-1-tridecene by *Pseudomonas fluorescens* SS101, which can promote plant yield. Synthesis of VOCs by bacteria has been reported to induce systemic resistance in plants and enhance their tolerance to biotic and abiotic stress [135]. Furthermore, the effect of co-inoculation of PGPR strains *Pseudomonas fluorescens*, *Bacillus subtilis*, and *P. putida* SJ04, with multiple plant growth-promoting traits on the *Mentha piperita* plant, was reported by del Rosario Cappellari et al. [139] to increase the concentration of the volatile organic compound and total phenolic compound when compared to an uninoculated plant.

In addition to the potential of plant growth-promoting microorganisms in enhancing water and nutrient uptake in plants, they also synthesize and secrete various compounds, among which are amino acids [140]. The plant produces root exudates that attract beneficial microbes in the soil towards the rhizosphere. Regulation of beneficial soil microorganisms surrounding the plant root and the physicochemical properties of the soil are regulated by the exudation of diverse chemical compounds from the plant root [108]. Thus, the type of microorganisms associated with the plant root determines the type of amino acid and root exudate to be secreted by the plant root. Hence, the variation in the population of plant growth-promoting microorganisms that adhere to the plant affects the type of amino acids, oxalic acids, flavonoids, and coumarins secreted by plants.

### 10. Effect of Biofertilizer on Phytopathogens and Pest

Plant diseases may be controlled through the application of chemical fungicides, insecticides, and herbicides. The application of these pesticides in pest management is a crucial aspect of modern agricultural practice. However, their excessive use is hazardous to the environment and poses a threat to human health and living organisms [141]. Thus, the use of beneficial microorganisms in controlling pests has gained more attention because

of its potential to be eco-friendly and cost-effective [142]. More importantly, research is emerging on the application of beneficial microorganisms as a substitute to control the negative effects of pesticides. Plant growth-promoting microorganisms have been subjected to various investigations for their implementation as biopesticides in protecting the environment and forestry. The application of some microbial strains in the formulation of biofertilizer to enhance plant yield also protects plants against pathogenic diseases, either directly by preventing the proliferation of plant pathogens or indirectly by competition for nutrients. Currently, a report has shown the effectiveness of microorganisms, such as *Azotobacter*, *Bacillus*, *Enterobacter*, *Paenibacillus*, and *Pseudomonas*, in reducing pesticide toxicity [143].

Interestingly, the symbiotic association of nitrogen-fixing microorganisms with a leguminous plant promotes the synthesis of cyanogenic defense compounds that prevent herbivore attacks on the plant [144]. One of the major factors that affect plant productivity is the attack by phytopathogens. Thus, the application of beneficial microorganisms that produce antimicrobial substances, such as chitinases and  $\beta$ -glucanases, in high concentration assist in limiting disease attack in plants [145]. *Pseudomonas fluorescens* and *Sinorhizobium* produce chitinase and  $\beta$ -glucanases when used in the formulation of biofertilizers and can suppress *Fusarium* wilt and soft rot in potato caused by *Fusarium udum* and *Erwinia carotovora* [134]. Recently, the application of *G. intraradices* has been reported by Deja-Sikora et al. [146] to improve potato yield and suppress the attack of the potato virus in the plant tissue. Similarly, Beris and Vassilakos [147] reported that inoculation of tomatoes with *G. mosseae* suppresses the effect of tomato yellow leaf curl Sardinia virus (TYLCSV). Some biofertilizers produce siderophores, which limit the amount of iron in the soil in the proximity of plant roots so that through competition for nutrients, the ability of disease-causing microorganisms is suppressed [148]. *Pseudomonas* and *Bacillus* with siderophore-producing ability have been reported by Devi et al. [149] to suppress *Fusarium* wilt in potato and maize. Similarly, Yasmin et al. [150] found that *Pseudomonas aeruginosa* was effective against bacterial blight in rice caused by *Xanthomonas oryzae* and *Rhizoctonia solani*, which is a major disease that affects rice in West Africa. Alaux et al. [151] reported that potato plants inoculated with *Rhizophagus irregularis* MUCL 41833 had enhanced plant defense against *Phytophthora infestans*, which is mediated by ERF3 through the involvement of the plant's ethylene signaling pathway.

## 11. Challenges with Biofertilizer

Though the application of beneficial soil microorganisms in the production of biofertilizers to enhance plant productivity is gaining more traction, and significant success has been recorded from its application over the past years, they have not been widely accepted on a large scale because of the difficulty of reproducing their beneficial effect on plants in a natural environment where there is variation in the environmental condition. The major challenges with the application of microbial biofertilizer are a lack of awareness on the eco-friendly importance of microbial biofertilizer among the communities of farmers, inadequate promotion and motivation by the agricultural extension worker to the farmers on the use of biofertilizer product, lack of availability of suitable carriers for biofertilizer formulation, lack of storage facilities to prevent contamination of the biofertilizer product, and extreme climatic conditions, which lead to inconsistency in the efficacy of biofertilizers on plant productivity in a natural environment. Further, the credibility in the application of biofertilizer products can be shattered by lack of labeling, e.g., expiry date and the name of microorganisms used in the production of the biofertilizer, and most biofertilizers are selective in their actions [152,153].

## 12. Prospects and Conclusions

The use of microbial biofertilizers as a key to modern agriculture is fundamental, based on its renewable, low cost, and eco-friendly potential in ensuring sustainable agriculture. Importantly, the application of biofertilizer as an integral component of agricultural

practice in promoting plant yield has gained more traction recently in meeting the demand of food production of the world populace. Employing mycorrhizal fungi and PGPR in the production of biofertilizers for rhizosphere management has recorded success in some developing countries and will continue to grow with time. Moreover, the new technology which involves the amendment of plant growth-promoting microorganisms with nanoparticles made from organic and inorganic material will continue to gain more attention with time.

In conclusion, overdependence on the use of chemical fertilizers has encouraged industries to produce chemicals that are toxic to human health. Thus, causing ecological imbalances. These drawbacks are combined with a high cost of production that is beyond the means of many farmers in the developing world. The application of biofertilizers is eco-friendly, relatively inexpensive, nontoxic, and possesses the significant potential to increase plant yield. Thus, the function of plant growth-promoting microorganisms and the application of biofertilizer made from viable microbial strains to the field bodes well for successful management of the rhizosphere for sustainable agriculture.

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