

# Microbial Insights into Biofortified Common Bean Cultivation

Alexander Machado Cardoso \* , Carlos Vinicius Ferreira da Silva and Vânia Lúcia de Pádua

Department of Biology, Rio de Janeiro State University—UERJ, Rio de Janeiro 23070200, Brazil; cviniciusbio@gmail.com (C.V.F.d.S.); vaniadepadua@gmail.com (V.L.d.P.)

\* Correspondence: alexander.cardoso@uerj.br or amcardosopf@yahoo.com.br

**Abstract:** Microorganisms play a fundamental role in sustainable agriculture, and their importance in common bean (*Phaseolus vulgaris*) cultivation cannot be underestimated. This review article aims to comprehensively explore the diverse roles of microorganisms in sustainable biofortified common bean cultivation. Biofortification refers to the process of increasing the nutrient content in crops, which helps combat deficiencies in iron, zinc, and vitamins in the human body. Biofortified beans have better agronomic characteristics and offer higher micronutrient content compared to conventional crops. We examine the contribution of various microbial communities in nitrogen fixation, soil structure improvement, nutrient recycling, and disease suppression. Understanding the interaction between beneficial microorganisms and biofortified common bean plants enables us to develop ecologically sound and sustainable approaches to optimize crop productivity and improve nutrition and livelihoods for millions of people worldwide while reducing the environmental impact of agricultural practices.

**Keywords:** beneficial microorganisms; crop cultivation; disease control; nitrogen fixation; plant growth promotion; rhizosphere



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## 1. Introduction

Common bean (*Phaseolus vulgaris*) is a staple crop widely cultivated worldwide due to its nutritional value and economic importance [1]. However, conventional agricultural practices often rely on synthetic inputs that can lead to environmental degradation and pose challenges for long-term sustainability. Harnessing the potential of microorganisms offers a promising pathway to foster sustainable common bean cultivation while preserving ecological balance [2]. Utilizing pesticides and chemical-mineral fertilizers undoubtedly leads to increased productivity in agriculture. However, it is essential to acknowledge that these fertilizers come with significant drawbacks—their high cost and potential for causing severe environmental contamination problems [3]. In addition, the usage of pesticides has proven to be detrimental to not only human health but also to other animals, insects and the overall quality of water and soil, disrupting their natural microbiota [4,5]. Given the critical state of our environment, it is imperative to recognize the urgency of the situation. This necessitates a shift towards adopting eco-friendly agricultural practices that prioritize sustainability. By focusing on promoting sustainable mechanisms, we can strive to increase crop yields even in challenging environments, all while supporting the economy to move forward with profitability [6].

Biofortification is a sustainable agricultural strategy that aims to address micronutrient malnutrition by increasing the mineral and vitamin content of food crops [7]. It can be achieved through conventional plant breeding, soil and foliar fertilizer application, and genetic engineering [8]. This approach is considered cost-effective, has less impact on the organoleptic properties of food crops, and can be implemented without significantly influencing the prices of foods. Biofortification is seen as a promising and collaborative technique that can improve global nutritional outcomes. It is also highlighted as a sustainable route to growing nutrient-rich crops under changing climates and is recognized as a

promising and sustainable mechanism for promoting nutrition and addressing micronutrient malnutrition [9,10]. This review discusses the microbial contribution to improved disease control, nitrogen fixation, soil structure improvement, nutrient recycling, and plant growth promotion. Understanding the symbiotic relationships between microorganisms and biofortified common beans is crucial for developing ecologically responsible and sustainable agricultural practices.

## 2. Rhizosphere

Microorganisms live in bulk soil or in association with roots, forming a complex community where a wide range of interactions takes place [11]. The soil is an environment with intricate characteristics that directly influence the survival, growth, multiplication, and other activities of different organisms [12]. The rhizosphere is a part of the soil directly influenced by the presence of roots, exhibiting different characteristics from the surrounding soil. It is the region where various interactions between microorganisms and plants occur [13]. The relationship between soil microbiota and environmental quality is extremely close. Additionally, the soil microbiota determines the temporary fixation of nutrients. The rhizosphere is a dynamic habitat where its dimensions are determined by the soil type and moisture composition [14]. Through the release of root exudates, the plant enriches the soil with a variety of organic compounds. The consumption of O<sub>2</sub> and release of CO<sub>2</sub> alter the soil characteristics in the vicinity of the root, modifying the root atmosphere [13,14].

Through the exudates released by the root, plants are able to select beneficial microorganisms, protecting them from infections caused by pathogens present in the environment [15]. The exudates consist of a collection of different substances released by the plant, including proteins, ions, water, enzymes, free oxygen, a diversity of primary and secondary metabolites with carbon in their composition, maltose, sucrose, xylose, rhamnose, glucose, fructose, ribose, arabinose, and oligosaccharides [16]. Plants can influence the composition of the microbial community in the rhizosphere through the released compounds, acting as chemotactic or repellent molecules [13]. As the patterns of exudates change, the rhizosphere undergoes alterations, and different microbial communities colonize the different rhizospheres. The physical and chemical modifications that roots produce create a unique ecosystem, where the growth of microbial communities can be either enhanced or inhibited [17]. The rhizosphere microbiome is structured according to the plant species, soil type, root morphology, exudate release and composition, and climate of the region [11]. Furthermore, variations in the microbiota can occur during different stages of plant development [18].

## 3. Plant Growth Promoting Bacteria

One of the sustainable strategies for an agricultural eco-friendly practice is based on plant growth-promoting bacteria (PGPR), which stimulate the development of host plants and significantly affect the structure of the rhizosphere bacterial community [19]. The plant growth-promoting bacteria are highly diverse and perform key functions for plant growth and defense mechanisms, which act directly or indirectly. In direct mechanisms, bacteria provide certain compounds to the plant or facilitate the uptake of soluble nutrients from the soil, for example, biological nitrogen fixation (BNF), phosphate solubilization, and the production of plant growth regulators such as auxins and cytokinins. In indirect mechanisms, bacteria also produce substances capable of mobilizing nutrients such as amino acids, siderophores, and organic acids that release phosphorus and metals. In addition, they can produce antibiotics and other substances, that influence the production of defense molecules in the plant or that affect the development of phytopathogenic microorganisms [20].

Interestingly, recent studies have described novel rhizobacterial molecules playing a key role in multi-trophic interactions with plants. These molecules, known as volatile organic compounds (VOCs), are low-molecular-weight lipophilic compounds with a low

boiling point and high vapor pressures that can be elicited and diffused through complex matrixes, such as cellular membranes, water, soil, and air [21], looking like signal transducers which form a cross-talk within and between organisms, below and even above the soil [22]. Many previous works have evaluated that VOCs have been shown to increase plant health, sometimes reproducing the effects of direct bacterial inoculation of roots [23]. Even so, plant development and the process of induced systemic resistance against pathogens and stress tolerance can be stimulated by VOCs. Some bacterial VOCs may produce a specific antimicrobial effect, inhibit pathogenic quorum sensing, biofilm, virulence, and increase plant tolerance to stress situations [24,25], and thus their use is favored in agribusiness as they increase biomass and plant productivity [26]. However, the role of rhizobacteria in the formation of VOCs is still unclear. Most rhizobacteria are unculturable, and their potential metabolic function analysis is difficult to reveal. Therefore, different culture-independent approaches such as metagenomics, transcriptomics, proteomics, and metabolomics are essential to investigate the rhizosphere microbiome [27].

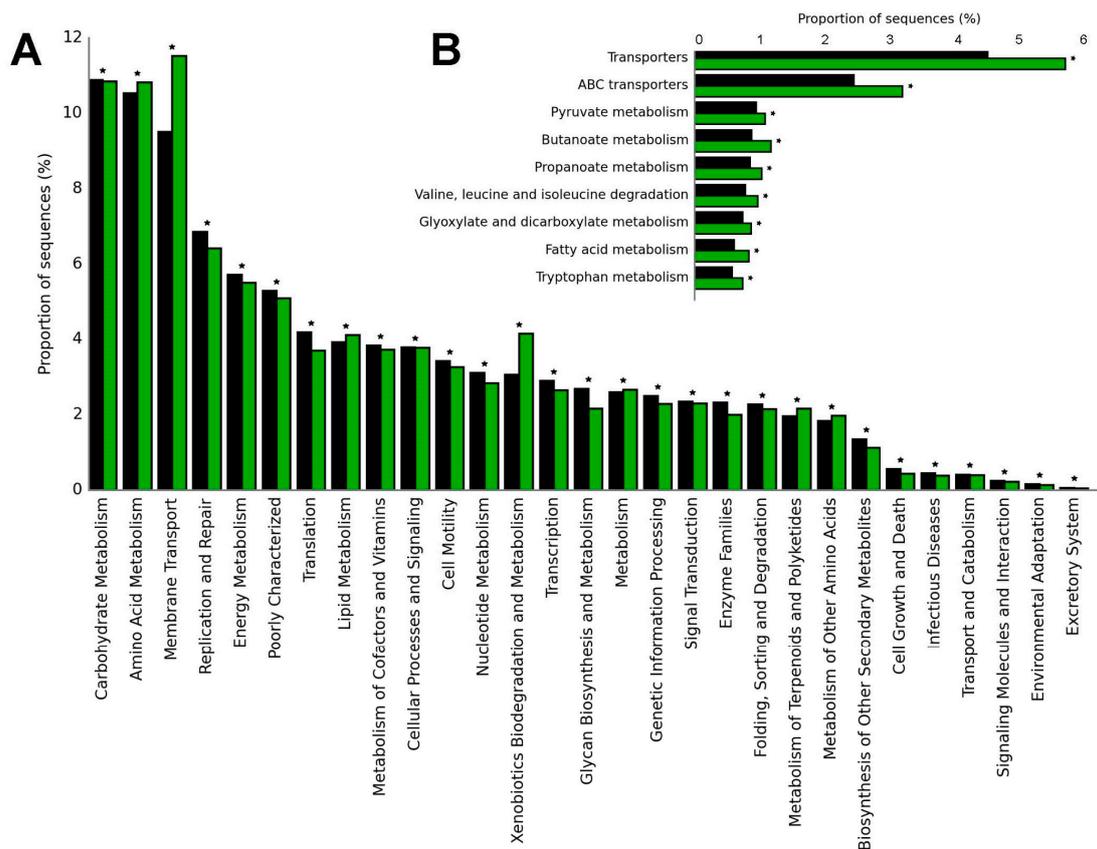
#### 4. Biofortification of Common Beans

Biofortified beans carry the potential to serve as a substantial source of essential minerals for undernourished populations, while common beans inherently contain a notable amount of nutrients. The primary aim of biofortification is to elevate their nutritional profile [28]. It is now clear that urban populations also need optimized diets, even though the overall objective of addressing nutritional deficiencies common in vulnerable communities fits in well with the targeted enhancement of nutritional mineral contents through biofortification. More than 1.5 billion people are thought to be iron deficient [29]. The consumption of iron-biofortified beans can help prevent and reverse iron deficiency, particularly in women and children. Studies have shown that regular consumption of these beans can lead to improved iron status, cognitive function, and physical performance in iron-deficient individuals [30]. Experiments utilizing biofortified beans in rats [31], pigs [32], and chicks [33] produced encouraging outcomes, which led to the start of human trials. A study on normal and high iron-biofortified beans was carried out among young women in Rwanda, most of whom were anemic or iron deficient. Following 4.5 months, the high iron bean group showed a statistically significant increase in total body iron ( $0.5 \text{ mg}\cdot\text{kg}^{-1}$ ), log serum ferritin ( $0.1 \text{ log } \mu\text{g}\cdot\text{L}^{-1}$ ), and hemoglobin levels ( $3.8 \text{ g}\cdot\text{L}^{-1}$ ) [34]. Other studies have reported increased neuron activity, better cognitive ability, and improved work capacity in this group [35–37].

Brazilian bean breeding programs have historically placed a significant emphasis on the development of bean cultivars enriched with high nutritional content. A noteworthy contribution to this endeavor is the creation of biofortified beans, exemplified by the common bean cultivar with a carioca grain type, known as BRS pontal (with elevated levels of essential nutrients such as iron and zinc), which has been recognized for its superior grain quality, resistance to prevalent diseases and drought conditions [38]. The intricate interplay between beans and specific microbial taxa introduces an additional dimension to biofortification strategies. Some studies suggest that certain bacterial species play a beneficial role in enhancing soil micronutrient availability and facilitate improved plant uptake and the subsequent accumulation of essential minerals in the grains [39]. Understanding and harnessing these microbial interactions offer a promising avenue for optimizing biofortification processes, thereby maximizing the nutritional benefits [40].

#### 5. Metabolic Potential

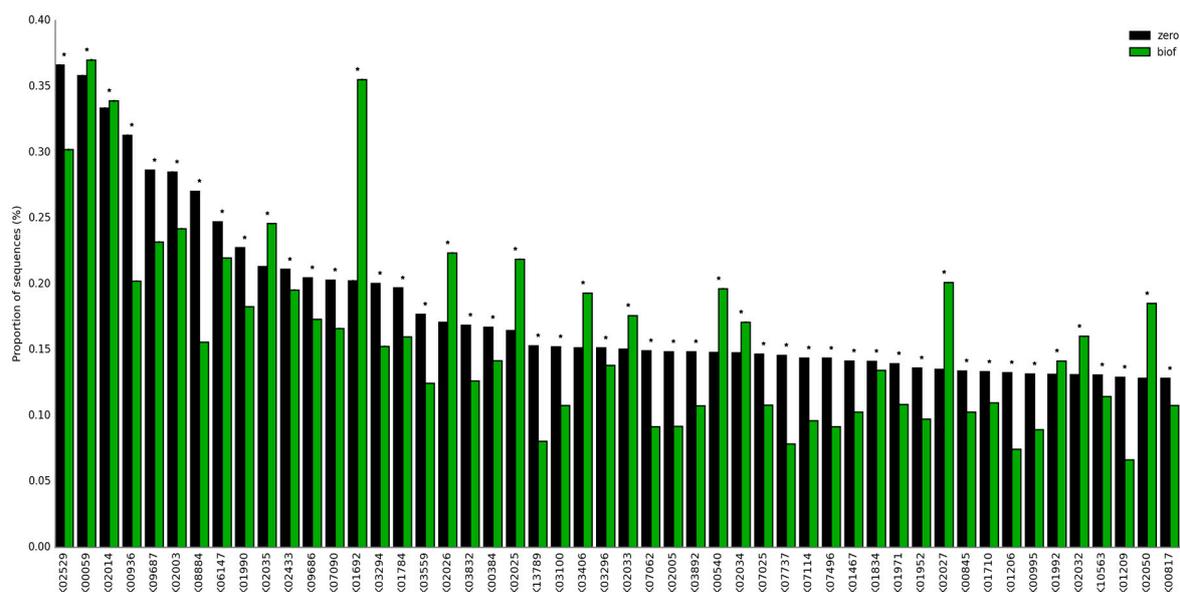
The potential biochemical functions of biofortified bean rhizobacteria can be revealed via a phylogenetic investigation of bacterial communities conducted by reconstructing unobserved states based on previous studies [40] and reference sequence databases [41]. Compared to bulk soils and rhizosphere, the analysis can predict high relative abundances of functional profiles in the bean rhizosphere related to membrane transport, amino acid, terpenoids, polyketides, and xenobiotic metabolism (Figure 1A).



**Figure 1.** Functional classification of the predicted genes. Functional classes were determined according to the second (A) or third (B) level of the KEGG annotations. Statistical analysis, data normalization, and determination of differentially abundant genes were then conducted using the STAMP program [42]. \*: statistical significance, *p* values were adjusted for multiple testing using the G-test, Fisher’s procedures, and Bonferroni correction in bulk soils (black) and rhizosphere (green) bean samples using the data from carioca beans [40].

An analysis of the predicted gene copy number of the tertiary functional levels showed that the predicted gene copy number for nine subfunctions, such as transporters, ABC transporters, valine, leucine and isoleucine degradation, pyruvate, butanoate, propanoate, tryptophan, fatty acid, glyoxylate, and dicarboxylate metabolism, increased in the rhizosphere compared to bulk soil samples (Figure 1B). In addition, a total of 4597 KOs (Kegg Orthologies) were identified with significant differences among the samples. Compared with the bulk soil, the relative abundances of KO2026, KO2025, KO2027, KO1992, and other genes related to transport system permease proteins in the rhizosphere were increased (Figure 2).

The relative abundances of quorum sensing and chemotaxis signal transduction system-related genes such as KO2035, KO3406, KO2034, KO2032, and KO2050 were enhanced. Increases in oxidoreductases (KO0059 and KO0540) and iron complex outer membrane receptor proteins (KO2014) were also observed. Interestingly, a recent study indicated that VOCs improved iron acquisition in plants [43]. High relative abundances of enoyl-CoA hydratase genes (KO1692) were detected, probably acting in the phenylacetate metabolic pathway [44]. Phenylacetate is a VOC naturally produced by many organisms with antimicrobial and antifungal activities, also behaving as a chemical signal in the rhizosphere to promote plant health, growth, and development [45–47].



**Figure 2.** Relative abundances of predicted genes. Statistical analysis, data normalization, and determination of differentially abundant genes were then conducted using the STAMP program [42]. \*: statistical significance,  $p$  values were adjusted for multiple testing using the G-test, Fisher's procedures, and Bonferroni correction in bulk soils (black) and rhizosphere (green) bean samples by using the data from carioca beans [40].

The identification and analysis of the emitted volatiles are usually accomplished using agar plate cultures such as bipartite Petri dish assays and gas chromatography coupled most often with mass spectrometry. Hundreds of bacterial VOCs have been identified, including alkanes, alkenes, alcohols, esters, ketones, sulfur compounds, and terpenoids [45,48]. Based on the predictive analysis and extensive literature search for microbial volatile organic compounds, the potential VOCs emitted by bacterial communities in bean rhizosphere may be acids (acetic, butanoate, dodecanoic, phenylacetate, isovalerate, propanoate), alcohols (benzylmethanol, heptanol, hexanols, isoamyl, sec-isoamyl), amines (dimethylhexadecylamine), ketones (1-(furan-2-yl)ethenone, 1-phenylethanone, 1-phenylpropane-1,2-dione, 4-methylpentan-2-one, aminoacetophenone, decan-2-one, heptan-4-one, nonan-2-one, tridecan-2-one, undecan-2-one), lactones (4-methyloxolan-2-one), pyrazines (2-methyl-5-propan-2-ylpyrazine, 2,5-dimethylpyrazine, 2,3,5-trimethylpyrazine, 2,3,5,6-tetramethylpyrazine, methylquinoxaline), terpenes (4,7,7-trimethyl-3-bicyclo[2.2.1]heptanyl) acetate, (5E)-6,10-dimethylundeca-5,9-dien-2-one, alphapinene), and sulfur compounds (4-methylsulfanylbutan-2-one, dimethyldisulphide, methylsulfanylmethane, methyl-sulfonylsulfanylmethane, (methyltrisulfanyl)methane, (methyldisulfa-nyl)-methylsulfanylmethane). Volatile metabolites released by microorganisms produce potential pesticides, fungicides, and bactericides and may contribute to sustainable crop protection and production [26], and acetaldehyde, butanoate, propanoate, pyrazines, terpenoids, polyketides, and xenobiotic derivatives are found to be the most frequently emitted compounds by bacteria [45,48–52].

Recently, bacterial community structures were described as being different between bulk soil and rhizosphere biofortified bean samples, presenting a high number of sequences affiliated with the genera *Burkholderia* [40]. Changes in the microbiota composition impact the various nutrients, minerals uptake, and synthesis of vitamins, amino acids, phytohormones that enhance plant growth, and defense against pathogenic organisms and predators. Such changes may be due to differences in the abundance of genes encoding enzymes that are involved in biochemical reactions leading to volatile compounds. Remarkably, *Burkholderia* species have been shown to emit these predicted compounds [48,53]. Moreover, the production of VOCs is widespread among rhizobacteria and strongly de-

depends on culture conditions. However, the identity of these molecules is still largely to be elucidated [54].

A recent study investigated sec-isoamyl alcohol (3-methyl-2-butanol) as an important volatile compound in the growth promotion of common bean *Phaseolus vulgaris* seedlings [54]. Also, many derivatives from amino acids metabolism such as the amino-containing lipid dimethylhexadecylamine related to bacterial quorum-sensing signals can modulate bacterial growth and plant morphogenesis, induce the iron uptake by roots, and regulate root exudation and defense responses [55–57]. Until now, the knowledge of VOCs on plant growth have been restricted to a few cultured species [45,48]. In fact, the biosynthesis of VOCs is not well investigated, and further experiments with labeled pre-cursors of several putative intermediates may elucidate the metabolic pathway and specifically address the function of these compounds released by rhizobacteria.

## 6. Biological Nitrogen Fixation

One of the main contributions of microorganisms in the cultivation of common beans is nitrogen fixation [58]. Species of *Rhizobium* and other bacteria establish a symbiotic relationship with the roots of common bean plants, converting atmospheric nitrogen into a usable form for the plants. Biological nitrogen fixation (BNF) is a natural process that involves the transformation of atmospheric nitrogen ( $N_2$ ) into ammonia ( $NH_3$ ), a form of nitrogen assimilated by plants [59]. This process is carried out by diazotrophic bacteria, known as rhizobia, which, in association with plants of the legume family, form specialized structures called nodules on the roots or stems, where BNF takes place (Figure 3). This interaction is termed symbiosis and involves the supply of fixed nitrogen by the bacteria to the plant, which, in turn, provides photoassimilates or organic carbon to the bacteria. This process reduces the dependency on chemical nitrogen fertilizers, helps mitigate nitrogen depletion in the soil, and promotes self-sufficiency in nitrogen supply for sustainable agriculture [60].



**Figure 3.** Visualization of nodules on bean roots during the process of biological nitrogen fixation (BNF), where bacteria establish a mutualistic relationship with the plants.

In the nodules (Figure 3), the process of BNF occurs, where bacteria, mainly belonging to the genera *Rhizobium*, *Bradyrhizobium*, and *Sinorhizobium*, establish a mutualistic relationship with the plants. The bacteria colonize the interior of the nodules and, in exchange for carbohydrates and organic compounds provided by the host plant, are capable of capturing atmospheric nitrogen and converting it into a form that plants can use as a nutrients [59]. Biological nitrogen fixation is essential for the nutrition of legume plants, since nitrogen is a fundamental element for the synthesis of proteins, DNA, and other vital compounds. The ability of bacteria to supply nitrogen to plants contributes to increased agricultural productivity and reduces the need for nitrogen fertilizers [61].

The direct relationship between BNF and iron bean biofortification is not clear. The information provided highlights the importance of nitrogen fixation and the challenges in breeding beans for higher iron concentrations without compromising yield. Further research may be needed to explore the potential connection between BNF and iron bean biofortification. Adopting farming techniques that promote nodule formation and activity is crucial to maximizing the benefits of BNF. Enhancing nitrogen fixation in the soil can be achieved by choosing legume varieties with a high capacity for symbiosis and by properly inoculating seeds with beneficial bacteria prior to planting [62]. Proper management of the symbiotic association with nitrogen-fixing bacteria is essential to ensure the productivity and sustainability of agricultural systems, contributing to more efficient and environmentally friendly agriculture. Another important practice is crop rotation with legumes in agricultural systems, which promotes nitrogen cycling and improves soil quality over time [63].

## 7. Improvement of Soil Structure

Mycorrhizal fungi and other beneficial microorganisms play a fundamental role in improving soil structure, providing significant benefits for the cultivation of plants [64]. These symbiotic interactions between microorganisms and plant roots have a positive impact on soil quality, reflecting in healthy plant growth and development [12]. They form a symbiotic relationship with the roots of common bean plants, establishing a network of hyphae around the roots that act as an extension of the plant's root system [65]. This symbiosis facilitates soil aggregation, creating structures called aggregates, which are composed of soil particles bound together by substances produced by microorganisms and plant roots. These aggregates improve soil structure, making it more porous and favoring water infiltration and aeration [12]. Moreover, mycorrhizal fungi help common bean plants to absorb nutrients more efficiently. They establish connections with the roots, expanding the plant's absorption area. As a result, common beans can obtain a greater amount of essential nutrients, such as nitrogen, phosphorus, and potassium, necessary for their healthy growth and development [65].

Other beneficial microorganisms, such as nitrogen-fixing bacteria and organic matter decomposers, also contribute to improving soil structure and nutritional enrichment. Nitrogen-fixing bacteria transform atmospheric nitrogen into a form that can be used by plants, reducing the need for chemical nitrogen fertilizers [59]. Meanwhile, bacteria and fungi that decompose organic matter break it down into simpler compounds, releasing nutrients to the plants and enhancing soil fertility [66].

## 8. Symbiotic Interactions

The symbiotic interactions between microorganisms and common bean plants not only improve the soil but also contribute to the crop's resilience to environmental stresses, such as drought and diseases. A well-structured and nutrient-enriched soil enables plants to better withstand adverse conditions and develop more robustly [67].

To optimize the benefits of these beneficial interactions, it is important to adopt sustainable agricultural practices that preserve and promote soil biodiversity. Avoiding excessive use of pesticides and implementing conservation management techniques, such as crop rotation and the use of mulch, help maintain soil health and the effectiveness of these symbiotic associations [12,63]. The role of mycorrhizal fungi and other beneficial microorganisms in improving soil structure is of the utmost importance for the productivity and sustainability of common bean cultivation [62]. These interactions enrich the soil, enhance nutrient absorption by plants, and strengthen resistance to environmental stressors. By promoting agriculture that values soil health and microbial biodiversity, we can ensure a more productive and sustainable agricultural future.

## 9. Nutrient Recycling

Microbial decomposition of organic matter in the soil is a fundamental process that plays a crucial role in supplying essential nutrients to common bean plants. In this process, microorganisms such as bacteria and fungi break down the organic matter present in the soil, releasing nutrients such as nitrogen, phosphorus, potassium, and other elements essential for healthy plant growth [68]. This natural nutrient recycling is highly beneficial for bean cultivation as it reduces dependence on external fertilizers. Chemical fertilizers, when applied excessively, can cause nutritional imbalances in the soil and have negative impacts on the environment, such as water contamination due to nutrient leaching [43]. By promoting microbial decomposition of organic matter, farmers can harness the nutrients present in the soil itself, saving financial resources and reducing the environmental impact. This practice aligns perfectly with the principles of sustainable agriculture as it fosters a more balanced nutrient management system, avoiding waste and maximizing the utilization of available resources [59,69].

To optimize microbial decomposition in the soil and promote efficient nutrient recycling, it is essential to adopt management practices that stimulate microbial activity. This includes using organic fertilizers, such as composting and animal manure, which provide a rich source of organic matter for microorganisms to degrade [70]. Furthermore, the adoption of conservation management techniques, such as soil mulching or no-till farming, creates a favorable environment for the development of the microbial community, protecting the soil from erosion and extreme temperature variations. However, it is important to note that microbial decomposition is influenced by environmental factors such as temperature, humidity, and soil pH. Therefore, monitoring these parameters and adjusting management practices according to local conditions is crucial to ensure the efficiency of nutrient recycling [43,71].

Microbial decomposition of organic matter is an essential process for sustainable nutrient management in common bean cultivation. Through this natural recycling, microorganisms contribute to the supply of essential nutrients, reducing the need for external fertilizers and promoting a more balanced and responsible approach to agriculture [72]. By adopting management practices that favor microbial activity in the soil, farmers can obtain the benefits of a more efficient and environmentally friendly production of biofortified common beans.

## 10. Biofertilizers for Disease Control

Biofertilizers exhibit substantial potential in managing soil-borne diseases. These supplements contain beneficial microbial species, such as *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus laterosporus*, *Trichoderma harzianum*, *Rhizobium*, and *Mycorrhiza*. By inhibiting and eliminating harmful bacteria, biofertilizers contribute to the reduction of soil-borne diseases [73]. The wide acceptance of biofertilizers is attributed to their dual benefits of promoting plant growth and safeguarding plants against soil-borne pathogens. Additionally, biofertilizers can stimulate indigenous soil bacteria such as *Pseudomonas* populations, enhancing the suppression of plant diseases [74].

Biofertilizers work to control soil-borne diseases through direct and indirect mechanisms. Direct mechanisms include nitrogen fixation, phosphate solubilization, micronutrient solubilization, and the production of phytohormones, which enhance plant growth and protect plants from pathogens. Indirect mechanisms involve the production of antibiotics, siderophores, and cyanide, which protect plants from the deleterious effects of pathogens. Additionally, biofertilizers act as bio-controllers of diseases by exhibiting antagonistic properties against soil-borne plant pathogens, inhibiting and killing harmful bacteria to reduce soil-borne diseases [75].

While microbial-based products, whether they are biopesticides or biofertilizers, have a lengthy history of controlling soil-borne pathogens, their utilization remains somewhat limited. This limitation is primarily linked to their perceived low and inconsistent efficacy, particularly under field conditions [76]. Nevertheless, when applied to soil or substrate,

biofertilizers demonstrate effectiveness in preventing and controlling soil-borne diseases. Furthermore, they optimize the taxonomic structure of microbial communities, fostering a relatively healthy microbial ecological environment. This environment, in turn, facilitates the emergence of numerous ecological functions within the soil and substrate [77].

## 11. Biocontrol Agents

Microorganisms such as certain bacteria and fungi may act as biocontrol agents, suppressing the growth and activity of harmful pathogens. This natural disease suppression reduces the dependency on chemical pesticides, helps mitigate the risk of pesticide resistance, and ensures a healthier agricultural ecosystem [78]. Preventing pest infestations in common bean crops using microbial insecticides like *Bacillus thuringiensis* (Bt) is a widely adopted practice in agricultural production [79].

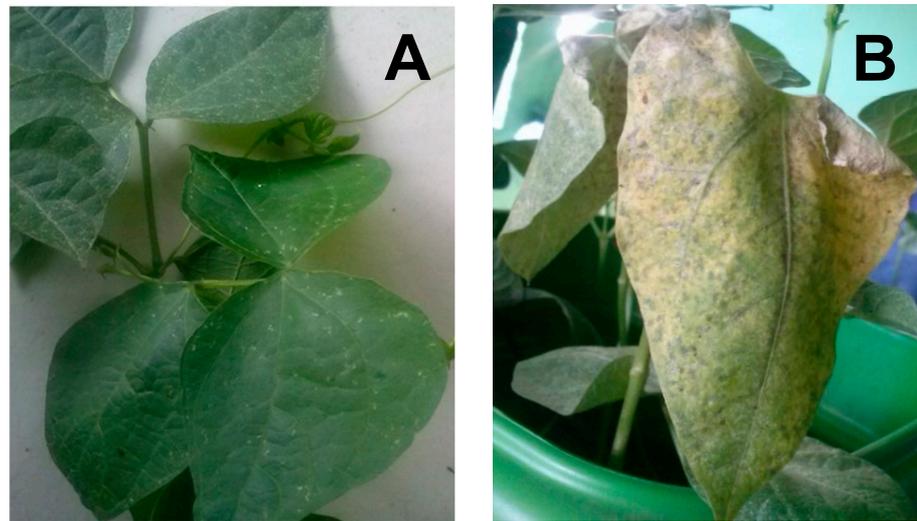
*Bacillus thuringiensis* is a gram-positive bacterium with the ability to form protein crystals, which, when degraded, release delta-endotoxin proteins that are toxic to various insect species while remaining harmless to mammals [80]. This approach has proven particularly effective in managing pest insects such as caterpillars from some lepidopteran species, like the fall armyworm (*Spodoptera frugiperda*) and the velvet bean caterpillar (*Anticarsia gemmatilis*), which cause significant damage to crops [81]. The use of Bt-based microbial insecticides offers an efficient and environmentally safe approach for controlling pests that affect common bean crops. This bacterium has been particularly useful in managing common bean pests like caterpillars from some lepidopteran species that can cause significant damage to plants [81].

One of the key benefits of using *Bacillus thuringiensis* as an insecticide is its highly selective action [82]. By applying this product to bean crops, its effect can be targeted solely against the target pests, reducing its impact on non-harmful organisms like pollinating insects and natural predators. Another important advantage is that Bt is biodegradable and does not leave toxic residues in the environment or crops, making it a sustainable option for agriculture [83]. However, it is crucial to follow good agricultural practices when using microbial insecticides in common bean crops. The constant monitoring of pest populations is important to determine the optimal timing of Bt application and to use recommended dosages. Additionally, integrating the use of Bt with other integrated pest management tactics, such as crop rotation, biological control, and monitoring traps, is recommended. The use of *Bacillus thuringiensis* as a microbial insecticide in common bean crops is a promising strategy for sustainable pest control in plants [84].

Insects can spread diseases to plants besides feeding on them. Bean golden mosaic virus disease (BGMVD) is caused by a virus belonging to the Geminiviridae family, transmitted in nature by vectors such as whiteflies (Aleyrodidae) and leafhoppers (Cicadellidae and Auchenorrhynca), which are cosmopolitan sap-sucking insects classified in the Hemiptera order [81]. The symptoms of the virus infection can vary according to the cultivar and the stage of plant development at the time of contamination, resulting in deformations and a reduction in the size of leaves, pods, and branches [85]. When infection occurs before or during flowering, it can lead to flower abortion and a significant reduction in the number of pods and grains, causing major economic losses [86]. The initial symptoms usually appear on the younger leaves, manifesting as small, bright yellow spots. Over time, these spots spread throughout the leaf blade or even throughout the entire plant (Figure 4), remaining limited by the veins, creating a characteristic mosaic appearance.

In addition to the visual symptoms, it is important to note that the golden mosaic virus can seriously affect crop yield, impacting the quality and quantity of produced grains [87]. Therefore, early monitoring and the adoption of appropriate control measures are crucial to mitigate the damage caused by this disease. Several strategies can be employed for the management of BGMVD, including the use of resistant or tolerant cultivars, effective control of vector insects through cultural practices, and integrated pest management. Also, it is essential to promote the cleanliness of cultivated areas, removing possible sources of

infection and applying preventive measures such as using physical barriers to prevent the entry of vectors into the fields [86].



**Figure 4.** Visual symptoms of plant disease. Healthy (A) and diseased (B) leaves showing the characteristic symptoms of bean golden mosaic virus disease, with bright yellow spots, transmitted by whiteflies and leafhoppers in biofortified common bean plants.

Lastly, continuous research is needed to understand the epidemiology of the BGMVD and other infections to develop more effective and sustainable disease control strategies. Only through an integrated and collaborative approach, involving producers, researchers, and government entities, can we protect our bean crops and ensure the food and economic security of our society. Furthermore, future studies on the role of the phyllosphere microbiome in plant disease defense and improving plant health and productivity will greatly contribute to the advancement of sustainable agriculture and enhanced food security [88].

In addition to investigating the role of the rhizosphere microbiome, it is crucial to explore the phyllosphere microbiome, which encompasses the leaves and aerial surfaces of plants. The phyllosphere microbiome constitutes a complex ecosystem that can directly influence plant health, disease resistance, and even agricultural productivity [89]. The phyllosphere provides a nutrient-rich environment, hosting various microbial species that interact with the host plant in unique ways. Some of these interactions can be beneficial, providing protection against pathogens and enhancing nutrient uptake, while others may be detrimental and cause diseases [90].

Recent studies indicate that bacteria, fungi, and yeasts in the phyllosphere can act as biocontrol agents, safeguarding plants against pathogen-induced diseases [89]. Additionally, these microorganisms can enhance a plant's ability to tolerate environmental stresses, such as high temperatures and drought, thereby improving its survival under adverse conditions. A notable example is the discovery of endophytic bacteria in the phyllosphere that can synthesize antimicrobial compounds, hindering the growth of pathogens [91]. Moreover, recent research demonstrates that the composition of the phyllosphere microbiome may be affected by the use of pesticides and other agricultural chemicals, underscoring the significance of sustainable agricultural practices to preserve this microbial ecosystem [89].

## 12. Conclusions

The diverse contributions of microorganisms in biofortified common bean cultivation highlight their immense potential to revolutionize sustainable agricultural practices. Integrating strategies based on microorganisms into cultivation systems can enhance crop productivity, reduce environmental impacts, and promote ecological balance. To harness these benefits, continuous research and the effective implementation of practices are essen-

tial, ensuring a successful transition towards a more sustainable and resilient agricultural future. Our research group has recently demonstrated a pronounced distinction in the bacterial communities between bulk soils and cultivated beans [40]. The comparison of these communities in biofortified and conventional beans unveiled notable differences, particularly in the prevalence of certain bacterial species. Notably, beans with elevated levels of iron and zinc exhibited an overrepresentation of the genera *Burkholderia* and *Rhodanobacter*. This intriguing finding suggests that biofortified bean varieties maintain a more favorable ecological balance compared to their conventional counterparts. The presence of *Burkholderia* and *Rhodanobacter* in higher proportions implies a potential symbiotic relationship, influencing the nutrient content of the beans. Such insights into the microbial communities associated with biofortified beans underscore the potential ecological advantages of these varieties over conventional ones. The implications of our results extend beyond the realm of microbial ecology. The observed ecological balance in biofortified beans hints at a more resilient and sustainable agricultural practice. These findings open new avenues for advancing sustainable bean production by considering the selection of inoculants that play a pivotal role in shaping the microbial community associated with the host plant. This intricate interplay between the plant and its associated microbiota has far-reaching effects on the physiology and overall development of the bean species.

### 13. Future Directions

Biofortification, the process of enhancing the nutritional content of crops, holds immense promise for addressing global malnutrition and improving public health. Several key directions emerge, each carrying significant implications for agriculture, nutrition, and sustainable development. One future direction involves the emerging field of precision agriculture that offers opportunities for targeted nutrient enhancement in specific plant tissues. By leveraging technologies such as CRISPR-Cas9, researchers can precisely manipulate the genes responsible for nutrient accumulation, allowing for a more tailored and efficient biofortification process. This approach has the potential to optimize nutrient delivery to meet specific dietary requirements. Future biofortification efforts should consider integrating traditional agricultural practices, specific bacterial inoculations, and indigenous knowledge. Collaborating with local communities can enhance the acceptance and sustainability of biofortified crops. Moreover, incorporating traditional crop varieties with desirable nutritional traits into breeding programs can help preserve genetic diversity. Also, it is important to note that climate change poses significant challenges to agriculture, affecting crop yields and nutrient content. Future biofortification initiatives must account for these challenges by developing crops that are not only nutrient-rich but also resilient to changing climatic conditions. This involves identifying and incorporating traits that enhance tolerance to heat, drought, and other climate-related stressors. The success of biofortification requires collaboration across diverse sectors, including agriculture, nutrition, health, and policy. Governments, non-governmental organizations, and private sectors should work together to create supportive policies, invest in research and development, and implement effective strategies to promote the adoption of biofortified crops. Furthermore, educating consumers about the benefits of biofortified crops is crucial for their widespread adoption. Future efforts should focus on raising awareness, dispelling misconceptions, and promoting the nutritional advantages of biofortified foods. Social marketing campaigns can play a pivotal role in influencing consumer behavior and preferences.

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