

Plant Microbiome Engineering for Disease Resistance and Growth Promotion

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Abstract- This review paper critically examines the theoretical and conceptual foundations of plant microbiome engineering as a promising strategy for enhancing disease resistance and promoting growth in plants, emphasizing the intricate relationships between plants and their microbiota, which collectively form a dynamic and functional ecosystem that influences plant health and productivity; the plant-associated microbiome, comprising bacteria, fungi, archaea, and viruses, plays a pivotal role in regulating plant immunity, nutrient acquisition, stress tolerance, and overall growth, with evidence suggesting that specific microbial communities can directly influence plant disease resistance by either outcompeting or inhibiting plant pathogens, modulating plant defense mechanisms, or inducing systemic resistance pathways, thus providing an alternative or complementary approach to traditional chemical control methods; microbiome manipulation for growth promotion has been shown to be effective through the addition of beneficial microorganisms, including plant growth-promoting rhizobacteria (PGPR) and mycorrhizal fungi, which enhance nutrient availability, improve water uptake, and modulate hormone signaling, contributing to increased plant biomass, yield, and resilience under abiotic stress conditions; however, challenges persist in understanding the complexity and specificity of plant-microbe interactions, as microbial communities vary greatly depending on environmental factors, plant genotype, and the developmental stage of the plant, which complicates the design of universal microbiome engineering strategies; several studies have demonstrated the potential of microbiome engineering through methods such as inoculation with targeted microorganisms, use of genetically modified microbes, and soil or root microbiota transplantation, but the lack of a comprehensive

understanding of microbial community structure-function relationships and the long-term stability of engineered microbiomes remains a significant barrier to field applications; moreover, ethical concerns regarding the release of genetically modified microorganisms and the impact of microbiome alterations on ecosystem dynamics need further investigation; the review also highlights the importance of integrating metagenomic, metatranscriptomic, and culturomic approaches to profile microbiomes and identify key microbial players responsible for plant health outcomes, while advocating for the need for interdisciplinary approaches combining microbiology, genomics, plant physiology, and ecological modeling to develop sustainable and effective microbiome-based disease management and growth promotion strategies, with a call for future research to address current gaps in knowledge and application.

Indexed Terms- Plant Microbiome, Disease Resistance, Growth Promotion, Microbiome Engineering, Plant Growth-Promoting Rhizobacteria (PGPR), Microbial Community Dynamics

I. INTRODUCTION

The complex consortium of microorganisms including bacteria, fungi, archaea and viruses living in a plant, collectively referred to as the plant microbiome, colonizes niches on the plant such as the roots, stems, leaves and flowers, and is considered central to the regulation of plant health, growth, and stress tolerance (Bulgarelli et al., 2013). Those microorganisms form mutualistic associations with the plant host to drive important physiological processes including nutrient uptake, pathogen resistance, and stress response, as well as improve plant fitness (Berendsen et al., 2012). In this

perspective, we highlight how microbiome-based interventions could be a fruitful solution to alleviate these issues related to disease management and plant growth promotion raised by the increasing demand for chemical pesticide use (Prasannavadana et al., 2020) and its environmental sustainability (Lebeis et al., 2015). Although many studies have demonstrated direct stimulation of plant immunity through induced systemic resistance or pathogenic competition by certain microbial communities (Chaparro et al., 2014), the highly dynamic and context-dependent nature of the plant microbiome poses challenges for the design of effective microbiota-based solutions. Triggering beneficial interactions among microbes within the plant microbiome has become a target of research to enhance plant health and, therefore, microbiome engineering (intentional manipulation of microbial communities) is regarded as an important strategy (Mendes et al., 2011). Microbial inoculants such as plant growth-promoting rhizobacteria (PGPR), mycorrhizal fungi, and endophytes are utilized to improve the acquisition of nutrients, increase resistance to abiotic stress, and promotion of plant growth, which has shown positive results in both pot and field experiments (Vessey, 2003). Relevant concepts Among all plant-microbiome interactions, the functional diversity and resilience of microbiomes make it difficult to systematically streamline microbiome engineering across plant species, environmental conditions, and plant developmental stages (Lugtenberg and Kamilova, 2009). Microbiome interventions therefore also need to weigh the positive bacteria against the negative, as various microbiota can mutate to pathogens under changed conditions (Porrás-Alfaro et al., 2014). Whereby the use of particular PGPR strains like *Bacillus subtilis* (*B. subtilis*) has been applied to improve disease resistance of crops such as tomato (Fukami et al., 2010) and the role of arbuscular mycorrhizal fungi (*Glomus* spp.) in other studies improvements in plant biomass and drought tolerance (Jansa et al., 2014). These successes notwithstanding, our incomplete knowledge of the molecular interplay mediated by the plant microbiome and the unpredictability of microbial communities have prevented microbiome engineering from being more widely adopted in agriculture (Compant et al., 2013). This emphasizes the need for a multidisciplinary integrated systems approach that incorporates insights

from genomics, plant physiology, microbiology and ecological modeling, to better understand the structure–function paradigms that underpin plant microbiomes, and to design more targeted microbiome design frameworks for disease resistance and growth promotion in plants (Schlatter et al., 2015).

- Concept of the plant microbiome and its role in plant health

The plant microbiome is an intricate assemblage of microorganisms, including bacteria, fungi, archaea, and viruses, that colonize above ground and below ground tissues, which play a key role in regulating plant health by influencing several physiological processes, such as nutrient acquisition, disease resistance, stress resilience, and growth promotion, in which microbial communities in the rhizosphere (the soil around plant roots) interact directly with the plant host to form beneficial relationships that can enhance plant fitness; for example, bacterial species, such as plant growth-promotion rhizobacteria *Pseudomonads* (*Bacillus subtilis* and *Pseudomonas fluorescens*), such as *Bacillus* species and *Pseudomonas* species also contribute to disease protection by inducing systemic acquired resistance through pathogen-associated molecular pattern-triggered immunity and the production of antimicrobial compounds and contribute to the stimulation of the secretion of plant root exudates that promote growth, while beneficial mycorrhizal fungi including *Glomus* spp. can enhance phosphorus uptake and drought resistance by modifying biological characteristics and root architecture, while sharing of soil benefits and active plant growth are now well demonstrated (Bulgarelli et al., 2013; Our, 2013; Jansa et al., 2014); further, the microbiome contributes to the performance of both plants and microbes by enriching plant growth through alteration of plant hormone concentrations, including auxins, cytokinins, and gibberellins, increasing biomass and yield in both normal and stressed conditions (Vessey, 2003), increasing salinity and heavy metal phytoremediation through the Secretion of osmoprotectants (through the osmoregulation of plant-bacteria interaction) and the secretion of detoxifying enzymes (Mendes et al., 2011); however, the microflora share of the crop-potential of the plant is virtually incomprehensible as the farm microbiome environment crops is exceedingly variable and easily disrupted if proper balance is not reach, and the

microbial community structure dynamically fluctuates due to various environmental perturbations, such as changes in soil microbial community composition and diseases initiated by risks that lead to the maintenance of harmful pathogenic microbial population in the rhizosphere are currently leading to the maintenance of dead, inducing the gradual degradation of the rhizosphere (Lebeis et al., 2015); thus, further work on, as well as a greater understanding on plant-microbe interactions established through effect of genomic bigger picture through integrated techniques and functional genomics would ideally contribute to add deduct ability microbiome engineering method not only for disease resistance, but also for promoting the growth of commercial plants consistent across crops and environments, (Schlatter et al., 2015).

- Importance of microbiome engineering and how microbial communities impact disease resistance and plant growth

Microbiome engineering is a powerful strategy to improve plant health and productivity by harnessing the natural associations between plants and microbial communities that are shown to impact disease resistance and growth promotion via mechanisms such as systemic acquired resistance (SAR) induction, ecological niche competition and plant growth-promoting compound synthesis (e.g., Compant et al., 2013), since beneficial microbes, i.e., *Pseudomonas fluorescens* and *Bacillus subtilis*, suppress plant pathogens in the rhizosphere by secreting antibiotics, enzymes, and siderophores to decrease soil-borne diseases such as root rot and wilt (Compant et al., 2013) and induce plant immune responses by activating defense-related genes (Lebeis et al., 2015), the aforementioned response has been observed in various crops including tomatoes and wheat (Lebeis et al., 2015) where microbial inoculants induced resistance against the pathogenic fungi *Fusarium* spp. and *Verticillium* spp.; similarly, microbes with direct plant growth-promoting characteristics can modulate plant hormone contents, such as azospirillum brasilense and fabricated by rhizobium spp. Auxins, cytokinins, and gibberellins produced by plant-growth-promoting rhizobacteria (PGPR) help regulate root architecture and increase nutrient uptake and have been shown to enhance crop yield (Vessey, 2003); arbuscular mycorrhizal fungi (AMF), including *Glomus* spp., also improve plant growth by enhancing

phosphorus acquisition and drought tolerance, forming symbiotic relationships with plant roots, and increasing soil aggregation, e.g., in maize and wheat (Jansa et al., 2014); however, microbiome engineering can only be effective if it takes into account the intricate dynamics of microbiomes (Mendes et al., 2011), which are regulated by environmental factors, plant genotype, and developmental stage and further, while there are promising results of microbiome-based strategies, the challenge remains that not only is the plant-microbe interaction complex but also the introduction of non-native or altered microbial communities can disrupt ecological balance (Porrás-Alfaro et al., 2014; Schlatter et al., 2015), i.e., if the balance is neglected, the establishment, persistence, and spread of plant pathogens can be favored.

- Literature review related to the study

Microbiome diversity and function play a crucial role in plant health, as a diverse microbial community consisting of bacteria, fungi, and other microorganisms inhabits various plant compartments, such as roots, stems, and leaves, where it facilitates a range of beneficial functions, including nutrient cycling, growth promotion, and disease suppression, with bacterial groups like *Pseudomonas*, *Bacillus*, and *Rhizobium*, and fungal species such as *Glomus* and *Trichoderma* often being the most studied for their beneficial effects, where they engage in mutualistic relationships with plants that enhance growth and stress tolerance (Berendsen et al., 2012; Bulgarelli et al., 2013); the microbiome's influence on disease resistance is particularly notable, as many beneficial microbes can directly suppress plant pathogens through mechanisms such as competition for resources and niches, production of antibiotics, and induction of plant defense responses, such as systemic acquired resistance (SAR) and induced systemic resistance (ISR), for example, *Bacillus subtilis* and *Pseudomonas fluorescens* have been shown to reduce the severity of soil-borne diseases like *Fusarium* wilt in tomatoes by secreting antifungal compounds and competing for space on the plant roots, while *Trichoderma* spp. are known to inhibit the growth of pathogens like *Rhizoctonia solani* through the production of hydrolytic enzymes and mycoparasitism (Compant et al., 2013; Vessey, 2003); microbiome manipulation strategies, including the introduction of specific microbial inoculants or bioinoculants, have garnered

attention as a promising means of enhancing plant health, as evidenced by successful trials in which microbial consortia tailored to specific crops and environmental conditions improved disease resistance and plant growth, such as the use of *Azospirillum* spp. to enhance nitrogen fixation in cereals or *Glomus* spp. to improve drought tolerance in maize and wheat (Jansa et al., 2014; Mendes et al., 2011); however, several challenges and limitations have hindered the widespread application of microbiome engineering, notably the unpredictability of microbial community responses to environmental factors, plant genotypes, and agricultural practices, as microbiomes are highly dynamic and context-dependent, making it difficult to achieve consistent and reproducible outcomes across different environments, as seen in inconsistent results from field trials where certain beneficial microbes failed to establish or perform as expected (Lebeis et al., 2015), additionally, the lack of comprehensive understanding of the specific functional roles and interactions of individual microbial taxa within the microbiome, especially under field conditions, limits the precision of microbiome engineering approaches, while the potential for unintended shifts in microbial communities toward pathogenic populations remains a concern, highlighting the need for careful monitoring and regulation in microbiome-based interventions (Porrás-Alfaro et al., 2014; Schlatter et al., 2015); thus, while previous studies have demonstrated the potential of microbiome manipulation for improving plant disease resistance and growth promotion, future research must focus on overcoming these limitations by developing standardized methodologies for microbiome engineering, improving our understanding of plant-microbe interactions, and addressing the ecological risks of manipulating microbial communities (Lugtenberg and Kamilova, 2009).

- Research gap related to the study

Despite significant advances in plant microbiome engineering, several critical research gaps remain, particularly regarding the detailed understanding of microbial community structure-function relationships, the ecological stability of engineered microbiomes, and the environmental and genetic factors that influence microbiome-mediated plant health; while some studies have demonstrated the beneficial effects of microbial inoculants, such as *Pseudomonas*

fluorescens and *Bacillus subtilis*, on plant disease resistance and growth promotion, these effects are often context-dependent, varying across plant species, environmental conditions, and soil types, highlighting the need for more nuanced and species-specific approaches to microbiome engineering (Lebeis et al., 2015; Mendes et al., 2011); further, while research has shown the importance of plant-associated microbiomes in modulating plant immunity and stress responses (Bulgarelli et al., 2013), a comprehensive understanding of the mechanisms by which microbiomes interact with plant immune systems remains limited, especially with regard to the identification of key microbial taxa and the molecular signaling pathways involved in these interactions (Chaparro et al., 2014); in addition, although some studies have provided promising results regarding the use of mycorrhizal fungi (*Glomus* spp.) and PGPR for improving nutrient uptake and drought tolerance in crops such as maize and wheat (Jansa et al., 2014), the variability in outcomes across different agricultural settings, particularly in terms of long-term stability and efficiency of these interventions under field conditions, remains poorly understood, necessitating further investigation into how microbial communities evolve and adapt over time in response to plant host and environmental changes (Schlatter et al., 2015); another significant gap is the lack of standardized methodologies for assessing and monitoring microbial community dynamics in natural and engineered systems, as current techniques, such as metagenomic and metatranscriptomic profiling, while informative, are still limited by challenges in capturing the full complexity and temporal fluctuations of microbial populations in the rhizosphere (Lugtenberg and Kamilova, 2009); moreover, the potential for unintended consequences from microbiome engineering, such as the disruption of native microbiota or the proliferation of opportunistic pathogens, raises concerns about the ecological risks associated with microbial inoculants, which have not been fully addressed in regulatory frameworks or safety assessments, further emphasizing the need for more rigorous ecological and safety evaluations before widespread implementation (Porrás-Alfaro et al., 2014); ultimately, bridging these gaps requires a multidisciplinary approach combining cutting-edge technologies in genomics, bioinformatics, and ecological modeling to unravel the complex dynamics

of plant-microbe interactions and optimize microbiome engineering strategies for sustainable agriculture (Fukami et al., 2010).

- Disease resistance enhancement with reference to reduced pathogen load and disease symptoms

Enhancing disease resistance through plant microbiome engineering has been shown to effectively reduce pathogen load and disease symptoms by leveraging beneficial microbial communities that either directly inhibit pathogen growth or stimulate the plant's immune system to mount a defense, with research demonstrating that the introduction of specific plant growth-promoting rhizobacteria (PGPR), such as *Pseudomonas fluorescens* and *Bacillus subtilis*, can significantly reduce pathogen populations in the rhizosphere by competing for space and nutrients, thus limiting pathogen access to the plant roots, and in some cases, these microbes produce antibiotics, such as 2,4-diacetylphloroglucinol, which directly inhibit the growth of pathogens like *Fusarium* spp. and *Pythium* spp. (Compant et al., 2013); further, microbiome engineering can stimulate the plant's own immune responses, inducing systemic acquired resistance (SAR) or induced systemic resistance (ISR), processes by which plants exhibit enhanced resistance to a wide range of pathogens after being exposed to specific microbial cues, with *Trichoderma* spp. being one such example, where it has been shown to induce the expression of defense-related genes such as *PR-1* and *chitinase*, leading to reduced susceptibility to *Rhizoctonia solani* and *Verticillium dahliae* (Bulgarelli et al., 2013; Vessey, 2003), while *Bacillus* spp. and *Enterobacter* spp. have been found to enhance disease resistance in crops like tomatoes and wheat through the production of siderophores and other antimicrobial compounds, reducing the incidence of root rot and leaf blight caused by *Fusarium* and *Xanthomonas* spp. (Mendes et al., 2011); moreover, the beneficial microbiomes can improve pathogen suppression by enhancing plant stress resilience, such as drought tolerance, which indirectly reduces plant susceptibility to pathogen attack, as stressed plants are often more vulnerable to infection, a mechanism demonstrated in maize and rice where beneficial microbes, including *Azospirillum brasilense*, were found to mitigate both drought stress and pathogen load, thereby improving plant health and yield under stressful conditions (Jansa

et al., 2014); however, while these approaches hold promise, the challenge remains in the unpredictability of microbiome composition and the specific interactions between microbial species and pathogens, as environmental conditions and plant genotype can significantly affect the establishment and efficacy of beneficial microbes, which underscores the importance of developing precise, tailored microbiome engineering strategies that take into account these variables to achieve consistent disease resistance and reduced pathogen load across different agricultural systems (Lebeis et al., 2015; Schlatter et al., 2015).

- Growth promotion effects with reference to Improved biomass, root-shoot ratio, and nutrient uptake

Microbiome engineering has been widely recognized for its potential to promote plant growth, enhancing key metrics such as biomass production, root-shoot ratio, and nutrient uptake, with studies demonstrating that the introduction of beneficial microbes, such as *Azospirillum brasilense* and *Rhizobium* spp., can significantly improve plant growth parameters by enhancing nitrogen fixation, thereby supporting higher biomass accumulation in crops like wheat and maize (Jansa et al., 2014; Mendes et al., 2011); additionally, the root-to-shoot ratio, a crucial indicator of plant health and resource allocation, has been shown to improve in response to inoculation with mycorrhizal fungi like *Glomus* spp., which form symbiotic relationships with plant roots, enhancing both nutrient and water uptake, particularly in nutrient-poor soils, with *Glomus* spp. contributing to improved root architecture by promoting root branching and elongation, thus increasing the root surface area available for nutrient absorption (Smith & Read, 2008); furthermore, beneficial bacteria such as *Bacillus subtilis* and *Pseudomonas fluorescens* have been found to stimulate plant growth through the production of phytohormones, including indole-3-acetic acid (IAA), which promotes cell division and elongation, thereby improving the root system's ability to take up essential nutrients like phosphorus, potassium, and micronutrients, leading to improved nutrient use efficiency and overall plant health (Compant et al., 2013; Vessey, 2003); several studies have also demonstrated that microbial communities in the rhizosphere can enhance plant responses to abiotic

stress, such as drought and salinity, by improving water retention and osmotic regulation, which in turn contributes to better biomass production and plant resilience (Chaparro et al., 2014); however, while these findings highlight the positive effects of microbiome engineering on growth promotion, challenges remain in optimizing microbial consortia to achieve consistent and reproducible improvements across diverse environmental conditions and crop types, underscoring the need for more research on the mechanistic interactions between microbes and plants to maximize the benefits of microbiome-based growth promotion strategies (Lebeis et al., 2015; Bulgarelli et al., 2013).

- Discussion related to the study

The study of plant microbiome engineering for disease resistance and growth promotion has garnered increasing attention due to its potential to revolutionize sustainable agricultural practices, yet challenges remain in optimizing microbiome-based strategies, particularly in achieving consistent outcomes across diverse environmental conditions, plant species, and agricultural practices, as the rhizosphere microbiome is highly dynamic and context-dependent, influenced by factors such as soil type, climate, and crop genotype (Mendes et al., 2011); while numerous studies have demonstrated that beneficial microbes, including *Pseudomonas fluorescens* and *Bacillus subtilis*, can significantly enhance plant growth and disease resistance by competing with pathogens, promoting nutrient uptake, and inducing plant immune responses, the success of these interventions often varies depending on the microbial consortia used, with some studies showing limited or inconsistent effects under field conditions (Compant et al., 2013; Lebeis et al., 2015); moreover, the mechanisms by which specific microbial taxa interact with plant host cells, particularly in the modulation of plant immune systems, remain insufficiently understood, limiting the ability to design targeted interventions that reliably enhance plant health across different crop species (Bulgarelli et al., 2013); further, while mycorrhizal fungi such as *Glomus spp.* have been shown to improve plant growth and stress tolerance by enhancing nutrient uptake and root architecture, the long-term sustainability and ecological impacts of introducing engineered microbial communities into natural ecosystems are

still under investigation, raising concerns about the unintended consequences of microbiome manipulation, such as the displacement of native microorganisms or the promotion of opportunistic pathogens (Schlatter et al., 2015); additionally, challenges in standardizing methods for assessing microbiome composition and function, particularly in terms of field applications, further complicate the scalability of microbiome engineering techniques, and despite promising theoretical and conceptual advancements, more robust methodologies and deeper ecological insights are needed to fully exploit the potential of microbiome engineering for crop protection and growth promotion (Lugtenberg & Kamilova, 2009; Porras-Alfaro et al., 2014); thus, while microbiome-based strategies show great promise, the successful deployment of microbiome engineering in agriculture will depend on overcoming these challenges and developing a more precise understanding of plant-microbe interactions under real-world conditions.

- Comparison with previous studies

In comparison to previous studies on plant microbiome engineering, recent advancements in microbiome manipulation for disease resistance and growth promotion highlight a more refined understanding of plant-microbe interactions, where early work primarily focused on the use of individual plant growth-promoting bacteria (PGPB) such as *Pseudomonas fluorescens* and *Bacillus subtilis* to control specific pathogens and improve growth, showing varying success depending on microbial strain and environmental conditions (Vessey, 2003; Compant et al., 2013), whereas more recent studies emphasize the use of microbial consortia that incorporate diverse microbial species to target multiple aspects of plant health, such as disease suppression, growth enhancement, and stress tolerance, as demonstrated by *Azospirillum spp.* in wheat and *Glomus spp.* in maize, which show synergistic effects in improving biomass and nutrient uptake through mutualistic relationships with plants (Mendes et al., 2011; Jansa et al., 2014); additionally, whereas early studies were limited by a narrow focus on pathogen inhibition through competition for resources and production of antimicrobial compounds, more recent research has broadened this scope by considering the role of microbiome-induced plant

immune responses, such as systemic acquired resistance (SAR) and induced systemic resistance (ISR), in enhancing disease resistance, as seen in work by Bulgarelli et al. (2013), where microbiome-induced SAR pathways were implicated in providing broad-spectrum protection against a variety of pathogens; however, despite these advancements, the complexity of microbiome composition and the variability of microbiome responses in different environments remain challenges, as early studies often overlooked these factors, and subsequent research, including studies by Lebeis et al. (2015), has shown that plant genotype, environmental factors, and soil microbiome composition can influence the efficacy of microbial interventions, often resulting in inconsistent outcomes across different agricultural systems, underscoring the need for more targeted, context-specific approaches; overall, while the trajectory from initial, single-microbe-based approaches to more complex microbial consortia reflects a positive trend toward optimizing microbiome engineering, significant gaps remain in achieving predictable, large-scale applications, as environmental variability and incomplete knowledge of microbial interactions continue to limit the broader adoption of microbiome engineering in agriculture (Schlatter et al., 2015; Lugtenberg & Kamilova, 2009).

- Mechanisms involved associated with beneficial microbes suppress disease and promote growth

Beneficial microbes in the plant microbiome employ a variety of mechanisms to suppress disease and promote growth, with one of the primary strategies being competitive exclusion, where microbes such as *Pseudomonas fluorescens* and *Bacillus subtilis* outcompete harmful pathogens for essential resources like nutrients and space in the rhizosphere, thereby reducing pathogen colonization and proliferation (Compant et al., 2013); additionally, certain microbes produce antimicrobial compounds, such as bacteriocins, siderophores, and antibiotics, that directly inhibit pathogen growth, with *Bacillus subtilis* producing surfactin and fengycin, which are known to suppress fungal pathogens like *Fusarium* spp. and *Pythium* spp. (Vessey, 2003); another crucial mechanism is the induction of systemic resistance in plants, where beneficial microbes trigger plant immune responses, such as systemic acquired resistance (SAR) or induced systemic resistance (ISR), which prime the plant's defense mechanisms and

prepare it to fight off future pathogen attacks, as shown by the role of *Trichoderma* spp. in inducing expression of defense-related genes like *PR-1* and *chitinase* (Bulgarelli et al., 2013); furthermore, beneficial microbes enhance plant growth by producing plant hormones like auxins (e.g., indole-3-acetic acid), cytokinins, and gibberellins, which stimulate cell division, root elongation, and nutrient uptake, with *Azospirillum brasilense* increasing root biomass and enhancing nitrogen uptake, leading to improved plant growth (Mendes et al., 2011); mycorrhizal fungi, such as *Glomus* spp., establish symbiotic relationships with plant roots, increasing nutrient uptake, especially phosphorus and micronutrients, and improving plant resilience to abiotic stresses, which indirectly enhances plant health and growth (Jansa et al., 2014); finally, certain microbial communities also play a key role in stress mitigation, helping plants tolerate environmental stressors like drought and salinity, thus maintaining higher growth rates and improved disease resistance under challenging conditions (Chaparro et al., 2014); overall, these multifaceted mechanisms demonstrate the potential of microbiome engineering in improving crop health and productivity, although the effectiveness of these strategies depends on the specific microbial consortia and environmental factors involved.

- Potential applications related to use in sustainable agriculture, biocontrol, and biofertilizers

The manipulation of the rhizosphere microbiome has broad applications for sustainable agriculture, biocontrol, and biofertilizers by providing a novel strategy to enhance plant health and promote systemic resistance against enemies while minimizing the use of chemical inputs; for instance, many studies show the effectiveness of beneficial microbes such as *Pseudomonas fluorescens* and *Bacillus subtilis* for biocontrol against a broad range of plant pathogens including several fungal, bacterial and nematode species by mechanisms such as competition for nutrients, pathogen inhibition via the production of antimicrobial compounds, and activation of plant defense responses like systemic acquired resistance (ISR) and SAR (Compant et al. and *Azospirillum* spp. They have been used to improve nitrogen fixation in legumes and non-leguminous crops, respectively, lowering reliance on synthetic nitrogen fertilizers and increasing soil fertility, which is reflected by increased

biomass and yield of crops such as maize and wheat (Mendes et al., 2011; Jansa et al., 2014); moreover, the application of mycorrhizal fungi such as *Glomus* spp. in sustainable agricultural practice has been shown to enhance nutrient uptake, especially phosphorus, in nutrient-poor soils, therefore reducing the need for phosphate fertilizers and increasing plant resilience to drought and salinity stresses, which is a key element of integrated pest management (IPM) strategies (Schlatter et al., 2015); the also ecological sustainability displayed by these approaches has been exemplified by the promotion of soil biodiversity, and conservation of beneficial microbial communities, which is essential for maintaining ecosystem balance and long term agricultural performance, therefore fully complying microbiome engineering to sustainable agriculture principles (Bulgarelli et al., 2013).

- Limitations of the study based on major Challenges such as microbial stability and environmental influences

Microbial stability is an important limiting factor determining the success of the microbiome engineering approach in providing disease resistance and growth promotion, as microbial communities are highly dynamic, and various environmental factors can have notable effects on the colonization and activity of introduced microbes (Mendes et al., 2011; Compant et al., 2013); for instance, environmental factors (soil pH, temperature, moisture, and nutrient status) can change the microbial composition and diminish the effectiveness of bioinoculants (*Pseudomonas fluorescens* and *Bacillus subtilis*) in greenhouse and field conditions (Mendes et al., 2011; Compant et al., 2013), making it difficult to predict the long-term benefits of microbiome interventions across different agricultural systems (Lebeis et al., 2015); furthermore, the complex and reciprocal interactions between introduced microbes and native microbiota may lead to counterproductive interactions (Bulgarelli et al., 2013); some of the mechanisms leading to undesirable outcomes might be the presence of significant interdependencies in microbial communities, the introduction of non-native species leading to ecological imbalances (Bulgarelli et al., 2013), exerted suppression of the host native beneficial microbes or promotion of pathogenic species (Bulgarelli et al., 2013); moreover, though several works have brought anecdotal evidence

demonstrating success in small-scale experiments, high explanatory power via controlled laboratory or in vitro settings have not translated to the shopfloor, with many microbiome interventions failing to exhibit durable disease suppression or growth promotion when applied on a large-extension spaced-out basis (Vessey, 2003; Jansa et al., 2014); thus clearly, gathering such knowledge is critical given that many microbiome manipulation strategies involve the introduction of live microbes or whole microbial consortia which will require appropriate handling, precise application techniques and often expensive production methods, making them commercially less valid and thus challenging for farmers to embrace (Schlatter et al., 2015); here, we discuss the limitations and challenges related to these three micro-biome engineering aspects in concern and show how overcoming these bottlenecks will open broader avenues towards resolving global agri-food problems via sustainable agriculture.

- Future research directions considering further exploration in plant microbiome engineering
- Future research directions in plant microbiome engineering for disease resistance and growth promotion should focus on further elucidating the intricate interactions between plant hosts and their associated microbial communities, with an emphasis on identifying specific microbial taxa that contribute to beneficial plant traits across a broader range of plant species and environmental conditions, as current studies have primarily focused on model species like *Arabidopsis thaliana* and *Solanum lycopersicum*, leaving a gap in understanding how microbiomes function in a wider array of crops (Bulgarelli et al., 2013); research should also aim to improve our understanding of the long-term stability and resilience of microbiome-based interventions in dynamic field environments, addressing how microbial communities adapt to environmental fluctuations such as soil pH, moisture, and temperature, which influence microbial persistence and function (Mendes et al., 2011); another key direction involves investigating the potential of synthetic microbiomes, which combine multiple beneficial microbes with complementary functions to create custom-tailored microbial consortia that can target multiple agricultural issues simultaneously, including disease suppression, nutrient uptake, and abiotic stress resistance, as demonstrated by studies

using *Pseudomonas* spp. and *Bacillus* spp. in synergistic mixtures (Schlatter et al., 2015); additionally, exploring the genetic engineering of plant microbiomes, such as through the integration of gene-editing technologies like CRISPR/Cas9, to enhance the expression of plant immune responses or improve microbe-plant interactions, could unlock new avenues for controlling plant diseases and promoting growth (Lebeis et al., 2015); a more granular focus on the mechanisms driving microbial diversity and its impact on disease suppression and growth promotion is essential, as most current studies still rely on simplified models that do not capture the full complexity of microbial interactions in natural ecosystems (Vessey, 2003); furthermore, given the environmental and ecological implications of microbiome manipulation, research should explore the potential risks of introducing non-native microbes, focusing on their impact on soil biodiversity and overall ecosystem health, thus balancing the benefits of microbiome engineering with its potential environmental consequences (Jansa et al., 2014); finally, large-scale field trials and long-term studies are crucial to determine the practical viability, scalability, and economic feasibility of microbiome engineering approaches in commercial agriculture, ensuring that these innovations can be translated from laboratory experiments to widespread agricultural practice.

CONCLUSION

The study of plant microbiome engineering for disease resistance and growth promotion underscores the critical role of beneficial microbes in enhancing plant health through mechanisms such as competitive exclusion of pathogens, production of antimicrobial compounds, and induction of plant immune responses, with key insights highlighting the contributions of *Pseudomonas fluorescens*, *Bacillus subtilis*, and *Trichoderma* spp. in suppressing plant diseases, while mycorrhizal fungi like *Glomus* spp. and nitrogen-fixing bacteria such as *Rhizobium* spp. improve nutrient uptake and biomass accumulation (Compant et al., 2013; Bulgarelli et al., 2013); practical applications of this research demonstrate that microbiome-based approaches can significantly enhance crop productivity by reducing reliance on chemical pesticides and synthetic fertilizers, thereby

promoting sustainable agricultural practices, as seen in successful field applications where bioinoculants like *Azospirillum brasilense* in cereals and microbial consortia in horticultural crops have resulted in increased resistance to *Fusarium* wilt and improved overall plant vigor (Mendes et al., 2011; Jansa et al., 2014); however, despite these promising advancements, challenges remain in ensuring the stability, scalability, and predictability of microbiome-based interventions under varying environmental conditions, necessitating further research into optimizing microbial consortia, understanding microbe-plant signaling pathways, and mitigating potential ecological risks associated with microbiome manipulation (Lebeis et al., 2015; Schlatter et al., 2015); future research should also explore the potential of synthetic microbiomes, genetic engineering of microbial strains, and integrative approaches that combine metagenomics, transcriptomics, and functional ecology to refine microbiome-based solutions for large-scale agricultural implementation, ensuring that microbial interventions remain effective across diverse soil types and climatic conditions while minimizing unintended disruptions to native microbial communities (Vessey, 2003); ultimately, microbiome engineering presents a transformative opportunity to enhance crop resilience, productivity, and sustainability, but achieving its full potential requires a deeper mechanistic understanding, more robust field validation, and interdisciplinary collaboration to develop precise, reliable, and ecologically sound microbiome-based agricultural strategies.

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