Impact of *Bacillus* Inoculation on Rhizosphere Bacterial Community Structure: A Review

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ABSTRACT

The rhizosphere is a specialized zone where plant roots interact with the soil microbiome. Among various beneficial microbes, the genus *Bacillus* stands out due to its diverse functionalities and potential to boost plant growth and resilience. *Bacillus* is a key genus of plant growth-promoting rhizobacteria (PGPR) known for enhancing nutrient availability, producing phytohormones, and inducing plant resistance to pathogens. Inoculating *Bacillus* species into the rhizosphere can significantly alter the bacterial community's structure and composition. These alterations are driven by the competitive and cooperative interactions between *Bacillus* and the native rhizosphere microorganisms. Incorporating soil microorganisms into the host plant's beneficial bacterial community improves soil nutrient cycling and nutrient use efficiency. Using microbial inoculants is an effective strategy to address crop succession challenges, enhance microbial community structure, and improve soil fertility, thereby promoting crop growth. This review thoroughly examines the current understanding of how *Bacillus* inoculation impacts rhizosphere bacterial community structure.

Keywords : biofertilizer, microbial community, nutrient availability, phytohormones

INTRODUCTION

The rhizosphere is a unique region where the interactions between plant roots and the soil microbiome occur [1]. Rhizosphere microorganisms are cruial in nutrient cycling, disease suppression, and overall plant health. Rhizosphere microorganisms are diverse and contain several major species such as bacteria, fungi, protozoa, archaea, viruses (phages), and nematodes [1,2]. Bacteria are among the most abundant and diverse microorganisms in the rhizosphere. They are pivotal in nutrient cycling processes such as nitrogen fixation and phosphorus and potassium solubilizations. They also play key roles in suppressing diseases and fostering plant growth through synthesizing various hormones and enzymes [3][4].

Among the various beneficial microbes, the genus *Bacillus* has garnered significant attention due to its versatile functionalities and potential to enhance a plant growth and resilience. *Bacillus* species have become significant biological control agents due to their capacity to produce antibiotics and durable endospores that can combat various plant pathogens [5]. *Bacillus* is plant growth promoting rhizobacteria (PGPR) that promotes plant growth through direct mechanisms (nitrogen fixation, phosphate solubilization, potassium solubilization, and phytohormones production) and indirect mechanisms (siderophore production, induced systemic resistance, and lytic enzymes production) [6].

The inoculation of *Bacillus* into the rhizosphere can lead to changes in the microbial community structure [7,8,9]. When microbial invasion occurs, three main outcomes are possible: (i) the invader may establish itself within the native microflora and induce changes in the microbial community composition, (ii) the soil's resilience may eliminate the invader, restoring the original conditions and maintaining the community as it was before the invasion, or (iii) the invader may establish itself, cause

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temporary shifts in the microbial community composition, and then the initial conditions are restored [10,11,12]. Once the bacterial inoculant becomes established in the microbiota, the overall density of the microbial community can increase, at least to the level of the inoculated taxon [13]. Plant inoculation with PGPR can increase biodiversity if the PGPR outcompetes the dominant taxa [14].

This review aims to explain the current state of knowledge and understanding the role of PGPR *Bacillus* on bacterial community structure. By synthesizing findings from various studies, we seek to identify common trends, elucidate underlying mechanisms, and highlight areas requiring further investigation. This review will contribute to a deeper understanding of how *Bacillus* inoculation can enhance agricultural productivity and sustainability.

RHIZOSPHERE AND ITS MICROBIAL COMMUNITY

Soil is a complex and dynamic environment where microbial communities regulate material circulation and energy flow, offer numerous ecosystem services, and play a crucial role in managing plant and agricultural ecological environments [15]]. The diversity and composition of the rhizosphere bacterial community depend on both plant species and soil properties [16,17]. The rhizosphere hosts a diverse array of microorganisms, many of which benefit plants by suppressing pathogenic invasions and aiding in nutrient acquisition from the soil [18,19].

Soil microorganisms are widely found among plant roots, and their incorporation into the host plant's beneficial bacterial community enhances soil nutrient cycling and improves nutrient use efficiency [20]. Plants invest significantly in root exudates to supply carbon compounds for nurturing their rhizosphere microbiota [19]. The plant rhizosphere harbors a vast number of microorganisms that play essential roles in modulating plant physiology and morphology, enhancing plant growth through phytohormone production, and protecting against phytopathogens [21].

Interactions between hosts and their microbiota, both direct and indirect, lead to inherent and induced changes in secondary metabolism and morphological structures [22]. Communication through signaling molecules, such as flavonoids [23], strigolactones [24], and sesquiterpenes [25], is important for the regulation of these interactions. Rhizosphere microbiotas can decrease the competitiveness of dominant plant species or boost the competitiveness of rare and subordinate plant species, thereby influencing plant community diversity [26].

Bacillus: CHARACTERISTICS AND ITS ROLE IN AGRICULTURE

Bacillus genus is Gram stain positive, obligate aerobes/facultative anaerobes, and spore-forming rods [27]. *Bacillus* is one of the predominant genera of plant growth promoting rhizobacteria (PGPR). *Bacillus* species can form long-lived, stress-tolerant spores and secrete metabolites that promote plant growth and prevent pathogen infections [28]. *Bacillus* spp. promote plant growth and yield under various environmental conditions through direct mechanisms (e.g., siderophore production, nitrogen fixation, phytohormone production, and nutrient solubilization) and indirect mechanisms such as the production of exo-polysaccharides (EPS), biofilm formation, hydrogen cyanide (HCN), and lytic enzymes [29].

Bacillus spp. secrete exopolysaccharides and siderophores that inhibit the movement of toxic ions and help ~~to~~ maintain the ionic balance, promote the movement of water in plant tissues, and inhibit the growth of pathogenic microbes [28]. *Bacillus* spp. produce antimicrobial metabolites that can substitute synthetic chemicals or supplement bio-pesticides and biofertilizers for controlling plant diseases [30]. *Bacillus* spp. secrete cyclic lipopeptides like iturin and surfactin, which contribute to disease suppression by serving as bi-functional molecules with antifungal properties and by triggering induced systemic resistance (ISR) [31]. *Bacillus* spp. secrete various catabolic enzymes, including proteases, chitinases, and glucanases, as well as peptide antibiotics and secondary metabolites, all of which contribute to pathogen suppression [32].

Bacillus species can fix atmospheric nitrogen and supply it to plants [33]. *Bacillus* species solubilize nutrients including phosphate, potassium, and zinc, enhancing nutrient absorption by plants [34,35,36]. These rhizobacteria secrete various organic acids, including oxalic, acetic, citric, adipic, butyric, malic, malonic, lactic, succinic, gluconic, glyconic, fumaric, and 2-ketogluconic acid, to solubilize nutrients in the soil [35].

PGPR are known for producing various phytohormones, such as auxins, cytokinins, and gibberellic acid, as secondary metabolites [37]. Auxin is an effective molecule that promotes plant growth under adverse environmental conditions by altering several cellular processes, including cell division and differentiation, and vascular bundle formation, ultimately leading to root elongation, increased root nodule formation, and seed formation [38][39]. *Bacillus* secrete cytokine hormones and volatile organic compounds (VOCs) that modify plant hormone networks, promoting cell division and growth [40]. *Bacillus* produce gibberellin which is also involved in different plant developmental processes and the regulation of many physiological processes [41].

IMPACT OF *Bacillus* INOCULATION ON BACTERIAL COMMUNITY STRUCTURE AND PLANT GROWTH

Soil microbial diversity is an important determinant of plant performance and productivity [42]. The inoculation of *Bacillus* species into the rhizosphere induces significant changes in the structure and composition of bacterial communities. These changes are driven by competitive and cooperative interactions between *Bacillus* and native rhizosphere microorganisms. Numerous studies have shown that inoculating with *Bacillus* can enhance the α diversity of microorganisms in the plant rhizosphere [7,43]. Inoculating with growth-promoting bacteria will result in the enrichment of beneficial bacterial populations in the rhizosphere soil [44]. Numerous studies have demonstrated that microbial inoculants can alter the microbial community in the plant rhizosphere and enhance soil fertility, thereby improving the soil environment, promoting crop growth, and reducing pollution from unsustainable farming practices [45,46]. *Bacillus* inoculation restructures the rhizosphere bacterial community by (i) enriching beneficial bacteria that enhance nutrient cycling and soil nutrient availability, thereby supporting plant growth and soil health [9], (ii) enriching nitrogen-cycling bacteria and enhancing microbial biomass nitrogen and organic nitrogen availability, thus promoting soil fertility, microbial diversity, and plant adaptability, even under stress conditions [8].

Bacillus species can modulate the rhizosphere microbiome by producing signaling molecules that influence microbial behavior, such as quorum sensing molecules that regulate biofilm formation and microbial colonization patterns. Applying microbial inoculants is regarded as an effective strategy to overcome the challenges of crop succession, improve microbial community structure, and sustain their beneficial functions [47,48].

The impact of *Bacillus* inoculation on the rhizosphere bacterial community is often assessed using high-throughput sequencing technologies, such as 16S rRNA gene sequencing, which provide detailed insights into the taxonomic and functional composition of microbial communities before and after *Bacillus* application. By promoting the growth and activity of beneficial microbial groups, *Bacillus* inoculation enhances nutrient acquisition and utilization by plants. Microbes can convert insoluble nutrients in soil and fertilizer into forms that plants can directly absorb and use through processes such as acidolysis, enzymolysis, and polysaccharide complex dissolution [49]. However, the effectiveness of *Bacillus* inoculation can vary depending on factors such as the specific *Bacillus* strain, plant species, soil type, and environmental conditions. Selecting the appropriate *Bacillus* strain and optimizing application methods are crucial for achieving consistent and significant benefits.

Table 1. Various Bacteria Inoculated into Plants and Their Effects

Bacteria	**Inoculated Plants**	**Effects on Bacterial Community Structure**	**References**
Bacillus velezensis YH-18, *Bacillus velezensis* YH-20	Peach	*Proteobacteria* and *Bacteroidetes* were significantly enriched, while *Acidobacteria*, *Verrucomicrobia*, *Latescibacteria*, and *Rokubacteria* were reduced	[9]
Bacillus subtilis, *Bacillus licheniformis*	Wheat	*Plantibacter*, *Lacibacter*, *Phyllobacterium* were enriched	[50]
Rhodopseudomonas palustris, *Bacillus subtilis*	Rice	*Proteobacteria*, *Bacteroidetes*, *Firmicutes*, and *Planctomycetes* were enriched	[51]
Bacillus amyloliquefaciens	Wheat	*Sphingomonas*, *Bacillus*, *Nocardioides*, *Rhizobium*, *Streptomyces*, *Pseudomonas* and *Microbacterium* were increased. Relative abundance of phytopathogenic fungi decreased	[43]
Bacillus amyloliquefaciens FH-1	Cucumber	Reduced the rhizosphere bacterial diversity, increased *Proteobacteria*, and decreased *Acidobacteria*	[52]
Bacillus sp.	Vetiver	*Acidobacteria* and *Bacteroidetes* were dominated	[8]
Bacillus mesonae H20-5	Tomato	Increased the bacterial species richness and diversity. *Actinobacteria* genera, including *Kineosporia*, *Virgisporangium*, *Actinoplanes*, *Gaiella*, *Blastococcus*, and *Solirubrobacter*, were enriched.	[7]

CONCLUSION

Bacillus inoculation in the rhizosphere significantly impacts plant growth and health by modifying bacterial community structures and enhancing beneficial interactions. *Bacillus* species, due to their resilience and diverse metabolic capabilities, compete with pathogens, produce antimicrobial compounds, and promote beneficial microbial functions. These interactions lead to improved nutrient availability, disease suppression, and enhanced plant growth through mechanisms like phytohormone production and induced systemic resistance. Studies using high-throughput sequencing have shown that *Bacillus* inoculation can increase the abundance of beneficial microbes and decrease harmful pathogens, resulting in a healthier rhizosphere. However, outcomes can vary based on factors such as *Bacillus* strain, plant species, and environmental conditions. Optimizing application strategies is essential for consistent benefits.

LIMITATION OF THE STUDY AND FUTURE DIRECTION

There may be some possible limitations in this study. This review primarily focuses on general mechanisms and outcomes of *Bacillus* inoculation without delving deeply into how spesific soil types and conditions. This review also does not explain the various methods of inoculating *Bacillus* into soil and does not discuss the potential long-term ecological impacts of *Bacillus* inoculation.

The future direction for the *Bacillus* inoculation involves carefully considering soil characteristics, particularly nitrogen (N) and phosphorus (P) content, as these factors significantly influence the efficacy and effects of *Bacillus* on plant growth and rhizosphere bacterial community structure. Soil N and P availability can impact nutrient cycling and microbial interactions, thereby modulating the outcomes of *Bacillus* inoculation. Considering *Bacillus* inoculation strategies to specific soil nutrient profiles will optimize plant growth-promotion results and enhance the stability and resilience of rhizosphere bacterial communities. Understanding how varying N and P levels interact with *Bacillus* species will enable the development of more effective and context-specific agricultural practices, ultimately leading to improved soil health and sustainable crop production.

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