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Updates on Rhizobacteria

Edited by Munazza Gull

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Meet the editor

Dr. Munazza Gull is currently working in the Biochemistry Department, Faculty of Science, King Abdul Aziz University, Saudi Arabia. She obtained an MSc in Plant Sciences from the University of Agriculture, Pakistan, in 1999. She received an MPhil in Microbial Plant Sciences from the same university in 2002. She also obtained a Ph.D. in Biotechnology from the National Institute of Biotechnology and Genetic Engineering (NIBGE) Faisalabad, Pakistan/Quaid i-Azam University Islamabad, Pakistan in 2008, where she worked as a researcher for many years. She has experience in microbial biotechnology and plant–microbe interactions. She previously worked with rhizobacteria, biocontrol, integrated disease management, and the production of secondary metabolites/bioproducts/antimicrobial compounds. Now, her research interests focus on plant/microbial potential utilization and exploration, DNA/protein fingerprinting in human disease management, molecular genetics, environmental/soil/water microorganisms, and phytological/industrial/biomedical productivity potential exploring both microorganisms and plants.

Contents

Preface

Plant growth-promoting rhizobacteria (PGPR) are root-associated bacteria representing many different genera and species that colonize the rhizosphere and rhizoplane and improve plant growth when artificially introduced onto seeds, seed pieces, or roots, or into soil. PGPR improve plant growth by one or more mechanisms, such as direct stimulation of plant growth, enhancement of nutrient uptake, suppression of plant pathogens, and/or induction of resistance in host plants against pathogens.

Many bacteria from the rhizosphere can influence plant growth and plant health positively, and we refer to them as PGPR. The beneficial effect of these bacteria has been variously attributed to their ability to produce various compounds, including phytohormones, organic acids, and siderophores, fixation of atmospheric nitrogen, phosphate solubilization, antibiotics that suppress deleterious rhizobacteria, or to some other unidentified mechanisms. Worldwide, rhizobacterial technology is gaining interest.

The rhizobacterial industry is a relatively new venture, just coming out of its infancy. Its potential is being tested, realized, and used. This book explores the hidden rhizobacterial potential through six chapters written by eminent academicians. It presents scientific information relevant to rhizobacterial functional and applied strategies for students, researchers, scientists, and industrialists.

I wish to thank to the chapter authors for their excellent and thoughtful contributions. I hope that this book will inspire readers to further explore new uses for rhizobacterial technology.

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Chapter 1

Plant Growth-Promoting Rhizobacteria in Management of Biotic and Abiotic Stresses

Soheila Aghaei Dargiri and Shahram Naeimi

Abstract

Plant Growth-Promoting Rhizobacteria (PGPR) modifies the activity of the relevant genes to affect the physiological traits, metabolites, pathways, and proteins of plants. Traditional methods for creating salt-tolerant crops are expensive, laborious, and occasionally difficult to adopt. It has been proposed that using microorganisms that encourage plant growth is a suitable and economical method of raising plant tolerance. These evocative microbes can act as a mediator between plants and their morphological, physiological, and molecular responses. Extensive research has been done on the signaling pathways used by hormones, plant receptors, and microbial signals to stimulate PGPR in plants. This chapter aims to increase comprehension of the convergence mechanisms used by these signaling molecules as well as the ambiguities of signaling activities that occur in the host as a result of interactions with PGPR under demanding environmental situations. In order to address biotic and abiotic stressors in agricultural areas and hence raise global food production, the use of rhizobacteria inoculants is a viable strategy.

Keywords: stress tolerance, cellular stress response, tolerant genes, signaling pathway, rhizobacteria

1. Introduction

Stress, as a term, refers to an external factor that exerts an impact on the vegetative development of the plant. The environmental conditions that trigger stress impede the plants' growth and progression, thereby curbing their capacity to propagate and transmit their genetic traits to the succeeding generations. In the natural milieu, stress can be attributed to both biotic and abiotic factors that operate in a concerted manner [1]. The ramifications of climate change on the outcomes of abiotic stress are manifold, imperiling the durability and efficacy of agricultural systems [2]. Abiotic and biotic stresses are significant ecological menaces that drastically diminish agricultural output. Abiotic stressors include a variety of environmental elements like heat, cold, salinity, and drought. But biotic stressors involve a wide variety of living things, including fungi, bacteria, viruses, nematodes, and insects [3].

Plants, in order to counteract stressful conditions, elicit particular responses that result in a reconfiguration at various levels, such as genetic and molecular, among others, as a means of safeguarding themselves from these stressors. At the cellular level, stress provokes alterations in cell division and cell cycle, alongside modifications in the endomembrane system, cellular vacuolization, and structural changes in the cell wall. Furthermore, plants adjust their metabolisms to adapt to diverse environmental stressors on a biochemical level. In recent times, numerous investigations have explored the relationship between stress response and the genetic makeup of plants [4]. Defense mechanisms in plants are often supported by microbial communities. The transition of plants from water to land established a crucial function for microorganisms, which encompassed safeguarding plants against various stressful conditions [5]. The soils in proximity to the roots have been identified as being hotspots for microbial activity. Plants have the ability to generate signals that promote the development of specific microbial communities and subsequently regulate their genetic and biochemical activity [6]. The act of housing bacterial communities, also known as PGPR, within the rhizosphere is a crucial aspect of plant growth and development. These rhizobacteria serve to provide aid to plants in mitigating stress, as well as offering overall assistance in plant vitality [7]. The utilization of plant growth-promoting rhizobacteria (PGPR) in the many signaling pathways of plant morphological, physiological, and molecular responses is examined in this chapter, along with the function that hormones, plant receptors, and microbial signals play. The convergence methods of these signaling molecules are also expounded upon, alongside an analysis of the uncertainties associated with host signaling activities. Consequently, an exploration of the interaction between PGPR and plants in stress-inducing environments is presented.

2. Stress in plants: Biotic and abiotic

Abiotic stress refers to a variety of environmental conditions, including heat, UV rays, salt, floods, droughts, and heavy metals. This array of stressors has caused significant loss of essential crop plants on a global scale [8]. On the other hand, biotic stress denotes the impairment brought about by various organisms, including insects, herbivores, nematodes, fungi, bacteria, and weeds [9]. The primary biotic and abiotic stressors on plants are depicted in **Figure 1**.

2.1 Stress on plants: negative effects of biotic and abiotic

The effects of the biotic variables are wide-ranging and include the death of the entire plant or its organs, a reduction in the bulk of the root, stem, leaf, or inflorescence, complete defoliation, holes, and cavities on plant parts, as well as other signs of eating [10]. The growth and development of plants are seriously threatened by abiotic stressors, which can include but are not limited to extremely hot or low temperatures, insufficient or excessive water availability, high saline levels, the presence of heavy metals, and exposure to UV radiation. The outcome is a significant decline in worldwide crop yields [11].

2.2 Plant stress management: Biotic and abiotic

Enhancing plant resilience necessitates emphasizing the impact of stressed conditions on these interactions over an extended duration, with a primary focus on the

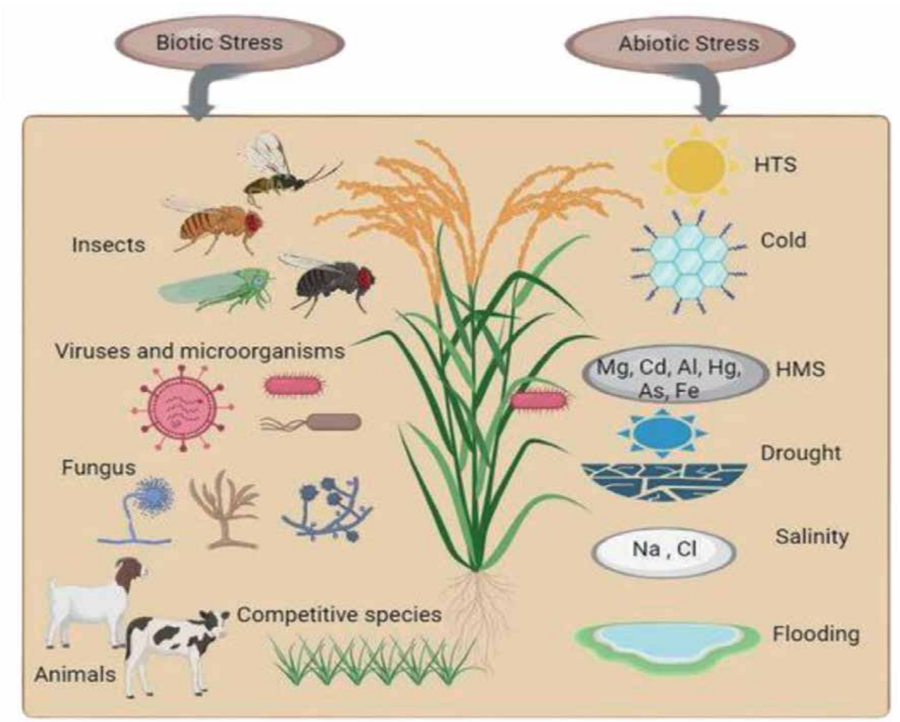

Figure 1.
Principal biotic and abiotic stressors that affect plants (it belongs to the authors study).

growth of crop species under varying pedoclimatic situations, consequent to diverse approaches pertaining to plant, soil, and water management. The consequences of the interplay between these overlapping factors are influenced by environmental factors. The effects of these interactions may be constructive or detrimental, contingent upon the extant circumstances, often resulting in them being inadequately stable for pragmatic implementation. To fully utilize these microorganisms in practical field settings, it is necessary to enhance our understanding of their interactions with the environment and plants. This can be accomplished by furnishing knowledge regarding the intricate feedback mechanisms operative between plants and microbes in the aftermath of stress events, primarily [12].

3. Rhizobacteria

Rhizobacteria, also referred to as root-associated bacteria, can have a parasitic, helpful, or neutral effect on a plant's growth. The term "rhiza" originates from the Greek language, where it denotes "root." Typically, rhizobacteria establish symbiotic relationships with numerous plants, thereby engendering mutualism. In scientific literature, rhizobacteria are frequently referred to as plant growth-promoting rhizobacteria (PGPRs). Joseph W. Kloepper introduced the term "PGPRs" for the first time in the later part of the 1970s [13].

PGPR typically comprises 2–5% of the rhizosphere microorganisms. The use of biofertilizer, which supplies a significant amount of the nitrogen supply to crops globally, makes this group of microbes extremely important. The interaction of PGPRs with host plants differs depending on the species. There are two important types of relationships: rhizospheric and endophytic. Rhizospheric connections entail PGPRs colonizing the host plant's root surface or superficial intercellular spaces, which frequently results in the development of root nodules [1].

3.1 Opportunity and attributes of plant growth-promoting rhizobacteria (PGRP)

Plant growth-promoting rhizobacteria (PGPR) are microorganisms that reside in plant roots and aid in plant growth by supplying increased mineral nutrition, producing plant hormones or other molecules that stimulate plant growth and strengthen the plant's defenses against biotic and abiotic stresses, or shielding plants from pathogens by reducing the survivability of pathogenic microorganisms [14].

3.2 Plant stress tolerance mediated by rhizobacteria

Through nutrient solubilization, nitrogen fixation, the production of phytohormones, siderophores, and biomolecules with pathogen-repelling properties, the group of microbes known as rhizobacteria can enhance plant growth and health [15]. To lessen plant stress, PGPR employs both direct and indirect mechanisms. The complex direct action of PGPR against plant abiotic stresses involves a number of features, including improved mineral acquisition, increased nutrient availability, improved water absorption, and facilitation of exopolysaccharide, biofilm, and the development of numerous organic solutes, including sugars, organic acids, amino acids, and polyamines [16]. Considerations for how PGPR can indirectly lessen plant stress include chemotaxis, phytohormone synthesis, and modulation, changes in their levels, activation of antioxidant defense mechanisms, 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, and the control of stress-responsive genes in plants. Together, these activities control a range of biological plant processes and aid in the establishment of local and remote rhizobacterial-induced abiotic stress tolerance in plants [14].

3.3 Rhizophore: signaling between plants and microbes

One of the key elements influencing the rhizomicrobiome is the cellular response shown by the microorganisms or plants that leads to metabolic transformation, catabolism, and chemical resistance [17]. Plants exhibit their capacity for adaptation by releasing root exudates into the rhizosphere, which enables them to choose the appropriate microbial communities while simultaneously discouraging the growth of detrimental communities. This selection process, also known as "niche colonization," enables the plants to create a healthy microbiome [18]. The directionality of communication allows us to distinguish between two different signaling groups in the rhizosphere: inter- and intraspecies microbial signaling and inter-kingdom signaling between microorganisms and plants. Acyl-homoserine lactones (AHLs or AI-1) and autoinducer peptides (AIPs), both of which are found in both Gram-positive and -negative bacteria, are grouped as two distinct kinds of quorum sensing signals in bacteria. Additionally, autoinducer type 2 (AI-2), a molecule with characteristics similar to both AHLs and AIPs, is present in both Gram-positive and -negative bacteria. Antibiotics, which have been found to encourage intra- or inter-species communication, are one of the numerous QS bacterial signal types [19].

3.3.1 Microorganisms

3.3.1.1 Inter- or intraspecies signaling

Microorganisms within the rhizomicrobiome engage in mutual communication via the synthesis of signaling molecules, which enables them to regulate their genetic expression [20]. Inter- or intraspecies communication among microorganisms is facilitated through the quorum sensing mechanism, which entails coordination that is dependent on cell density. Quorum sensing, a cellular interaction mechanism, involves the generation, emission, and identification of chemical signals, also referred to as autoinducers (AIs) [21]. These AIs control the gene expression of specific bacterial processes, such as biofilm formation, adhesion, and motility, upon detection by the receiver, propagation, virulence, metabolism, and symbiotic aggregation [22]. Acyl-homoserine lactone (AHLs or AI-1) and autoinducer peptides (AIPs) are two families used to categorize quorum sensing signals in bacteria, the former being present in Gram-negative bacteria and the latter in Gram-positive bacteria. AHLs and AIPs share characteristics with autoinducer type 2 (AI-2), which is present in both Gram-positive and Gram-negative bacteria [23].

Volatile organic compounds (VOCs) constitute a crucial category of signaling molecules. They are synthesized and released by microbes for the purpose of long-range communication within a microbial community, as well as in microbe–plant interaction [23]. Volatile organic compounds (VOCs) are a set of small molecular weight lipophilic compounds, with a range of 100–500 Da. Through unique metabolic processes that depend on the genotype of the relevant species, several bacterial and fungal species create these compounds [24]. According to reports, volatile organic compounds (VOCs) generated by bacteria include sulfury, terpenoids, alkanes, ketones, and alkenes [25]. Studies have shown that VOCs operate as antimicrobial QS signaling molecules and have an important impact on microbial activity, including but not limited to virulence, stress resistance, and biofilm formation [26]. In addition to these functions, published research has shown that VOC signals also regulate plant growth, notably root architecture and hormone signaling, as well as plant immunity to biotic and abiotic stresses [27]. Additional signaling routes involving microbial VOCs will become clearer with more study. Therefore, these intricate signaling pathways among rhizosphere microorganisms play a crucial role in the formation of the rhizomicrobiome by attracting specific bacteria via inter- or intraspecies communication.

3.3.1.2 Interkingdom

The communication between microbial and botanical species, known as interkingdom signaling, which either induces or suppresses gene expression, has a profound impact on plant growth. Interkingdom signaling can be categorized into two groups: microbe–plant signaling and plant-microbe signaling, depending on the direction of the stimulus.

3.3.1.2.1 Microbe: plant signaling

Microorganisms produce and release signals in the field of microbe–plant signaling that cause symbiotic interactions with the plant. Specific modifications in the plant transcriptome can be induced by rhizosphere-originating signals from the microbial population. Similar to how plants manufacture phytohormones, PGPR also creates these signaling molecules. The signals that stimulate the growth of plants have the ability to govern their developmental procedures, and furthermore, they can bestow plants with the capacity to withstand abiotic and biotic stressors.

The majority of the work now in existence on the interaction between microorganisms and plants has focused on interactions that have positive effects, like fostering plant development and reinforcing plant defenses against biotic and abiotic challenges. The microorganisms in the rhizosphere that interact favorably with plants are mycorrhiza, rhizobia, plant growth-promoting bacteria, and fungi (PGPR or PGPF) [17]. Microbe-associated molecular patterns (MAMPs), such as flagellin, chitin, and lipopolysaccharides, are usually referred to as microbial signals and can be detected by plants. Pattern recognition receptors (PRRs) are activated throughout the perception process, and this activation triggers a local defense response by way of a hormone signaling network. The resulting immune responses are produced as a consequence of this intricate mechanism [28]. Interkingdom communication has been reported to involve quorum sensing signals. Bacterial QS signals like N-butanoyl homoserine lactone and N-hexanoyl homoserine lactone (AHLs), which are recognized by plants, encourage the formation of a symbiotic interaction between the bacteria and the plants. Additionally, changes in hormone levels in plants promote root growth. Previous investigations back up these results [29]. DSF, yet another bacterial QS molecule, is responsible for triggering innate immunity in a variety of plants by the recognition of dangerous microorganisms via PRRs [30]. Bacterial QS molecules not only cause physiological changes in plants, but they also cause the release of chemicals that are similar to the pathogenic microorganisms' QS molecules [31].

3.3.1.2.2 Signaling compounds

Microbial populations that include parasitic, commensal, and mutualistic bacteria find a home in plants. The plant's reaction to these microbial signals is the secretion of chemical compounds known as "root exudates." Both high- and low-molecular-weight compounds, including proteins and mucilage, as well as organic acids, sugar, aliphatic acids, fatty acids, amino acids, flavonoids, and secondary metabolites, are present in these exudates [32]. The biology of the rhizosphere is significantly influenced by the interaction between plants and microorganisms, which is made possible by the production of phytochemicals [33]. The released compounds draw rhizospheric microbes to the plant roots, where they engage in either harmful or symbiotic relationships. Of all the plant-microbe interactions that have been examined, the relationship between legumes and nitrogen-fixing bacteria has been the most extensively studied. The establishment of a symbiotic association is defined by a sequence of signals that culminate in the formation of root nodules. These nodules function as a nutrient source for rhizospheric bacteria, who reciprocate by providing the plant with a readily available form of nitrogen. Over the past decade, there has been significant research into the signals that plants emit to promote nodule formation [34].

VOCs, many plant organs, including leaves, flowers, fruits, and roots, emit volatile organic compounds [35]. About 1% of all secondary metabolites found in plants are classified as volatile organic compounds (VOCs), which include terpenoids, fatty acids, phenylpropanoids, and amino acids. The propensity of VOCs to permeate plant membranes with ease and subsequently disperse into the surrounding air or soil is the mechanism by which this occurs. They facilitate the attraction of pathogenic root colonizers toward the soil while simultaneously hindering their proliferation [36]. The dispersion of signaling compounds, comprising of volatiles, root exudates, and strigulates, emanating from plants, serves as a wide-ranging mechanism to stimulate bacterial receptor proteins, which, in turn, triggers a microbial response that regulates the gene expression. It is a well-established fact that the discerned chemical signals have a significant impact on the growth and defense mechanisms of plants. The intricate and specific nature of these phytochemicals leads to modifications or alterations within the rhizomicrobiome.

3.4 Hormone signaling pathway and rhizobacteria

Hormones, also referred to as plant growth regulators, are chemical substances that have a significant impact on the development and differentiation of plant cells, tissues, and organs. Additionally, these substances act as essential chemical messengers that enable communication between various cellular units [37]. The preponderance of scholarly inquiry has assessed the phytohormonal production of flora to facilitate their resilience in the face of non-living environmental pressures. Presently, it is incontrovertible that the rhizomicrobiome of plants, which encompasses rhizobacteria that stimulate plant growth, also engenders phytohormones which control how quickly plants develop [38]. Their various mechanisms of action have been divided into two categories: direct mechanisms, which include nitrogen fixation, phosphate solubilization, siderophore production, and phytohormone production, and indirect mechanisms, which include parasitism, induced systemic resistance, antibiosis, induced competition for nutrients, and production of a variety of metabolites [39].

3.5 Plant hormones and rhizobacteria as signaling molecules

Plants and rhizobacteria both have the ability to make phytohormones, which operate as essential bioregulators for a variety of cellular mechanisms and signaling pathways in plants. Numerous phytohormones, including auxins (AUX), cytokinin (CK), abscisic acid (ABA), ethylene (ET), salicylic acid (SA), jasmonic acid (JA), gibberellic acids (GA), strigulates (SL), and brassinosteroids (BRs), are included in this group [40].

3.6 Rhizobacterial hormone signaling and interaction with plant hormones

It has become clear that phytohormones, which are produced by both plants and plant growth-promoting rhizobacteria (PGPR), have a role in the resilience of plants to abiotic stresses in addition to serving other important roles. The plant growth-promoting rhizobacteria (PGPR) can control how well plants tolerate abiotic stress by modulating plant hormones and physiological responses. Studies have been conducted concerning the hormonal signaling of rhizobacteria and its interaction with phytohormones, which serve as mediators of the plant's capacity to endure abiotic stress [41]. The complex interaction of numerous signaling molecules including ABA, ET, SA, and JA along with AUX and GA has a significant impact on the genetic pathways that control plants' capacity to endure stress [42]. In order to coordinate the actions of several genes and the regulators that control them in response to stressful stimuli, hormonal intercommunication is essential. Exogenous phytohormones have the same ability to change the hormone levels in plants as phytohormones produced by microbes. Plant hormonal balance can be altered by microbes by either generating growth regulators or inducing their production within the plant [43]. The differences between signaling molecules synthesized by PGPR and plants are shown in **Figure 2**.

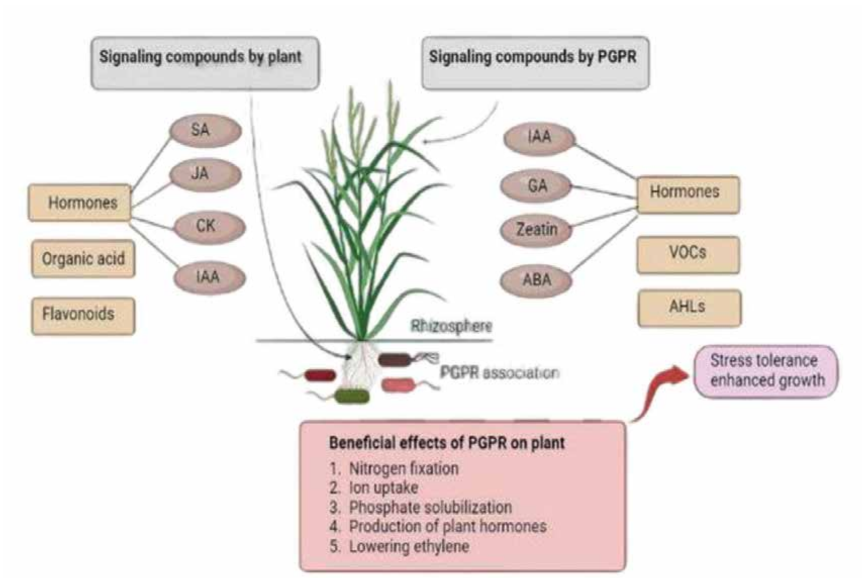

Figure 2.
To establish a mutually advantageous association in the rhizosphere, the presence of signaling molecules produced by both PGPR and plants is imperative. The PGPR is responsible for producing a variety of hormones such as indole acetic acid (IAA), GA, zeatin, and ABA. Additionally, it produces VOCs including 2-heptanol, 2-endecanone, and pentadecane, in addition to ACC deaminase. Additionally, it generates acyl-homoserine lactones (AHLs), such as 3-oxo-C6HL and 3-oxo-C8HL, and cyclopeptides (CDPs). These compounds cause the activation of plant signaling cascades, which eventually boost plant development and increase its ability to withstand stress. The creation of signaling molecules is induced by PGPR, which is comparable to how growth hormones like SA, JA, CK, and IAA modulate plant signaling and stress responses. This relationship promotes plant growth by providing essential minerals through nitrogen fixation, ion uptake of necessary components including Fe, Zn, and micronutrients, as well as the solubilization of phosphate (it belongs to the authors study).

4. Stress management in plants using rhizobacteria

4.1 Stress management mechanism facilitated by PGPR

Plants are commonly subjected to various environmental challenges, including but not limited to salinity, heavy metals, and water scarcity. To adapt to these unfavorable conditions, plants commonly modify their root morphology. It has been suggested that phytohormones like auxin and ethylene significantly alter the root architecture. Indole acetic acid (IAA), a phytohormone that is essential for increasing cellular elongation and controlling root growth in the lower sections of the plant, is unquestionably produced mostly in the aerial regions of the plant body, particularly the shoot [44]. Given that these bacterial strains can produce Indole-3-acetic acid (IAA) at a level that promotes root growth and the production of lateral root hairs, it is not surprising that plants that are associated with Plant Growth Promoting Rhizobacteria (PGPR) may exhibit similar patterns of root growth [45]. The process of encouraging root growth comprises an increase in surface area, which benefits the plant organism by facilitating increased absorption of water and nutrients [46]. In order to control plant development under stress, endophytic rhizobacteria must produce the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase [47]. It is interesting that ACC deaminase is used by rhizobacteria to speed up the breakdown of ACC (1-aminocyclopropane-1-carboxylic acid), producing ammonia as a nitrogen source. For the creation of ethylene, ACC acts as a precursor molecule. Because of this, the root system is improved by the hydrolysis of ACC, which also lowers ethylene production and increases auxin (IAA) levels [48].

The activity of a number of enzymes is required for the regulation of plant body under broad-spectrum environmental stress. These enzymes are ascorbate peroxidase (APX), catalase (CAT), proteinase inhibitors, phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO), peroxidase (PO), superoxide dismutase (SOD), and lipoxygenase (LOX). According to reports, all of these enzymes have been linked to induced acquired resistance (ISR) [49]. Phytoalexins and phenolic compounds can be produced when certain kinds of enzymes are activated, providing immediate defense against pathogenic interactions. Volatile organic compounds (VOCs), Flagellin, LCOs, cyclodipeptides (CDPs), and lipopolysaccharides (LPS) are only a few examples of the signal molecules that rhizobacteria make that are capable of being recognized by plant receptors (PRs). Prior exposure to the ethylene response mutant (ethylene receptor1/ER1) and jasmonic acid response mutant (jasmonic acid receptor1/JAR1) causes an increase in sensitivity to ethylene and jasmonic acid, respectively, which results in the production of several defense compounds. The natural buildup of salicylic acid (SA) and its related signaling pathway are crucial in controlling the emergence of systemic acquired resistance (SAR). A crucial issue to take into account is the existence of pathogen-related proteins (PR proteins), which have a significant impact on the formation of SAR. The downstream effects of the ISR and SAR pathways differ when a regulatory mutation known as non-expressor pathogen-related genes 1 (NPR1) is present despite being regulated by various substances [50]. NPR1 is capable of expertly controlling the production of proteins that are crucial for resisting pathogens via the SAR pathway, the JA and ethylene-mediated ISR pathway, and other pathways that are solely dependent on SA for their expression.

4.1.1 Biotic stress management by rhizobacteria

Similarly, in response to PGPR, plants produce signaling molecules such as plant growth hormones (SA, JA, CK, and IAA), which support their signaling and stress response. Through nitrogen fixation, ion absorption (Fe, Zn, and micronutrients), and phosphate solubilization, the associated PGPR increases plant development [51]. The PGPR produces hydrolytic enzymes, siderophores, and antibiotics and modulates plant ethylene levels as defense strategies against plant diseases [52]. Through the mechanism of cell wall alteration via lignin deposition, the PGPR displays a defense response [53]. The fact that plants activate some signaling pathways in response to PGPR and pathogenic microorganisms suggests that these pathways are regulated, balanced, and overlap with one another. These pathways, which interact with one another, are regulated by hormones. In recent years, it has been possible to identify a large number of regulatory molecules involved in the interaction between the pathways leading to systemic acquired resistance (SAR) and induced systemic resistance (ISR).

4.1.2 Abiotic stress management by rhizobacteria

The Plant Growth-Promoting Rhizobacteria (PGPR) strain KT 2440 of *Pseudomonas putida* is a highly significant PGPR with major application in agriculture. Because of its in-depth analysis, a wide range of defenses against non-living elements have been developed. The magnificent exopolysaccharide (EPS) is a remarkable defensive barrier that emerges on the root surface, warding off the malevolent Na + ions that attempt to invade the root cells. This awe-inspiring EPS is the result of the tireless efforts of the plant growth-promoting rhizobacteria (PGPR). This exceptional creation not only protects the root but also imparts an incredible tolerance to salt stress. The enzyme EptA's synthesis of EPS is governed by a gene, which is however not the only determinant. The paramount regulatory factors, namely PmrA and PmrB, work in tandem to stimulate the expression of the eptA gene through a dual-component signaling pathway. This intricate mechanism thus ensures the proper regulation of EPS production. PmrA, a remarkable cytoplasmic response regulator, stands at the forefront of this regulatory signaling cascade. PmrB, on the other hand, stands guard as a sensor kinase embedded in the membrane, ever vigilant and ready to perceive any changes in its surroundings [54]. The activation of this enzyme is believed to be triggered by Vanadate and various other signals, including high metal ions, low pH, and PmrB kinase. This results in the phosphorylation of PmrA and the activation of the eptA gene and eventually leads to the synthesis of EPS [55]. The minuscule microorganisms generate EPS as a reaction to a plethora of environmental factors, imparting the bacterium with safeguarding traits against that particular duress. Inquisitive scrutiny has established that the ABA and SA communication routes function as pivotal arbiters in response to both non-living and living adversities. The pathways of plant signaling are diverse and versatile, encompassing mitogen-activated protein kinase (MAPK) as well as calcium signaling. These pathways are capable of initiating numerous other plant signaling pathways, while also influencing the expression of various stress response genes [56].

5. Convergence of signaling molecules

Lipo-Chito oligosaccharides, sometimes referred to as nodulation factors, are exquisite signaling substances that plant growth promoting rhizobacteria (PGPR) can produce with skill. The presence of these compounds instigates the flourishing of nodules and plays a pivotal role in fostering the harmonious coexistence between the roots of flora and rhizobacteria [57]. The growth of lateral roots in host plants can be significantly influenced by a variety of factors, among which is LCO. The manner in which stressful conditions impact this phenomenon can be directly attributed to these factors. Particularly noteworthy are the LCOs produced by bacterial associations, such as those containing oligosaccharide-binding LysM sites in lysin motif receptor-like kinases (LysM-RLKs), which have the remarkable ability to be detected by plant receptors. This interaction triggers the initiation of signal transduction pathways that ultimately lead to the activation of nodulation [58]. The amalgamation of LCOs and chitiologosaccharides (Cos) has the potential to augment the efficacy of particular pathways. This action is attained by triggering the inflow of calcium (Ca^{2+}) through the plasma membrane, producing reactive oxygen species (ROS), and activating MAPK (mitogen-activated protein kinase) pathways. Plant defense systems can be activated by this interaction between chitiologosaccharides (Cos) and LYK/CERK1 receptors [59].

The pathway of MAPK assumes a pivotal role in the conveyance of signals during symbiotic interactions, as demonstrated by the earlier-mentioned discoveries. Numerous additional studies have also demonstrated that flora containing LCOs enhance the genes that bind to calmodulin. This supports the combination of LCO and CaMB and their conceivable involvement in the calcium signaling pathway in host plants. The proteins binding to calmodulin (CaMB) are exquisitely perceptive to calcium (Ca^{2+}), and they possess the capability to oversee a diverse range of target proteins. Furthermore, the emergence of MYB44, a result of the amalgamation of acyl-homoserine lactones (AHLs) and G-protein coupled receptor (GPCR), has the potential to stimulate and spur the growth of roots. The culmination of these discoveries alludes to an unwavering alignment amidst PGPR and plant communication pathways. This alignment will undeniably be pivotal in safeguarding plant fitness and amplifying fortification mechanisms against phytopathogens and environmental adversities. The depiction of the crossing point between rhizobia and plant signaling pathways is vividly portrayed in **Figure 3**. Nitric oxide (NO) acts as a connecting agent between the PGPR and the host plant's signaling, according to a number of thorough research studies. In the presence of oxygen, bacterial nitric oxide synthase (bNOS), which is found in certain rhizobacteria, can catalyze the conversion of l-arginine to l-citrulline, producing NO [60]. Anaerobic denitrification, which is carried out by bacteria that are free to move around, is another route for nitric oxide to be produced. The nitrate reductase, nitrite reductase, NO reductase, and N2O reductase enzymes work together to convert nitrate (NO3) to nitrogen [60]. The process of denitrification can also take place in aerobic conditions by utilizing periplasmic nitrate reductase (Nap) rather than the usual membrane-bound respiratory nitrate reductase (Nar). Heterotrophic nitrification is a distinct method of bacterial NO production that converts ammonia into hydroxylamine (NH_2OH), NO_2, and NO_3. NO, a lipophilic diffusible bioactive molecule, has the capability to participate in numerous signaling pathways, including those that are elicited by stress and those that are implicated in development [60].

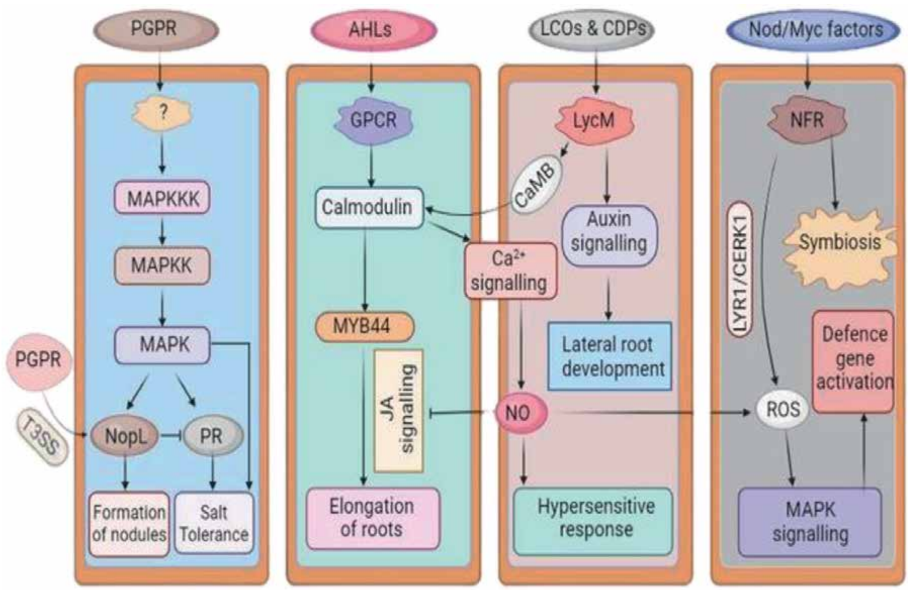

Figure 3.
The interplay between rhizobacteria and the host plant organism gives rise to an array of advantageous physiological pathways via intricate signaling mechanisms (it belongs to the authors study).

6. Conclusion

The utilization of PGPR denotes a collection of bacterial strains that are employed to alleviate stress reactions in plants. Novel research on hormones, functioning as signaling molecules in plant-rhizobacteria interactions and overseeing biotic and abiotic stress, unlocks fresh pathways for investigating the advantageous associations in plants. Future investigations hold the potential to unravel a plethora of knowledge regarding the intricate molecular pathways that are identified by the realms of molecular biology and proteomic analyses concerning the perception of plant hormones. These investigations may shed light on the significant influence of phytohormones on plants' reactions to biotic and abiotic stresses. In the realm of environmental agriculture, there exist numerous genetic modification techniques with recognizable efficacy. The CRISPR-Cas genome editing, for instance, is a viable option for crops and commercial biotech goods pertinent to PGPR, albeit facing several challenges. However, by fostering mutually advantageous collaboration between industry and research, one can leverage omics-based genetic tools to enhance PGPR and effectively meet the pressing need for sustainable biofertilizers.

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References

[1] Kumar R, Swapnil P, Meena M, Selpair S, Yadav BG. Plant growth-promoting rhizobacteria (PGPR): Approaches to alleviate abiotic stresses for enhancement of growth and development of medicinal plants. Sustainability. 2022;**14**(23):1-16

[2] Kaviani Rad A, Balasundram SK, Azizi S, Afsharyzad Y, Zarei M, Etesami H, et al. An overview of antibiotic resistance and abiotic stresses affecting antimicrobial resistance in agricultural soils. International Journal of Environmental Research and Public Health. 2022;**19**(8):1-27

[3] Cohen SP, Leach JE. Abiotic and biotic stresses induce a core transcriptome response in rice. Scientific Reports. 2019;**9**(1):1-11

[4] Singh AA, Ghosh A, Agrawal M, Agrawal SB. Secondary metabolites responses of plants exposed to ozone: An update. Environmental Science and Pollution Research. 2023;**30**(38):88281-88312

[5] Lyu D, Msimbira LA, Nazari M, Antar M, Pagé A, Shah A, et al. The coevolution of plants and microbes underpins sustainable agriculture. Microorganisms. 2021;**9**(5):1-13

[6] Backer R, Rokem JS, Ilangumaran G, Lamont J, Praslickova D, Ricci E, et al. Plant growth-promoting rhizobacteria: Context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. Frontiers in Plant Science. 2018;**9**(1473):1-17

[7] Hasan A, Tabassum B, Hashim M, Khan N. Role of plant growth promoting rhizobacteria (PGPR) as a plant growth enhancer for sustainable agriculture: A review. Molecules. 2023;**1**:1-14

[8] Umar OB, Ranti LA, Abdulbaki AS, Bola A, Abdulhamid A, Biola M, et al. Stresses in plants: Biotic and abiotic. Current Trends in Wheat Research. 2021:1-115

[9] Roberts MR, López SA. Plant epigenetic mechanisms in response to biotic stress. Epigenetics in Plants of Agronomic Importance: Fundamentals and Applications: Transcriptional Regulation and Chromatin Remodelling in Plants. 2019:65-113

[10] Ekwomadu TI, Mwanza M. Fusarium fungi pathogens, identification, adverse effects, disease management, and global food security: A review of the latest research. Agriculture. 2023;**13**(9):1810

[11] Yadav S, Modi P, Dave A, Vijapura A, Patel D, Patel M. Effect of abiotic stress on crops. Sustainable Crop Production. 2020;**3**(17):5-16

[12] de Vries FT, Griffiths RI, Knight CG, Nicolitch O, Williams A. Harnessing rhizosphere microbiomes for drought-resilient crop production. Science. 2020;**368**(6488):270-274

[13] Vessey JK. Plant growth promoting rhizobacteria as biofertilizers. Plant and Soil. 2003;**255**(8):571-586

[14] Rosier A, Medeiros FH, Bais HP. Defining plant growth promoting rhizobacteria molecular and biochemical networks in beneficial plant-microbe interactions. Plant and Soil. 2018;**428**:35-55

[15] Ahemad M, Kibret M. Mechanisms and applications of plant growth

promoting rhizobacteria: Current perspective. Journal of King Saud University-Science. 2014;**26**(1):1-20

[16] Kazerooni EA, Maharachchikumbura SS, Adhikari A, Al-Sadi AM, Kang S-M, Kim L-R, et al. Rhizospheric bacillus amyloliquefaciens protects Capsicum annuum cv. Geumsugangsan from multiple abiotic stresses via multifarious plant growth-promoting attributes. Frontiers in Plant Science. 2021;**12**(669693):1-19

[17] Venturi V, Keel C. Signaling in the rhizosphere. Trends in Plant Science. 2016;**21**(3):187-198

[18] Bai Y, Müller DB, Srinivas G, Garrido-Oter R, Potthoff E, Rott M, et al. Functional overlap of the Arabidopsis leaf and root microbiota. Nature. 2015;**528**(7582):364-369

[19] Aslam M, Pei P, Ye P, Li T, Liang H, Zhang Z, et al. Unraveling the diverse profile of N-acyl homoserine lactone signals and their role in the regulation of biofilm formation in Porphyra haitanensis-associated Pseudoalteromonas galatheae. Microorganisms. 2023;**11**(9):1-18

[20] del Carmen O-MM, Fadiji AE, Babalola OO, Glick BR, Santoyo G. Rhizobiome engineering: Unveiling complex rhizosphere interactions to enhance plant growth and health. Microbiological Research. 2022;**263**(127137):1-15

[21] Nag M, Lahiri D, Banerjee J, Chowdhury TR. Natural Products: Alternate Therapeutic as Quorum Sensing (QS) Inhibitors. Taylor & Francis, CRC Press; 2023

[22] Jamil F, Mukhtar H, Fouillaud M, Dufossé L. Rhizosphere signaling: Insights into plant–rhizomicrobiome interactions for sustainable agronomy. Microorganisms. 2022;**10**(5):1-26

[23] Zhu X, Chen W-J, Bhatt K, Zhou Z, Huang Y, Zhang LH, et al. Innovative microbial disease biocontrol strategies mediated by quorum quenching and their multifaceted applications: A review. Frontiers in Plant Science. 2023;**13**(1063393):1-18

[24] Kanchiswamy CN, Malnoy M, Maffei ME. Bioprospecting bacterial and fungal volatiles for sustainable agriculture. Trends in Plant Science. 2015;**20**(4):206-211

[25] Kai M, Effmert U, Piechulla B. Bacterial-plant-interactions: Approaches to unravel the biological function of bacterial volatiles in the rhizosphere. Frontiers in Microbiology. 2016;**7**(108):1-14

[26] Schmidt R, Cordovez V, De Boer W, Raaijmakers J, Garbeva P. Volatile affairs in microbial interactions. The ISME Journal. 2015;**9**(11):2329-2335

[27] Bitas V, Kim H-S, Bennett JW, Kang S. Sniffing on microbes: Diverse roles of microbial volatile organic compounds in plant health. Molecular Plant-Microbe Interactions. 2013;**26**(8):835-843

[28] Morris G, Gevezova M, Sarafian V, Maes M. Redox regulation of the immune response. Cellular & Molecular Immunology. 2022;**19**(10):1079-1101

[29] Schikora A, Schenk ST, Hartmann A. Beneficial effects of bacteria-plant communication based on quorum sensing molecules of the N-acyl homoserine lactone group. Plant Molecular Biology. 2016;**90**:605-612

[30] Kakkar A, Nizampatnam NR, Kondreddy A, Pradhan BB, Chatterjee S.

Xanthomonas campestris cell–cell signalling molecule DSF (diffusible signal factor) elicits innate immunity in plants and is suppressed by the exopolysaccharide xanthan. Journal of Experimental Botany. 2015;**66**(21):6697-6714

[31] Corral-Lugo A, Daddaoua A, Ortega A, Espinosa-Urgel M, Krell T. Rosmarinic acid is a homoserine lactone mimic produced by plants that activates a bacterial quorum-sensing regulator. Science Signaling. 2016;**9**(409):1-10

[32] Sasse J, Martinoia E, Northen T. Feed your friends: Do plant exudates shape the root microbiome? Trends in Plant Science. 2018;**23**(1):25-41

[33] Zhang Y, Ruyter-Spira C, Bouwmeester HJ. Engineering the plant rhizosphere. Current Opinion in Biotechnology. 2015;**32**:136-142

[34] Oldroyd GE. Speak, friend, and enter: Signaling systems that promote beneficial symbiotic associations in plants. Nature Reviews Microbiology. 2013;**11**(4):252-263

[35] Li S, Yuan X, Xu Y, Li Z, Feng Z, Yue X, et al. Biogenic volatile organic compound emissions from leaves and fruits of apple and peach trees during fruit development. Journal of Environmental Sciences. 2021;**108**:152-163

[36] Kujawa J, Kujawski W, Cyganiuk A, Dumée LF, Al-Gharabli S. Upgrading of zirconia membrane performance in removal of hazardous VOCs from water by surface functionalization. Chemical Engineering Journal. 2019;**374**:155-169

[37] Santhi C, Rajesh M, Ramesh S, Muralikrishna K, Gangaraj K, Payal G. Genome-wide exploration of auxin response factors (ARFs) and their expression dynamics in response to abiotic stresses and growth regulators in coconut *(Cocos nucifera* L.). Plant Gene. 2021;**28**:100344

[38] Kalimuthu R, Suresh P, Varatharaju G, Balasubramanian N, Rajasekaran K, Shanmugaiah V. Isolation and characterization of indole acetic acid [IAA] producing tomato rhizobacterium pseudomonas sp VSMKU4050 and its potential for plant growth promotion. International Journal of Current Microbiology and Applied Sciences. 2019;**8**(6):443-455

[39] Upadhyay S, Singh G, Singh D. Mechanism and understanding of PGPR: An approach for sustainable agriculture under abiotic stresses. International Journal of Plant Sciences. 2016;**134**(2):24-75

[40] Vocciante M, Grifoni M, Fusini D, Petruzzelli G, Franchi E. The role of plant growth-promoting rhizobacteria (PGPR) in mitigating plant's environmental stresses. Applied Sciences. 2022;**12**(3):1231

[41] Kang S-M, Khan AL, Waqas M, Asaf S, Lee KE, Park YG, et al. Integrated phytohormone production by the plant growth-promoting rhizobacterium bacillus tequilensis SSB07 induced thermotolerance in soybean. Journal of Plant Interactions. 2019;**14**(1):416-423

[42] Khan N, Bano A, Ali S, Babar MA. Crosstalk amongst phytohormones from planta and PGPR under biotic and abiotic stresses. Plant Growth Regulation. 2020;**90**:189-203

[43] Cai T, Meng X, Liu X, Liu T, Wang H, Jia Z, et al. Exogenous hormonal application regulates the occurrence of wheat tillers by changing endogenous hormones. Frontiers in Plant Science. 2018;**9**(1886):1-17

[44] Takatsuka H, Umeda M. Hormonal control of cell division and elongation along differentiation trajectories in roots. Journal of Experimental Botany. 2014;**65**(10):2633-2643

[45] Keswani C, Singh SP, Cueto L, García-Estrada C, Mezaache-Aichour S, Glare TR, et al. Auxins of microbial origin and their use in agriculture. Applied Microbiology and Biotechnology. 2020;**104**:8549-8565

[46] Vejan P, Abdullah R, Khadiran T, Ismail S, Nasrulhaq BA. Role of plant growth promoting rhizobacteria in agricultural sustainability—A review. Molecules. 2016;**21**(5):1-17

[47] Glick BR. Plant growth-promoting bacteria: Mechanisms and applications. Scientifica. 2012;**2012**(1):1-15

[48] Naing AH, Maung TT, Kim CK. The ACC deaminase-producing plant growth-promoting bacteria: Influences of bacterial strains and ACC deaminase activities in plant tolerance to abiotic stress. Physiologia Plantarum. 2021;**173**(4):1992-2012

[49] Kamle M, Borah R, Bora H, Jaiswal AK, Singh RK, Kumar P. Systemic acquired resistance (SAR) and induced systemic resistance (ISR): Role and mechanism of action against phytopathogens. Fungal Biotechnology and Bioengineering. 2020;**1**:457-470

[50] Annapurna K, Kumar A, Kumar LV, Govindasamy V, Bose P, Ramadoss D. PGPR-induced systemic resistance (ISR) in plant disease management. Bacteria in Agrobiology: Disease Management. 2013;**1**:405-425

[51] Chauhan A, Saini R, Sharma JC. Plant growth promoting rhizobacteria and their biological properties for soil enrichment and growth promotion. Journal of Plant Nutrition. 2021;**45**(2):273-299

[52] Neeraja C, Anil K, Purushotham P, Suma K, Sarma P, Moerschbacher BM, et al. Biotechnological approaches to develop bacterial chitinases as a bioshield against fungal diseases of plants. Critical Reviews in Biotechnology. 2010;**30**(3):231-241

[53] Jha Y. Induction of anatomical, enzymatic, and molecular events in maize by PGPR under biotic stress. Role of Rhizospheric Microbes in Soil: Volume 1: Stress Management and Agricultural Sustainability. 2018;**1**:125-141

[54] Hamed SM, Mohamed HO, Ashour HM, Fahmy LI. Comparative genomic analysis of strong biofilm-forming *Klebsiella pneumoniae* isolates uncovers novel IS Ecp 1-mediated chromosomal integration of a full plasmid-like sequence. Infectious Diseases. 2023;**56**:1-19

[55] Novović K, Jovčić B. Colistin resistance in Acinetobacter baumannii: Molecular mechanisms and epidemiology. Antibiotics. 2023;**12**(3):516

[56] Edel KH, Kudla J. Integration of calcium and ABA signaling. Current Opinion in Plant Biology. 2016;**33**: 83-91

[57] Choudhary S, Singh A, Rani M. Rhizosphere: Hotspot of soil-microbes-plant interaction. A Monthly Peer Reviewed Magazine for Agriculture and Allied Sciences. 2022;**80**(1):1-511

[58] Rush TA, Tannous J, Lane MJ, Gopalakrishnan Meena M, Carrell AA, Golan JJ, et al. Lipo-chitooligosaccharides induce specialized fungal metabolite profiles that modulate bacterial growth. Msystems. 2022;**7**(6):2379-5077

[59] Demidchik V, Shabala S, Isayenkov S, Cuin TA, Pottosin I. Calcium transport across plant membranes: Mechanisms and functions. New Phytologist. 2018;**220**(1):49-69

[60] Grover M, Bodhankar S, Sharma A, Sharma P, Singh J, Nain L. PGPR mediated alterations in root traits: Way toward sustainable crop production. Frontiers in Sustainable Food Systems. 2021;**4**(618230):1-28

Chapter 2

Nitrogen Fixation by Rhizobacterial Nif Mechanism: An Advanced Genetic Perspective

Nazeef Idris Usman and Muazzam Muazu Wali

Abstract

The global population's rising nutritional needs pose a challenge, demanding a 70% boost in agricultural efficiency to feed 10 billion people by 2050. This task is complicated by limited arable land and the imperative to reduce agrochemical usage. To overcome this, harnessing rhizobacteria and comprehending nif gene mechanisms to enhance nitrogen fixation is crucial. Nif genes encode enzymes, converting atmospheric nitrogen into vital ammonia found in diverse prokaryotes. Nitrogen-fixing bacteria, categorized as autogenous, symbiotic, and combined, collaborate with plants or independently fix nitrogen. Nitrogenase enzymes, represented by Mo, V, and Fe forms, enable this conversion. Nif operons, like nifRLA, nifHDK, nifENB, nifJ, nifUSVM, and nifWF, are pivotal in nitrogen fixation, synthesizing components, and regulating enzymes. Biotech advancements, like 2A peptides and gene manipulation, show promise in boosting crop yields. Translating rhizobacterial nitrogen fixation to cereals could revolutionize agriculture and global food security.

Keywords: *Klebsiella pneumoniae*, nif operon, nitrogenase, ammonia, pyruvate flavodoxin oxidoreductase

1. Introduction

The world population and its nutritional demand are gradually increasing in order to meet the dietary requirements of an estimated world population approaching 10 billion individuals by 2050, agricultural efficiency needs to be enhanced by approximately 70% [1]. This demanding task must be accomplished without expanding the available arable land and while maintaining or reducing the use of harmful agrochemicals such as fertilizers and pesticides [2]. Utilizing rhizobacteria and understanding nif gene mechanism in enhancing their nitrogen-fixing mechanism to increase agricultural yield is important in solving this ever-growing problem.

Nitrogen-fixing (nif) gene encodes enzymes responsible for the conversion of atmospheric nitrogen. These genes can be found as a core gene in diazotrophs, in free-living anaerobic bacteria capable of nitrogen fixation, such as *Klebsiella pneumoniae*, *Azotobacter vinelandii Rhodospirillum rubrum*, and *Rhodobacter capsulatus*, These genes are arranged in an operon [3]. They can also be found on plasmids together with other genes [4].

Nif gene clusters, which contain nitrogenase and various related enzymes, can be found in a wide range of prokaryotes, encompassing both bacteria and archaea [5]. On a global scale, the composition of prokaryotic communities involved in nitrogen fixation is primarily influenced by climatic conditions, with the majority of these groups showing a positive association with consistently warm or seasonally humid climates. Among the various soil characteristics, pH and nitrogen content have frequently been observed to exhibit the strongest correlations with the diversity of nitrogen-fixing groups [6]. Rhizobacteria can be categorized into three groups: nitrogen-fixing bacteria that can produce their own nitrogen, nitrogen-fixing bacteria that form a symbiotic relationship with plants, and nitrogen-fixing bacteria that can utilize both atmospheric nitrogen and other sources of nitrogen. To put a base for understanding the molecular mechanism of nitrogen fixation, we have to understand the forms of bacterial nitrogen fixation. Bueno Batista and Dixon, [3, 7] characterized nitrogen-fixing bacteria into autogenous, symbiotic, and combined nitrogen-fixing bacteria.

2. Autogenous and symbiotic nitrogen-fixing bacteria

Autogenous nitrogen-fixing bacteria refer to bacteria that are independent and capable of fixing nitrogen. *Azotobacter vinelandii,* cyanobacteria (or blue-green algae) Anabaena, and Nostoc are bacteria that fall into this category and exhibit notable nitrogenase activity and expression. It can efficiently perform nitrogen fixation even in the presence of oxygen, making it a prominent bacterium for studying autogenous nitrogen-fixing bacteria [3]. *Azotobacter vinelandii* requires oxygen and carries out nitrogen fixation using the Mo nitrogenase that is sensitive to oxygen. However, in situations where there is a limited availability of molybdenum (Mo), *Azotobacter vinelandii* can also fix nitrogen through alternative forms of nitrogenase known as the Vnf and Anf systems. These systems are genetically distinct from each other and provide additional pathways for nitrogen fixation when Mo is scarce [8]. Symbiotic nitrogen-fixing bacteria invade and multiply the rhizosphere then induce the formation of root nodules; the plant cell and the bacteria engage in an intimate association where the bacteria convert nitrogen to ammonia within the nodules while the plants provide shelter for the bacteria. Rhizobia species are a very good example of symbiotic bacteria [9]. We have reached a point where our knowledge of biological nitrogen fixation has advanced significantly, allowing us to consider the possibility of utilizing synthetic biology techniques to engineer symbiotic relationships. However, it's important to note that currently, symbiotic nitrogen fixation is predominantly observed in legumes within agricultural systems [10].

3. Rhizobacterial nif mechanism

3.1 The *nif* operons and genes

The set of genes responsible for the conversion of atmospheric nitrogen is known as the *nif genes.* In *Klebsiella pneumoniae*, these twenty (20) genes are enclosed within seven *nif operons* [11]. They play a crucial role in nitrogen fixation and are primarily present in nitrogen-fixing bacteria [12]. *Klebsiella pneumoniae* exists as a free-living bacterium, within its chromosome, there is a specific region spanning 24 kilobases (24-Kb) that contains a total of 20 *nif genes* [13]. These nif genes code for the entire nitrogen-fixing mechanism and its regulation. The important parts of this mechanism are the nitrogen complex, RNA polymerase sigma 54 factor, and the Pyruvate Flavodoxin oxidoreductase.

3.2 Nitrogenase enzyme complex

Nitrogenase is an intricate enzyme found in bacteria, responsible for converting dinitrogen (N_2) into ammonia (NH_3) through an ATP-dependent process. The enzyme is typically composed of two proteins: the catalytic molybdenum-iron protein (MoFeP) and the iron protein (FeP), which acts as its specific reductase [14]. Due to its remarkable stability, the conversion of dinitrogen (N_2) into ammonia (NH_3) by the nitrogenase enzyme complex demands a considerable amount of energy input [15]. Various nitrogen-fixing bacteria harbor three types of nitrogenase: molybdenum (Mo) nitrogenase, vanadium (V) nitrogenase, and iron-only (Fe) nitrogenase. Among them, molybdenum nitrogenase is the most extensively studied and well-characterized type, commonly found in diazotrophs like legume-associated rhizobia [16].

The nitrogenase (nif) genes encompass a range of components, including the nitrogenase structural genes responsible for forming the nitrogenase enzyme [17]. Additionally, there are genes involved in activating the Fe protein, molybdenum nitrogenase biosynthesis, iron-molybdenum cofactor biosynthesis, electron donation to nitrogenase, and regulatory genes essential for the expression of nif genes [12]. The nif regulon consists of factors that can activate or inhibit the production of proteins required for nitrogen fixation based on the prevailing environmental conditions [3].

Nitrogenase exists in three distinct isoforms, each containing a different metal in the M-Cluster. In molybdenum nitrogenase, the molybdenum occupies the M-Cluster. Another isoform, known as vanadium nitrogenase, substitutes molybdenum with vanadium in the M-Cluster. The third variant, termed Iron Nitrogenase or Iron Nitrogenase, has iron in place of molybdenum in its M-Cluster [18]. The most studied molybdenum nitrogenase is presented in **Figure 1** below.

3.2.1 Molybdenum (Mo) nitrogenase

The predominant role of molybdenum nitrogenase lies in facilitating the major portion of biological nitrogen fixation, a prokaryotic metabolic process crucial for shaping the global biogeochemical cycles of nitrogen and carbon [19]. The

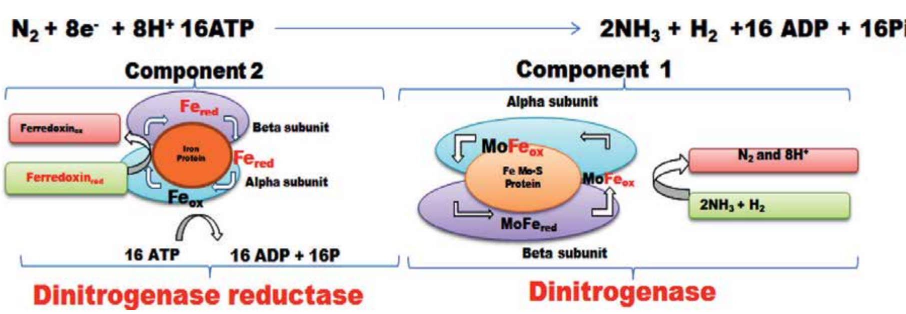

Figure 1.
Molybdenum nitrogenase. Collectively built from our study using paint Microsoft software.

molybdenum version of nitrogenase (Mo-N2ase) has undergone thorough characterization and exhibits the highest level of catalytic activity. The Mo-N2ase system comprises two-component metalloproteins that work together to catalyze the ATP-dependent transformation of N_2 into NH_3. These components are the molybdenum iron protein and the iron protein. The MoFe protein is a tetramer made up of α2β2 subunits and contains two metalloclusters, namely the P-cluster and the R-homocitrate FeMo-cofactor (FeMoCo) cluster [20].

3.2.2 Vanadium (V) nitrogenase

Vanadium nitrogenases are enzymes capable of reducing atmospheric nitrogen into a biologically useful form. The enzymes also possess the unique ability to convert carbon monoxide (CO) into hydrocarbons, similar to the industrial Fischer-Tropsch process that turns CO and hydrogen into liquid hydrocarbons, a process that can only be performed by vanadium nitrogenase enzyme [21]. Most importantly, they serve as a substitute for molybdenum nitrogenase in situations where molybdenum is unavailable. Although it represents a significant biological use of vanadium, living organisms seldom rely on vanadium nitrogenase [22].

3.2.3 The iron only nitrogenase

Fe-only nitrogenase functions without the need for any additional heterometals other than iron, yet its specific architecture and cofactor structure have remained undisclosed thus far [22]. Fe-only nitrogenases consist of two components, namely the Fe protein (AnfH) and the FeFe protein (AnfDGK). However, compared to the other two nitrogenases (Mo and V nitrogenases), there is relatively limited knowledge regarding the biosynthesis and catalytic properties of the Fe-only nitrogenase. Among the three types of nitrogenases, the Fe-only nitrogenase is considered the simplest, as it relies on fewer gene products for its function [23].

3.3 RNA polymerase and the sigma 54 factor

In prokaryotes, the regulation of transcription frequently entails direct interaction between regulatory proteins and RNA polymerase. In the case of Sigma 54 RNA polymerase holoenzyme, regulatory proteins associated with enhancer regions located distantly from the promoter interact with the polymerase through DNA looping. The s54-dependent nifA promoter in *Klebsiella pneumoniae* is activated when the growth conditions are limited [24].

3.4 Pyruvate flavodoxin oxidoreductase

Pyruvate flavodoxin oxidoreductase is encoded by nifJ gene from operon nifJ, which is an oxidoreductase enzyme responsible for transferring electrons from pyruvate to flavodoxin, thus reducing nitrogenase [25]. This enzyme facilitates the conversion of pyruvate and acetyl-CoA and is commonly known as pyruvate: ferredoxin oxidoreductase (PFOR). It utilizes three substrates: pyruvate, CoA, and oxidized ferredoxin, and converts them into three products: acetyl-CoA, CO2, and reduced ferredoxin [26].

4. The *nif* mechanism and regulation

4.1 NifRLA operon

This operon activates the nigfA gene using RNA polymerase sigma 54 protein and kick starts the transcription of structural proteins in NifHDK [27]. The NifA protein is classified within the group of enhancer-binding proteins responsible for activating gene expression alongside RNA polymerase, specifically the specialized sigma factor σ54 (RpoN). This interaction enables the polymerase core to recognize −24/−12 promoters [28]. But the mechanism depends on the availability or absence of oxygen and ammonia. These environmental factors determine the activation of all the nif genes [29]. From nifRLA operon, *nifL gene* is deactivated in the absence of oxygen. Its primary function is to inhibit the transcription of nifA gene. The flavoprotein contain FAD that serves as a redox sensing cofactor that transduces molecules responsible for conveying the oxygen status to the nifL protein [30]. The fumarate nitrate functions here as a reduction regulator. In the absence of oxygen, the nifL protein exists in its reduced form with $FADH_2$ as the cofactor, and in this state, it cannot inhibit the activity of the nifA protein [3]. However, in the presence of oxygen, the nifL protein becomes oxidized with FAD as the cofactor, leading it to inhibit the nifA protein and consequently shutting down all other operons [24]. When a significant concentration of ammonium ions is found in the environment, it suppresses the transcription of both nitrogenase and other nif genes. NH4+ serves as a co-repressor for glutamine synthetase by covalently modifying it through adenylylation. This modified enzyme then attaches to the nifR region of the nifRLA operon, leading to the inhibition of nifL and nifA gene transcription. Consequently, σ-RNA polymerase is unable to initiate the transcription of other genes [31]. Under conditions of limited nitrogen availability during growth, the inhibitory effect of nifL protein on the nifA protein is counteracted by the Glnk protein's antagonistic action on the nifL proteins [32]. The repressor binding site encoded by nifR situated between the promoter of the nifRLA operon deactivates the entire operon when there is availability of ammonia in the environment [29, 30, 33].

4.2 NifHDK operon

This operon contained structural genes designated as nifH, nifD, and nifK [27], The nitrogenase enzyme complex is formed through the expression of several genes found in the nif operon. The nifD and nifK genes code for dinitrogenase, while nifH encodes for dinitrogenase reductase. The process of N2 fixation is tightly controlled at the genetic level, involving transcriptional and translational modifications of the nifHDK gene products [34].

4.3 NifEN and nifBQ

The nifEN and nifBQ genes are responsible for encoding cofactor assembly proteins that act as molecular scaffolds for the formation of Fe-Mo clusters. While not all alternative nitrogenases were found to have their own cofactor synthesis genes (nifENB), the phylogenetic analysis focused solely on the Mo-Fe nitrogenase due to this limitation. But nifEN is tolerant to varied expression levels [35]. Additionally, organisms with fused nifEN and nifNB genes were identified based on their sequence length [17]. NifB and nifEN are believed to be the exclusive nif-specific components essential for assembling an active nitrogenase, and their roles cannot be replaced by other enzyme activities within the host cell. NifB acts as an enzyme dependent on S-adenosylmethionine, responsible for the rearrangement and catalytic transformation of two [4Fe-4S] clusters into a [8Fe-9S-C] cluster known as nifB-co. Subsequently, this nifB-co cluster is bound and transferred to nifEN [25]. Arguably, nifQ on its own, because of limited information, is declared unessential for the assembly of Mo-Fe protein in *Klebsiella pneumoniae* but Bennett [25, 36, 37] reported that nifQ together with nifY, nifO, nifZ can code for nitrogenase cofactor in *Azotobacter, Rhodobacter, and Klebsiella oxytoca.*

4.4 NifJ operon

The operon comprises solely the nifJ gene, which encodes the pyruvate-flavodoxin-oxidoreductase protein. This protein facilitates the conversion of pyruvate, flavodoxin, or ferrodoxin to acetyl CoA while transferring electrons to nitrogenase enzymes [37, 38].

4.5 NifUSVM operon

The genes nifU and nifS encode the components of nitrogenase responsible for assembling [2Fe-2S] and [4Fe-4S] clusters essential for growth in nitrogen-fixing conditions. NifUS plays a crucial role in providing [Fe-S] clusters, which serve as metabolic building blocks for the biosynthesis of FeMo-Co. Surprisingly, in nifUS mutants, the expression of nifB was reduced, but the assembly of nifB's [Fe-S] clusters was compensated by other non-nif machinery responsible for [Fe-S] cluster assembly. This indicates that nifUS is not indispensable for synthesizing active nifB [39], as presented in **Figure 2**.

NifV is responsible for producing homocitrate, which serves as the cofactor in the active site. Additionally, Fe-S clusters are highly susceptible to damage caused by reactive oxygen species, leading to the oxygen sensitivity observed in numerous enzymes containing Fe-S cofactors [1, 25]. In the presence of nifM, the monomeric subunit of the Fe protein comprised approximately 10% of the entire cell protein. On the other hand, when nifM was absent, the nifH protein constituted 4.7% of the whole-cell protein, but it displayed no observable activity in whole-cell extract assays. The role of nifM is to convert the nifH peptide into the functional Fe protein of nitrogenase. Without the presence of nifM, only an inactive form of the nifH polypeptide is produced [40].

4.6 NifWF operon

NifW was observed to interact with an apo-form of the MoFe protein lacking FeMo-Co, containing immature P-clusters, but its specific biochemical role remains unidentified [41]. The binding of the accessory protein NifW is associated with a reduction in the distance between the clusters, along with slight alterations in their coordination. These findings suggest that NifW plays a conformational role in P-cluster biosynthesis, bringing the two [4Fe:4S] precursors closer together before their fusion, which could be vital in complex cellular environments [42]. The nifF gene facilitates the transfer of electrons from the nifJ protein to the Fe protein of nitrogenase.

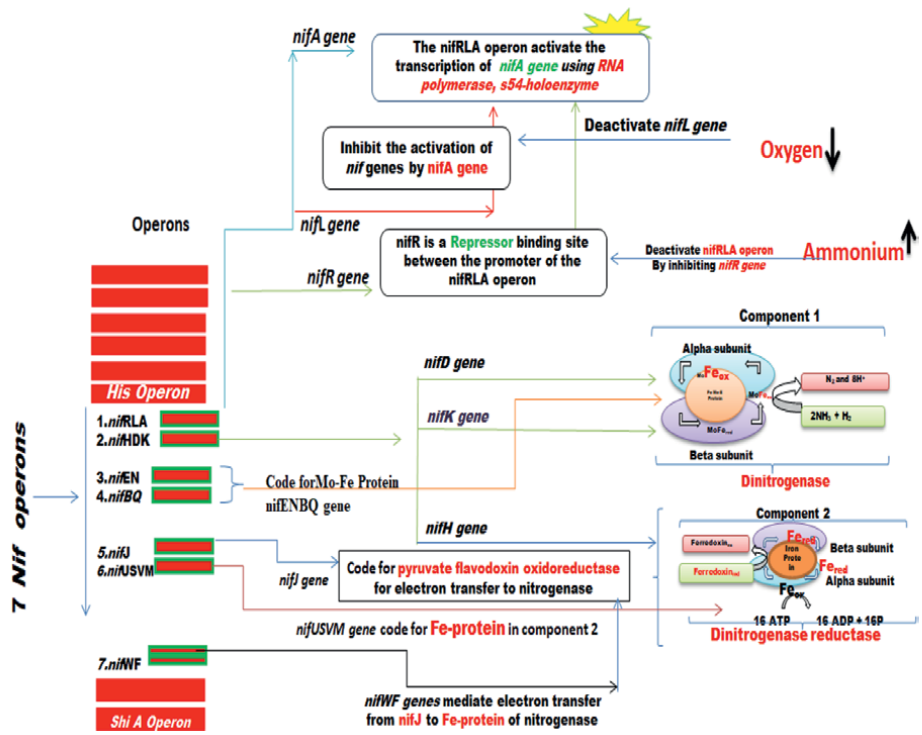

Figure 2.
Mechanism of nitrogen fixation and regulation. Built from our study.

4.7 NifT, nifY and nifZ

NifT and nifZ genes from different nif operons were termed unessential and/or undetermined by Poudel *et al.* [38]. This is because they cannot technically code for anything on their own, many scholars combine them under the function of an entire operon, not a specific gene. However, nifY code is a chaperone for the apo Mo-fe protein in the molybdenum nitrogenase complex [36, 37].

5. Recent advances in the manipulation of nif genes to optimize crop yield

To satisfy the ever-increasing population, there is a need to optimize crop production and increase yield using biotechnology. Using soil bioavailable microorganism transformation into nitrogen-fixing bacteria or optimizing the average concentration of nitrogen in the soil by manipulating the nif regulon will go a long way in solving food shortage in the world. Scientists advance in achieving this through:

1. Utilizing 2A peptides to simultaneously express multiple proteins involved in the synthesis of the complex nitrogenase system. When nif proteins are connected using 2A peptides, successful co-expression occurs in *Saccharomyces cerevisiae*. The inclusion of a 2A tail in nifH, nifB, and nifD does not impact the activity of these nif proteins, except for nifK, where the presence of the 2A tail results in a complete loss of activity. Co-expression of nine nif proteins (nifH, nifB, nifD,

nifK, nifE, nifN, nifX, hesA, and nifV) from *Paenibacillus polymyxa* (WLY78) is effectively achieved in yeast. Moreover, yeast nifH, co-expressed with nifH from *P. polymyxa*, and nifS and nifU from *Klebsiella oxytoca* demonstrate the activity of the Fe protein [12].

2. Manipulating nitrogenase gene expression in heterologous hosts to improve activity and oxygen tolerance and potentially engineer synthetic symbiotic relationships with plants through engineering nif clusters and engineering nitrogenase clusters in native and heterologous hosts, inducible expression in response to fixed nitrogen and oxygen, engineering symbiotic relationships between legumes and rhizobacters, and engineering electron carriers [25].

3. Mimicking microbial fixing mechanism of rhizobacteria in cereal crops to create cereals that can fix nitrogen and burst crop yield [25, 43, 44].

6. Conclusion

In conclusion, addressing the growing challenge of meeting the nutritional demands of an approaching world population of 10 billion by 2050 requires a significant enhancement in agricultural efficiency. This challenge must be met while avoiding the expansion of arable land and minimizing the use of harmful agrochemicals. To overcome these hurdles, harnessing the potential of rhizobacteria and understanding the mechanisms of the nitrogen-fixing (Nif) genes become pivotal.

The nif genes, responsible for the conversion of atmospheric nitrogen into biologically useful forms, play a central role in nitrogen fixation. These genes are found in diverse prokaryotes, and their arrangement varies from core operons to plasmid-located clusters. Nitrogenase, the enzyme complex encoded by nif genes, is a sophisticated machinery consisting of various components, each with distinct roles in catalyzing the conversion of nitrogen. Molybdenum nitrogenase is the most widely studied and characterized variant, but vanadium and iron-only nitrogenases also contribute to this process.

The regulation of nif gene expression is intricately linked to environmental factors such as oxygen and nitrogen availability. Complex regulatory networks involving proteins like nifA, nifL, and glnk modulate gene expression based on these factors. Furthermore, the assembly of active nitrogenase involves several cofactor assembly proteins, such as nifEN and nifB, which are crucial for forming functional Fe-Mo clusters.

Recent advancements in genetic engineering have opened avenues to optimize crop yields through the manipulation of nif genes. Techniques like utilizing 2A peptides to co-express multiple nif proteins and engineering nitrogenase gene expression in heterologous hosts show promise in improving nitrogen fixation efficiency and oxygen tolerance. Additionally, the possibility of creating synthetic symbiotic relationships between plants and nitrogen-fixing bacteria offers exciting prospects for enhancing agricultural productivity.

In a world where food security is a pressing concern, understanding the intricate mechanisms of nif genes and leveraging the potential of nitrogen-fixing bacteria stand as promising strategies to address the challenge of feeding a growing global population while ensuring sustainability and environmental responsibility.

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Conflict of interest

I declare that there is no conflict of interest related to the content presented in this document. The information and perspectives shared are solely intended to provide an objective overview of the subject matter.

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References

[1] Haskett TL, Tkacz A, Poole PS. Engineering rhizobacteria for sustainable agriculture. The ISME Journal. 2021;**15**(4):949-964. DOI: 10.1038/s41396-020-00835-4

[2] Cordell D, White S. Tracking phosphorus security: Indicators of phosphorus vulnerability in the global food system. Food Security. 2015;**7**(2):337-350. DOI: 10.1007/s12571-015-0442-0

[3] Zhang W, Chen Y, Huang K, Wang F, Mei Z. Molecular mechanism and agricultural application of the NifA–NifL system for nitrogen fixation. International Journal of Molecular Sciences. 2023;**24**(2):907. DOI: 10.3390/ijms24020907

[4] González D, Robas M, Fernández V, Bárcena M, Probanza A, Jiménez PA. Comparative Metagenomic Study of Rhizospheric and Bulk Mercury-Contaminated Soils in the Mining District of Almadén. Frontiers in Microbiology. 7 Mar 2022;**13**:797444. DOI: 10.3389/fmicb.2022.797444. PMID: 35330761; PMCID: PMC8940170

[5] Alleman AB, Peters JW. Mechanisms for generating low potential electrons across the metabolic diversity of nitrogen-fixing bacteria. Applied and Environmental Microbiology. 2023;**89**(5):e00378-e00323. DOI: 10.1128/aem.00378-23

[6] Sepp S-K, Vasar M, Davison J, Oja J, Anslan S, Al-Quraishy S, et al. Global diversity and distribution of nitrogen-fixing bacteria in the soil. Frontiers in Plant Science. 2023;**14**:1100235. DOI: 10.3389/fpls.2023.1100235

[7] Bueno Batista M, Dixon R. Manipulating nitrogen regulation in diazotrophic bacteria for agronomic benefit. Biochemical Society Transactions. 2019;**47**(2):603-614. DOI: 10.1042/BST20180342

[8] Hamilton TL, Ludwig M, Dixon R, Boyd ES, Dos Santos PC, Setubal JC, et al. Transcriptional profiling of nitrogen fixation in Azotobacter vinelandii▿. Journal of Bacteriology. 2011;**193**(17):4477-4486. DOI: 10.1128/JB.05099-11

[9] Delavaux CS, Weigelt P, Magnoli SM, Kreft H, Crowther TW, Bever JD. Nitrogen-fixing symbiotic bacteria act as a global filter for plant establishment on islands. Communications Biology. 2022;**5**(1):1209. DOI: 10.1038/s42003-022-04133-x

[10] Mus F, Crook MB, Garcia K, Garcia Costas A, Geddes BA, Kouri ED, et al. Symbiotic nitrogen fixation and the challenges to its extension to nonlegumes. Applied and Environmental Microbiology. 2016;**82**(13):3698-3710. DOI: 10.1128/AEM.01055-16

[11] Lie TJ, Kuo YP, Leite M, Costa KC, Harwood CS, Leigh JA. A genetic study of Nif-associated genes in a Hyperthermophilic methanogen. Microbiology Spectrum. 2022;**10**(1):e02093-e02021. DOI: 10.1128/spectrum.02093-21

[12] Wang M, Shang Y, Liu X, Chen S. Assembly of nitrogenase biosynthetic pathway in Saccharomyces cerevisiae by using polyprotein strategy. Frontiers in Microbiology. 2 Mar 2023;**14**:1137355. DOI: 10.3389/fmicb.2023.1137355. PMID: 36937264; PMCID: PMC10017450

[13] Burén S, Jiménez-Vicente E, Echavarri-Erasun C, Rubio LM.

Biosynthesis of Nitrogenase cofactors. Chemical Reviews. 2020;**120**(12):4921-4968. DOI: 10.1021/acs.chemrev.9b00489

[14] Owens CP, Tezcan FA. Chapter Twelve—Conformationally gated electron transfer in Nitrogenase. Isolation, purification, and characterization of Nitrogenase from Gluconacetobacter diazotrophicus. In: David SS, editor. Methods in Enzymology. Vol. 599. Academic Press; 2018. pp. 355-386. DOI: 10.1016/bs.mie.2017.09.007

[15] Greed S. Unveiling the final nitrogenase. Nature Reviews Chemistry. 2023;**7**(6):Article 6. DOI: 10.1038/s41570-023-00507-9

[16] Okubo T, Piromyou P, Tittabutr P, Teaumroong N, Minamisawa K. Origin and evolution of nitrogen fixation genes on Symbiosis Islands and plasmid in Bradyrhizobium. Microbes and Environments. 2016;**31**(3):260-267. DOI: 10.1264/jsme2.ME15159

[17] Koirala A, Brözel VS. Phylogeny of Nitrogenase structural and assembly components reveals new insights into the origin and distribution of nitrogen fixation across bacteria and archaea. Microorganisms. 2021;**9**(8):1662. DOI: 10.3390/microorganisms9081662

[18] Garcia AK, Kolaczkowski B, Kaçar B. Reconstruction of Nitrogenase Predecessors Suggests Origin from Maturase-Like Proteins. Genome Biology and Evolution. 2022;**14**(3):evac031. DOI: 10.1093/gbe/evac031

[19] Hernandez JA, George SJ, Rubio LM. Molybdenum trafficking for nitrogen fixation. Biochemistry. 2009;**48**(41):9711-9721. DOI: 10.1021/bi901217p

[20] Threatt SD, Rees DC. Biological nitrogen fixation in theory, practice, and reality: A perspective on the molybdenum nitrogenase system. FEBS Letters. 2023;**597**(1):45-58. DOI: 10.1002/1873-3468.14534

[21] Hickman D. Vanadium Nitrogenase at Atomic Resolution. ChemistryViews. 2020. Available from: https://www.chemistryviews.org/details/ezine/11274016/Vanadium_Nitrogenase_at_Atomic_Resolution/

[22] Harwood CS. Iron-only and vanadium Nitrogenases: Fail-safe enzymes or something more? Annual Review of Microbiology. 2020;**74**:247-266. DOI: 10.1146/annurev-micro-022620-014338

[23] López-Torrejón G, Burén S, Veldhuizen M, Rubio LM. Biosynthesis of cofactor-activatable iron-only nitrogenase in Saccharomyces cerevisiae. Microbial Biotechnology. 2021;**14**(3):1073-1083. DOI: 10.1111/1751-7915.13758

[24] Danson AE, Jovanovic M, Buck M, Zhang X. Mechanisms of σ54-dependent transcription initiation and regulation. Journal of Molecular Biology. 2019;**431**(20):3960-3974. DOI: 10.1016/j.jmb.2019.04.022

[25] Bennett EM, Murray JW, Isalan M. Engineering Nitrogenases for synthetic nitrogen fixation: From pathway engineering to directed evolution. Biodesign Research. 2023;**5**:0005. DOI: 10.34133/bdr.0005

[26] Katsyv A, Schoelmerich MC, Basen M, Müller V. The pyruvate:Ferredoxin oxidoreductase of the thermophilic acetogen, *Thermoanaerobacter kivui*. FEBS Open Bio. 2021;**11**(5):1332-1342. DOI: 10.1002/2211-5463.13136

[27] Milenkov M, Thummer R, Glöer J, Grötzinger J, Jung S, Schmitz RA.

Insights into membrane association of Klebsiella pneumoniae NifL under nitrogen-fixing conditions from mutational analysis. Journal of Bacteriology. 2011;**193**(3):695-705. DOI: 10.1128/JB.00775-10

[28] Salazar E, Díaz-Mejía JJ, Moreno-Hagelsieb G, Martínez-Batallar G, Mora Y, Mora J, et al. Characterization of the NifA-RpoN regulon in rhizobium etli in free life and in Symbiosis with Phaseolus vulgaris. Applied and Environmental Microbiology. 2010;**76**(13):4510-4520. DOI: 10.1128/AEM.02007-09

[29] Steunou A-S, Jensen SI, Brecht E, Becraft ED, Bateson MM, Kilian O, et al. Regulation of nif gene expression and the energetics of N2 fixation over the diel cycle in a hot spring microbial mat. The ISME Journal. 2008;**2**(4):Article 4. DOI: 10.1038/ismej.2007.117

[30] Schmitz RA, Klopprogge K, Grabbe R. Regulation of nitrogen fixation in Klebsiella pneumoniae and Azotobacter vinelandii: NifL, transducing two environmental signals to the nif transcriptional activator NifA. Journal of Molecular Microbiology and Biotechnology. 2002;**4**(3):235-242

[31] Ospina-Betancourth C, Acharya K, Sanabria J, Curtis TP. Low inhibitory effect of ammonia on the nitrogen-fixing activity of a sludge enriched with nitrogen-fixing bacteria. Bioresource Technology Reports. 2021;**14**:100655. DOI: 10.1016/j.biteb.2021.100655

[32] Gosztolai A, Schumacher J, Behrends V, Bundy JG, Heydenreich F, Bennett MH, et al. GlnK facilitates the dynamic regulation of bacterial nitrogen assimilation. Biophysical Journal. 2017;**112**(10):2219-2230. DOI: 10.1016/j.bpj.2017.04.012

[33] Genetics and regulation of N2 Fixation. Biology LibreTexts. 2017. Available from: https://bio.libretexts.org/Bookshelves/Microbiology/Microbiology_(Boundless)/05%3A_Microbial_Metabolism/5.15%3A_Nitrogen_Fixation/5.15E%3A_Genetics_and_Regulation_of_N_Fixation

[34] Watanabe T, Horiike T. The evolution of molybdenum dependent nitrogenase in cyanobacteria. Biology. 2021;**10**(4):Article 4. DOI: 10.3390/biology10040329

[35] Temme K, Zhao D, Voigt CA. Refactoring the nitrogen fixation gene cluster from Klebsiella oxytoca. Proceedings of the National Academy of Sciences. 2012;**109**(18):7085-7090. DOI: 10.1073/pnas.1120788109

[36] Rubio LM, Ludden PW. Maturation of nitrogenase: A biochemical puzzle. Journal of Bacteriology. 2005;**187**(2):405-414. DOI: 10.1128/JB.187.2.405-414.2005

[37] Rubio LM, Ludden PW. Biosynthesis of the iron-molybdenum cofactor of Nitrogenase. Annual Review of Microbiology. 2008;**62**(1):93-111. DOI: 10.1146/annurev.micro.62.081307.162737

[38] Poudel S, Colman DR, Fixen KR, Ledbetter RN, Zheng Y, Pence N, et al. Electron transfer to Nitrogenase in different genomic and metabolic backgrounds. Journal of Bacteriology. 2018;**200**(10):e00757-e00717. DOI: 10.1128/JB.00757-17

[39] Zhao D, Curatti L, Rubio LM. Evidence for nifU and nifS participation in the biosynthesis of the iron-molybdenum cofactor of Nitrogenase*. Journal of Biological Chemistry. 2007;**282**(51):37016-37025. DOI: 10.1074/jbc.M708097200

[40] Baysal C, Burén S, He W, Jiang X, Capell T, Rubio LM, et al. Functional expression of the nitrogenase Fe protein in transgenic rice. Communications Biology. 2020;**5**(1):Article 1. DOI: 10.1038/s42003-022-03921-9

[41] Nonaka A, Yamamoto H, Kamiya N, Kotani H, Yamakawa H, Tsujimoto R, et al. Accessory proteins of the nitrogenase assembly, NifW, NifX/NafY, and NifZ, are essential for diazotrophic growth in the nonheterocystous cyanobacterium *leptolyngbya boryana*. Frontiers in Microbiology;**10**. Available from: https://www.frontiersin.org/articles/10.3389/fmicb.2019.00495

[42] Stappen CV, Jiménez-Vicente E, Pérez-González A, Yang Z-Y, Seefeldt LC, DeBeer S, et al. A conformational role for NifW in the maturation of molybdenum nitrogenase P-cluster. Chemical Science. 2020;**13**(12):3489-3500. DOI: 10.1039/D1SC06418E

[43] Making Real a Biotechnology Dream: Nitrogen-Fixing Cereal Crops. MIT News: Massachusetts Institute of Technology. Available from: https://news.mit.edu/2020/making-real-biotechnology-dream-nitrogen-fixing-cereal-crops-0110

[44] Warmack RA, Maggiolo AO, Orta A, et al. Structural consequences of turnover-induced homocitrate loss in nitrogenase. Nature Communications. 2023;**14**:1091. DOI: 10.1038/s41467-023-36636-4

Chapter 3

Roles of Rhizobacteria in Cereals

Champa Wijekoon and Vinuri Weerasinghe

Abstract

The rhizosphere is a dynamic ecosystem consisting of a plethora of microorganisms. The rhizosphere microbiome plays diverse roles in cereal plants. Among them, the bacterial population associated with roots including exophyte microbes and endophytes has a direct impact on plant development and health. In this chapter, we describe the rhizosphere bacterial microbiome in cereals, meta-genomics studies, isolation and identification of rhizobacterial endophytes and exophytes in different cereal plants, characterization of cereal rhizobacteria, and the potential roles of the rhizobacteria in cereal crops. The potential roles of these microbes will be pathogenic, parasitic, neutral, growth-promoting, stress-tolerant, biocontrol, etc. Overall, this chapter will explore the recent research advances and updates in rhizobacteria in cereal crops.

Keywords: rhizosphere, rhizobacteria, cereals, plant growth-promoting rhizobacteria, biocontrol, endophytes, exophytes, microbiome

1. Introduction

The rhizosphere is the thin layer or narrow region of soil (approx. 1 mm) surrounding the roots of a plant, and it is directly influenced by root activities [1, 2]. The rhizosphere consists of three main zones, namely, endo-rhizosphere, rhizoplane, and ecto-rhizosphere [1]. The endo-rhizosphere is the innermost zone of the rhizosphere which includes plant root tissues such as the cortex and endodermis. Ecto-rhizosphere is the outer-most zone which comprises soil particles adjacent to the roots. The rhizoplane consists of the cortex, epidermis, and mucilage. It is the root surface that interacts with soil microbes and soil particles [1]. Bacteria inhabiting the rhizosphere, i.e., rhizobacteria, are found in all three zones of the rhizosphere. The endo-rhizosphere is inhabited by root endophytes (microbes living inside the roots) while the exophytes (microbes living outside of the roots) inhabit the ecto-rhizosphere [3]. Epiphytic (surface-dwelling) microbes colonize the rhizoplane (**Figure 1**) [4]. Rhizosphere is home to many organisms including bacteria, fungi, actinomycetes, protozoa, algae, nematodes, and arthropods [1, 5]. The rhizosphere microbiome plays different roles in cereal plants, however, bacterial microbes in them have sparked great importance for further studies. Rhizosphere microbial studies have been carried out in different cereal crops including rice, wheat, oat, barley, maize, rye, and sorghum [2, 5–25].

Root epidermal cells release root exudates which consist of an array of compounds, including organic acid ions, inorganic ions, siderophores, sugars, vitamins, amino acids, purines, nucleosides, and enzymes, to the soil [1]. Polysaccharide mucilage is produced by the root cap [17]. Soil microbes exploit these compounds as carbon and

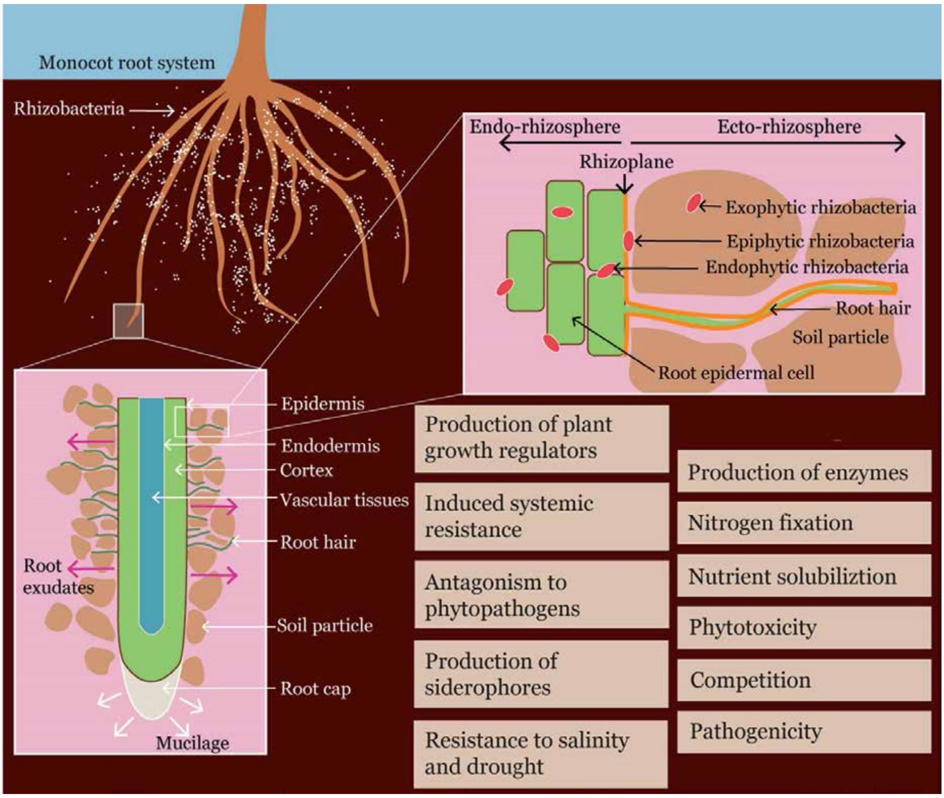

Figure 1.
The structure and interactions of the cereal rhizosphere.

energy sources [2]. As a result, the microbial density in the rhizosphere increases up to 10 to 100 times than the bulk soil. The rhizosphere is therefore called the second genome [1, 26] or an extended genome [20] of plants. The majority of the rhizosphere community is bacteria, with approx. 10^4 bacterial species/g of soil [1].

The rhizosphere microbial community is mainly considered substrate-driven, and it may vary with the plant genotype, plant developmental stage, and environmental factors [1, 17]. The root exudates influence plant-rhizobacteria and rhizobacteria-rhizobacteria interactions [2]. The plant-rhizobacteria interactions could be pathogenic, parasitic, neutral, growth-promoting, stress-tolerant, and antimicrobial [1]. Root exudates may attract beneficial bacteria. For example, plant growth-promoting rhizobacteria (PGPR) could be recruited through a process called chemotaxis. These PGPRs may boost plant growth and provide a protective function under abiotic and biotic stresses [2, 3]. On the other hand, the secretion of antimicrobial compounds by roots may inhibit microbial growth [1]. Some root exudates are involved in antibiosis (microbial inhibition due to antibiotics and toxins), parasitism (microbial inhibition due to cell wall-degrading enzymes), and systemic resistance (microbial inhibition by inducing plant defense mechanisms) [1].

Different mechanisms have been proposed to explain the exophytic and endophytic microbial profiles. The exophytic bacteria are prone to frequent fluctuations, thus highly dependent on environmental conditions, host genotype, and the soil microbial community. It is suggested that autolysis of root epidermal cells facilitates exo-bacterial invasion in internal plant tissues. Successful colonization of the plant root tissues could make them stable root endophytes [1, 3]. However, plant endogenic microflora that have co-evolved with the plant host could be present prior to exophytic invasions [3]. The endophytic community may influence the exophytic profile by modifying the root morphology [3].

In addition to plant-rhizobacteria interactions, communication among rhizobacteria is important for establishing a healthy rhizosphere. Signaling molecules called autoinducers produced by bacteria in response to a stimulation or at a particular stage of life, interact with receptors of other bacterial cells. A certain bacterial cell density is required to trigger bacterial gene expression that is responsible for cell-cell communication. This process is defined as quorum-sensing [1].

Rhizobacterial interactions with plants are categorized as positive/beneficial, negative/harmful, or neutral [2, 3, 17]. The positive mechanisms of plant growth-promoting rhizobacteria have been elucidated by previous researchers which could be classified broadly as direct and indirect [2, 17]. The production of plant growth regulators, enzymes and siderophores, solubilization of minerals, and symbiotic nitrogen fixation, directly influence plant growth, hence defined as direct mechanisms. Reports show that some PGPRs may use multiple mechanisms for accomplishing plant growth enhancement [17]. Mechanisms such as antagonistic activity against phytopathogens and induced systemic resistance have indirect effects on plant growth promotion [2, 17]. Negative interactions of rhizobacteria with plants include phytopathogenicity that adversely affects plant health [3].

Different species of rhizobacteria have been reported over the past few decades. Some examples include *Azospirillum, Klebsiella, Pseudomonas, Azotobacter, Alcaligens, Enterobacter, Burkholderia, Arthobacter, Serratia, Bacillus* and *Stenotrophomonas* [2]. Certain strains of PGPR belonging to *Bacillus, Enterobacter, Burkholderia, Acinetobacter, Alcaligenes, Arthrobacter, Azospirillum, Azotobacter, Beijerinckia, Erwinia, Flavobacterium, Rhizobium* and *Serratia* are utilized in a global scale to enhance crop productivity [13]. Diazotrophic soil bacteria such as *Rhizobium* are members of the PGPR [2]. Siderophore-producing bacteria are involved in iron sequestration for plants [6, 27]. Some of the PGPRs such as *Rhizobium, Azospirillum, Pseudomonas, Flavobacterium, Arthrobacter,* and *Bacillus* have been identified to enhance tolerance to abiotic stresses such as high salinity [27]. Some PGPR produces secondary metabolites such as hydrogen cyanide, 2,4-diacetylphloroglucinol, and antibiotics (e.g., phenazine) that play a role in antagonism [27]. Chromium stress in wheat is alleviated by *Pseudomonas* sp. whereas barley inoculated with *Arthrobacter mysorens* 7 and *Flavobacterium* sp. L-30 helps survival in lead-contaminated soil [8]. Certain rhizobacteria are available for commercial use as soil formulations, i.e., bioinoculants. Some examples are *Arthrobacter mysorens* 7, *Flavobacterium* sp. L30, and *Klebsiella mobilis* CIAM 880 [8].

This chapter will review the rhizobacteria present in different cereal crops, their roles, applications in agriculture, challenges, and future directions. In addition, this will further discuss the rhizosphere bacterial microbiome, identification and characterization studies of rhizobacteria, and the potential roles of the rhizobacteria in cereals such as maize, rice, wheat, barley, rye, and sorghum.

2. Identification and characterization of rhizobacteria

Rhizobacteria in the cereal rhizosphere are identified and characterized in numerous ways. They could be mainly categorized into culture-independent and culture-dependent approaches. Culture-dependent methods involve bacterial isolation, identification, and characterization. Culture-independent methods usually include meta-analyses such as meta-genomics, meta-transcriptomics, and meta-proteomics [4]. Bacterial identification and characterization in cereals can be carried out on isolated colonies or on root and/or soil samples as a whole [5, 11, 20]. Rhizobacteria are characterized using bioassays that target their biochemical properties and pathways. **Figure 2** summarizes the methods used in the identification and characterization of rhizobacteria.

2.1 Identification of rhizobacteria

2.1.1 Culture-dependent identification methods

In the studies of culture-based methods, a sample of the rhizosphere is suspended in a sterile solution (water/buffer) under aseptic conditions. The diluted rhizosphere suspension could then be plated on bacterial culture media such as tryptic soy agar [7, 21] and Luria-Bertani agar [19]. Single bacterial colonies are purified using subsequent culturing steps [5, 19]. Conventionally, culturable bacteria are identified mainly using phenotypic characters such as colony color, texture, size, form, margin, elevation, microscopic features, etc. [7, 12]. Microscopic techniques allow for determining the cell morphology, and more importantly the habitat and colonization patterns of bacteria [4]. Finally, bacteria are identified with reference to taxonomic keys. The disadvantages of conventional methods are the inability to capture large quantities of microorganisms, especially in the soil, and the reliance on culturable isolates. Many microorganisms would have remained unknown unless for the development of molecular identification techniques [4].

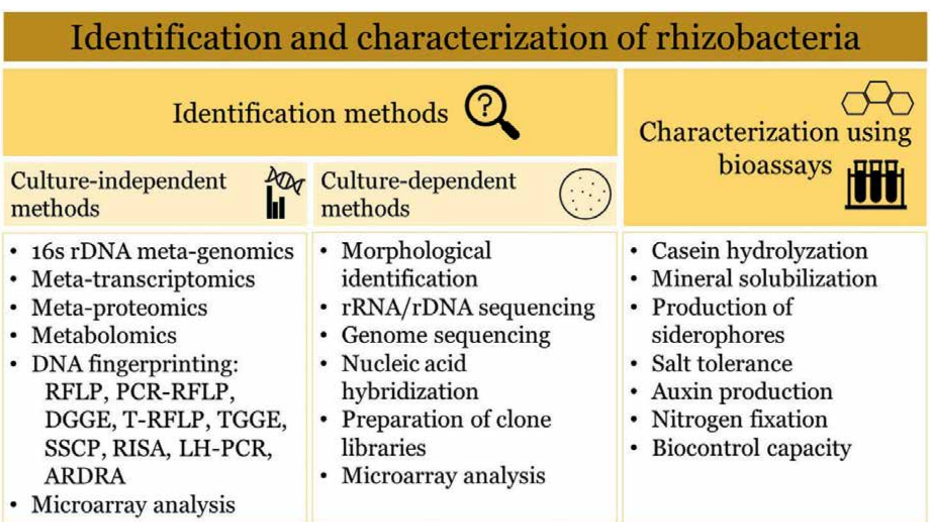

Figure 2.
The methods of identification and characterization of rhizobacteria.

Purified cultivable bacterial colonies could further be isolated and studied using molecular identification. The first step of molecular identification is nucleic acid (DNA/RNA) extraction, followed by polymerase chain reactions (PCR) [4]. Bacteria are identified by amplifying the universal bacterial 16S rRNA gene [4]. **Table 1** provides a summary of different target regions of the 16S rRNA gene used in previous studies with their respective primer pairs [5, 9, 11, 24, 25]. PCR techniques become useful for the functional identification of rhizobacteria as they allow to targeting of PGP traits with specific primers. For example, primers have been designed to target the gene encoding dinitrogenase reductases (*nifH*), and ammonia monooxygenase (*amoA*) [4].

Next, PCR products are sequenced, mainly using the Sanger sequencing technique. Taxonomy is assigned with reference to 16S databases such as GreenGenes [25] and SILVA [20, 21]. One limitation of the 16S rDNA identification is the interference from chloroplast 16S rDNA and mitochondrial 18S rDNA, resulting in inaccurate molecular identification [29]. Therefore, generally, mitochondria, chloroplasts, archaea, and eukaryote-associated sequences are removed after classification [25]. Different pipelines are used for sequence analysis, for example, the Quantitative Insights Into Microbial Ecology (QIIME) analysis [20, 21]. DNA sequence identification is usually followed by a phylogenetic analysis. The evolutionary relatedness among bacterial isolates is determined by constructing phylogenetic trees according to various algorithms [19, 21, 28].

Some other molecular identification methods include hybridization/probing, fingerprinting, preparation of clone libraries, and microarrays [4]. Nucleic acid

Amplified region	Primers	Size of the amplified region	Reference
V3–V4 region of the 16S rRNA gene	341F (5′-CCTAYGGGRBGCASCAG-3′) and 806R (5′-GGACTACNNGGGTATCTAAT-3′)	N/A	[19]
16S rRNA gene rrs	T7-PA and PH	1.5 kb	[11]
16S rRNA gene	fD1 (5′-AGAGTTTGATCCTGGCTCAG-3′) and rD1 (5′-AAGGAGGTGATCCAGCC-3′)	1.5 kb	[21]
16S rRNA gene	27F and 1492R	N/A	[19]
V4 region of the 16S rRNA gene	515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACVSGGGTATCTAAT-3′)	N/A	[25]
16S rRNA gene partial sequence	pA (5′-AGAGTTTGATCCTGGCTCAG-3′) and pH (5′-AAGGAGGTGATCCAGCCGCA-3′)	N/A	[28]
16S rRNA gene	27f (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1401r (5′-CGGTGTGTACAAGACCC-3′)	1.4 kb	[10]
16S rRNA gene	63F primer (5′-CAGGCCTAACACATGCAA GTC-3′), 20F primer (5′-AGAGTTTGATCATGGCTCAG-3′) and 1500R primer (5′-GGTTACCTTGTTACGACTT-3′)	N/A	[16]

N/A: Not available.

Table 1.
Regions of the 16S rRNA gene used for molecular identification of rhizobacteria.

hybridization (DNA-DNA or DNA-RNA) is based on the affinity of DNA or RNA fragments to respective probes. DNA fingerprinting is another molecular technique used in bacterial identification where organisms are differentiated according to their unique DNA. Some of the fingerprinting methods successfully used are restriction fragment length polymorphism (RFLP), polymerase chain reaction-restriction fragment length (PCR-RFLP), denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (T-RFLP), temperature gradient gel electrophoresis (TGGE), single-strand conformational polymorphisms (SSCP), ribosomal internal spacer analysis (RISA), length heterogeneity-PCR (LH- PCR), and amplified ribosomal DNA restriction analysis (ARDRA) [4, 22, 29]. In order to examine wheat rhizobacteria [9], conducted a DGGE analysis by amplifying the region from 8 to1492 bp and the V3 region of the 16S rRNA gene using the primer pairs, GM3f-GM4r and 338f-520r, respectively. The total DNA from the rhizosphere of barley was extracted by [10], and targeted for PCR amplification of 16S rRNA genes, followed by a T-RFLP analysis.

DNA microarrays, commonly known as DNA chips or bio-chips can be used to investigate specific genes of rhizobacteria or specific taxa within a community [4]. To identify bacterial taxa enriched in the course of barley monoculture [10], compared the rhizobacterial communities from three vegetation cycles, using a taxonomic 16S rRNA microarray containing 1033 probes. The data showed similar and distinct taxa in the three cycles [10]. Another study tested the effect of the evolutionary changes in eukaryotes on the composition of their associated microbiome using chloroplast sequences of Poaceae genotypes, namely, maize, sorghum, and wheat [11]. Their 16S rRNA taxonomic microarray analysis study revealed the correlation between rhizobacterial communities and Poaceae genotypes. They could also identify distinct bacterial taxa involved in cooperation (e.g., *Rhodospirillales* and *Bacillales*), symbiosis (e.g., *Rhizobiales*), and parasitism (e.g., *Agrobacterium* and *Xanthomonas*). In some studies, PCR products are transferred into vectors and cloned to prepare DNA clone libraries. Clone libraries can comprise complementary DNA (cDNA) or genomic DNA [4].

Another well-established method for identifying cultivable bacterial strains is the fatty acid analysis. It is a fast and cheap method, and a large number of conservative characteristics can be subjected to quantitative analysis [7]. The authors analyzed the fatty acid profiles of 1188 isolates of rhizobacteria in barley. The analysis separated the isolates into three distinct groups, with *Pseudomonas*, *Cytophaga,* and Gram-positive bacteria, respectively, as the predominant bacterial taxa. Bacterial communities such as rhizobacteria are further analyzed using metabolomics which measures the metabolic profile, including primary and secondary metabolites, at a specific time point. This approach is based on analytical techniques such as nuclear magnetic resonance (NMR), gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-mass spectrometry (LC-MS) [4].

2.1.2 Culture-independent identification methods

Culture-independent identification techniques do not necessarily need culturable bacterial isolates and are often carried out on a community of bacteria (e.g. soil samples and plant tissues), based on meta-analyses. Meta-analyses provide information on bacterial communities and their interactions. Some of the approaches are, meta-genomics, meta-transcriptomics, meta-proteomics, and metabolomics. Meta-genomics allows the identification and diversity and abundance calculations of both culturable and unculturable microbiomes. The bacterial abundance is measured in relation to the copy number of the 16S rRNA gene [19], and the degree of gene expression is examined using transcriptomics [11]. Transcriptomics is useful in identifying active genes or pathways. The gene expression level can be quantified by bioinformatics software [25]. Meta-proteomics provides a protein analysis at the bacterial community level [4].

Several sophisticated high-throughput sequencing techniques, i.e., next-generation sequencing methods, have been developed in the recent past for fast, efficient, and simultaneous identification of multiple organisms. **Table 2** provides some high-throughput sequencing techniques used in meta-genomics studies for the characterization of bacteria on a community scale.

The analysis of sequence data includes various statistical and bioinformatics methods. In addition to taxonomical classification, the sequence data can be classified as core, shared, and unique taxa [25]. Statistical software such as R [25] and IBM SPSS [16] are used to analyze data using several statistical models [20]. Statistical models allow calculations of alpha diversity (within-sample richness) and beta diversity (between-sample dissimilarity) [19, 24, 25]. Bacterial correlation network analyses highlight the interactions among bacterial taxa, and determine the keystone taxa that are critical for maintaining community structure and function under different soil management practices [25].

2.2 Characterization of rhizobacteria

Rhizobacteria can be characterized according to their biochemical properties. Bacterial colonies isolated from cereal crops could be grown in selective or differential media, and identified based on their substrate-specificity [30]. Staining techniques, particularly Gram staining and endospore staining, aided with microscopic observations are also used for characterization [31]. A less time-consuming method of biochemical characterization is the use of the BIOLOG system. This method provides a phenotypic fingerprint of bacteria by assessing a microorganism

Sequencing platform	PCR amplified region	PCR primers	Reference
Illumina MiSeq	V3-V4 hypervariable region of the 16S rRNA gene	341F (5′-CCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′)	[21]
Ion Torrent	N/A	N/A	[25]
Illumina MiSeq	16S rRNA gene	338F and 806R with specific primers synthesized using barcodes	[20]
PacBio RS II	Full length of 16S rRNA genes	27F and 1492R	[5]
Illumina Miseq and HiSeq	N/A	341F (5′-CCTACACGACGCTCGGCATCTTCGGATTTA-3′) and 805R (5′-GACTGGAGTTCCTTGGCACCCGAATTCCA-3′)	[24]

N/A: Not available.

Table 2.
High-throughput sequencing techniques used for meta-genomics studies of bacterial communities.

up to the species level using carbon source utilization assays and chemical sensitivity assays in special microplates [28].

Assessing the functional roles of bacteria is not always possible since only the culturable rhizobacteria can be tested using bioassays [29]. However, several biochemical tests have been developed to qualitatively and/or quantitatively assess the biochemical properties of bacteria, for example, carbon sources and the production of enzymes. In an attempt to biochemical characterization of rhizobacteria [21], conducted several bioassays to determine plant growth-promoting traits. Rhizobacteria were tested for casein hydrolyzation, phosphate solubilization, potassium solubilization, zinc solubilization, production of siderophores, and salt tolerance. All the assays were conducted in agar plates incorporated with selective/inhibitory reagents. The number of positive isolates was counted for each functional assay [21]. The ability of rhizobacteria to produce IAA, fix nitrogen, and control *Fusarium graminearum*, *F. proliferatum*, *F. verticillioides,* and *F. boothii* were analyzed by [19]. **Table 3** provides a brief description of

Bioassay	Special constituents of the medium	Observation of a positive isolate	Reference
Hydrolyzation of casein	5% skimmed milk powder, 0.5% pancreatic digest of casein, 0.25% yeast extract, 0.1% D-glucose	A clear halo in the medium surrounding the bacterial colony	[21]
Solubilization of phosphate	Tricalcium phosphate and Pikovskayas agar	A halo zone in the medium surrounding the bacterial colony	[21]
Solubilization of potassium	Potash feldspar and Pikovskayas agar	A halo zone in the medium surrounding the bacterial colony	[21]
Solubilization of zinc	Zinc oxide and Pikovskayas agar	A halo zone in the medium surrounding the bacterial colony	[21]
Production of Siderophores: iron solubilization	Chrome azurol S (CAS) and hexadecyltrimethylammonium bromide (HDTMA)	An orange halo in the medium surrounding the bacterial colony	[21, 28]
Salt tolerance	Different concentrations of sodium chloride (NaCl)	High growth/colony count compared to the control	[21]
Antagonistic activity - Dual culture	N/A	Pathogen growth inhibition	[19]
IAA production	Nutrient broth supplemented with tryptophan	Development of red color in the supernatant of the bacterial culture broth with the addition of orthophosphoric acid and Salkowaskis reagent	[28]
	LB broth supplemented with L-tryptophan (200 mg/l)	Optical density of the cell-free supernatant after adding Salper reagent	[16]
Nitrogen fixation	Nitrogen-free malate (NFM/NFB) medium: malic acid, KH_2PO_4, $MgSO_4.7H_2O$, NaCl, $CaCl_2$, Na_2MoO_4, $MnSO_4.H_2O$, EDTA, 0.5% bromothymol blue, biotin, KOH	Development of blue color in the NFB medium	[2]

N/A: Not available.

Table 3.
Assays for biochemical characterization of rhizobacteria.

some bioassays used in bacterial characterization. Software packages such as PICRUSt provide predicting models for functional profiles of bacterial communities based on the 16S rRNA gene sequences [24], which are applicable for unculturable bacteria as well.

3. Contribution of cereal rhizobacteria to face current challenges in agriculture

The global population is estimated to be nearly 10 billion over the next two decades, and a constant supply of food is essential to feed them [32]. It is challenging for researchers, breeders, and farmers to meet these demands within the existing arable lands while minimizing the use of agrochemicals [32]. Cereals are prone to many diseases by pests and pathogens, leading to yield loss. Climate change and human activities contribute to the development of undesirable environments with high salinity, high temperature, water stress, and less fertile soils. Cereals such as maize, rice, wheat, barley, rye, and sorghum, are staple foods of the majority of the world population, and their cultivation covers a large proportion of arable lands. Plant growth-promoting rhizobacteria from cereals have therefore been studied as a sustainable, eco-friendly approach in various agricultural aspects such as soil nutrient supplements and biocontrol agents [2, 6]. There is evidence for PGPR inoculations to enhance crop yield, and plant growth, and alter the microbial population in the soil (**Table 4**). However, their mechanisms are yet to be fully understood, and efficient application methods are yet to be developed [1]. This section highlights the importance of probing for beneficial rhizobacteria with examples from previous studies on plant hormone production, mineral solubilization, biocontrol properties, managing biotic and abiotic stress situations, and bioremediation.

3.1 Phytohormone production and mineral solubilization

Most PGPRs have the ability to produce indole-3-acetic acid (IAA), a type of auxin that stimulates root growth and the formation of lateral roots and root hairs [16]. Cytokinins, gibberellins, and inhibitors of ethylene may also be responsible for altered root morphology, facilitating nutrient uptake [15]. A mixture of *Azospirillum brasilense* with *Bradyrhizobium japonicum* could increase seed germination and initial development of maize [17]. Application of the rhizobacteria, *Pseudomonas fluorescens* BRM-32111 and *Burkholderia pyrrocinia* BRM-32113 on upland rice seeds in Brazil increased biomass, leaf area, root length, root biomass, chlorophyll content, rate of carbon assimilation, rubisco carboxylation efficiency and water use efficiency in seedlings under greenhouse conditions [14].

Crop-associated indigenous nitrogen-fixers may be agronomically important since they could supply a portion of the crop's total nitrogen requirement [29]. Mineral solubilization by PGPR is another key tool for increasing nutrient uptake efficiency. The PGPR is adapted to fulfill the requirements of both nutrient-deficient and excessive soils. The effect of five nitrogen-fixing bacteria (*Bacillus licheniformis* RC02, *Rhodobacter capsulatus* RC04, *Paenibacillus polymyxa RC05*, *Pseudomonas putida* RC06, and *Bacillus* OSU-142), and two phosphate-solubilizing bacteria (*Bacillus megaterium* RC01 and *Bacillus* M-13) was tested in barley under greenhouse conditions by [33]. *Bacillus* M-13 and *B. megaterium* RC01 significantly increased the phosphate availability in soil. All the tested PGPR strains were diazotrophic. Among them, *Bacillus*

Rhizobacteria	Inoculated cereal crop	Potential role/s	Reference
Brevibacterium frigoritolerans, *Bacillus thuringiensis*, and *Bacillus velezensis*	Wheat (China)	Increase plant height, root length, dry weight, and fresh weight	[23]
Pseudomonas sp.	Wheat	Tolerant to chromium (Cr)	[8]
Bacillus megaterium M3, *Bacillus subtilis* OSU142, *Azospirillum brasilense* Sp245, and *Raoultella terrigena*	Wheat and barley	Increase root and shoot dry weight under cold stress	[31]
Bacillus amyloliquefaciens and *Microbacterium oleovorans*	Maize	Protection against *Fusarium verticillioides*	[2]
Azospirillum brasilense + *Bradyrhizobium japonicum*	Maize	Increase seed germination and initial development of seedlings	[17]
Sinorhizobium sp. A15, *Bacillus* sp. A28, *Sphingomonas* sp. A55 and *Enterobacter* sp. P24	Maize	Increase IAA synthesis and early plant growth rate under conservation tillage	[19]
Pseudomonas fluorescens BRM-32111 + *Burkholderia pyrrocinia* BRM-32113	Upland rice (Brazil)	Increase biomass, leaf area, root length, root biomass, chlorophyll content, rate of carbon assimilation, rubisco carboxylation efficiency, water use efficiency, and tolerance to allelochemicals in seedlings under greenhouse conditions	[14]
Stenotrophomonas maltophilia, *Enterobacter* sp., *Bacillus* sp., *Ochrobactrum haematophilum* and *Pseudomonas aeruginosa*	Rice	Limit the mycelial growth of *Rhizoctonia solani* and *Fusarium oxysporum* Increase germination percentage, seed vigor index, total dry biomass, and nitrogenase activity	[2]
Arthrobacter, Klebsiella, Pseudomonas, Bacillus, Proteus and *Staphylococcus*	Barley	Increase bioremediation of cadmium (Cd)-contaminated soils	[8]
Arthrobacter mysorens 7, *Flavobacterium* sp. L30, and *Klebsiella mobilis* CIAM 880	Barley	Solubilize Cd Increase root elongation in Cd-contaminated soil Nitrogen fixation	[8]
Arthrobacter mysorens 7 and *Flavobacterium* sp. L-30	Barley	Tolerant to lead (Pb)	[8]
Pseudomonas putida	Fall rye (Secale cereal)	Tolerant to petroleum hydrocarbon (PHC)	[12]
Bacillus and *Sphingomonas*	N/A	Mineralize heavy-metals Degrade organic pollutants	[24]
Geobacter	N/A	Involved in iron and sulfate cycles Degrade (anaerobic) polycyclic aromatic hydrocarbons (PAHs)	[24]

N/A: Not available.

Table 4.
Potential PGP roles of rhizobacteria in cereal crops.

OSU-142 performed the best. Further experiments revealed that the inoculation of nitrogen-fixers in barley had a significant impact on the uptake of nitrogen (N), iron (Fe), manganese (Mn), and zinc (Zn) [33].

3.2 Biocontrol agents

Biotic agents such as phytopathogens and pests are a major threat to cereal production. Even though synthetic pesticides are efficient in disease management, continuous use may lead to negative impacts such as increasing disease resistance, soil and water toxicity, and accumulation of residues in food chains [18]. Biocontrol agents are organisms that significantly reduce pest and pathogen levels. They are assumed to be less harmful due to their biological origin and hence considered as an alternative to environmentally deleterious agrochemicals. Biocontrol agents can be applied by seed dipping, spraying, through irrigation, or as solid inoculants [30].

Several biocontrol agents have been experimented. Some PGPR such as *Bacillus amyloliquefaciens* and *Microbacterium oleovorans* can protect maize against *Fusarium verticillioides* when applied in the form of seed coatings. Based on that, [2] conducted a similar experiment using five selected rhizobacterial isolates as inoculations on rice seeds. The isolates: *Stenotrophomonas maltophilia*, *Enterobacter* sp., *Bacillus* sp., *Ochrobactrum haematophilum*, and *Pseudomonas aeruginosa*, exhibited multiple PGP attributes and successfully limited the mycelial growth of two fungal pathogens, *Rhizoctonia solani* and *Fusarium oxysporum,* in vitro. PGPR-inoculated plants had a substantially higher germination percentage, seed vigor index, and total dry biomass compared to the control [2]. Further, the *nifH* gene responsible for regulating nitrogen fixation was also detected in rhizobacterial isolates with significant nitrogenase activity [2].

3.3 Managing abiotic stress

Some of the major abiotic stresses on cereals are salinity, drought, low and high temperatures, and nutrient deficiencies [22, 26]. High saline content in the soil leads to osmotic stress, nutrient deficiency, ion toxicity, hormonal imbalance, and oxidative stress in plants. As a consequence, photosynthesis, protein synthesis, lipid metabolism, and stomatal closure are interfered, leading to lower crop yields [13]. Salt-tolerant rhizobacteria enhance root and shoot biomass, nutrient and water uptake, chlorophyll content, and disease resistance under saline conditions [13]. Some PGPRs form biofilms which provide plants protection from external stresses, while supporting their growth and increasing crop quality [13].

The rhizosphere of a halotolerant barley cultivar (*Hordeum maritimum* With.) was investigated by [16], in search for halotolerant rhizobacteria. Three halotolerant bacterial strains: *Bacillus mojavensis* S1, *B. pumilus* S2, and *Pseudomonas fluorescens* S3, were isolated and inoculated on a salt-sensitive barley cultivar. The bacteria showed an increase in IAA and proline production under salinity stress, which corresponds to plant adaptation in saline conditions. In a study in China by [23], wheat plants inoculated with three salt-tolerant plant growth-promoting rhizobacteria: *Brevibacterium frigoritolerans*, *Bacillus thuringiensis*, and *Bacillus velezensis*, increased in plant height, root length, dry weight, and fresh weight. Another group of scientists in China examined the rhizosphere bacterial diversity and soil metabolome of sea rice SR86 seedlings, over a range of salinity levels [5]. A rhizobacterial co-occurrence network of SR86 seedlings described keystone taxa involved in coping with salt stress. Further analysis on the network explained the potential contribution of keystone taxa, and specific metabolites in salt tolerance. Four rhizobacterial strains capable of alleviating salt stress and promoting seedling growth under salinity stress were isolated, characterized, and inoculated on SR86 plants. The inoculants were re-isolated and characterized for verification. Bacterial phyla, namely, Proteobacteria, Firmicutes, Desulfobacterota, and Verrucomicrobiota were detected in PGPR-applied soil [5].

In another study [31], investigated the performance of PGPR applications compared to a fertilizer treatment in wheat and barley plants under cold stress. The PGPR used were, *Bacillus megaterium* M3, *Bacillus subtilis* OSU142, *Azospirillum brasilense* Sp245, and *Raoultella terrigena*. The authors observed a statistically significant difference between PGPR inoculations and the fertilizer treatment with respect to root and shoot dry weight.

3.4 Plant acclimatization for toxicity/phytoremediation

Due to a lack of agricultural lands to meet the needs of the growing population, environments that are generally considered undesirable for cultivation may also be used for crop production. Heavy metals are common soil contaminants. Microbial activity under heavy-metal stress can change the pH of soil and consequently, its absorption and adsorption characteristics [8, 34]. However, high levels of heavy metals can cause significant changes in the microbial community structure [24, 34].

The effect of PGPR on barley plant growth in Cd-polluted soil was tested by [8] as a measure of increasing barley yield in Cd-polluted soils. Three commercially available PGPR: *Arthrobacter mysorens* 7, *Flavobacterium* sp. L30 and *Klebsiella mobilis* CIAM 880 were found to be successful Cd solubilizers, out of which *K. mobilis* CIAM 880 was the most effective. In addition, all three PGPRs were able to fix nitrogen and actively colonize the barley root system and rhizosphere which resulted in significant root elongation in Cd-contaminated soil. The authors used several measurements such as Cd accumulation in bacteria, gas chromatography analysis, IAA production, and a mathematical model simulation to propose the complex mechanism of bacterial Cd tolerance [8]. Moreover, rhizobacteria of the genera, *Arthrobacter*, *Klebsiella*, *Pseudomonas*, *Bacillus*, *Proteus*, and *Staphylococcus* were proposed as good candidates for accelerated bioremediation of soils heavily contaminated with Cd [8].

Research has been conducted to investigate the transformations of heavy metals (HMs) and polycyclic aromatic hydrocarbons (PAHs) in rhizosphere soils, and the adaptive responses of the rhizobacterial community in rice in China [24]. The dominant genera of rhizobacterial community in HM-PAH co-contaminated paddy rhizosphere soil were *Bacillus*, *Massilia*, *Sphingomonas*, and *Geobacter*, and their higher abundances appeared at the tillering stage and heading stage of rice. *Bacillus* and *Sphingomonas* are known to mineralize heavy metals and degrade organic pollutants whereas *Geobacter* is known for contributing to iron or sulfate cycles, and anaerobically degrading PAHs [24]. These phenomena explain their richness in HM-PAH co-contaminated paddy rhizosphere soil. In a similar study, fall rye (Secale cereal) grown in petroleum hydrocarbon (PHC)-impacted soil treated with two strains of *Pseudomonas putida* showed a gradual decrease in the detrimental effects of PHC over 3 years [12]. The two *Pseudomonas* strains were introduced to the rhizosphere by seed treatment, and field trials were conducted at an oil refinery land farm in Canada. Transcriptomics data revealed significant upregulation of two genes under stress conditions. *P. putida* is also known as a PGPR that produces auxin.

Another soil contaminant is allelochemicals which are the secondary metabolites of plants and microorganisms that accumulate due to long-term consecutive cropping systems. High concentrations of allelochemicals have detrimental effects on plant growth and development. During a study in Brazil, [14] observed allelochemicals in rice, maize, and sorghum plantations. The introduction of PGPR tolerant to allelochemicals was suggested as a method to overcome this challenge [14]. Phytoremediation is another strategy that uses rhizobacteria that have the ability to sequester, degrade, and transform contaminants [12, 29].

3.5 Cultural practices

Cultural practices, for example, the use of fertilizer and antimicrobials, tillage, crop rotations, intercropping, etc., can influence the rhizosphere composition, either enhancing or depleting beneficial bacteria [22]. Under conservation tillage, maize seeds inoculated with PGPR significantly promoted the early plant growth rate which promotes high grain yield [19]. The PGPR strains, *Sinorhizobium* sp. A15, *Bacillus* sp. A28, *Sphingomonas* sp. A55 and *Enterobacter* sp. P24 used for inoculations was previously isolated from the rhizosphere of maize grown in the same area [19]. The PGPR isolates were capable of IAA synthesis. These PGPR significantly increased the abundance and species richness of rhizobacteria which was supported by a 16S rRNA analysis. The molecular analysis further described changes in certain bacterial classes and genera in response to certain PGPR inoculations, indicating the role of PGPR in coordinating the ecological functions of the rhizosphere [19]. For example, the relative abundance of the class Alphaproteobacteria and the genera *Sphingomonas*, *Candidatus solibacter*, and *Bryobacter* were significantly reduced with all four PGPR inoculations while a significantly high relative abundance of the genus *Streptomyces* was observed in all inoculation except A15 [19]. A similar study in China by [20], confirmed that the crop growth stage, long-term tillage practices, and short implementation years of crop rotation significantly affected the phylogenetic diversity of root-associated bacteria in wheat. The work was supported by co-occurrence networks and a correlation analysis. Acidobacteria, Actinobacteria, Chloroflexi, and Proteobacteria were the four dominant phyla present in all samples [20]. The variations in the wheat rhizosphere composition under nitrogen-phosphorous-potassium fertilizer treatments were evaluated by [21]. According to the results of greenhouse experiments, fertilizer addition decreased the proportion of nutrient-solubilizing bacteria in wheat.

4. Challenges of rhizobacteria applications in agriculture

Once beneficial rhizobacteria with PGP traits are successfully identified, the next step is to implement methods for their application under field conditions. Attempts have been made to genetically engineer PGP traits to rhizobacteria. Beneficial traits such as nitrogen fixation, phosphorous solubilization, IAA biosynthesis, naringenin biosynthesis, biocontrol, and rhizoremediation, have become successful in the transfer and optimization within some rhizobacteria [32]. However, PGPR colonization and performance under real field conditions have not always been consistent [26]. As discussed in previous sections, the rhizobacterial population is highly dependent on environmental factors, host factors as well as cultural practices. **Figure 3** outlines the challenges and future directions of PGPR in agriculture.

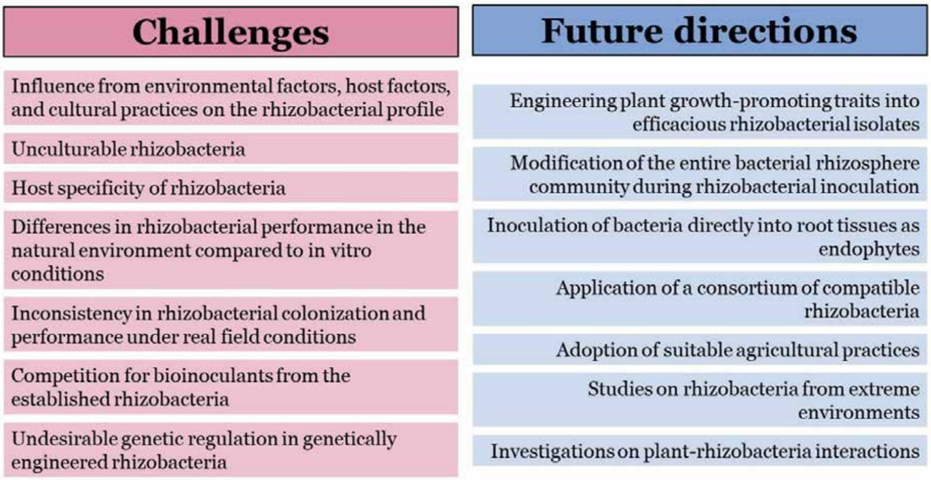

Figure 3.
Challenges and future directions of plant growth-promoting rhizobacteria in agriculture.

The performance of rhizobacteria in the natural environment may vary from that under in vitro conditions. Further, not all rhizosphere bacteria can successfully be cultured in synthetic media. For effective results, the PGPR should be able to survive in foreign soils while competing with the established microbial community [26]. Moreover, host specificity plays a role in successful rhizobacterial colonization. For example, attempts to modify non-host soils with *Rhizobium* have not always been successful. Biocontrol agent inoculations have met varying degrees of failure in disease control, therefore, their use in pest management is not well established [3]. Sometimes, undesirable genetic regulation might repress PGP traits that are genetically engineered to rhizobacteria. Therefore, bioinoculants should be prepared addressing the above issues.

So far, several approaches have been proposed. One strategy is to engineer and transfer PGP traits into selected efficacious rhizobacterial isolates [32]. Another suggestion is to modify the entire bacterial rhizosphere community during inoculation. By inoculating bacteria directly into root tissues as endophytes, it is expected that they will later spread to the soil or control root characteristics and eventually manipulate the rhizosphere community [3]. The application of a consortium of compatible PGPR rather than one bacterial isolate alone has a better influence on improving the adaptability of plants exposed to stress conditions [22, 26]. Crop rotations and intercropping, especially with leguminous crops can influence the development of favorable associations of exophytes and endophytes with plants [3, 26]. The cereal-legume cropping system exchanges PGPR, particularly the diazotrophs from legumes, and may promote rhizobacterial community diversity, soil health, and plant growth. This is a promising strategy to optimize resource-use efficiency and crop yield in less fertile agricultural lands [22]. Exploiting PGPR from extreme environments is another approach to address biotic and abiotic stresses in cereals. Further exploring and improving knowledge on plant-PGPR interactions, particularly at the molecular level altogether will help determining efficient ways of utilizing cereal rhizobacteria as eco-friendly, sustainable agricultural applications.

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5. Conclusion

The cereal rhizosphere is an excellent source of bacteria possessing various functions. Rhizobacterial exophytes and endophytes have a direct impact on cereal plant health and nutrition. Several techniques are used to identify and characterize rhizobacteria, mainly characterized by culture-dependent and culture-independent approaches. Exploiting the potential roles of these microbes will lead to effective, sustainable, and eco-friendly applications in agriculture. Beneficial cereal rhizobacteria will be eco-friendly alternatives for sustainable agriculture.

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Conflict of interest

The authors declare no conflict of interests.

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References

[1] Bashir O, Khan K, Hakeem KR, Mir NA, Rather GH, Mohiuddin R. Soil microbe diversity and root exudates as important aspects of rhizosphere ecosystem. In: Hakeem K, Akhtar M, editors. Plant, Soil and Microbes. Cham: Springer; 2016. pp. 337-357. DOI: 10.1007/978-3-319-29573-2_15

[2] Mir MI, Hameeda B, Quadriya H, Kumar BK, Ilyas N, Zuan ATK, et al. Multifarious indigenous diazotrophic rhizobacteria of rice (*Oryza sativa* L.) rhizosphere and their effect on plant growth promotion. Frontiers in Nutrition. 2022;**8**(781764):1-14. DOI: 10.3389/fnut.2021.781764

[3] Sturz AV, Nowak J. Endophytic communities of rhizobacteria and the strategies required to create yield enhancing associations with crops. Applied Soil Ecology. 2000;**15**:183-190

[4] Enespa CP. Tool and techniques study to plant microbiome current understanding and future needs: An overview. Communicative & Integrative Biology. 2022;**15**(1):209-225. DOI: 10.1080/19420889.2022.2082736

[5] Wang G, Weng L, Huang Y, Ling Y, Zhen Z, Lin Z, et al. Microbiome-metabolome analysis directed isolation of rhizobacteria capable of enhancing salt tolerance of sea rice 86. Science of the Total Environment. 2022;**843**(156817):1-13. DOI: 10.1016/j.scitotenv.2022.156817

[6] Ehsan S, Qureshi A, Chaudhary N, Ali A, Niaz A, Javed H, et al. Effect of fluorescent-producing rhizobacteria on cereal growth through siderophore exertion. Journal of Applied Research in Plant Sciences. 2023;**4**(2):601-611. DOI: 10.38211/joarps.2023.04.02.168

[7] Olsson S, Alstrom S, Persson P. Barley rhizobacterial population characterised by fatty acid profiling. Applied Soil Ecology. 1999;**12**(3):197-204. DOI: 10.1016/S0929-1393(99)00011-6

[8] Pishchik VN, Vorobyev NI, Chernyaeva II, Timofeeva SV, Kozhemyakov AP, Alexeev YV, et al. Experimental and mathematical simulation of plant growth promoting rhizobacteria and plant interaction under cadmium stress. Plant and Soil. 2002;**243**:173-186

[9] Roesti D, Gaur R, Johri BN, Imfeld G, Sharma S, Kawaljeet K, et al. Plant growth stage, fertiliser management and bio-inoculation of arbuscular mycorrhizal fungi and plant growth promoting rhizobacteria affect the rhizobacterial community structure in rain-fed wheat fields. Soil Biology & Biochemistry. 2006;**38**:1111-1120. DOI: 10.1016/j.soilbio.2005.09.010

[10] Schreiner K, Hagn A, Kyselkova M, Moenne-Loccoz Y, Welzl G, Munch JC, et al. Comparison of barley succession and take-all disease as environmental factors shaping the rhizobacterial community during take-all decline. Applied and Environmental Microbiology. 2010;**76**(14):4703-4712. DOI: 10.1128/AEM.00481-10

[11] Bouffaud M, Poirier M, Muller D, Moënne-Loccoz Y. Root microbiome relates to plant host evolution in maize and other Poaceae. Environmental Microbiology. 2014;**16**(9):2804-2814. DOI: 10.1111/1462-2920.12442

[12] Gurska J, Glick BR, Greenberg BM. Gene expression of Secale cereale (fall Rye) grown in petroleum hydrocarbon (PHC) impacted soil with and without plant growth-promoting rhizobacteria

(PGPR), *pseudomonas putida*. Water, Air, and Soil Pollution. 2015;**226**(308):1-19. DOI: 10.1007/s11270-015-2471-x

[13] Kasim WA, Gaafar RM, Abou-Ali RM, Omar MN, Hewait HM. Effect of biofilm forming plant growth promoting rhizobacteria on salinity tolerance in barley. Annals of Agricultural Science. 2016;**61**(2):217-227. DOI: 10.1016/j.aoas.2016.07.003

[14] Rêgo MCF, Cardoso AF, Ferreira TDC, de Filippi MCC, Batista TFV, Viana RG, et al. The role of rhizobacteria in rice plants: Growth and mitigation of toxicity. Journal of Integrative Agriculture. 2018;**17**(12):2636-2647. DOI: 10.1016/S2095-3119(18)62039-8

[15] Nguyen ML, Spaepen S, du Jardin P, Delaplace P. Biostimulant effects of rhizobacteria on wheat growth and nutrient uptake depend on nitrogen application and plant development. Archives of Agronomy and Soil Science. 2019;**65**(1):58-73. DOI: 10.1080/03650340.2018.1485074

[16] Mahmoud OMB, Hidri R, Taamalli OWT, Abdelly C, Djebali N. Auxin and proline producing rhizobacteria mitigate salt-induced growth inhibition of barley plants by enhancing water and nutrient status. South African Journal of Botany. 2020;**128**:209-217. DOI: 10.1016/j.sajb.2019.10.023

[17] dos Santos RM, Diaz PAE, Lobo LLB, Rigobelo EC. Use of plant growth-promoting rhizobacteria in maize and sugarcane: Characteristics and applications. Frontiers in Sustainable Food Systems. 2020;**4**(136):1-15. DOI: 10.3389/fsufs.2020.00136

[18] Nelly N, Khairul U, Putri AY, Hamid H, Syahrawati M. Isolation and selection of maize plants rhizobacteria with the potential of entomopathogens against *Spodoptera litura* (Lepidoptera: Noctuidae). Biodiversitas. 2020;**21**(2):753-758. DOI: 10.13057/biodiv/d210243

[19] Chen L, Hao Z, Li K, Sha Y, Wang E, Sui X, et al. Effects of growth-promoting rhizobacteria on maize growth and rhizosphere microbial community under conservation tillage in Northeast China. Microbial Biotechnology. 2021;**14**(2):535-550. DOI: 10.1111/1751-7915.13693

[20] Li T, Li Y, Shi Z, Wang S, Wang Z, Liu Y, et al. Crop development has more influence on shaping rhizobacteria of wheat than tillage practice and crop rotation pattern in an arid agroecosystem. Applied Soil Ecology. 2021;**165**:1-10. DOI: 10.1016/j.apsoil.2021.104016

[21] Reid TE, Kavamura VN, Abadie M, Torres-Ballesteros A, Pawlett M, Clark IM, et al. Inorganic chemical fertilizer application to wheat reduces the abundance of putative plant growth-promoting rhizobacteria. Frontiers in Microbiology. 2021;**12**(642587):1-16. DOI: 10.3389/fmicb.2021.642587

[22] Chamkhi I, Cheto S, Geistlinger J, Zeroual Y, Kouisni L, Bargaz A, et al. Legume-based intercropping systems promote beneficial rhizobacterial community and crop yield under stressing conditions. Industrial Crops and Products. 2022;**183**(114958):1-15. DOI: 10.1016/j.indcrop.2022.114958

[23] Huang Z, Wang C, Feng Q, Liou R, Lin Y, Qiao J, et al. The mechanisms of sodium chloride stress mitigation by salt-tolerant plant growth promoting rhizobacteria in wheat. Agronomy. 2022;**12**(543):1-14. DOI: 10.3390/agronomy12030543

[24] Yi S, Li F, Wu C, Ge F, Feng C, Zhang M, et al. Co-transformation of

HMs-PAHs in rhizosphere soils and adaptive responses of rhizobacteria during whole growth period of rice (*Oryza sativa* L.). Journal of Environmental Sciences. 2023;**132**:71-82. DOI: 10.1016/j.jes.2022.07.017

[25] Chinta YD, Araki H. Cover crop amendments and lettuce plant growth stages alter rhizobacterial properties and roles in plant performance. Microbial Ecology. 2023;**86**:446-459. DOI: 10.1007/s00248-022-02090-w

[26] Benmrid B, Ghoulam C, Zeroual Y, Kouisni L, Bargaz A. Bioinoculants as a means of increasing crop tolerance to drought and phosphorus deficiency in legume-cereal intercropping systems. Communications Biology. 2023;**6**(1016):1-15. DOI: 10.1038/s42003-023-05399-5. Available from: https://www.nature.com/commsbio

[27] Adesemoye AO, Egamberdieva D. Beneficial effects of plant growth-promoting rhizobacteria on improved crop production: Prospects for developing economies. In: Maheshwari DK, Saraf M, Aeron A, editors. Bacteria in Agrobiology: Crop Productivity. Berlin Heidelberg: Springer; 2013. pp. 45-64. DOI: 10.1007/978-3-642-37241-4_2

[28] Kumar K, Amaresan N, Bhagat S, Madhuri K, Srivastava RC. Isolation and characterization of rhizobacteria associated with coastal agricultural ecosystem of rhizosphere soils of cultivated vegetable crops. World Journal of Microbiology and Biotechnology. 2011;**27**:1625-1632. DOI: 10.1007/s11274-010-0616-z

[29] Lacava PT, Azevedo JL. Endophytic bacteria: A biotechnological potential in agrobiology system. In: Maheshwari DK, Saraf M, Aeron A, editors. Bacteria in Agrobiology: Crop Productivity. Berlin Heidelberg: Springer; 2013. pp. 1-44. DOI: 10.1007/978-3-642-37241-4_1

[30] Kumar U, Dangar TK. Functional role of plant growth promoting endo- and rhizobacteria in major cereal crops. Popular Kheti. 2013;**1**(1):37-40. DOI: 10.13140/RG.2.2.33583.84642

[31] Turan M, Gulluce M, Şahin F. Effects of plant-growth-promoting rhizobacteria on yield, growth, and some physiological characteristics of wheat and barley plants. Communications in Soil Science and Plant Analysis. 2012;**43**(12):1658-1673. DOI: 10.1080/00103624.2012.681739

[32] Haskett TL, Tkacz A, Poole PS. Engineering rhizobacteria for sustainable agriculture. The International Society for Microbial Ecology Journal. 2021;**15**:949-964. DOI: 10.1038/s41396-020-00835-4

[33] Çakmakçi R, Dönmez MF, Erdoğan Ü. The effect of plant growth promoting rhizobacteria on barley seedling growth, nutrient uptake, some soil properties, and bacterial counts. Turkish Journal of Agriculture and Forestry. 2007;**31**(3):189-199

[34] Gupta R, Khan F, Alqahtani FM, Hashem M, Ahmad F. Plant growth-promoting rhizobacteria (PGPR) assisted bioremediation of heavy metal toxicity. Applied Biochemistry and Biotechnology. 2023:1-29. DOI: 10.1007/s12010-023-04545-3

Chapter 4

Plant Growth-Promoting Rhizobacteria (PGPR): A Potential Alternative Tool for Sustainable Agriculture

Ashmita Ghosh, Ritwik Acharya, Shubhajit Shaw and Debnirmalya Gangopadhyay

Abstract

Soil is an important natural resource that nurtures living microbial communities and improves plant productivity, thus ensuring food security. The chemical fertilizers used during the last few decades though improved plant productivity so rapidly; however, it is indiscriminate use results in poor soil health and less agricultural productivity, affecting food security and human health worldwide. There is an urgent need of biological agents, such as plant growth-promoting rhizobacteria (PGPR), which may serve as better alternative to solve this problem. PGPR plays an important role to increase soil fertility, plant growth promotion, and suppression of phytopathogens for the development of eco-friendly sustainable agriculture. The present study provides a critical overview on PGPR, its mechanism and function, and significance as a potential alternative tool for sustainable agriculture. An attempt has been made to propose an eco-friendly model integrating PGPR with various sectors, such as human health, agriculture, and food industry for its effective commercialization. The study might be helpful to identify the prospects and challenges of PGPR to fully integrate them into sustainable agriculture practices.

Keywords: food security, PGPR, soil, sustainable agriculture, eco-friendly model

1. Introduction

The agricultural sector significantly contributes to a substantial portion of a country's wealth, particularly in developing nations. The agriculture sector not only generates employment opportunities but also ensures food security. The increased use of chemical fertilizers though enhanced agricultural productivity; however, their excessive application has negatively impacted the soil health. This has led to a decline in agricultural output, posing a significant threat to both human well-being and global food security. Presently, there is a growing emphasis on maximizing agricultural output through the efficient utilization of limited resources while adopting a holistic approach to minimize adverse environmental effects. This approach not only balances environmental health and productivity but also enhances resilience in the face of changing climatic conditions, safeguarding the well-being of both present and future generations [1]. PGPR represents an alternative tool for sustainable agriculture, offering several benefits. It is a group of beneficial bacteria, which forms a symbiotic relationship with the plants and mostly found in the rhizosphere and the soil region around plant roots. PGPR not only fosters a more balanced and resilient ecosystem within the soil but also improves nutrient availability, enhances stress tolerance in plants, and increases crop productivity [2]. It contributes to the growth, development, and overall health of plants through various mechanisms such as nitrogen fixation, nutrient solubilization, and disease suppression [3]. Some of the PGPRs such as *Azobacter*, *Azosprillum*, *Bacillus*, *Enterobacter*, *Klebsiella*, *Pseudomonas*, *Variovorax*, and *Serratia* are play important roles in the agricultural field [4].

Of late, the role of PGPR in agriculture sector has gained wide popularity since promotion of such biological agent not only align well with the principles of sustainable agriculture but also uphold ecological balance while fulfilling the food requirements of an expanding global population. However, there are various challenges involve in effective utilization of this PGPR in agriculture. These challenges might involve the careful screening of PGPR strains, evaluating their complex interactions with native soil microbes, assessing their adaptability to diverse environmental conditions, and understanding potential long-term impacts, etc. On the other hand, the process of bringing PGPR to the commercial market involves obtaining necessary regulatory approvals, ensuring the ability to scale up production processes, developing efficient methods of application, and ensuring the sustained viability of PGPR in field conditions. The present study provides a critical overview on PGPR, its mechanism and function, and significance as a potential alternative tool for sustainable agriculture. An attempt has been made to propose an eco-friendly model integrating PGPR with various sectors such as human health, agriculture, and food industry for its effective commercialization. The study might be helpful to identify the prospects and challenges of PGPR to fully integrate them into sustainable agriculture practices.

2. PGPR and its classification

The rhizosphere, the region surrounding plant roots, is characterized by elevated microbial activity, fostering a limited but crucial reservoir of macro- and micronutrients. PGPR establishes colonies within this zone, contributing to the plant's growth and development [5]. PGPR can be classified in many ways (**Figure 1**). For instance, PGPR based on their interaction with plants can be divided into symbiotic bacteria and free-living rhizobacteria. Their classification is also influenced by their residing locations; intracellular PGPR (iPGPR), exemplified by *Rhizobia* sp. and *Frankia* sp., inhabit plant cells, form nodules, and are localized within specialized structures. On the contrary, extracellular PGPR (ePGPR) inhabits the external environment of plant cells, lacking nodules but still actively promoting plant growth. Furthermore, PGPRs are categorized based on their functional roles into four types such as biofertilizers (enhancing nutrient availability for plants), phytostimulators (facilitating plant growth, often through phytohormone production), rhizoremediators (involved in the breakdown of organic pollutants), and biopesticides (aiding in disease control primarily through the synthesis of antibiotics and antifungal metabolites) [6]. PGPR encompasses a variety of genera, including but not limited to *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Pseudomonas*, *Acetobacter*, *Micrococcus*,

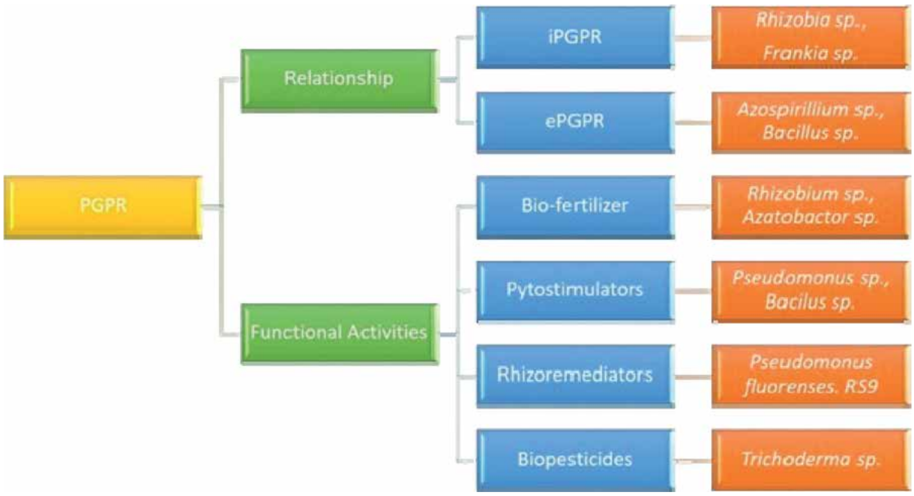

Figure 1.
Classification of PGPR.

Burkholderia, *Bacillus*, *Paenibacillus*, *Agrobacterium*, *Caulobacter*, *Chromobacterium*, *Erwinia*, *Flavobacterium*, *Serratia*, *Rhizobium*, and some belonging to the *Enterobacteriaceae* family [7].

3. PGPR-assisted zone: the rhizosphere

The term “"rhizosphere” was coined by German plant biologist and agronomist Lorenz Hiltner in 1904 to describe the interface between plants and roots. The term is derived partially from the Greek word “rhiza,” meaning root [8]. Hiltner’s definition characterizes the rhizosphere as the region surrounding a plant root, hosting a distinct community of microorganisms influenced by substances released by plant roots. The rhizosphere is composed of three main zones: the endorhizosphere, rhizoplane, and ectorhizosphere (**Figure 2**). The endorhizosphere refers to the root tissue, including the endodermis and cortical layers. The rhizoplane is the root surface where bacteria and soil particles attach, encompassing layers such as the mucilaginous polysaccharide layer, cortex, epidermis, and the ectorhizosphere, which adheres to the soil around the root [9].

The rhizosphere serves as a habitat where plants, soil, microbes, and soil microfauna engage in extensive interactions. These interactions are pivotal for biochemical exchanges and the sharing of signal molecules between plants and rhizobacteria. These interactions profoundly impact plant growth and production. Rhizobacteria, known as rhizosphere-competent bacteria, actively colonize plant roots throughout various plant growth phases, indicating the presence of rhizobacteria. The rhizobacterial populations particularly under abiotic stress associated with roots play a crucial role in maintaining plant health. Plant-microbe interactions occur within the rhizosphere, where both beneficial and potentially harmful microorganisms coexist. The composition of the rhizobacterial community in the rhizosphere varies with changes in soil properties [10]. Naturally, interactions among rhizobacteria in the rhizosphere significantly influence soil health and enhance its nutritional condition, both of

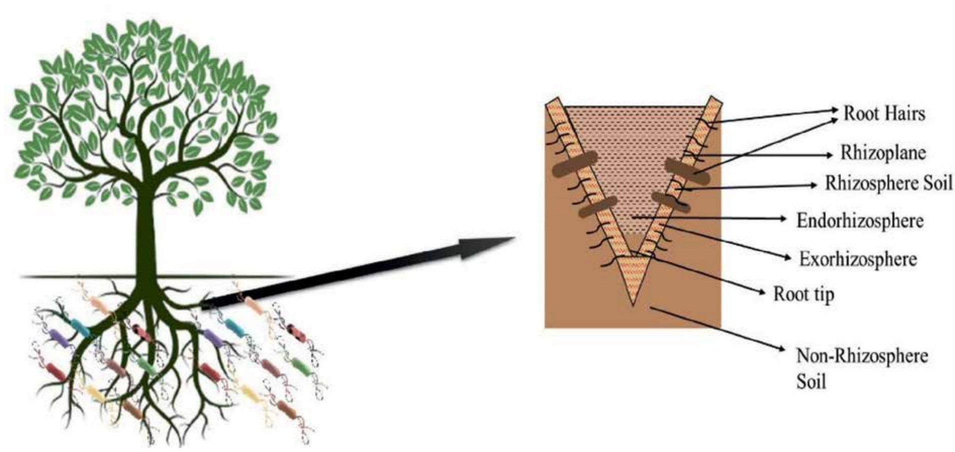

Figure 2.
PGPR-assisted zone: the rhizosphere.

which are vital for improved plant growth [11]. The exchange of resources between shoots and roots relies on the growth and proliferation of roots, facilitated by abundant interactions between roots, the rhizosphere, and rhizobial bacteria. A robust rhizosphere-rhizobacterial interaction shields root exudates, which comprise various chemical substances attracting microorganisms to the root [12, 13]. This interaction mediated by root exudation plays a pivotal role in plant-microbe interactions by facilitating root colonization and stimulating root growth.

The rhizosphere is enriched with utilizable carbon sources due to rhizodeposition, a process involving the release of organic substances by plant roots, including amino acids, fatty acids, sterols, growth factors, organic acids, and sugars. The rhizosphere hosts an intricate microbial community comprising saprophytes, endophytes, epiphytes, pathogens, and beneficial microorganisms such as bacteria, fungi, nematodes, protozoa, algae, and more [14]. According to Yadav et al., 1,200,106 bacteria/g dry soil are found in the rhizosphere, which is significantly higher than fungi (12,105 fungi/g dry soil), algae (5105 algae/g dry soil), and actinomycetes (46,106 actinomycetes/g dry soil) [15]. Root exudation, secretion, and deposition contribute various organic compounds, making the rhizosphere richer in nutrients than the bulk soil. This richness fosters an active and enhanced microbial community in the root zone, leading to the phenomenon known as the rhizosphere effect. The rhizosphere effect is quantified as the R:S ratio, where R represents the total number of microorganisms in the rhizosphere, and S represents the corresponding amount in the bulk soil. This ratio serves as a measure of microbial activity, with a higher R:S ratio indicating increased activity in the rhizosphere [6, 9].

4. Mechanism of PGPR and functions

PGPR employs both direct and indirect mechanisms to stimulate plant growth (**Figure 3**). These mechanisms are vital for enhancing nutrient availability, promoting hormonal balance, and fortifying plants against various stresses. By utilizing these direct and indirect mechanisms, PGPR contributes to a holistic approach in promoting plant growth and health, making them valuable allies in sustainable agriculture.

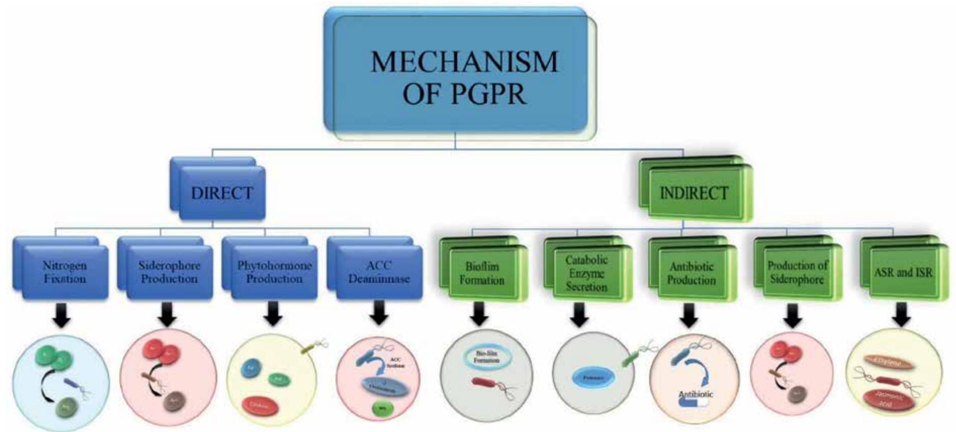

Figure 3.
Mechanism of action of PGPR.

4.1 Direct method

In case of direct method, PGPR generally enhances plant growth either by aiding in the acquisition of crucial resources such as nitrogen, phosphorus, and essential minerals or by modulating the levels of plant hormones. The specific processes involved in these mechanisms are detailed below:

4.1.1 Nitrogen fixation

Organisms capable of fixing nitrogen can be classified into two main groups namely, symbiotic nitrogen-fixing bacteria, exemplified by members of the Rhizobiaceae family, which establish symbiotic relationships with leguminous plants (e.g., *rhizobia* with legumes) and nonleguminous trees (e.g., *Frankia*) and nonsymbiotic nitrogen-fixing bacteria, including free-living, associative, and endophytic types, such as *cyanobacteria*. However, nonsymbiotic nitrogen-fixing bacteria only moderately fulfill the nitrogen requirements of their host plants [16]. In the case of leguminous plants, the roots form a symbiotic partnership with nitrogen-fixing *rhizobia* from the Rhizobiaceae family, which are *α-proteobacteria*. This symbiosis leads to the development of nodules, where *rhizobia* colonizes as intracellular symbionts, resulting from a complex interplay between the host plant and the symbiotic bacteria. Diazotrophs, a subset of plant growth-promoting rhizobacteria, fix atmospheric nitrogen (N_2) in nonleguminous plants, forming a non-obligate relationship with the host plants. The process of nitrogen fixation involves a complex enzyme, comprising the iron protein dinitrogenase reductase and dinitrogenase with a metal cofactor. Dinitrogenase reductase generates electrons with high reducing power, facilitating the conversion of N_2 to NH_3 by dinitrogenase. Three distinct nitrogen-fixing systems have been identified based on the metal cofactor viz., Mo-nitrogenase, V-nitrogenase, and Fe-nitrogenase. The structural composition of the nitrogen-fixing system varies among different bacterial genera. The predominant biological nitrogen fixation is carried out by the molybdenum nitrogenase, which is present in all diazotrophs.

Nitrogen fixation genes, referred to as nif genes, are present in both symbiotic and free-living systems. These genes, encoding nitrogenase (nif) enzymes, include structural components, genes related to Fe protein activation, iron-molybdenum cofactor biosynthesis, electron donation, and regulatory genes essential for enzyme synthesis and function. Typically organized in a 20–24 kb cluster with seven operons expressing 20 distinct proteins in diazotrophs, nif genes play a crucial role in nitrogen fixation [16]. Within this gene cluster, the nifDK and nifH genes encode two-component proteins of the molybdenum nitrogenase enzyme complex. NifDK, a heterotetrameric ($\alpha 2\beta 2$) protein, consists of two ab dimers linked by twofold symmetry. Each α-subunit (NifD) within the active site of NifDK contains an iron-molybdenum cofactor (FeMo-co) [17]. In Rhizobium and other diazotrophs, the symbiotic activation of nif genes is contingent upon low oxygen levels, regulated by another set of genes known as fix genes, found in both symbiotic and free-living nitrogen fixation systems. Significantly, nitrogen fixation demands a substantial amount of energy, requiring at least 16 mol of ATP for each molecule of fixed nitrogen. Phosphate solubilization is a crucial process as phosphorus is the second most important plant growth-limiting nutrient after nitrogen. Despite the abundance of phosphorus in soils, its accessibility to plants is often limited. Key phosphate-solubilizing bacteria include *Azotobacter*, *Bacillus*, *Beijerinckia, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Microbacterium, Pseudomonas, Rhizobium*, and *Serratia* [7]. Inorganic phosphorus is typically solubilized through the action of low molecular weight organic acids produced by various soil microorganisms. Conversely, organic phosphorus is mineralized through the activity of phosphatases, which catalyze the hydrolysis of phosphoric esters. It is noteworthy that certain bacterial strains can exhibit both phosphate solubilization and mineralization capabilities. Apart from providing phosphorus to plants, phosphate-solubilizing bacteria enhance plant growth by improving the efficiency of biological nitrogen fixation (BNF) and increasing the availability of other trace elements through the synthesis of essential plant growth-promoting substances [5, 18].

4.1.2 Siderophore production

Iron is an essential element for virtually all forms of life, with the exception of certain lactobacilli; hence, all known microorganisms require iron for their survival. In aerobic environments, iron is predominantly present as Fe^{3+}, and it tends to form insoluble hydroxides and oxyhydroxides, making it inaccessible to both plants and microbes [19]. Bacteria typically acquire iron through the production of low-molecular-mass iron chelators known as siderophores, which exhibit high association constants for complexing iron. Most siderophores are water-soluble and can be categorized as extracellular or intracellular.

In general, rhizobacteria vary in their ability to utilize siderophores produced by others of the same genus (homologous siderophores), while some can use siderophores from different genera of rhizobacteria (heterologous siderophores) [18]. In the presence of iron limitation, the Fe^{3+-}siderophore complex on the bacterial membrane undergoes reduction to Fe^{2+}, which is then released into the cell through a gating mechanism connecting the inner and outer membranes in both Gram-negative and Gram-positive rhizobacteria. The siderophore may be degraded or recycled during this reduction process [19]. Siderophores, in the context of iron deficiency, act as solubilizing agents for iron sourced from minerals or organic substances [20]. Beyond their role in iron complexation, siderophores can also form stable complexes with other heavy metals such as Al, Cd, Cu, Ga, In, Pb, and Zn, as well as radionuclides, such as U and Np. The binding of a siderophore to a metal increases the concentration of soluble metal [19]. Consequently, bacterial siderophores play a crucial role in alleviating stresses on plants caused by elevated levels of heavy metals in the soil.

4.1.3 Phytohormone production

The microbial synthesis of the phytohormone auxin, specifically indole-3-acetic acid (IAA), has long been recognized. Generally, IAA released by rhizobacteria can impact various plant developmental processes as the plant's endogenous pool of IAA may be influenced by the externally acquired IAA secreted by soil bacteria [16, 21]. Notably, IAA serves as a reciprocal signaling molecule, influencing gene expression in diverse bacteria, underscoring its critical role in rhizobacteria-plant interactions [22]. Moreover, the down-regulation of IAA as a signaling molecule is associated with plant defense mechanisms against various phytopathogenic bacteria. This is evidenced by the heightened susceptibility of plants to bacterial pathogens upon the exogenous application of IAA or exposure to IAA produced by the pathogen [22]. IAA has been implicated in nearly every facet of plant growth, development, and defense responses. The intricate complexity of IAA biosynthesis, transport, and signaling pathways reflects its diverse functional roles [23].

In general, IAA influences plant processes such as cell division, extension, and differentiation, stimulates seed and tuber germination, accelerates xylem and root development, regulates vegetative growth, initiates lateral and adventitious root formation, mediates responses to light, gravity, and fluorescence, and influences photosynthesis, pigment formation, biosynthesis of various metabolites, and stress resistance. Rhizobacteria-produced IAA is expected to impact these physiological processes by altering the plant auxin pool. Furthermore, bacterial IAA enhances root surface area and length, facilitating the plant's access to soil nutrients. Additionally, rhizobacterial IAA loosens plant cell walls, promoting increased root exudation, which, in turn, provides additional nutrients to support the proliferation of rhizosphere bacteria [16]. Consequently, rhizobacterial IAA is recognized as a pivotal effector molecule in plant–microbe interactions, playing roles in both pathogenesis and phyto-stimulation [22].

4.1.4 1-aminocyclopropane-1-carboxylate (ACC) deaminase

Ethylene, a crucial plant growth hormone, is essential for normal plant growth and development [23]. This hormone is produced internally by nearly all plants and is also generated by various biotic and abiotic processes in soils, playing a vital role in inducing diverse physiological changes in plants. Aside from its role as a plant growth regulator, ethylene is recognized as a stress hormone [24]. Under stressful conditions such as salinity, drought, waterlogging, heavy metals, and pathogenicity, the endogenous production of ethylene significantly increases, leading to severe impacts on overall plant growth. Elevated concentrations of ethylene can cause defoliation and other cellular processes that may result in decreased crop performance [7, 24]. PGPR that produces the enzyme ACC deaminase contributes to plant growth and development by reducing ethylene levels. This reduction in ethylene levels is associated with increased salt tolerance and the mitigation of drought stress [25, 26]. Currently, bacterial strains displaying ACC deaminase activity have been identified across various genera, including *Acinetobacter, Achromobacter, Agrobacterium, Alcaligenes, Azospirillum, Bacillus, Burkholderia, Enterobacter, Pseudomonas, Ralstonia, Serratia,* and *Rhizobium*, among others. These rhizobacteria take up the ethylene precursor ACC and convert it into 2-oxobutanoate and NH_3 through the action of ACC deaminase.

ACC deaminase producers play a significant role in alleviating stress induced by various phytopathogenic microorganisms (such as viruses, bacteria, and fungi), as well as stressors such as polyaromatic hydrocarbons, heavy metals, radiation, wounding, insect predation, high salt concentration, drought, extremes of temperature, high light intensity, and flooding [16]. Consequently, the notable effects observed upon seed/root inoculation with ACC deaminase-producing rhizobacteria include enhanced plant root elongation, promotion of shoot growth, and improvements in rhizobial nodulation, as well as increased uptake of nitrogen (N), phosphorus (P), and potassium (K). Additionally, there is an observed enhancement in mycorrhizal colonization in various crops [16, 25, 27, 28].

4.2 Indirect mechanisms

The indirect mechanisms of PGPR involve functions aimed at alleviating inhibitory effects on plant growth and development caused by various pathogens. These functions often include the use of biocontrol agents, which have specific roles in suppressing or managing pathogenic organisms that can negatively impact plants. The details of these indirect mechanisms may vary, encompassing processes such as antagonism, competition for resources, and the induction of systemic resistance in plants. Overall, the goal is to enhance plant health and productivity by mitigating the harmful effects of pathogens indirectly through the actions of PGPR described below:

4.2.1 Biofilm formation

Recent investigation has revealed that biofilm development in the rhizosphere plays a significant role in rhizobacteria's modes of action against root diseases. The presence of high numbers of bacterial cells in biofilms causes the release of different compounds such as toxins and antibiotics in their periphery, which inhibits phytopathogens in the soil [29].

4.2.2 Catabolic enzyme secretion

Various microbial species can release catabolic enzymes (proteases, -1,3-glucanase, and chitinases) and small compounds, which can help to control soil-borne plant diseases. Electron microscopy studies provide specifics of the antagonist effect on fusarium hyphae, revealing the clear anomaly of mycelial growth, which can be related to the influence of cell wall-degrading enzymes produced by rhizobacteria, such as chitinases [30].

4.2.3 Antibiotic production

Antibiotics and other chemicals harmful to phytopathogens have been isolated from *Bacillus* strain metabolites. This has been demonstrated in research on *Bacillus megaterium*, which can colonize roots and reduce Rhizoctoniasolani [31].

4.2.4 Production of siderophores

Rhizobacteria produce siderophores as secondary implications. These molecules have the ability to bind Fe^{3+} ions, which are required for metabolism and cell proliferation. In this manner, bacteria that colonize plant roots might compete for available iron in the soil and may impede the growth of other rhizosphere microbes. Siderophore-producing rhizobacteria can inhibit the growth of harmful microorganisms in the root zone [32].

4.2.5 Acquired systemic resistance and induced systemic resistance

Plants have a natural basal defense system against phytopathogens, but other systems can be triggered or generated to boost plant resistance [33]. Acquired systemic resistance (ASR) and induced systemic resistance (ISR) are the two most commonly researched types of resistance induction. When plants are exposed to an inducer agent (such as a pathogenic organism), defense mechanisms are activated at the induction site, which exhibits alterations (necrosis), as well as other distant sites, resulting in the plant being systemically protected against subsequent infections caused by a broad spectrum of pathogens [34]. ASR is accompanied by a rise in salicylic acid content and the accumulation of proteins associated with pathogenesis (PRPs), which are plant defense mechanisms. ISR can be initiated by nonpathogenic microorganisms in the rhizosphere and does not involve the salicylic acid signaling pathway or the generation of PRPs; rather, this type of resistance is activated by the jasmonic acid and ethylene resistance-signaling pathway.

5. PGPR: a novel approach to sustainable agriculture

Global food production increases in the twentieth century were essentially based on two broad areas of progress: chemical inputs (commercial fertilizers and pesticides) and genetic alterations *via* targeted breeding and gene manipulation. However, the continued use of chemicals, fertilizers, and pesticides, as well as the resultant negative impacts on the environment, has shifted public opinion. Scientists are experimenting with several strategies to boost crop output in a sustainable manner, including the use of phytomicrobiome members, which is now being recognized as a "fresh" green revolution [35]. The use of beneficial microorganisms on food crops has been extensively researched, but their application in the field is quite limited. The introduction of phytomicrobiome members in agricultural systems as a sustainable solution for disease management and nutrient supplementation could mitigate the negative effects associated with the overuse of chemical inputs (fertilizers and pesticides) [36]. Furthermore, members of the phytmicrobiome have been used as an effective technique to alleviate specific biotic and abiotic challenges that may impair crop growth and productivity [37].

5.1 PGPR in abiotic stress management

Any unfavorable environmental conditions that may affect the functional diversity of microbes and also the physicochemical properties of soil can dictate abiotic stress. Numerous drastic conditions, including heavy metal toxicity, salinity, drought, and flooding affecting the plant microbiome and the surrounding ecology, are abiotic stress [38].

5.2 PGPR in biotic stress management

Living organisms, particularly bacteria, viruses, fungi, insects, and nematodes, cause biotic stress in plants. Such stress directly interacts with host nutrition, resulting in plant death. Biotic stress causes both pre- and postharvest loss. Although few microbes participate in pathogen biological control, PGPR is known to provide protection from a variety of diseases *via* mechanisms such as bacteriocin, antibiosis, volatile organic compound (VOC) production, and lysis *via* the extracellular enzyme [39]. Microbial stimulants have been shown to be efficient in suppressing a range of plant pathogens, resulting in sound harvest growth.

5.3 Co-metabolism of PGPR

Rhizospheric microbial metabolites are thought to be critical to ecological success. Based on their substrate consumption patterns, many rhizomicrobes that share this environment play essential ecological roles [40]. If two strains have comparable substrate uptake characteristics, the fittest will survive, resulting in the competitive exclusion of the less fit strain [41]. A rhizobacteria strain frequently acts in such a way that it excretes a unique compound that was not present in the native root. This results in the creation of a novel niche that cross-feeding strains may occupy [42].

5.4 PGPR as biofertilizer

Biofertilizers are live formulations of beneficial bacteria that aid in nutrient availability through biological activity and so improve soil health and consequently soil microflora. Plant growth-promoting microorganisms (PGPM) are the key component of this biofertilizer. This PGPM can be divided into three key groups such as arbuscularmycorrhizal organisms (AMF), plant development advancing rhizobacteria (PGPR), and nitrogen-fixing rhizobia [43], all of these are beneficial to plant development and sustenance. Nonetheless, it has been reported that PGPR has been used as a biofertilizer all over the world, contributing to increased yields and soil quality. As a result of the expected PGPR commitment, it may prompt sustainable agribusiness. Such biofertilizers are available in both solid and liquid formulations. There are three forms of liquid formulations: root inoculation, seed inoculation, and soil inoculation [44].

6. Prospects and challenges

PGPR offers promising avenues for sustainable agriculture and ecosystem health, yet they come with inherent challenges. They hold the potential to enhance plant growth, nutrient uptake, and disease resistance through mechanisms such as nitrogen fixation, phosphate solubilization, and production of growth-promoting substances. Harnessing these benefits could lead to reduced reliance on chemical fertilizers and pesticides, fostering environmentally friendly agricultural practices. However, successful implementation faces hurdles such as variability in PGPR effectiveness across different plant species and environments, as well as competition with native soil microbes. Ensuring consistent and reliable results requires a deep understanding of the intricate interactions between PGPR, plants, and soil. Furthermore, developing cost-effective and scalable production methods for PGPR inoculants, ensuring their compatibility with other agricultural practices, and navigating regulatory approvals pose significant challenges. Bridging the gap between research findings and on-field application necessitates collaboration among microbiologists, agronomists, and farmers. Overcoming these challenges could unlock the full potential of PGPR, driving sustainable agriculture while mitigating concerns about food security and environmental impact.

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This section describes an environmentally friendly approach using PGPR as an alternative tool to increase crop productivity ensuring sustainable agriculture. In this context, an eco-friendly model integrating PGPR with various sectors, such as human health, agriculture, and food industry, is proposed for its effective commercialization (**Figure 4**). At its core, PGPR acts as a linchpin, establishing beneficial connections between these sectors. In agriculture, PGPR-enhanced crops not only yield more but also require fewer synthetic agrochemicals, reducing environmental impact. The model extends to human health as well, where PGPR-associated crops can contribute to more nutritious diets, potentially lowering the incidence of diet-related illnesses. Moreover, the use of PGPR in soil enrichment aligns with sustainable land management practices, mitigating soil degradation and erosion. In the food industry, the utilization of PGPR-enriched crops can foster a supply of quality raw materials while reducing the ecological footprint of food production. The model *via* synergistically linking these sectors fosters circular economies where agricultural waste can be utilized as biofertilizer, reducing the need for external inputs, while PGPR-influenced agriculture generates higher-quality produce for both human consumption and industrial processing. Collaboration across sectors fuels innovation, allowing for the exploration of novel applications, such as PGPR-based probiotics for both humans and animals. However, realizing this model demands addressing challenges, such as scaling up PGPR production, ensuring regulatory compliance for human consumption, and implementing effective knowledge transfer across sectors. This integrated model through strategic partnerships, interdisciplinary research, and informed policymaking can potentially capitalize the symbiotic relationships facilitated by PGPR to drive sustainable practices, benefiting ecosystems, human health, and the global economy.

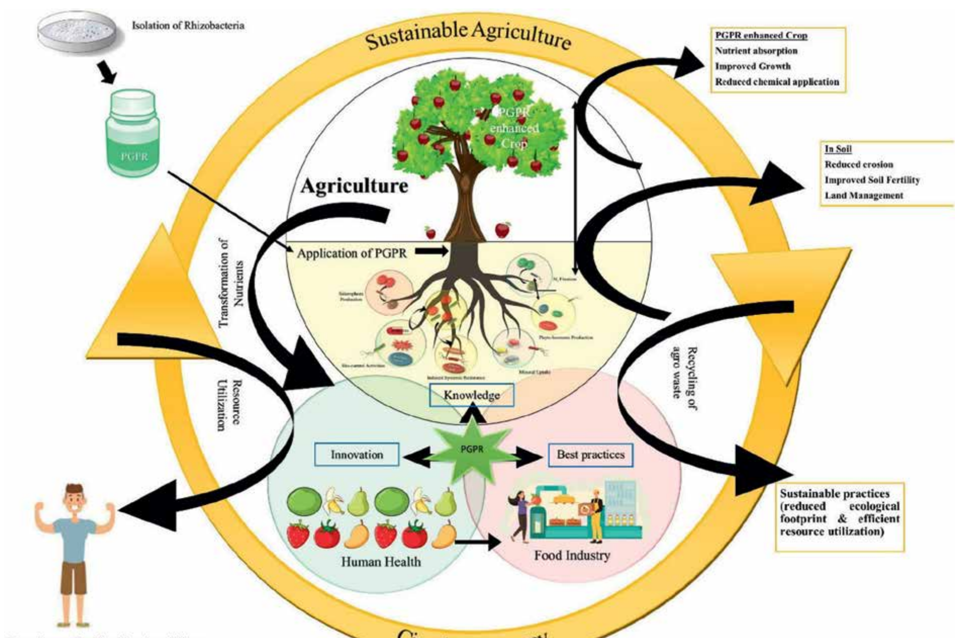

Figure 4.
An eco-friendly model integrating PGPR with other sectors, such as human health, agriculture, and food industry, is proposed for its effective commercialization.

7. Conclusion

The use of bacterial fertilizers has resulted in considerable improvements in plant growth, health, and yield. The PGPR stimulation mechanism might be direct or indirect. PGPR, showcasing a range of activities directed toward promoting plant growth, also exhibits bioremediation capabilities by detoxifying pollutants, such as heavy metals and pesticides. Additionally, they play a role in controlling various phytopathogens, functioning as biopesticides. In various crop studies, PGPR has demonstrated remarkable results. Biological control of plant diseases by microbial-based products has enough potential for a global market share of about 15–20% with an annual growth rate of around 15%. The use of microbial-based products is an environmentally friendly approach and is the best way to reduce the use of chemical fertilizers. The productive efficacy of a particular PGPR can be further improved by optimizing and acclimating to the soil conditions. In the future, it is anticipated that PGPR will replace chemical fertilizers, pesticides, and synthetic growth regulators, given their numerous detrimental effects on sustainable agriculture. Further, research and elucidation of mechanisms for PGPR-mediated phyto-stimulation will open the door to discover more effective rhizobacteria-technological strains that may work in a variety of agri-ecological environments. Since, its discovery, PGPR has shown great promise as a major contributor to sustainable agriculture development. However, much remains to be done in terms of both explorations and implementation. Explorations, which involve understanding the mechanism at the same time as implementation, require a great deal of optimization in field application. PGPR should be encouraged and prioritized as a bioremediation tool for biocontrol. PGPR has all the potential to act as biofertilizer, which could work in a better ecosystem with increased productivity. However, further understanding of the PGPR process could aid in the identification of more particular strains for successful implementation at the industrial level, thus could reduce the application of chemical fertilizers safeguarding the ecosystem.

Conflict of interest

The authors declare no conflict of interest.

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References

[1] Gupta G, Parihar SS, Ahirwar NK, Snehi SK, Singh V. Plant growth promoting rhizobacteria (PGPR): Current and future prospects for development of sustainable agriculture. Journal of Microbial & Biochemical Technology. 2015;**7**(2):096-102. DOI: 10.4172/1948-5948.1000188

[2] Nadeem SM, Naveed M, Ahmad M, Zahir ZA. Rhizosphere bacteria for crop production and improvement of stress tolerance: Mechanisms of action, applications, and future prospects. In: Arora NK, editor. Plant Microbes Symbiosis: Applied Facets. India: Springer; 2015. pp. 1-36. DOI: 10.1007/978-81-322-2068-8_1

[3] Egamberdieva D, Shrivastava S, Varma A, editors. Plant-growth-promoting Rhizobacteria (PGPR) and Medicinal Plants. Vol. 42. Cham: Springer; 2015. 442 p. DOI: 10.1007/978-3-319-13401-7

[4] Rai PK, Singh M, Anand K, Saurabh S, Kaur T, Kour D, et al. Role and potential applications of plant growth-promoting rhizobacteria for sustainable agriculture. In: Rastegari AA, Yadav AN, Yadav N, editors. New and Future Developments in Microbial Biotechnology and Bioengineering. Amsterdam: Elsevier; 2020. pp. 49-60

[5] Ahmad F, Ahmad I, Khan MS. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiological Research. 2008;**163**:173-181. DOI: 10.1016/j.micres.2006.04.001

[6] Antoun H, Prévost D. Ecology of plant growth promoting rhizobacteria. In: Siddiqui ZA, editor. PGPR: Biocontrol and Biofertilization. Dordrecht: Springer; 2005. pp. 1-38

[7] Bhattacharyya PN, Jha DK. Plant growth-promoting rhizobacteria (PGPR): Emergence in agriculture. World Journal of Microbiology and Biotechnology. 2012;**28**:1327-1350. DOI: 10.1007/s11274-011-0979-9

[8] Hartmann A, Rothballer M, Schmid M. Lorenz Hiltner, a pioneer in rhizosphere microbial ecology and soil bacteriology research. Plant and Soil. 2008;**312**:7-14. DOI: 10.1007/s11104-007-9514-z

[9] Prashar P, Kapoor N, Sachdeva S. Rhizosphere: Its structure, bacterial diversity and significance. Reviews in Environmental Science and Bio/Technology. 2014;**13**:63-77. DOI: 10.1007/s11157-013-9317-z

[10] Pérez-de-Luque A, Tille S, Johnson I, Pascual-Pardo D, Ton J, Cameron DD. The interactive effects of arbuscularmycorrhiza and plant growth-promoting rhizobacteria synergistically enhance host plant defences against pathogens. Scientific Reports. 2017;**7**(1):16409. DOI: 10.1038/s41598-017-16697-4

[11] Badri DV, Weir TL, van der Lelie D, Vivanco JM. Rhizosphere chemical dialogues: Plant–microbe interactions. Current Opinion in Biotechnology. 2009;**20**(6):642-650. DOI: 10.1016/j.copbio.2009.09.014

[12] el ZaharHaichar F, Santaella C, Heulin T, Achouak W. Root exudates mediated interactions belowground. Soil Biology and Biochemistry. 2014;**77**:69-80. DOI: 10.1016/j.soilbio.2014.06.017

[13] Semchenko M, Saar S, Lepik A. Plant root exudates mediate neighbour recognition and trigger complex

behavioural changes. New Phytologist. 2014;**204**:631-637. DOI: 10.1111/nph.12930

[14] Avis TJ, Gravel V, Antoun H, Tweddell RJ. Multifaceted beneficial effects of rhizosphere microorganisms on plant health and productivity. Soil Biology and Biochemistry. 2008;**40**(7):1733-1740. DOI: 10.1016/j.soilbio.2008.02.013

[15] Yadav BK, Akhtar MS, Panwar J. Rhizospheric Plant-Microbe Interactions: Key Factors to Soil Fertility and Plant Nutrition. In: Arora NK, editor. Plant Microbes Symbiosis: Applied Facets. India: Springer; 2015. pp. 127-145

[16] Glick BR. Plant growth-promoting bacteria: Mechanisms and applications. Scientifica. 2012;**2012**:1-15. DOI: 10.6064/2012/963401

[17] Rubio LM, Ludden PW. Biosynthesis of the iron-molybdenum cofactor of nitrogenase. Annual Review of Microbiology. 2008;**62**:94-106. DOI: 10.1146/annurev.micro.62.081307.162737

[18] Zaidi A, Khan M, Ahemad M, Oves M. Plant growth promotion by phosphate solubilizing bacteria. Acta Microbiologica et Immunologica Hungarica. 2009;**56**(3):263-284

[19] Rajkumar M, Ae N, Prasad MN, Freitas H. Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. Trends in Biotechnology. 2010;**28**(3):142-149. DOI: 10.1016/j.tibtech.2009.12.002

[20] Indiragandhi P, Anandham R, Madhaiyan M, Sa TM. Characterization of plant growth–promoting traits of bacteria isolated from larval guts of diamondback moth Plutellaxylostella (Lepidoptera: Plutellidae). Current Microbiology. 2008;**56**:327-333. DOI: 10.1007/s00284-007-9086-4

[21] Spaepen S, Vanderleyden J, Remans R. Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiology Reviews. 2007;**31**(4):425-448. DOI: 10.1111/j.1574-6976.2007.00072.x

[22] Spaepen S, Vanderleyden J. Auxin and plant-microbe interactions. Cold Spring Harbor Perspectives in Biology. 2011;**3**:a001438. DOI: 10.1101/cshperspect.a001438

[23] Santner A, Calderon-Villalobos LI, Estelle M. Plant hormones are versatile chemical regulators of plant growth. Nature Chemical Biology. 2009;**5**:301-307. DOI: 10.1038/nchembio.165

[24] Saleem M, Arshad M, Hussain S, Bhatti AS. Perspective of plant growth promoting rhizobacteria (PGPR) containing ACC deaminase in stress agriculture. Journal of Industrial Microbiology and Biotechnology. 2007;**34**(10):635-648. DOI: 10.1007/s10295-007-0240-6

[25] Nadeem SM, Zahir ZA, Naveed M, Arshad M. Preliminary investigations on inducing salt tolerance in maize through inoculation with rhizobacteria containing ACC deaminase activity. Canadian Journal of Microbiology. 2007;**53**(10):1141-1149. DOI: 10.1139/W07-081

[26] Zahir ZA, Munir A, Asghar HN, Shaharoona B, Arshad M. Effectiveness of rhizobacteria containing ACC deaminase for growth promotion of peas (Pisumsativum) under drought conditions. Journal of Microbiology and Biotechnology. 2008;**18**:958-963

[27] Shaharoona B, Naveed M, Arshad M, Zahir ZA. Fertilizer-dependent efficiency

of Pseudomonads for improving growth, yield, and nutrient use efficiency of wheat (*Triticum aestivum* L.). Applied Microbiology and Biotechnology. 2008;**79**:147-155. DOI: 10.1007/s00253-008-1419-0

[28] Nadeem SM, Zahir ZA, Naveed M, Arshad M. Rhizobacteria containing ACC-deaminase confer salt tolerance in maize grown on salt-affected fields. Canadian Journal of Microbiology. 2009;**55**(11):1302-1309. DOI: 10.1139/W09-092

[29] Kwon JW, Kim SD. Characterization of an antibiotic produced by *Bacillus subtilis* JW-1 that suppresses Ralstoniasolanacearum. Journal of Microbiology and Biotechnology. 2014;**24**(1):13-18. DOI: 10.4014/jmb.1308.08060

[30] Zhao YQ, Wu YH, Fu Y, Zhao XX, Chen JG. Activity pathogenic effect of cell wall degrading enzyme in tobacco target spot pathogen Rhizoctoniasolani. Tobacco Science and Technology. 2014;**11**:84-88

[31] Zheng XY, Sinclair JB. The effects of traits of Bacillus megateriumonseed and root colonization and their correlation withthe suppression of Rhizoctonia root rot of soybean. BioControl. 2000;**45**(2):223-243. DOI: 10.1023/A:1009998304177

[32] Kumar A, Bahadur I, Maurya BR, Raghuwanshi R, Meena VS, Singh DK, et al. Does a plant growth-promoting rhizobacteria enhance agricultural sustainability. Journal of Pure and Applied Microbiology. 2015;**9**:715-724

[33] Bonas U, Lahaye T. Plant disease resistance triggered by pathogen-derived molecules: Refined models of specific recognition. Current Opinion in Microbiology. 2002;**5**(1):44-50. DOI: 10.1016/S1369-5274(02)00284-9

[34] Romeiro RS. PGPR and systemic induction of resistance against plant pathogens. Summa Phytopathologica. 2000;**26**:177-184

[35] Lyu D, Zajonc J, Pagé A, Tanney CA, Shah A, Monjezi N, et al. Plant holobiont theory: The phytomicrobiome plays a central role in evolution and success. Microorganisms. 2021;**9**:1-12. DOI: 10.3390/microorganisms9040675

[36] Antar M, Lyu D, Nazari M, Shah A, Zhou X, Smith DL. Biomass for a sustainable bioeconomy: An overview of world biomass production and utilization. Renewable and Sustainable Energy Reviews. 2021;**139**:110691. DOI: 10.1016/j.rser.2020.110691

[37] Khan N, Bano A, Ali S, Babar MA. Crosstalk amongst phytohormones from planta and PGPR under biotic and abiotic stresses. Plant Growth Regulation. 2020;**90**:189-203. DOI: 10.1007/s10725-020-00571-x

[38] Mohanty P, Singh PK, Chakraborty D, Mishra S, Pattnaik R. Insight into the role of PGPR in sustainable agriculture and environment. Frontiers in Sustainable Food Systems. 2021;**5**:667150. DOI: 10.3389/fsufs.2021.667150

[39] Hamid B, Zaman M, Farooq S, Fatima S, Sayyed RZ, Baba ZA, et al. Bacterial plant biostimulants: A sustainable way towards improving growth, productivity, and health of crops. Sustainability. 2021;**13**:2856. DOI: 10.3390/su13052856

[40] Ghoul M, Mitri S. The ecology and evolution of microbial competition. Trends in Microbiology. 2016;**24**:833-845. DOI: 10.1016/j.tim.2016.06.011

[41] Jacoby RP, Kopriva S. Metabolic niches in the rhizospheremicrobiome: New tools and approaches to analyse

metabolic mechanisms of plant–microbe nutrient exchange. Journal of Experimental Botany. 2019;**70**:1087-1094. DOI: 10.1093/jxb/ery438

[42] Ponomarova O, Gabrielli N, Sévin DC, Mülleder M, Zirngibl K, Bulyha K, et al. Yeast creates a niche for symbiotic lactic acid bacteria through nitrogen overflow. Cell Systems. 2017;**5**:345-357. DOI: 10.1016/j.cels.2017.09.002

[43] Vejan P, Abdullah R, Khadiran T, Ismail S, Nasrulhaq BA. Role of plant growth promoting rhizobacteria in agricultural sustainability—A review. Molecules. 2016;**21**:573. DOI: 10.3390/molecules21050573

[44] Lopes MJ, Dias-Filho MB, Gurgel ES. Successful plant growth-promoting microbes: Inoculation methods and abiotic factors. Frontiers in Sustainable Food Systems. 2021;**5**:606454. DOI: 10.3389/fsufs.2021.606454

Chapter 5

Parameter Affinity Estimation of Rhizobacterial Cocktail Formulations for Hydrocarbon Degradation Using Locally Available Substrates in Crude Oil-Impacted Soil

Joseph E. Agbaji, Enobong Effiong and Godwin C. Iheanacho

Abstract

This chapter focuses on the estimation of parameter affinity in rhizobacterial cocktail formulations for bio-recovery of crude oil-impacted soil. The topic relied on a study investigating the utilization of locally available substrates in ecologically disturbed ecosystems, with a focus on the rhizosphere of weeds growing on aged crude oil-impacted soil in the Niger Delta region. The identified rhizobacterial isolates: *Achromobacter agilis, Pseudomonas fluorescens, Bacillus thuringiensis*, and *Staphylococcus lentus*, are renowned for significant biodegradative potentials. The researchers assessed the impact of different parameters on growth dynamics of these isolates. By utilizing agro-residues like corn chaff as carbon source, corn steep liquor for nitrogen, and poultry droppings for phosphorus, as sources of limiting nutrients, the researchers varied factors like nutrient availability, pH levels, and temperature to estimate the affinity of these parameters for growth formulations and bioremediation capabilities by fitting the substrate utilization data into a Growth Kinetics Models. Data obtained revealed the isolates' affinity for different substrates and provide valuable insights for optimizing the composition and performance of rhizobacterial cocktails for efficient hydrocarbon degradation in crude oil-impacted soil. Additionally, they underscored the potential of locally available substrates and microbial flora as effective tools for bio-recovery of crude oil-impacted soil.

Keywords: rhizobacterial, cocktails, hydrocarbon degradation, affinity, parameter estimation, bio-recovery, ecosystem

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1. Introduction

1.1 Rhizobacteria

Rhizobacteria comprise a diverse group of bacteria that confer numerous beneficial effects on plant health and growth. The rhizosphere, the region of the soil closely influenced by plant roots, creates a nutrient-rich environment or ecosystem that fosters a diverse array of bacteria and fungi, many of which exhibit potential benefits for plants.

In some peer-reviewed literature, these bacteria have been referred to as Plant Growth Promoting Rhizobacteria (PGPR) due to their proven capacity to mitigate the proliferation of pathogenic microorganisms detrimental to plant health [1, 2].

Primarily characterized as Gram-negative, rod-shaped bacteria, rhizobacteria often possess a single or no flagellum. They can exhibit aerobic chemoheterotrophic behavior, utilizing both organic and inorganic resources. A subset of these bacteria is capable of nitrogen fixation, either in a symbiotic or free-living capacity, thus contributing to plant nitrogen nutrition. Notable microbial species within this context include *Trichoderma* and *Pseudomonas sp*., recognized for their multifaceted roles encompassing antagonism, competition, and antibiosis [3]. Other significant taxa encompass *Alcaligenes*, *Azospirillum*, *Arthrobacter*, *Acinetobacter*, *Azoarcus*, *Bradyrhizobium*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Pantoea*, *Paenibacillus*, *Rhizobium Azorhizobium*, *Bradyrhizobium*, *Allorhizobium*, *Sinorhizobium*, *Methylobacterium*, *Frankia*, *and Mesorhizobium*.

The functional range of some of these rhizobacterial strains encompasses abiotic stress tolerance, enzymatic production, synthesis of organic compounds, nutrient solubilization to facilitate plant uptake, modulation of plant growth regulators, and the synthesis of Siderophores [4–6]. Moreover, during the process of nodulation in plants, select bacterial strains actively contribute to nitrogen fixation [4]. According to Becker et al. [7], these bacterial communities constitute a pivotal niche within the phytomicrobiome of most plants, forming an intricately interwoven and structured microcosm inhabited by terrestrial organisms adeptly adapted to their environment. However, the thriving of these microorganisms in their respective niches is influenced by a range of factors, including the availability of essential nutrients required for metabolic activities in proximity to plant roots. In return, plants influence the rhizobacterial community through the exudation of chemical compounds, a process that can exert both antagonistic and stimulatory effects [7–9].

Furthermore, Kumar et al. [10] suggest that the realm of rhizobacteria encompasses a spectrum of microorganisms, encompassing not only saprophytes, but also endophytes, epiphytes, pathogens, and numerous beneficial microbes. A subset of these microorganisms, referred to as intracellular Plant Growth Promoters or rhizomicrobiota, engages in direct interactions with plants by existing as endophytes. Concurrently, a substantial portion of these microbes flourish outside plant tissues, collectively referred to as exophytes. This group populates the exterior of plant roots, constituting a diverse community across the rhizoplane, rhizosphere, and phyllosphere [11].

2. Bioremediation cocktail

Bioremediation represents an advanced form of biodegradation and biomineralization, wherein living organisms, encompassing plants and animals, alongside their derivatives, are harnessed to diminish or transform harmful substances into less hazardous and more valuable forms [12, 13]. Predominantly, microbes and their metabolic products have been harnessed for the mitigation of deleterious pollutants in the environment [14]. This technology is recognized for its cost-effectiveness, eco-friendliness, technological viability, and scalability. These attributes have been pivotal in driving the attention and engagement of environmental enthusiasts worldwide.

Whilst bioremediation techniques have often been lauded for their cost-efficiency [15], it is noteworthy that certain costs may be incurred due to factors such as mechanical and chemical treatments, containment, procurement of exogenous strains, nutrients, and suitable substrates, as well as the application of surfactants. Contemporary strategies like landfilling and land farming have influenced the scalability of the process, particularly in cases involving the physical management of pollution [16]. Nevertheless, when juxtaposed against conventional methodologies, these approaches tend to be more economical [17, 18]. The categories of these technologies exhibit minimal intrusion or disruption of the environmental framework and can be classified as *Ex-situ* and *In-situ*, predicated on the treatment location and technological prerequisites. The former may necessitate pollutant excavation to an alternative site for potential treatment, whereas the *In-situ* approach is characterized by non-invasive interventions. Notably, the treatment of most organic pollutants occurs on-site, rendering it less obtrusive and more manageable—referred to as In-Situ treatment technology (On-site). In instances involving groundwater treatment, a technical methodology such as pump-and-treat is employed (Off-site Treatment Technology).

Interactions between pollutants and the speciation of concern can disrupt the physicochemical attributes of environmental matrices, potentially leading to nutrient leaching [19–21]. The integration of indigenous organisms, with minimal human intervention [13]—often termed nature-assisted treatment—has spurred innovations in Remediation by Natural Attenuation (RENA). The degradation efficiency and kinetics of hydrocarbons tend to follow a sequence: *n-alkanes* → *branched alkanes* → low molecular weight aromatics → *cycloalkanes* → *polycyclic aromatics* [22]. Whilst various microbes partially oxidize aliphatic hydrocarbons, complete metabolism is facilitated by *Flavobacterium* and select members of the Gammaproteobacteria. Cyclic hydrocarbons, including benzene, might exhibit steric hindrances that influence their responsiveness to bioremediation technologies.

The concept of the rhizobacterial cocktail involves the formulation of exogenous microbial consortia tailored to fulfil nutrient and microbial requisites within diverse biotechnological contexts. Developing a rhizobacterial cocktail necessitates rigorous screening, strain selection, optimization of nutritional provisions, and the incorporation of delivery technologies [12]. In a related study, Shinwari et al. [23] engineered a system employing a consortium of rhizobacterial cultures to remediate metal-impacted soil. These formulations can be administered via batch or feed-batch strategies, effectively catering to specific environmental objectives, such as bioremediation or the degradation of intricate compounds. Bioaugmentation and biostimulation constitute pivotal strategies underpinning cocktail development.

3. Pollution of environmental media

The intensification of industrialization, population growth, and routine human activities has led to an increased demand for secure and cheaper energy source like petroleum hydrocarbon or crude oil, a high carbon polluting source to several media [24, 25]. Pollution, in its essence, represents the inadvertent introduction of harmful and unwanted toxic substances into the environment. Any substance capable of inducing detrimental effects on living organisms is appropriately classified as a pollutant.

Pollutants are categorized into organic or inorganic classes based on their underlying chemical composition [26]. Inorganic pollutants, comprising heavy metals and radioactive isotopes, are non-biodegradable, whilst organic pollutants are biodegradable. A pollutant can trigger a range of adverse effects, encompassing teratogenic, carcinogenic, mutagenic, and other severe deleterious outcomes. Notably, the residues of certain concerning pollutants exhibit recalcitrance or persistence within the environment, subsequently impeding the recovery of polluted matrices [27]. The persistence of pollutants in the environment is intrinsically linked to their xenobiotic nature, allowing them to endure over time.

Crude oil stands as a pivotal economic driver for numerous nations. Incidents of oil leaks and spills are frequently attributed to various activities including drilling, transportation, distribution, and storage [17]. Instances such as oil well blowouts, tanker accidents, and pipeline vandalism contribute to the release of over 0.5% of produced oil back into ecosystems as pollutants [28]. Notably, the Niger Delta region of Nigeria has emerged as a significant hub for soil and water pollution, arising from both exploration and exploitation activities [29]. This extensive pollution has led to the substantial depletion of the region's natural diversity.

Scientific evidence attests that certain organisms, particularly higher plants, synthesize hydrocarbons in various forms, such as waxes, exudates, oils, and organic materials. Whilst these compounds contribute to the overall hydrocarbon content of the soil, they have minimal impact on the biogenic levels of soil hydrocarbon content [30, 31]. Numerous reports have documented the detrimental effects of various spills on the biodiversity of affected ecosystems [32]. These spill-related incidents are largely attributed to anthropogenic factors, often stemming from the failure of transport infrastructure, such as pipelines or acts of deliberate sabotage.

The pollution of arable land exerts negative repercussions on crop yield, fertility, and productivity [33–35]. Uquetan et al. [36] have identified the influence of crude oil and hydrocarbons on crop productivity and yield. They emphasize that hydrocarbons within crude oil-contaminated soil disrupt the soil's physical, chemical [37], and microbiological [13, 38–40] properties. These disruptions significantly contribute to diminished crop productivity, particularly impacting the functional roles of soil organisms. Chukwu and Udoh report that concentrations of crude oil exceeding 3% w/w in any medium can result in the loss of metabolic capabilities in animals and plants. Enzyme activity inhibition can consequently hinder the growth of vital cash crops, such as maize, cassava, and vegetables. The study conducted by Udoh and Chukwu [37] highlights the significant influence of hydrocarbons on soil physicochemical attributes. Consequently, the decline of soil's rich biodiversity, as measured over time, is elucidated in their study, which compares results from investigations in 2020 and 2008 to evaluate the potential utility of soil pre-exposed to pollution. The study reveals that the impact of soil pollution diminishes with time, concurrent with a reduction in the intensity of impact.

4. Isolate selection: rhizobacterial flora in crude oil-impacted soil

Strain selection serves as a critical process aimed at harnessing specific microbes with superior potential for generating desired products at enhanced yields compared to their counterparts. Distinct reference benchmarks and methodologies are deployed to differentiate these strains from the myriad of other microorganisms coexisting within their habitat. Often, these strains occupy analogous niches within their microenvironment [41]. This procedure has emerged as a pivotal strategy in the field of bioaugmentation.

The isolation and selection of strains from the rhizosphere region of plants necessitate that bacteria originate from the root vicinity, thus precluding the inadvertent isolation of non-target organisms. This process mandates the utilization of batch enrichment procedures. The utilization of plants possessing robust phytoremediation attributes offers a valuable avenue for isolating bacterial strains that exhibit a heightened potential for hydrocarbon degradation or an adeptness to survive on exudates and waxes. Contemporary techniques include the use of enzyme assays or nucleic acid presence to discern the composition of rhizobacterial flora. Microbial strains may actively (assimilatory) or passively (dissimilatory) partake in the processes of degradation or fermentation.

5. Agro-waste as substrates for bioremediation

Agro-waste, also referred to as agro-residues, refers to the byproducts stemming from agricultural processes, which may lack inherent value or utility in the final product. Agricultural waste, synonymous with "agro-waste" or agro-residues, encompasses spent materials originating from the processing of food, food products, animals, and animal products. Primarily comprised of plant materials, these byproducts result from their transformation into more valuable derivatives. The concept of recycling and repurposing these bioresources has not been fully embraced, signaling that the challenges arising from inadequate waste management in developing countries are far from resolved [42].

Manures, plant chaff, stalks, and leaves stand as archetypal instances of agro-waste, often discarded or rarely repurposed. Many of these agro-residues encompass relatively insoluble biopolymers like cellulose and lignin, alongside soluble components including biomolecules and their constituent units [43]. Mismanagement of agro-waste poses risks of environmental degradation, health issues, and diminished esthetic value [44]. Within the agro-industry, substantial quantities of waste and residues are generated, presenting significant waste management challenges for these facilities. Strategies such as burning, burying, dumping, and landfilling are commonly employed for handling these agro-residues [45].

Characterized by their composition, agro-waste harbors appreciable nutritional and anti-nutritional elements that remain untapped [46]. Numerous food industries produce substantial volumes of agro-waste, with noteworthy examples including pomegranate peels, lemon peels, green walnut husks, and palm kernel shells. A wide array of organic waste holds potential for bioenergy production and serves as a medium amendment for cultivating valuable resources. The ascendancy of agro-waste as feedstocks and substrates for microbial product synthesis underscores their capacity to provide essential nutrients [40, 47]. Biotechnological applications leverage agro-waste for nutrient supply in biostimulation processes, as immobilization matrices for starter cultures or inocula, and as supplements for lipid biosynthesis [48]. Notable materials, including banana peels, yam peels, potato peels, cassava peels, rice husks, sugarcane bagasse, and oil palm residues, serve as sources of carbon whilst concurrently acting as conditioners and absorbents (**Table 1**) [56, 57].

Contributors	Agro-waste utilized	Application
[49]	Egg shells and cocoa peats	Immobilization of *Janibacter* sp.
[50]	Spent Mushroom Compost	Biostimulate and biotransform heavy metal-polluted soil.
[51]	Bone Char	Biostimulation of nutrient
[12]	Corn Steep liquor, Poultry droppings, Bone Char	Design of bioremediation cocktail for bioremediation
[52]	Sugarcane bagasse	Biotreatment of halogenic-organic pollutant
[47]	Groundnut shell, Sugarcane straw, and melon husk	Immobilization of starter cultures for biostimulation and treatment of refinery waste
[53]	Bone char and Poultry Manure	Biostimulation efficiency using kinetic and model analysis
[54]	Plantain peels and Guinea corn Chaffs	Stimulation of Indigenous soil microbes for bioremediation
[55]	Goat Manure (*Capra aegrageus hricus*)	Biostimulation of crude oil-polluted soil

Table 1.
Bioremediation case studies using agro-waste.

6. Nutrients from agro-waste

6.1 Carbon

Carbon stands as one of the most abundant elements in nature, existing in both organic and inorganic forms. Plant-derived carbon sources are readily accessible, particularly from carbohydrate-rich food products. Cereal-derived waste emerges as a practical and cost-effective reservoir of carbohydrates, thus serving as an essential carbon source. Cereal varieties such as wheat, rice, maize, oat, millet, barley, rye, and sorghum boast lignocellulosic biomass, presenting a cost-efficient carbon pool for diverse industrial applications, including microbial metabolism stimulation and fermentation processes [58]. Notably, wheat bran, derived from wheat processing, embodies the fibrous outer pericarp layer of wheat grains left after milling. This material is rich in complex polysaccharides, such as cellulose, hemicellulose, and pentosan, thus serving as valuable carbon proxies [59]. Rice bran's proximate composition showcases its carbohydrate content (34–62%) and crude fiber (7–11%) [58, 60]. Additionally, sugarcane bagasse constitutes a carbon reservoir with cellulose (45%), hemicellulose (32%), and lignin (17%) [61].

6.2 Nitrogen

Bacteria contribute to the fixation of nitrogen, which plants absorb in the form of nitrates for synthesizing proteins and other essential macromolecules. Fixed nitrate and ammonia play pivotal roles in animal nutrition, particularly in algae and higher plant metabolism. Urea emerges as a highly accessible nitrogen source, reacting with water to produce ammonia, thus rendering the enclosed nitrogen available to plants. Nitrate originating from urea serves as a bioavailable and readily utilizable nitrogen source in various bioprocesses. Notably, run-off from animal farms remains a sought-after reservoir of nitrates and phosphates due to the prevalence of sewage, atmospheric deposition, urban run-off, and industrial wastewater in these effluents [62]. Improper management of nitrate and phosphate-rich sources can result in surface water eutrophication [63].

6.3 Phosphate

Phosphate, a fundamental component of fertilizers, is ubiquitously present in rocks and can be found in soil pre-exposed to leaching or pollution from industrial activities. This nutrient plays a pivotal role in the growth of plants and animals, influencing cell division and metabolism, and constitutes a key component of nucleic acids. Seepage from phosphate-rich effluents has been implicated in causing algal blooms [64–66], and on soil, it can lead to serious health hazards. Valuable sources of phosphate within agro-waste include wheat bran, bone char, and cow dung ash. Both industrial and domestic effluents have been recognized as phosphate sources, with potential implications for water pollution [67]. As highlighted by Fuentes et al. [68], elevated phosphate levels in water can precipitate toxin proliferation, leading to adverse health effects, such as kidney damage and osteoporosis. Additionally, algal biomass, particularly digestate, has been identified as another phosphate-rich feedstock (**Tables 2** and **3**).

Agro-waste	Total Phosphate content (g/kg)
Cow dung	2.94–4.02
Poultry manure	23.6–27.8
Pig manure	16.22–29.7
Municipal Solid Waste MSW (Compost)	2.9–5.6 5.0–8.0
Sewage sludge	38.3
Wastewater	2.09–3.43

Table 2.
Phosphate content of some agro-waste [68].

Nitrogen content			
Sample	**NO_3-N**	**Total Nitrogen**	**% Nitrogen**
	mg/l	mg/l	
Corn Steep Liquor (after filtration)	1.22	17.50	
Corn Steep Liquor (24 hrs. Soaked)	2.14	20.00	
Corn Steep Liquor (Blended & 12 hrs Soak; Prior to filtration)	3.31	30.95	
Millet Steep Liquor (24 hrs Soaked)	0.70	21.10	
Millet Steep Liquor (after filtration)	2.47	37.30	
Millet Steep Liquor (Blended & 12 hrs Soak; Prior to filtration)	5.24	48.30	

Nitrogen content			
Sample	**NO_3-N**	**Total Nitrogen**	**% Nitrogen**
Guinea Corn Steep Liquor (24 hrs. Soaked)	3.31	5.00	
Guinea Corn Steep Liquor (after filtration)	1.08	9.85	
Guinea Corn Steep Liquor (Blended & 12 hrs Soak; Prior to filtration)	2.18	10.15	
Blood (Cow)			39.20
Urine (Cow)			2.49
Carbon content			
	Moisture	TOC	
	%	%	
Corn Chaff	18.35	99.54	
Guinea Corn Chaff	11.21	98.67	
Millet Chaff	12.1	98.98	
Phosphorus content			
	Phosphate	Phosphorus	
	mg/kg	mg/mg	
Cow Bone Char	17.71	5.78	
Crab Char	10.67	3.48	
Shrimp Char	6.94	2.26	
Chicken Droppings	Level (%)		
1. Nitrate (as NO3)	0.18		
2. Phosphate (as PO4)	2.42		
3. Total Phosphorus (as P)	9.5		
4. Total Nitrogen (as N)	1.03		
5. Total Ammonia (as NH3)	< 0.01		
6. Potassium (as K)	1.55		
7. Total Organic Carbon ©	23.41		
8. Carbonates (CO3)	0.38		
Potassium content			
		Concentration (ppm)	
Wood Ash	470.992		
Plantain Peel Char	176.037		

Source: Agbaji [19].

Table 3.
Proximate composition of agro-waste residues Agbaji [19].

7. Theoretical model for determining kinetic parameters of bacterial growth in batch culture

In laboratory setting, the growth kinetics parameters of rhizobacteria [7] were determined through the assessment of total viable counts and incubation durations using first-order kinetics. Batch culture, conducted within a closed system containing a limited initial substrate, facilitated the exploration of microbial growth behavior [19]. The study employed an inocula of rhizobacteria, which was introduced into a Bushnell Haas medium (Mineral Salt Medium), supplemented with 1.0 ml crude oil as the sole carbon source to align with the kinetics. The inoculated rhizobacteria were monitored across growth phases, with cell biomass and growth indices displaying exponential increments at a constant maximum rate during the log phase [7]. The specific growth rate was determined by the linear gradient of a sigmoidal growth-versus-time plot [69, 70].

Mathematically, the first-order rate equation is given by:

$$r = \frac{dN}{dt} = \mu N \quad (1)$$

where, N = Microbial biomass (CFU/ml), t = the time/duration (hours), and μ = specific growth rate of (hours^{-1}).

Integration of Eq. (1), within the limit; at t = 0, N = N_0 and at t = t, N = N:

$$\ln(N / N_0) = \mu t \quad (2)$$

To deduce the specific growth rate (μ) of rhizobacterial isolates for each batch culture, an amendment of 1.0% w/w crude oil was made to simulate the pollutant. The graph of ln(N/N0) against time t was plotted, and the slope determined the specific growth rate at the initial crude oil concentration. The generation time (t_g), representing the time for cell number to double, was calculated from Eq. (2) as:

When N = 2 N_0; t – t_0 equal tg. Substituting for N and t, Eq. (2) becomes

$$t_g = \ln 2 / \mu = 0.693 / \mu \quad (3)$$

7.1 Effects of substrate utilization on kinetic parameters of bacterial growth model

The study explored the impact of varying concentrations of corn chaff substrate (0.0 to 25.0 gL^{-1}), corn steep liquor (0 to 50% v/v), and poultry droppings (0.0 to 2.5 gL^{-1}) on kinetic parameters. These agro-waste concentrations were employed as substrates for cultivating *Achromobacter agilis, Pseudomonas fluorescens*, *Bacillus thuringiensis*, and *Staphylococcus lentus*, with their specific growth rates calculated. The intrinsic physiological properties of microorganisms depend on the substrate and growth temperature [71]. This study highlighted the use of agro-waste for rhizobacterial cultivation, demonstrating their high affinity and growth rate using agro-substrates. Carbon substrate (corn chaff), nitrogen substrate (corn steep liquor), and phosphorus substrate (poultry droppings) served as limiting nutrients, incorporated in the mineral salt medium.

A decline in growth rate and cessation due to substrate depletion were characterized using the Monod equation, introduced by Jacques Monod in 1942. This model relates specific growth rate (μ) to residual growth-limiting substrate (S) concentration, represented as:

$$ì = \frac{\mu_m s}{k_s + s} \quad (4)$$

Here, μ and μm denote specific growth rate and maximum specific growth rate, respectively, whilst S signifies substrate concentration, and Ks represents substrate saturation or utilization constant.

This study was designed to identify agro-waste utilization by rhizobacterial cultivation and estimation of the maximum specific growth rate (μm), and KS, the half-saturation or utilization constant, which is defined as the substrate concentration at which growth occurs at one-half the value of μm and is a demonstration of high growth affinity of the organism for agro-substrates.

Both μ_m and K_S reflect the organism's intrinsic properties, substrate, and growth temperature.

Inverting Eq. (4), the equation below results

$$\frac{1}{\mu} = \frac{1}{\mu_m} + \left(\frac{K_s}{\mu_m}\right)\left(\frac{1}{s}\right) \tag{5}$$

This equation corresponds to the Lineweaver-Burk plot. For each agro-waste substrate utilizer, a plot of the inverse of the specific growth rate (1/μ) against the inverse of the initial substrate concentration (1/S) was constructed. The resulting slope and intercept were used to estimate maximum specific growth rates and substrate saturation constants. The study's findings encompassed various growth phases, with observed dynamics contributing to a comprehensive understanding of bacterial growth behavior.

8. A case study of the iterations of agro-waste on rhizobacterial growth rate

8.1 Kinetic of bacterial growth rate analysis

The hydrocarbon degradative potential of the bacterial isolates was assessed using both viable plate count and optical density (OD) methods, as illustrated in **Figure 1**. The bacterial strains employed in this investigation encompass *Achromobacter agilis*, *Pseudomonas fluorescens*, *Bacillus thuringiensis*, *and Staphylococcus lentus.*

A graphical representation of the growth dynamics is presented in **Figure 1**, depicting the cell count or biomass concentration of the aforementioned bacterial isolates measured in colony-forming units (cfu/ml) and optical density (OD) across time in hours. The semi-logarithmic plot provides insights into the different growth phases—lag, log, stationary, and death. The lag phase, although not overt, can be attributed to the bacteria's physiological adaptation from prior subcultures and the presence of a substantial initial inoculum size [71]. Notably, the lag time for bacterial growth ranges from zero to a few hours of incubation time. Furthermore, the stationary phase, aligning with the asymptote where bacterial biomass reaches its maximum, occurs around day five to six. This observation is of significance in light of the achieved half-life of 6 days after a 56-day treatment of hydrocarbon-polluted soil using a bioremediation cocktail formulated from these isolates.

8.1.1 The experimental growth rate model

The exponential growth phase's experimental growth rate of biomass within the batch system was characterized by Eq. 2: ln(N/N0) = μt = > lnN = lnN0 + μt, where the linear equation's slope equates to the specific growth rate. Applying this equation

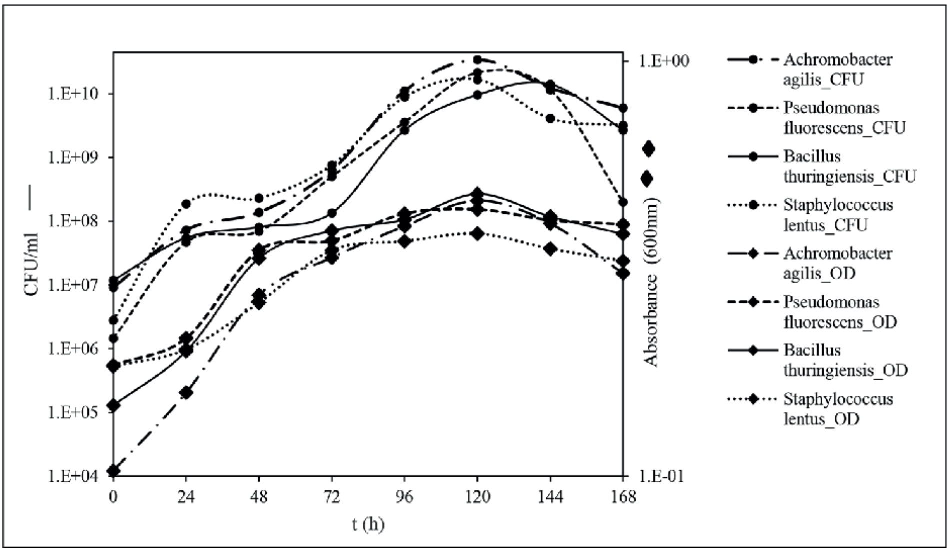

Figure 1.
Bacterial hydrocarbon degradation potential growth curve (source: Author study-Agbaji [19]).

to the colony-forming unit data from **Figure 1** yields the linear plot displayed in **Figure 2**. In this context, the specific growth rate (μ) of each isolate is identical to the slope of its corresponding growth model's linear equation.

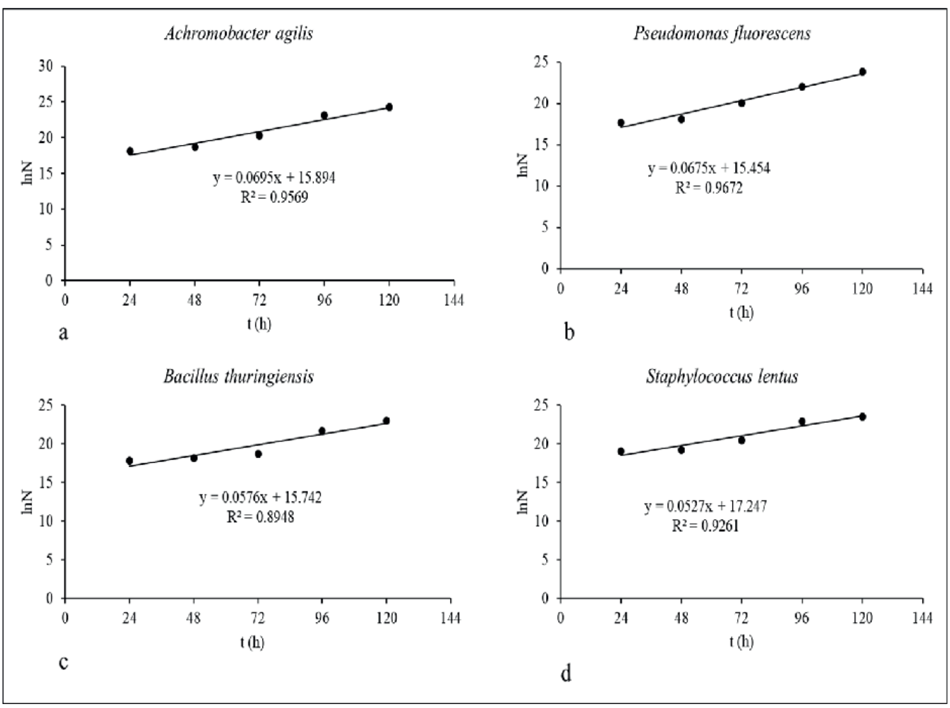

Figure 2.
Exponential growth logarithm vs. time (source: Author study-Agbaji [19]).

8.1.2 Calculation of generation time and kinetic parameters

Utilizing Eq. 3, with the specific growth rate, the generation time was computed. The results of these computations, along with the lag time (λ) and asymptote (A) derived from the semi-logarithmic plot in **Figure 1**, were summarized in **Table 4**. The summary highlights the specific growth rates of bacterial isolates in Bonny light crude oil, following the order: *Achromobacter agilis* > *Pseudomonas fluorescens* > *Bacillus thuringiensis* > *Staphylococcus lentus.* This sequence also correlates with the isolates' generation times. Notably, *Pseudomonas fluorescens* exhibits the shortest lag time, followed by *Achromobacter agilis*, *Bacillus thuringiensis*, and *Staphylococcus lentus*. Concerning the asymptote, representing the stationary phase characterized by maximum bacterial biomass, *Achromobacter agilis* displays the highest biomass, succeeded by *Pseudomonas fluorescens*, *Staphylococcus lentus*, and *Bacillus thuringiensis.*

A graphical representation of the natural logarithm versus time for the exponential growth of the bacterial isolates is shown in **Figure 2**. The slope of each line within the graph corresponds to the specific growth rate (μ).

8.2 Growth responses of rhizobacterial species using agro-waste substrate

The preceding Section 8.1 presents the laboratory experimental results that underpin the parameter estimation process. These experiments were conducted using high-grade laboratory nutrients as sources, laying the foundation for the subsequent selection of rhizobacterial species with significant growth potential. However, in the context of this study chapter, these laboratory-grade limiting nutrients were replaced with nutrients sourced from agro-waste materials. This innovative approach allows the study to estimate the parameter affinity of the selected rhizobacteria for these agro-waste substrates, thereby bridging the gap between controlled laboratory conditions and real-world application scenarios.

8.2.1 Growth responses of rhizobacterial species using corn chaff as the sole carbon source

The influence of initial corn chaff concentrations, ranging from 0.0 to 2.5 g dL^{-1} as delineated in **Table 5**, was investigated to ascertain its impact on the growth indices of rhizobacterial strains. Specifically, this analysis encompassed *Achromobacter agilis*, *Pseudomonas fluorescens*, *Bacillus thuringiensis*, and *Staphylococcus lentus*. The variation in initial corn chaff concentrations served as a basis for evaluating the specific growth

	μ	**t_g**	**λ**	**Asymptote**	**R^2**
	h^{-1}	**h**	**h**	**Cfu/ml**	**Value**
Achromobacter agilis	0.070	9.973	13.8	3.42E+10	0.957
Pseudomonas fluorescens	0.068	10.269	12.6	2.15E+10	0.967
Bacillus thuringiensis	0.058	12.034	15.4	1.41E+10	0.895
Staphylococcus lentus	0.053	13.153	17.5	1.67E+10	0.926

μ = specific growth rate; t_g = generation time; λ = lag time of growth, and R^2 = goodness of best fit.
Source: Author study-Agbaji [19].

Table 4.
Summary of estimated kinetic parameters of batch bacterial growth model.

Carbon substrate (Corn Chaff)	Achromobacter agilis	Pseudomonas fluorescens	Bacillus thuringiensis	Staphylococcus lentus
(S_{cc}) Conc.	μ	μ	μ	μ
g dL^{-1}	h^{-1}	h^{-1}	h^{-1}	h^{-1}
0.0	0.0000	0.0000	0.0000	0.0000
0.5	0.0539	0.0644	0.0494	0.0679
1.0	0.0619	0.0694	0.055	0.0712
1.5	0.0629	0.0718	0.0586	0.0742
2.0	0.0643	0.0773	0.0598	0.0759
2.5	0.0559	0.0592	0.0588	0.0626

S_{cc} = corn chaff substrate; h = hour; g = gram; dL = deciLitre.
Source: Author study-Agbaji [19].

Table 5.
Varied carbon substrate concentrations (corn chaff) and corresponding specific growth rate (μ) values for Rhizobacterial isolates, applied in the formulation of bioremediation cocktail.

rate of these rhizobacteria. The resultant specific growth rate data obtained from the bacterial isolates cultivated on corn chaff substrate are tabulated in **Table 5** and subsequently depicted in the Monod model plot presented in **Figure 3**.

Figure 3 illustrates the intricate interplay between the carbon substrate, represented by corn chaff, and the growth behavior exhibited by the individual

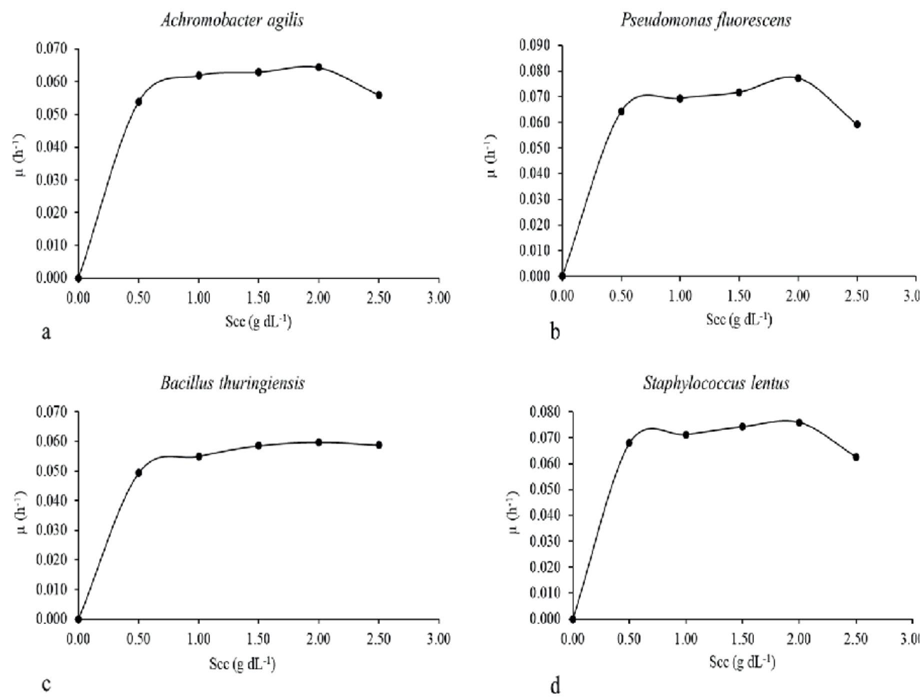

Figure 3.
Impact of carbon substrate (corn chaff) on growth patterns of Rhizobacterial isolates (source: Author study-Agbaji [19]).

rhizobacterial isolates—namely, (a) *Achromobacter agilis*, (b) *Pseudomonas fluorescens*, (c) *Bacillus thuringiensis*, and (d) *Staphylococcus lentus*. This graphical representation is a visualization of the Monod model plot.

8.2.2 Growth responses of rhizobacterial species using corn steep liquor as the sole nitrogen source

In this phase of investigation, the focus shifted to evaluating the impact of initial corn steep liquor concentrations, spanning from 0 to 50 ml dL^{-1} as delineated in **Table 6**, on the growth indices of specific rhizobacterial strains. The rhizobacterial isolates subjected to analysis encompassed *Achromobacter agilis*, *Pseudomonas fluorescens*, *Bacillus thuringiensis*, and *Staphylococcus lentus*. This experimental approach aimed to scrutinize the relationship between initial corn steep liquor concentrations and the specific growth rate exhibited by the aforementioned bacterial isolates. The resulting specific growth rate data, obtained from the bacterial isolates' utilization of corn steep liquor as a nitrogen substrate, are methodically presented in **Table 6**. This dataset served as the foundation for the ensuing construction of the Monod model plot illustrated in **Figure 4**.

Figure 4 visually portrays the intricate interplay between the nitrogen substrate, represented by corn steep liquor, and the ensuing growth patterns exhibited by individual rhizobacterial isolates—specifically, (a) *Achromobacter agilis*, (b) *Pseudomonas fluorescens*, (c) *Bacillus thuringiensis*, and (d) *Staphylococcus lentus*. This visual representation is a realization of the Monod model plot, elucidating the dynamic relationship between initial corn steep liquor concentrations and the growth behavior of these isolates.

8.2.3 Growth responses of rhizobacterial species using poultry droppings as the exclusive phosphorus source

In the context of this segment, the investigation turned its focus towards comprehending the impact of varying initial concentrations of poultry droppings, ranging from 0.0 to 0.25 g dL^{-1} as illustrated in **Table 7**, on the growth indices of specific

Nitrogen substrate (Corn Steep Liquor)	Achromobacter agilis	Pseudomonas fluorescens	Bacillus thuringiensis	Staphylococcus lentus
(S_{csl}) Conc.	μ	μ	μ	μ
ml dL^{-1}	h^{-1}	h^{-1}	h^{-1}	h^{-1}
0	0.0000	0.0000	0.0000	0.0000
10	0.0818	0.0648	0.0505	0.0477
20	0.0890	0.0840	0.0720	0.0636
30	0.0668	0.0707	0.0681	0.0524
40	0.0638	0.0613	0.0668	0.0496
50	0.0418	0.0581	0.0658	0.0460

S_{csl} = corn steep liquor; substrate; h = hour; ml = milliliter; dL = deciLitre.
Source: Author study-Agbaji [19].

Table 6.
Varied nitrogen substrate concentrations (corn steep liquor) and corresponding specific growth rate (μ) values for Rhizobacterial isolates, applied in the formulation of bioremediation cocktail.

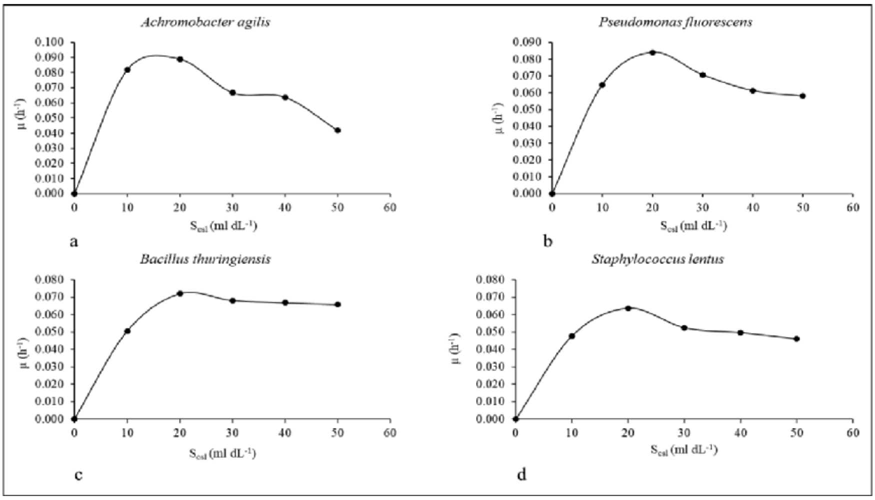

Figure 4.
Impact of nitrogen substrate (corn steep liquor) on growth patterns of Rhizobacterial isolates (source: Author study-Agbaji [19]).

Phosphorus substrate (Poultry droppings)	Achromobacter agilis	Pseudomonas fluorescens	Bacillus thuringiensis	Staphylococcus lentus
(S_{pd}) Conc.	μ	μ	μ	μ
g dL^{-1}	h^{-1}	h^{-1}	h^{-1}	h^{-1}
0.00	0.0000	0.0000	0.0000	0.0000
0.05	0.0497	0.0591	0.0580	0.0472
0.10	0.0630	0.0626	0.0628	0.0580
0.15	0.0727	0.0636	0.0647	0.0636
0.20	0.0755	0.0680	0.0666	0.0641
0.25	0.0783	0.0688	0.0672	0.0654

S_{pd} = poultry droppings substrate; h = hour; g = gram; dL = deciLitre.

Table 7.
Varied phosphorus substrate concentrations (poultry droppings) and corresponding specific growth rate (μ) values for Rhizobacterial isolates, employed in the formulation of bioremediation cocktail.

rhizobacterial strains. The selected bacterial isolates subjected to analysis were *Achromobacter agilis*, *Pseudomonas fluorescens*, *Bacillus thuringiensis*, *and Staphylococcus lentus*. The primary objective was to scrutinize the relationship between the initial concentration of poultry droppings and the specific growth rate exhibited by these diverse bacterial isolates. The resultant specific growth rate data arising from the utilization of poultry droppings as a phosphorus substrate by the bacterial isolates were methodically documented in **Table 7**. These data points were subsequently plotted against the respective initial concentrations of poultry droppings, culminating in the construction of the Monod model plot portrayed in **Figure 5**. The graphical representation provided by **Figure 5** depicts a characteristic trend, wherein the specific growth rate exhibited an upward trajectory concomitant with the escalation of the initial concentration of poultry droppings.

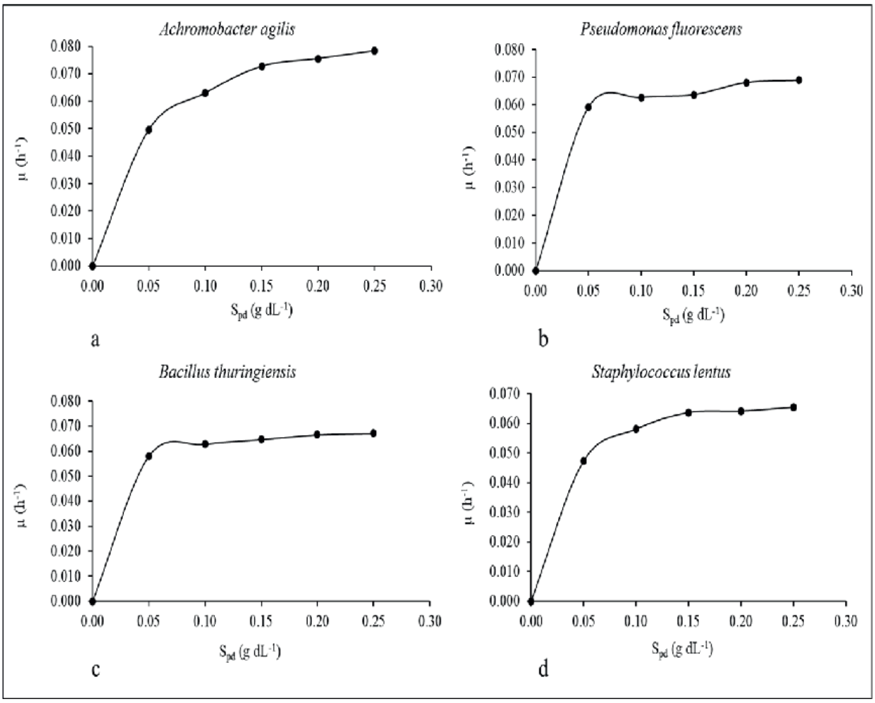

Figure 5.
Influence of phosphorus substrate (poultry droppings) on growth patterns of Rhizobacterial isolates (source: Author study-Agbaji [19]).

Figure 5 visually conveys the intricate interplay between the phosphorus substrate, represented by poultry droppings, and the ensuing growth patterns manifested by individual rhizobacterial isolates—namely, (a) *Achromobacter agilis*, (b) *Pseudomonas fluorescens*, (c) *Bacillus thuringiensis*, and (d) *Staphylococcus lentus*. This graphical representation serves as a tangible embodiment of the Monod model plot, elucidating the dynamic relationship between initial poultry droppings concentrations and the growth behavior of these isolates. Notably, the graphical trend showcases a discernible elevation in specific growth rate in tandem with the increasing initial concentration of poultry droppings.

8.3 Estimation of kinetic parameters using the Monod model

The pursuit of estimating the fundamental kinetic parameters, namely the maximum specific growth rate (μm) and the substrate utilization constant (KS), as defined in Eq. 4, necessitated the conversion of the datasets from **Tables 5–7** into a corresponding set of values tabulated in **Tables 8–10**. Subsequently, these derived values were employed to generate graphical representations conforming to the Lineweaver-Burk equation (Eq. 5), offering valuable insights into the parameter affinities. The implications of this process are encapsulated within the Lineweaver-Burk plots presented in **Figures 6–8**. These plots predominantly capture data points representative of the exponential growth phase, aligning with the observations gleaned from the Monod model plots illustrated in **Figures 3–5**, exclusively for each distinct agro-waste substrate (**Table 11**).

Carbon (Corn chaff)	Achromobacter agilis	Pseudomonas fluorescens	Bacillus thuringiensis	Staphylococcus lentus
$1/S_{cc}$	1/μ	1/μ	1/μ	1/μ
$dL\ g^{-1}$	h	h	h	h
0.00	0.000	0.000	0.000	0.000
2.00	18.553	15.528	20.243	14.728
1.00	16.155	14.409	18.182	14.045
0.67	15.898	13.928	17.065	13.477
0.50	15.552	12.937	16.722	13.175
0.40	17.889	16.892	17.007	15.974

Source: Author study-Agbaji [19].

Table 8.
Inverted values of the range of initial carbon (corn chaff) substrate concentration and their specific growth rates (μ) values from the results of **Table 5**.

Nitrogen (Corn steep liquor)	Achromobacter agilis	Pseudomonas fluorescens	Bacillus thuringiensis	Staphylococcus lentus
$1/S_{csl}$	1/μ	1/μ	1/μ	1/μ
$dL\ ml^{-1}$	h	h	h	h
0.00	0.000	0.000	0.000	0.000
0.10	12.225	15.432	19.802	20.964
0.05	11.236	11.905	13.889	15.723
0.03	14.970	14.144	14.684	19.084
0.03	15.674	16.313	14.970	20.161
0.02	23.923	17.212	15.198	21.739

S_{csl} = corn steep liquor substrate; h = hour; ml = milliliter; dL = deciLitre.
Source: Author study-Agbaji [19].

Table 9.
Inverted values of the range of initial nitrogen (corn steep liquor) substrate concentration and their specific growth rates (μ) values from the results of **Table 6**.

Phosphorus (Poultry droppings)	Achromobacter agilis	Pseudomonas fluorescens	Bacillus thuringiensis	Staphylococcus lentus
$1/S_{pd}$	1/μ	1/μ	1/μ	1/μ
$dL\ g^{-1}$	h	h	h	h
0.0	0.000	0.000	0.000	0.000
20.0	20.121	16.920	17.241	21.186
10.0	15.873	15.974	15.924	17.241
6.7	13.755	15.723	15.456	15.723
5.0	13.245	14.706	15.015	15.601
4.0	12.771	14.535	14.881	15.291

Source: Author study-Agbaji [19].

Table 10.
Inverted values of the range of initial phosphorus (poultry droppings) substrate concentration and their specific growth rates (μ) values from the results of **Table 7**.

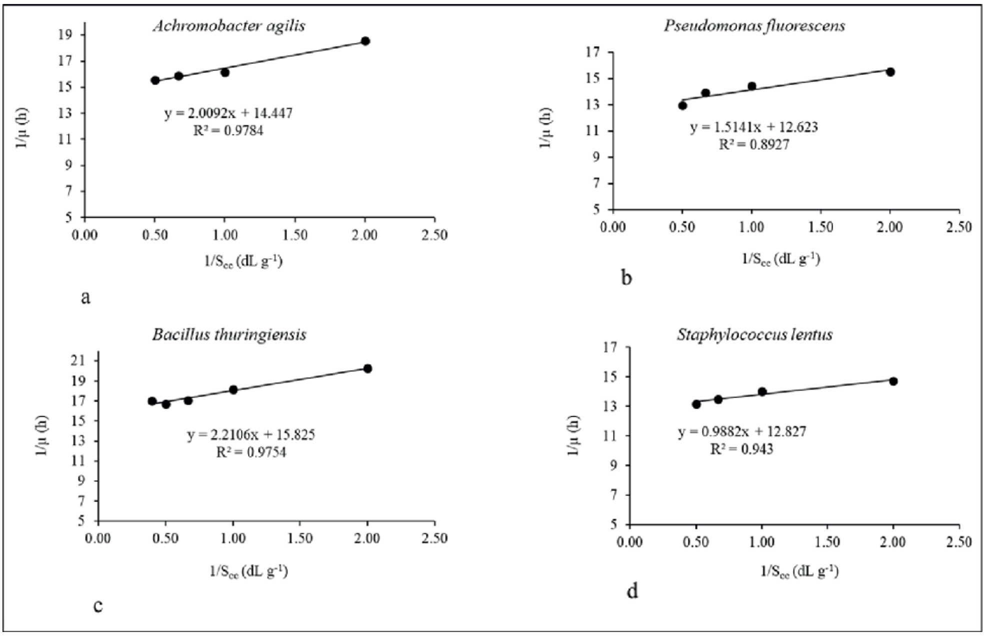

Figure 6.
The Lineweaver-Burk plot for the estimation of μ_m and K_S from the intercept and slope of the linear equation (corn chaff) (source: Author study-Agbaji [19]).

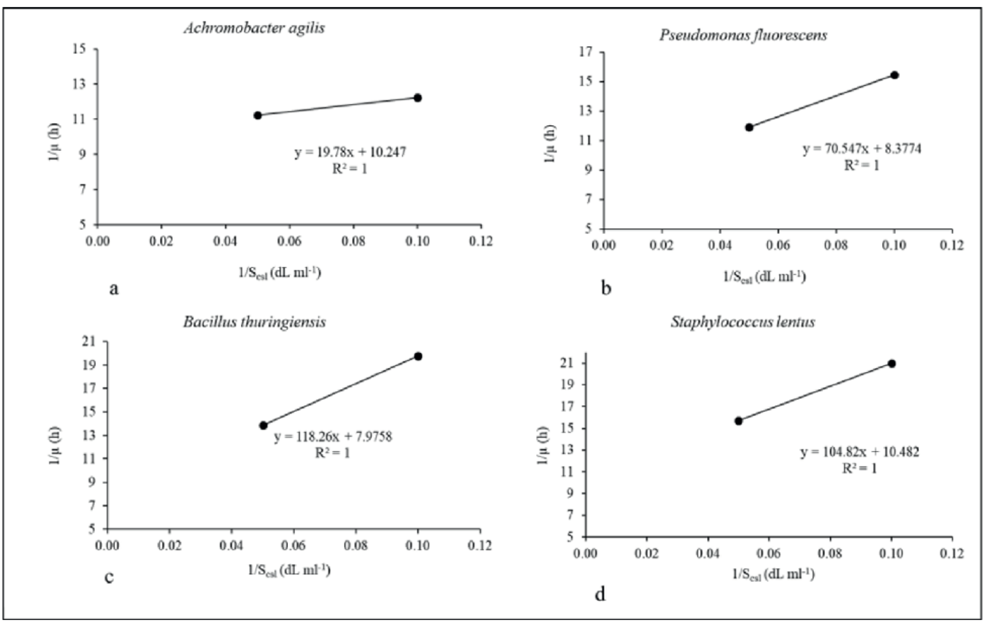

Figure 7.
The Lineweaver-Burk plot for the estimation of μ_m and K_S from the intercept and slope of the linear equation (corn steep liquor) (source: Author study-Agbaji [19]).

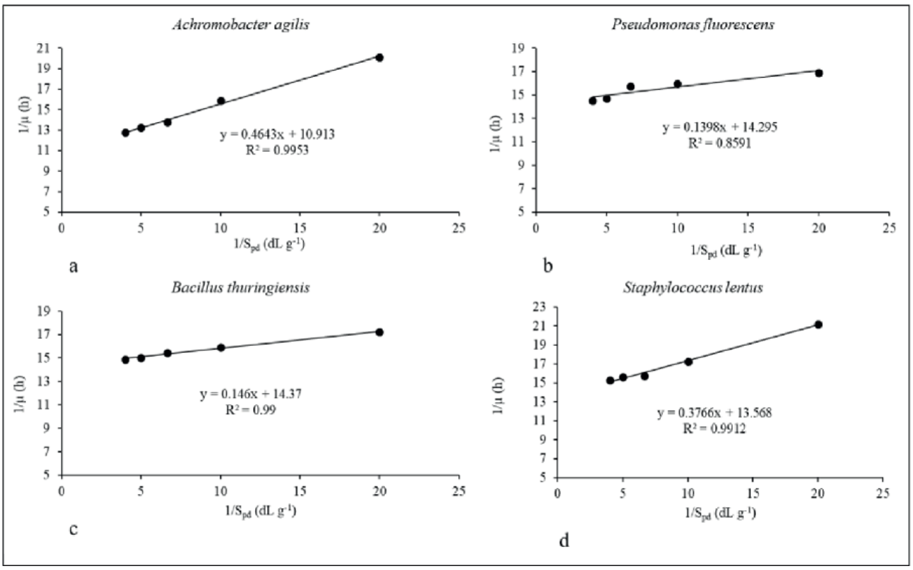

Figure 8.
The Lineweaver-Burk plot for the estimation of μ_m and K_S from the intercept and slope of the linear equation (poultry droppings). (source: Author study-Agbaji [19]).

Carbon substrate	μ_m	K_S	R^2 Value
Corn Chaff	h^{-1}	$g\ dL^{-1}$	
Achromobacter agilis	0.069	0.139	0.978
Pseudomonas fluorescens	0.079	0.120	0.893
Bacillus thuringiensis	0.063	0.140	0.975
Staphylococcus lentus	0.078	0.077	0.943

Table 11.
Parameter affinity estimates; maximum specific growth rate (μ_m) and substrate utilization constant (K_S) for bacterial utilization of corn chaff as a carbon nutrient source.

8.3.1 Interpretation of parameter affinity from the Monod and Lineweaver-Burk plots

The analysis of the estimated kinetic parameters, derived from both the Monod and Lineweaver-Burk plots, provides significant insights into the substrate affinities and growth characteristics of the bacterial isolates under various agro-waste substrates. The affinities of the bacterial isolates for different substrates are detailed below:

For Corn Chaff as the Carbon Source (**Table 11**): The calculated KS values in **Table 11** illustrate that the bacterial isolates exhibit a pronounced affinity for corn chaff as a carbon substrate. The order of affinity is found to be *Staphylococcus lentus* > *Pseudomonas fluorescens* > *Achromobacter agilis* > *Bacillus thuringiensis*. Remarkably, *Pseudomonas fluorescens* exhibits the highest maximum specific growth rate, followed by *Staphylococcus lentus*, *Achromobacter agilis*, and then *Bacillus thuringiensis*.

For Corn Steep Liquor as the Nitrogen Source (**Table 12**): In contrast, **Table 12** demonstrates considerably higher KS values, indicative of diminished affinity for corn steep liquor as a nitrogen substrate. *Bacillus thuringiensis* displays the highest maximum specific growth rate amongst the bacterial isolates, followed by *Pseudomonas fluorescens*, *Achromobacter agilis*, *and Staphylococcus lentus* in sequence. The kinetic values underscore the variations in these indices as predictive factors for modeling the bioremediation potential of the bacterial isolates in hydrocarbon-polluted soil.

For Poultry Droppings as the Phosphorus Source (**Table 13**): The analysis of **Table 13** unveils low KS values, signifying a robust affinity for poultry droppings as a phosphorus substrate. The hierarchy of affinity is *Pseudomonas fluorescens* > *Bacillus thuringiensis* > *Staphylococcus lentus* > *Achromobacter agilis*. *Achromobacter agilis*, however, demonstrates a comparatively higher growth rate in comparison to the other three isolates.

Observations from the Monod and Lineweaver-Burk plots: The Monod model, depicted in **Figure 3**, indicates that *Achromobacter agilis*, *Pseudomonas fluorescens*, *Bacillus thuringiensis*, *and Staphylococcus lentus* all exhibit a strong affinity for corn chaff as a carbon substrate. Notably, within the concentration range beyond 0.5 to 2.0 g dL^{-1}, the substrate concentration surpasses requirements, leading to maximal growth at the specific growth rate (μm) and representing the exponential growth phase of bacterial dynamics.

Similarly, **Figure 4** illustrates that the bacterial isolates manifest limited affinity for corn steep liquor as a nitrogen source. Specifically, concentrations above 10 ml

Nitrogen substrate	μ_m	K_S	R^2 **Value**
Corn steep liquor	h^{-1}	ml dL^{-1}	
Achromobacter agilis	0.098	1.930	1.0
Pseudomonas fluorescens	0.119	8.421	1.0
Bacillus thuringiensis	0.125	14.827	1.0
Staphylococcus lentus	0.095	10.000	1.0
Source: Author study-Agbaji [19].			

Table 12.
Estimated maximum specific growth rate (μ_m) and substrate utilization constant (K_S) for bacterial utilization of corn steep liquor as nitrogen nutrient source.

Phosphorus substrate	μ_m	K_S	R^2 **Value**
Poultry droppings	h^{-1}	g dL^{-1}	
Achromobacter agilis	0.092	0.043	0.995
Pseudomonas fluorescens	0.070	0.010	0.859
Bacillus thuringiensis	0.070	0.010	0.990
Staphylococcus lentus	0.074	0.028	0.991
Source: Author study-Agbaji [19].			

Table 13.
Estimated maximum specific growth rate (μ_m) and substrate utilization constant (K_S) for bacterial utilization of poultry droppings as phosphorus nutrient source.

dL^{-1} for *Achromobacter agilis* and 20 ml dL^{-1} for *Pseudomonas fluorescens*, *Bacillus thuringiensis*, and *Staphylococcus lentus* indicate excessive substrate levels, inducing growth at the maximum specific growth rate (μm), characteristic of the exponential growth phase.

Finally, **Figure 5** highlights the propensity of *Achromobacter agilis*, *Pseudomonas fluorescens*, *Bacillus thuringiensis*, and *Staphylococcus lentus* to prefer poultry droppings as a phosphorus substrate. Within the range of 0.05 to 0.25 g dL^{-1}, the substrate concentration sufficiently meets the demands for maximum growth rate (μm), representing the exponential growth phase. Conversely, concentrations below 0.05 g dL^{-1} are limiting and inadequate to sustain growth at the maximal specific growth rate (μm).

9. Conclusion

In the face of persistent global environmental pollution, stemming from improper waste disposal and inadvertent pollutant release, innovative solutions are essential. The culmination of the research in this book chapter has illuminated the potential of bioremediation cocktails, comprising rhizobacterial flora sourced from impacted areas and readily available agro-waste materials, as a practical and cost-effective strategy for addressing contamination challenges. By amalgamating insights from various facets of study, we can draw comprehensive conclusions that underscore the significance and versatility of this approach.

The study investigation delved into the critical process of isolating and selecting strains of rhizobacteria from crude oil-impacted soil. This stringent procedure involved careful consideration of factors, such as niche specificity, growth kinetics, and hydrocarbon-degrading potential. Through meticulous strain selection, the study demonstrated the pivotal role of rhizobacteria in bioaugmentation, presenting a promising avenue for eco-recovery efforts.

The utilization of agro-waste as substrates for bioremediation has emerged as a practical means to address waste management challenges whilst simultaneously fostering microbial growth. This novel approach capitalizes on the abundant organic matter present in materials like corn chaff, poultry droppings, and corn steep liquor. The study investigations have unveiled the intricate interplay between agro-waste composition, microbial growth kinetics, and pollutant degradation potential. The identification of optimal concentrations for corn chaff, corn steep liquor, and poultry droppings further refines our understanding of the potential of these substrates as drivers of efficient bioremediation.

Central to the study research is the determination of kinetic parameters for bacterial growth in batch culture. Through rigorous experimentation and data analysis, the study quantified growth rates, lag times, and maximum biomass levels for *Achromobacter agilis*, *Pseudomonas fluorescens*, *Bacillus thuringiensis*, and *Staphylococcus lentus*. This comprehensive assessment allowed practitioners to infer the intricate relationships between these parameters, providing crucial insights into the growth dynamics of these bacterial species.

Furthermore, the application of Monod and Lineweaver-Burk models facilitated the estimation of affinity parameters, shedding light on the bacterial isolates' preferences for specific substrates. This mechanistic understanding of substrate affinity and utilization provides valuable guidance for the formulation of effective bioremediation cocktails. The pivotal role of these models in predicting bacterial behavior underscores their applicability in designing tailored strategies for pollutant cleanup.

Following the consolidation of the study findings, it becomes evident that the synthesis of rhizobacterial-based bioremediation cocktails with locally sourced agro-waste holds significant promise for diverse applications. Beyond pollution mitigation, this approach has implications for ecosystem restoration, waste management, and sustainable environmental stewardship. The synergistic amalgamation of cutting-edge research and practical application paves the way for scalable, impactful, and eco-friendly solutions that contribute to a healthier, more resilient planet. In the ever-evolving landscape of environmental conservation, bioremediation cocktails and agro-waste utilization stand as beacons of innovation and hope.

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References

[1] Jeyanthi V, Kanimozhi S. Plant growth promoting rhizobacteria (PGPR)-prospective and mechanisms: A review. Journal of Pure and Applied Microbiology. 2018;**12**(2):733-749

[2] Lynch JM. Beneficial microbes in the rhizosphere - with special reference to wheat. Plant and Soil. 2005;**194**(1-2):1-3

[3] Prathap M, Ranjitha HB. Potential role of plant growth-promoting rhizobacteria in plant defense against soil-borne pathogens. Plant Science Today. 2015;**2**(4):180-185

[4] Kumar V, Saharan BS, Sharma D, Choudhary M. Role of plant growth-promoting rhizobacteria in the remediation of metal-contaminated soils. In: Bioremediation of Industrial Waste for Environmental Safety. Singapore: Springer; 2020. pp. 87-100

[5] Garcia JL, Patel BKC, Ollivier B. Taxonomic, phylogenetic, and ecological diversity of methanogenic archaea. Anaerobe. 2015;**34**:1-3

[6] Chaudhary P, Sharma R, Singh S, Nain L. Bioremediation of PAH by Streptomyces sp. Bulletin of Environmental Contamination and Toxicology. 2011;**86**(3):268-271

[7] Backer R, Rokem JS, Ilangumaran G, Lamont J, Praslickova D, Ricci E, et al. Plant growth-promoting rhizobacteria: Context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. Frontiers in Plant Science. 2018;**9**:1473

[8] Zhang R, Vivanco JM, Shen Q. The unseen rhizosphere root–soil–microbe interactions for crop production. Current Opinion in Microbiology. 2017;**37**:8-14

[9] Smith DL, Gravel V, Yergeau E. Signaling in the phytomicrobiome. Frontiers in Plant Science. 2017;**8**:611

[10] Kumar A, Verma JP, Singh M. Plant growth promoting rhizobacteria (PGPR): A sustainable approach towards improving soil and plant health. In: Microbial Interventions in Agriculture and Environment. Singapore: Springer; 2021. pp. 45-65

[11] Oleńska E, Małek W, Wójcik M, Swiecicka I, Thijs S, Vangronsveld J. Beneficial features of plant growth-promoting rhizobacteria for improving plant growth and health in challenging conditions: A methodical review. Science of the Total Environment. 2020;**743**:140682

[12] Agbaji JE, Nwaichi EO, Abu GO. Optimization of bioremediation cocktail for application in the eco-recovery of crude oil polluted soil. AAS Open Research. 2020;**3**(7):1-25

[13] Abu GO. Process and phenomenal microbiology: How microbes were created to create jobs for mankind. University of Port Harcourt. 2017;**141**(1):5-123

[14] Udume OA, Abu GO, Stanley HO, Vincent-Akpu IF, Momoh Y, Eze MO. Biostimulation of petroleum-contaminated soil using organic and inorganic amendments. Plants. 2023;**12**(3):431

[15] Nwankwegu AS, Orji MU, Onwosi CO. Studies on organic and in-organic biostimulants in bioremediation of diesel-contaminated arable soil. Chemosphere. 2016;**162**:148-156

[16] Ojewumi ME, Emetere ME, Babatunde DE, Okeniyi JO. In situ

bioremediation of crude petroleum oil polluted soil using mathematical experimentation. International Journal of Chemical Engineering. 2017;**2017**:5184760

[17] Ali H, Khan E, Ilahi I. Environmental chemistry and ecotoxicology of hazardous heavy metals: Environmental persistence, toxicity, and bioaccumulation. Journal of Chemistry. 2019;**2019**:2-14. DOI: 10.1155/2019/6730305

[18] Ron EZ, Rosenberg E. Enhanced bioremediation of oil spills in the sea. Current Opinion in Biotechnology. 2014;**27**:191-194

[19] Agbaji JE, Nwaichi EO, Abu GO. Optimized production of bioremediation agent for application in the eco-recovery of oil spill site. In: Paper Presented at the SPE Nigeria Annual International Conference and Exhibition, Virtual. OnePetro.org; Aug 2020. DOI: 10.2118/203654-MS

[20] Gaskin SE, Bentham RH. Rhizoremediation of hydrocarbon-contaminated soil using Australian native grasses. Science of the Total Environment. 2010;**408**(17):3683-3688

[21] Nwilo CP, Badejo TO. Impacts and Management of oil Spill Pollution along the Nigerian Coastal Areas. Lagos, Nigeria: Department of Survey & Geoinformatics, University of Lagos; 2005. pp. 567-570. DOI: 10.7901/2169-3358-2005-1-567

[22] Stepanova AY, Gladkov EA, Osipova ES, Gladkova OV, Tereshonok DV. Bioremediation of soil from petroleum contamination. PRO. 2022;**10**(6):1224

[23] Shinwari KI, Jan M, Shah G, Khattak SR, Urehman S, Daud MK, et al. Seed priming with salicylic acid induces tolerance against chromium (VI) toxicity in rice (*Oryza sativa* L.). Pakistan Journal of Botany. 2015;**47**(SI):161-170

[24] Popoola LT, Yusuff AS, Adeyi AA, Omotara OO. Bioaugmentation and biostimulation of crude oil contaminated soil: Process parameters influence. South African Journal of Chemical Engineering. 2022;**39**(1):12-18

[25] Saadoun I. Isolation and characterization of bacteria from crude petroleum oil contaminated soil and their potential to degrade diesel. Journal of Basic Microbiology. 2002;**42**:420-428

[26] Alori ET, Gabasawa AI, Elenwo CE, Agbeyegbe OO. Bioremediation techniques as affected by limiting factors in soil environment. Frontiers in Soil Science. 2022;**2**:937186

[27] Bhandari S, Poudel DK, Marahatha R, Dawadi S, Khadayat K, Phuyal S, et al. Microbial enzymes used in bioremediation. Journal of Chemistry. 2021;**2021**:1-17

[28] Saravana KP, Amruta SR. Analysis of biodegradation pathway of crude oil by Pseudomonas sp. isolated from marine water sample. Archives of Applied Science Research. 2013;**5**(4):165-171

[29] Mnif S, Chebbi A, Mhiri N, Sayadi S, Chamkha M. Biodegradation of phenanthrene by a bacteria consortium enriched from Sercina oilfield. Process Safety and Environmental Protection. 2017;**107**:44-53

[30] Biache C, Mansuy-Huault L, Faure P. Impact of oxidation and biodegradation on the most commonly used polycyclic aromatic hydrocarbon (PAH) diagnostic ratios: Implications for the source identifications. Journal of Hazardous Materials. 2014;**267**:31-39

[31] Gennadiev AN, Pikovskii YI, Zhidkin AP, Kovach RG, Koshovskii TS, Smirnova MA, et al. Factors and features of the hydrocarbon status of soils. Eurasian Soil Science. 2015;**48**:1193-1206

[32] Atashgahi S, Sánchez-Andrea I, Heipieper HJ, van der Meer JR, Stams AJ, Smidt H. Prospects for harnessing biocide resistance for bioremediation and detoxification. Science. 2018;**360**(6390):743-746

[33] Agarry SE, Ogunleye OO. Box-Behnken design application to study enhanced bioremediation of soil artificially contaminated with spent engine oil using biostimulation strategy. International Journal of Energy and Environmental Engineering. 2012;**3**:1-14

[34] Tang J, Lu X, Sun Q, Zhu W. Aging effect of petroleum hydrocarbons in soil under different attenuation conditions. Agriculture, Ecosystems & Environment. 2012;**149**:109-117

[35] Ng EL, Lwanga EH, Eldridge SM, Johnston P, Hu HW, Geissen V, et al. An overview of microplastic and nanoplastic pollution in agroecosystems. Science of the Total Environment. 2018;**627**:1377-1388

[36] Uquetan UI, Osang JE, Egor AO, Essoka PA, Alozie SI, Bawan AM. A case study of the effects of oil pollution on soil properties and growth of tree crops in Cross River State, Nigeria. International Research Journal of Pure and Applied Physics. 2017;**5**(2):19-28

[37] Udoh BT, Chukwu ED. Post-impact assessment of oil pollution on some soil characteristics in Ikot Abasi, Niger Delta region, Nigeria. Journal of Biology, Agriculture and Healthcare. 2014;**4**(24):111-119

[38] Obieze CC, Chikere CB, Adeleke R, Selvarajan R, Ntushelo K, Akaranta O. Field-scale biostimulation shifts microbial community composition and improves soil pollution recovery at an artisanal crude oil refining site. International Journal of Environmental Studies. 2022;**26**:1-20

[39] Chikere CB, Tekere M, Adeleke R. Microbial communities in field-scale oil-polluted soil remediation using 16S rRNA amplicon sequencing. International Journal of Environmental Studies. 2021;**78**(3):410-426

[40] Effiong E, Agwa O, Abu GO. Niche-proxies of hydrocarbon-impacted rhizosphere soil of weeds of Bodo in Gokana, Rivers state, Nigeria. Archives of Current Research International. 2020;**19**(4):1-14

[41] Singer AC, van der Gast CJ, Thompson IP. Perspectives and vision for strain selection in bioaugmentation. Trends in Biotechnology. 2005;**23**(2):74-77

[42] Devianti D, Yusmanizar Y, Syakur S, Munawar A, Yunus Y. Organic fertilizer from agricultural waste: Determination of phosphorus content using near infrared reflectance. IOP Conference Series: Earth and Environmental Science. 2021;**644**:012002. DOI: 10.1088/1755-1315/644/1/012002

[43] Chakraborty D, Chatterjee S, Althuri A, Mohan SV. Sustainable enzymatic treatment of organic waste in a framework of circular economy. Bioresource Technology. 2022;**370**:128487

[44] Iheukwumere CL. The use of agro-wastes as recovery tool of soil contaminated with spent engine oil: Using Okra (*Abelmoschus Esculentus*) growth indices as indicator [thesis]. 2018

[45] Sadh PK, Duhan S, Duhan JS. Agro-industrial wastes and their utilization using solid-state fermentation: A review. Bioresources and Bioprocessing. 2018;**5**(1):1-15

[46] Graminha EBN, Gonçalves AZL, Pirota RDPB, Balsalobre MAA, Da Silva R, Gomes E. Enzyme production by solid-state fermentation: Application to animal nutrition. Animal Feed Science and Technology. 2008;**144**(1-2):1-22

[47] Iheanacho GC, Ibiene AA, Okerentugba PO. Treatment of Port Harcourt refinery effluent by a bacterial consortium immobilized on agro-based bio carriers. Asian Journal of Biotechnology and Bioresource Technology. 2019;**5**(4):1-10

[48] Effiong E, Agwa OK, Abu GO. Algal-biomass production from Chlorella sp. using hot and cold water infusions of poultry droppings. Asian Journal of Biotechnology and Bioresource Technology. 2019;**4**(4):1-9

[49] Kalsi A, Celin SM, Bhanot P, Sahai S, Sharma JG. A novel eggshell-based bio formulation for remediation of RDX (hexahydro-1, 3, 5-trinitro-1, 3, 5-triazine) contaminated soil. Journal of Hazardous Materials. 2021;**401**:123346

[50] Dabrowska M, Debiec-Andrzejewska K, Andrunik M, Bajda T, Drewniak L. The biotransformation of arsenic by spent mushroom compost–an effective bioremediation agent. Ecotoxicology and Environmental Safety. 2021;**213**:112054

[51] Dike CC, Shahsavari E, Surapaneni A, Shah K, Ball AS. Can biochar be an effective and reliable biostimulating agent for the remediation of hydrocarbon-contaminated soils? Environment International. 2021;**154**:106553. ISSN 0160-4120. DOI: 10.1016/j.envint.2021.106553

[52] Sadañoski MA, Benítez SF, Fonseca MI, Velázquez JE, Zapata PD, Levin LN, et al. Mycoremediation of high concentrations of polychlorinated biphenyls with pleurotus sajor-caju LBM 105 as an effective and cheap treatment. Journal of Environmental Chemical Engineering. 2019;**7**(6):103453

[53] Omotosho J, Momoh OLY, Ikebude CF. Application of bone char in bioremediation of crude oil polluted soil: Optimization and kinetic analysis. Journal of Research Information in Civil Engineering. 2018;**15**(4):2361-2374

[54] Akpe AR, Ekundayo AO, Aigere SP, Okwu GI. Bacterial degradation of petroleum hydrocarbons in crude oil-polluted soil amended with cassava peels. American Journal of Research Communication. 2015;**3**(7):99-118

[55] Nwogu TP, Azubuike CC, Ogugbue CJ. Enhanced bioremediation of soil artificially contaminated with petroleum hydrocarbons after amendment with Capra aegagrus hircus (goat) manure. Biotechnology Research International. 2015;**2015**:657349

[56] Hamoudi-Belarbi L, Hamoudi S, Belkacemi K, Nouri LH, Bendifallah L, Khodja M. Bioremediation of polluted soil sites with crude oil hydrocarbons using carrot peels waste. Multidisciplinary Digital Publishing Institute Journal of Environment. 2018;**5**(124):1-11

[57] Aghalibe CU, Igwe JC, Obike AI. Studies on the removal of petroleum hydrocarbon (PHCs) from a crude oil impacted soil amended with cow dung, poultry manure and NPK fertilizer. Chemistry Research Journal. 2017;**2**(4):22-30

[58] Naik B, Kumar V, Rizwanuddin S, Chauhan M, Gupta AK, Rustagi S, et al.

Agro-industrial waste: A cost-effective and eco-friendly substrate to produce amylase. Food Production, Processing and Nutrition. 2023;**5**(1):30

[59] Curti E, Carini E, Bonacini G, Tribuzio G, Vittadini E. Effect of the addition of bran fractions on bread properties. Journal of Cereal Science. 2013;**57**(3):325-332

[60] Alauddina M, Islama J, Shirakawaa H, Kosekib T, Ardiansyahc KM, Komaia M. Rice bran as a functional food: An overview of the conversion of rice bran into a superfood/functional food. Superfood and Functional Food. An Overview of Their Processing and Utilization. 2017;**14**(1):291-305. DOI: 10.5772/66298

[61] Karp SG, Woiciechowski AL, Soccol VT, Soccol CR. Pretreatment strategies for delignification of sugarcane bagasse: A review. Brazilian Archives of Biology and Technology. 2013;**56**:679-689

[62] Gizaw A, Zewge F, Kumar A, Mekonnen A, Tesfaye M. A comprehensive review on nitrate and phosphate removal and recovery from aqueous solutions by adsorption. AQUA—Water Infrastructure, Ecosystems and Society. 2021;**70**(7):921-947

[63] Berkessa YW, Mereta ST, Feyisa FF. Simultaneous removal of nitrate and phosphate from wastewater using solid waste from factory. Applied Water Science. 2019;**9**:1-10

[64] Mor S, Chhoden K, Ravindra K. Application of agro-waste rice husk ash for the removal of phosphate from the wastewater. Journal of Cleaner Production. 2016;**129**:673-680

[65] Husain K, Ansari RA, Ferder L. Pharmacological agents in the prophylaxis/treatment of organophosphorous pesticide intoxication. Indian Journal of Experimental Biology. 2010;**48**(7):642-650

[66] Peltzer PM, Lajmanovich RC, Sánchez-Hernandez JC, Cabagna MC, Attademo AM, Bassó A. Effects of agricultural pond eutrophication on survival and health status of *Scinax nasicus* tadpoles. Ecotoxicology and Environmental Safety. 2008;**70**(1):185-197

[67] Williams KF, Agwa OK. Optimization of chlorella-biomass production using domestic and restaurant waste water as a potential feedstock. Microbiology Research Journal International. 2021;**31**(12):14-22

[68] Fuentes B, Bolan N, Naidu R, Mora MDLL. Phosphorus in organic waste-soil systems. Journal of Soil Science and Plant Nutrition. 2006;**6**(2):64-83

[69] Kova K, Egli T. Growth kinetics of suspended microbial cells: From single- substrate-controlled growth to mixed-substrate kinetics. Microbiology and Molecular Biology Reviews. 1998;**62**(3):646-666

[70] Talaiekhozani A, Jafarzadeh N, Fulazzaky MA, Talaie MR, Beheshti M. Kinetics of substrate utilization and bacterial growth of crude oil degraded by pseudomonas. Journal of Environmental Health Science and Engineering. 2015;**13**(64):1-8

[71] Maier RM, Pepper IL, Gerba CP. Environmental Microbiology. San Diego: Academic Press; 2009. p. 397

Chapter 6

Emerging Knowledge and Latest Applications of Rhizobacteria

Maxine Atuheirwe

Abstract

This book chapter explores the emerging knowledge and latest applications of rhizobacteria in various fields, including agriculture, environmental remediation, and biotechnology. Rhizobacteria, a diverse group of bacteria that colonize the rhizosphere, has shown immense potential in promoting plant growth, enhancing nutrient uptake, and combating plant pathogens. This chapter provides an overview of the recent advancements in understanding the mechanisms of rhizobacteria-plant interactions and highlights their practical applications in sustainable agriculture, soil health improvement, and ecosystem restoration. Furthermore, it discusses the potential of rhizobacteria in the bioremediation of pollutants and their role in enhancing plant stress tolerance. The chapter concludes by identifying future research directions and the potential impact of rhizobacteria in addressing global challenges related to food security, environmental sustainability, and human health.

Keywords: rhizobacteria, rhizobacteria-plant interactions, mechanisms, induced resistance, plant stress

1. Introduction

1.1 Definition and importance of rhizobacteria

Rhizobacteria, also known as plant growth-promoting rhizobacteria (PGPR), are a diverse group of bacteria that reside in the rhizosphere-soil region directly surrounding plant roots [1, 2]. These bacteria have a mutually beneficial relationship with plants and play a vital role in promoting plant growth and health [2]. Rhizobacteria have gained significant attention in agriculture and environmental research due to their numerous beneficial effects on plants [1].

The importance of rhizobacteria lies in their ability to enhance plant growth and improve plant health through various mechanisms [3, 4]. One of the key functions of rhizobacteria is their ability to fix atmospheric nitrogen and make it available to plants in a usable form [5]. Nitrogen fixation is crucial for plant growth, as nitrogen is an essential nutrient required for the synthesis of proteins, enzymes, and chlorophyll [3]. By converting atmospheric nitrogen into ammonium, rhizobacteria contribute to the overall nitrogen availability in the soil, thereby promoting plant growth and development [3, 4].

In addition to nitrogen fixation, rhizobacteria also can solubilize and mineralize other nutrients such as phosphorus, potassium, and iron, making them more accessible to plants [6]. These bacteria produce enzymes and organic acids that break down complex organic matter in the soil, releasing nutrients that would otherwise be unavailable to plants [3]. This nutrient solubilization and mineralization by rhizobacteria contribute to improved nutrient uptake and utilization by plants, leading to enhanced growth and productivity [7].

Rhizobacteria also exhibit plant growth-promoting traits through the production of phytohormones such as auxins, cytokinins, and gibberellins [8]. These hormones play essential roles in regulating plant growth and development, including cell division, elongation, and differentiation [8]. By producing these hormones, rhizobacteria can stimulate root growth, enhance nutrient absorption, and improve overall plant vigor [8]. Furthermore, rhizobacteria possess the ability to suppress plant pathogens and protect plants against various diseases [9]. They do so through multiple mechanisms, including the production of antibiotics, competition for nutrients and space, induction of systemic resistance, and the secretion of enzymes that degrade the cell walls of pathogens [9]. This biocontrol activity of rhizobacteria helps reduce the dependence on chemical pesticides and promotes sustainable agricultural practices [9].

Another crucial aspect of rhizobacteria is their role in enhancing plant tolerance to abiotic stresses such as drought, salinity, and heavy metal toxicity [10]. These bacteria produce compounds called osmoprotectants and enzymes that scavenge harmful reactive oxygen species, thereby reducing stress-induced damage to plants [10]. By improving plant stress tolerance, rhizobacteria can enable plants to survive and thrive under adverse environmental conditions [9].

In summary, rhizobacteria are beneficial bacteria that colonize the rhizosphere and interact with plant roots, promoting plant growth, health, and stress tolerance. Their ability to fix nitrogen, solubilize nutrients, produce phytohormones, suppress pathogens, and enhance stress tolerance makes them invaluable in sustainable agriculture, ecological restoration, and environmental remediation. Harnessing the potential of rhizobacteria holds promise for reducing chemical inputs, improving crop productivity, and promoting environmentally friendly agricultural practices.

2. Scope and objectives of the chapter

2.1 Rhizosphere: the interface for interaction

The rhizosphere is the dynamic interface where plant roots and the surrounding soil interact, and it serves as a crucial site for the establishment and functioning of rhizobacteria-plant interactions [11, 12]. This specialized zone is characterized by a high concentration of organic compounds, root exudates, and microbial activity, creating a unique microenvironment that supports diverse microbial populations, including rhizobacteria [11].

Rhizobacteria play a fundamental role in shaping the rhizosphere and influencing plant health and development [11]. The interaction between rhizobacteria and plants in this zone is a complex and dynamic process that involves a range of mechanisms and signals [9, 12, 13]. One of the primary drivers of rhizobacteria-plant interactions is the release of root exudates by plants [14]. These exudates consist of a diverse array of organic compounds, including sugars, amino acids, organic acids, enzymes, and secondary metabolites [14]. Root exudates act as an energy source for rhizobacteria, attracting them to the rhizosphere [11]. Rhizobacteria, in turn, respond to these exudates by colonizing the root surface or entering the root system, establishing a close association with the plant [3].

Rhizobacteria exhibit various modes of colonization within the rhizosphere [7]. They can attach to the root surface, forming biofilms or colonizing root hairs, or they can enter the root tissues through natural openings or wounds [7]. Some rhizobacteria are even capable of penetrating the root cell walls and entering the intracellular spaces, forming endophytic associations [4]. The specific colonization strategies employed by rhizobacteria depend on the bacterial species and the plant host [7]. Once established in the rhizosphere or within the plant tissues, rhizobacteria engage in intricate interactions with plants [6, 7]. These interactions can be mutualistic, where both the bacteria and the plant benefit, or they can be commensalism, where the bacteria benefit without harming the plant [6]. Rhizobacteria promote plant growth and health through a variety of mechanisms [8].

One of the key mechanisms employed by rhizobacteria is the production of plant growth-promoting substances, such as phytohormones [8]. These substances include auxins, cytokinins, and gibberellins, which regulate various aspects of plant growth and development [8]. Rhizobacteria can stimulate root elongation, lateral root formation, and nutrient uptake by producing these phytohormones, thereby enhancing plant growth [15]. In addition to phytohormone production, rhizobacteria contribute to plant nutrient acquisition [6]. They can solubilize and mineralize nutrients present in the soil, making them more accessible to plants [6]. By secreting enzymes and organic acids, rhizobacteria break down complex organic matter and release nutrients such as phosphorus, potassium, and iron, which are vital for plant growth [4, 7]. This nutrient solubilization and mineralization increase nutrient availability in the rhizosphere, supporting plant nutrition [7]. Furthermore, rhizobacteria can protect plants against pathogens by producing antimicrobial compounds or inducing systemic resistance [9]. They can also compete with pathogenic microorganisms for space and nutrients, limiting their growth and colonization [2]. Through these mechanisms, rhizobacteria help suppress plant diseases and enhance plant defense mechanisms [9]. Moreover, rhizobacteria can assist plants in tolerating abiotic stresses [10]. They can produce osmoprotectants or enzymes that scavenge reactive oxygen species, mitigating the damage caused by drought, salinity, or heavy metal toxicity [10]. Rhizobacteria-induced stress tolerance enables plants to cope with adverse environmental conditions and maintain productivity [10].

In conclusion, the rhizosphere serves as the interface for interaction between rhizobacteria and plants. Root exudates attract rhizobacteria, which then colonize the rhizosphere or enter the plant tissues. Rhizobacteria-plant interactions in the rhizosphere contribute to plant growth, nutrient acquisition, disease suppression, and stress tolerance. Understanding and harnessing these interactions offer great potential for improving crop productivity, reducing chemical inputs, and promoting sustainable agricultural practices.

2.2 Mechanisms of rhizobacteria-plant interactions

The interactions between rhizobacteria and plants are mediated through a variety of mechanisms, allowing for a range of beneficial effects on plant growth, health, and stress tolerance [6, 12, 13]. Understanding these mechanisms is essential for harnessing the potential of rhizobacteria in agriculture and environmental applications. Here are some key mechanisms involved in rhizobacteria-plant interactions:

Induced systemic resistance (ISR): rhizobacteria can stimulate the plant's innate defense mechanisms through the ISR [12]. They trigger a systemic response in plants, leading to enhanced resistance against pathogens [12]. Rhizobacteria can produce compounds that activate plant defense pathways, resulting in the accumulation of antimicrobial substances, strengthening of cell walls, and production of defense-related proteins [12].

Phytohormone production: rhizobacteria can synthesize and release phytohormones, such as auxins, cytokinins, and gibberellins [6]. These hormones play critical roles in plant growth and development [6]. Rhizobacteria-produced phytohormones can stimulate root elongation, lateral root formation, and nutrient uptake, promoting overall plant growth and vigor [6].

1. Nutrient solubilization and mineralization: rhizobacteria possess the ability to solubilize and mineralize nutrients in the rhizosphere, making them more available for plant uptake [13]. Through the secretion of enzymes and organic acids, rhizobacteria break down complex organic matter and release nutrients, such as phosphorus, potassium, and iron [13]. This nutrient solubilization and mineralization contribute to improved plant nutrition and growth [7].

2. Nitrogen fixation: certain rhizobacteria, such as nitrogen-fixing bacteria, can convert atmospheric nitrogen (N_2) into a plant-usable form, ammonium (NH_4^+) [6]. This process, known as nitrogen fixation, provides plants with a vital nutrient required for various metabolic processes, including protein synthesis [6]. Rhizobacteria that establish symbiotic relationships with leguminous plants form specialized structures called nodules, where nitrogen fixation occurs [4].

3. Biocontrol of pathogens: rhizobacteria can act as biocontrol agents by suppressing plant pathogens [9]. They produce antimicrobial compounds, including antibiotics, volatile organic compounds, and lytic enzymes, that inhibit the growth and activity of pathogenic microorganisms [9]. Rhizobacteria can also compete with pathogens for space and nutrients, limiting their colonization and establishment on plant surfaces [8].

Stress tolerance enhancement: rhizobacteria play a role in improving plant tolerance to abiotic stresses, such as drought, salinity, and heavy metal toxicity [10]. They can produce osmoprotectants, which help plants maintain cellular water balance under water-deficit conditions [16]. Rhizobacteria can also produce enzymes that scavenge reactive oxygen species, reducing oxidative stress caused by environmental factors. By enhancing stress tolerance, rhizobacteria enable plants to withstand adverse conditions and maintain productivity [16].

4. Root colonization and biofilm formation: rhizobacteria establish intimate associations with plant roots by colonizing the root surface or entering the root tissue [11, 16]. They can form biofilms, which are complex microbial communities encased in a self-produced matrix, on root surfaces. Biofilms provide protection and enhanced nutrient availability for rhizobacteria, while also influencing root architecture and function [11].

These mechanisms collectively contribute to the beneficial effects of rhizobacteria on plant growth, nutrition, disease suppression, and stress tolerance [16]. The specific mechanisms employed by rhizobacteria can vary depending on the bacterial species, plant host, and environmental conditions [17]. Exploring and harnessing these mechanisms offers opportunities for sustainable agriculture, improved crop productivity, and ecological restoration.

2.3 Signaling pathways and induced systemic resistance

Signaling pathways and induced systemic resistance concerning rhizobacteria induced systemic resistance (ISR) is a defense mechanism in plants that is activated by certain beneficial microorganisms, including rhizobacteria [12]. Through signaling pathways, plants can perceive the presence of rhizobacteria and initiate a systemic response that enhances their resistance to various pathogens and pests [12]. Understanding the signaling pathways involved in ISR is crucial for harnessing the potential of rhizobacteria in sustainable agriculture [18].

Here are the key signaling pathways and their role in induced systemic resistance:

1. Jasmonic acid (JA) pathway: the jasmonic acid pathway is one of the major signaling pathways involved in the ISR [18]. When plants perceive the presence of rhizobacteria or their elicitors, it triggers the biosynthesis of jasmonic acid, a plant hormone involved in defense responses [18]. The accumulation of jasmonic acid leads to the activation of downstream defense-related genes, including those involved in the production of defensive compounds such as protease inhibitors and volatile organic compounds [18]. These compounds contribute to the plant's resistance against pathogens and pests.

2. Salicylic acid (SA) pathway: the salicylic acid pathway is another essential signaling pathway associated with the ISR [19]. It plays a crucial role in defense against biotrophic pathogens, such as fungi and bacteria When plants recognize rhizobacteria or their elicitors, it triggers the accumulation of salicylic acid, which acts as a signaling molecule in the defense response [19]. Salicylic acid induces the expression of defense-related genes, including pathogenesis-related (PR) genes, which produce proteins with antimicrobial properties [19]. Activation of the salicylic acid pathway results in enhanced resistance against a wide range of pathogens [19].

3. Ethylene (ET) pathway: ethylene is a gaseous plant hormone that plays a significant role in various physiological processes, including defense responses [20]. In the context of ISR, rhizobacteria can induce the production of ethylene in plants, leading to the activation of defense mechanisms [20]. Ethylene influences plant growth and defense by regulating the expression of genes involved in stress responses, cell wall fortification, and the production of defense-related metabolites [20]. Ethylene signaling contributes to the overall resistance of plants to pathogens and pests [20].

4. Systemin-mediated pathway: the systemin-mediated pathway is involved in long-distance signaling and communication between different plant tissues [21]. In response to rhizobacteria or their elicitors, plants can produce a small peptide called systemin, which acts as a signaling molecule [22]. Systemin is transported to other parts of the plant, where it triggers a cascade of events leading to the activation of defense responses [22]. This pathway plays a role in systemic acquired

resistance (SAR) and can enhance plant resistance to a broad spectrum of pathogens [23]. These signaling pathways intricately interact and engage in crosstalk to coordinate a comprehensive defense response in plants [23]. The recognition of rhizobacteria or their elicitors triggers the activation of these pathways, leading to the induction of defense-related genes and the production of antimicrobial compounds, enzymes, and other defensive metabolites [22].

These systemic responses provide long-lasting protection to plants against various pathogens and pests, even in parts of the plant that are not directly exposed to beneficial microorganisms [12, 20]. By understanding these pathways, researchers can further unravel the molecular mechanisms underlying ISR and optimize the use of rhizobacteria for sustainable crop protection. Harnessing the potential of rhizobacteria-induced systemic resistance offers a promising avenue for reducing the reliance on chemical pesticides, promoting environmentally friendly agricultural practices, and enhancing crop productivity.

2.4 Applications of rhizobacteria in agriculture

Rhizobacteria, or plant growth-promoting rhizobacteria (PGPR), have gained significant attention in agriculture due to their beneficial effects on plants [2]. The applications of rhizobacteria in agriculture are diverse and hold great promise for sustainable and environmentally friendly practices [4]. Here are some key applications of rhizobacteria in agriculture:

1. Enhanced nutrient availability: rhizobacteria can solubilize and mineralize nutrients in the soil, making them more available to plants [7]. By secreting enzymes and organic acids, rhizobacteria break down complex organic matter and release essential nutrients such as phosphorus, potassium, and iron [7]. This enhances nutrient uptake by plants, reduces the reliance on synthetic fertilizers, and promotes efficient nutrient utilization.

2. Nitrogen fixation: certain rhizobacteria, known as nitrogen-fixing bacteria, can form symbiotic associations with leguminous plants [2]. These bacteria colonize specialized structures called root nodules and convert atmospheric nitrogen into a usable form for plants [7]. This biological nitrogen fixation reduces the need for nitrogen fertilizers, improves soil fertility, and enhances plant growth and productivity [6].

3. Disease suppression: rhizobacteria can suppress plant diseases through various mechanisms. They produce antimicrobial compounds, such as antibiotics and siderophores, that inhibit the growth of plant pathogens [12, 20]. Rhizobacteria also compete with pathogens for nutrients and space, limiting their colonization and spread [12, 20]. Additionally, rhizobacteria can induce systemic resistance in plants, activating their defense mechanisms and enhancing their ability to withstand pathogen attacks [12].

4. Stress tolerance: rhizobacteria play a crucial role in enhancing plant tolerance to abiotic stresses, such as drought, salinity, and heavy metal toxicity [12]. They produce osmoprotectants, enzymes, and antioxidants that mitigate stress-

induced damage and improve plant survival under adverse conditions [20]. Rhizobacteria-induced stress tolerance helps plants maintain productivity and adapt to challenging environments [12, 20].

5. Plant growth promotion: rhizobacteria promote plant growth through various mechanisms. They produce phytohormones, such as auxins, cytokinins, and gibberellins, which regulate plant growth and development [6]. Rhizobacteria can stimulate root elongation, lateral root formation, and nutrient uptake, leading to enhanced plant growth and vigor [7]. Furthermore, they improve soil structure and nutrient cycling, contributing to overall plant health and productivity [2].

6. Environmental remediation: rhizobacteria have applications in environmental remediation and the restoration of degraded ecosystems. They can degrade or detoxify pollutants, such as hydrocarbons, heavy metals, and pesticides, through the production of enzymes and metabolic activities [24]. Rhizobacteria facilitate the breakdown and transformation of these pollutants, reducing their harmful effects on the environment and promoting soil and water quality [24].

7. Biofertilizers and biocontrol agents: rhizobacteria are utilized as biofertilizers and biocontrol agents in sustainable agricultural practices [25]. Biofertilizers containing nitrogen-fixing bacteria or nutrient-solubilizing bacteria provide a natural and eco-friendly alternative to synthetic fertilizers, reducing nutrient runoff and environmental pollution [25]. Biocontrol agents based on rhizobacteria can effectively manage plant diseases and pests, reducing the reliance on chemical pesticides and promoting ecological balance in the agroecosystems [25].

These applications of rhizobacteria in agriculture demonstrate their potential for improving crop productivity, reducing chemical inputs, enhancing soil health, and promoting sustainable farming practices. Incorporating rhizobacteria-based strategies in agriculture can contribute to food security, environmental sustainability, and the development of resilient farming systems. Ongoing research and innovation in this field continue to expand the range of applications and optimize the utilization of rhizobacteria for maximum agricultural benefits.

2.5 Rhizobacteria in environmental remediation

Rhizobacteria, or plant growth-promoting rhizobacteria (PGPR), have shown significant potential in environmental remediation, which involves the restoration and cleanup of polluted or contaminated environments [26]. These beneficial bacteria can play a crucial role in the degradation, detoxification, and removal of various pollutants, making them valuable tools for addressing environmental challenges [27]. Here are the key aspects of using rhizobacteria in environmental remediation:

1. Pollutant degradation: rhizobacteria can degrade a wide range of pollutants, including hydrocarbons, pesticides, heavy metals, and organic contaminants [26]. They produce enzymes, such as dehydrogenases, oxidases, and hydrolases, that can break down complex organic molecules into simpler and less toxic forms [26]. By utilizing these enzymatic activities, rhizobacteria can contribute to the degradation and mineralization of pollutants in the environment [26].

2. Detoxification and transformation: rhizobacteria can detoxify pollutants by transforming them into less toxic or non-toxic compounds [27]. For example, certain rhizobacteria can degrade pesticides into harmless metabolites through enzymatic reactions [27]. They can also immobilize heavy metals by transforming them into less bioavailable forms, reducing their toxicity and potential for environmental contamination [27].

3. Phytoextraction and phytostabilization: rhizobacteria can facilitate the process of phytoextraction and phytostabilization in contaminated environments [28]. Phytoextraction involves the use of plants to absorb and accumulate pollutants from the soil, while phytostabilization aims to reduce the mobility and bioavailability of contaminants [28]. Rhizobacteria can enhance these processes by promoting plant growth, improving nutrient uptake, and facilitating the mobilization and transformation of pollutants, thereby aiding in the removal or immobilization of contaminants [28].

4. Bioremediation and biodegradation: rhizobacteria-based bioremediation is a strategy that harnesses the metabolic capabilities of bacteria to degrade or remove pollutants from contaminated sites [27]. In this approach, rhizobacteria are introduced into the contaminated environment to enhance the natural microbial degradation processes [27]. The bacteria can stimulate the growth of indigenous microorganisms, provide enzymes and co-factors required for pollutant degradation, and create favorable conditions for biodegradation to occur [27].

5. Soil and water quality improvement: rhizobacteria play a crucial role in improving soil and water quality in contaminated environments [26, 29]. By degrading or transforming pollutants, they can reduce the concentrations of toxic compounds, thereby enhancing soil fertility and promoting the growth of beneficial organisms [27]. Rhizobacteria can also enhance nutrient cycling, improve soil structure, and contribute to the overall health and resilience of ecosystems [29].

6. Bioaugmentation and biostimulation: bioaugmentation involves the deliberate introduction of selected rhizobacteria strains into contaminated environments to enhance pollutant degradation [29]. These strains are chosen based on their specific metabolic capabilities and compatibility with the target contaminants [30]. Biostimulation, on the other hand, aims to stimulate the activity of indigenous rhizobacteria through the addition of nutrients or other growth-promoting substances [31]. Both bioaugmentation and biostimulation approaches can enhance the effectiveness of remediation efforts and accelerate the degradation of [30].

The application of rhizobacteria in environmental remediation offers a sustainable and eco-friendly approach to addressing pollution and restoring contaminated environments. Their potential for pollutant degradation, detoxification, phytoextraction, and bioremediation makes them valuable tools in the cleanup of contaminated soils, sediments, and water bodies. Ongoing research and development in this field continue to improve our understanding of rhizobacteria's capabilities and optimize their use in environmental remediation practices.

2.6 Rhizobacteria for biotechnological applications

Rhizobacteria, or plant growth-promoting rhizobacteria (PGPR), have gained significant interest in biotechnology due to their diverse and beneficial properties. These bacteria possess unique characteristics that make them valuable for various biotechnological applications. Here are some key areas where rhizobacteria are utilized in biotechnology:

1. Agriculture and crop improvement: rhizobacteria have substantial applications in agriculture for enhancing crop productivity and sustainability [4]. They can promote plant growth by fixing atmospheric nitrogen, solubilizing nutrients, producing phytohormones, and improving nutrient uptake and utilization [4]. These capabilities make rhizobacteria ideal candidates for developing biofertilizers and bioinoculants, which can reduce the reliance on chemical fertilizers and enhance soil fertility [4]. Additionally, rhizobacteria-based biocontrol agents can protect plants from diseases and pests, reducing the need for synthetic pesticides [4].

2. Bioremediation and environmental cleanup: rhizobacteria play a vital role in environmental biotechnology by assisting in the cleanup and restoration of polluted or contaminated environments [27]. Their ability to degrade various pollutants, including hydrocarbons, pesticides, and heavy metals, makes them valuable tools in bioremediation processes [27]. Rhizobacteria can be used in bioaugmentation or biostimulation strategies to enhance the natural degradation processes and facilitate the removal or transformation of pollutants in soil, water, and sediments [27].

3. Phytoremediation enhancement: phytoremediation is a plant-based approach for removing contaminants from the environment [32]. Rhizobacteria can enhance the effectiveness of phytoremediation by improving plant growth, nutrient uptake, and pollutant degradation [32]. They can promote the establishment of beneficial plant-microbe interactions, such as mycorrhizal associations, which further enhance the plants' ability to remediate contaminated sites [32]. Rhizobacteria can also facilitate the mobilization and transformation of pollutants, aiding in their uptake and detoxification by plants [32].

4. Biocontrol agents and biological pest management: rhizobacteria have the potential to be used as biocontrol agents for managing plant diseases and pests [24]. They can produce antimicrobial compounds, compete with pathogens for resources, induce systemic resistance in plants, and interfere with pathogen signaling pathways [24]. Rhizobacteria-based biocontrol agents offer a sustainable and environmentally friendly alternative to chemical pesticides, reducing the impact on ecosystems and human health [24].

5. Bioprospecting for novel bioactive compounds: rhizobacteria represent a rich source of bioactive compounds with potential applications in medicine, pharmaceuticals, and other industries [33]. These bacteria produce diverse secondary metabolites, enzymes, and other bioactive substances that can have antimicrobial, anticancer, antifungal, or antioxidant properties [33]. Bioprospecting efforts aim to identify and characterize these bioactive compounds for various biotechnological applications, including drug development, industrial enzymes, and biocatalysis [33].

6. Plant-microbe interactions and synthetic biology: studying the interactions between rhizobacteria and plants provides insights into the molecular mechanisms underlying beneficial plant-microbe associations [6, 11]. Advances in synthetic biology and genetic engineering allow researchers to manipulate these interactions and engineer plants with enhanced traits. For example, introducing specific genes from rhizobacteria into plants can confer stress tolerance, improve nutrient uptake, or enhance disease resistance [6, 11]. These approaches have the potential to revolutionize crop improvement and contribute to sustainable agriculture [6, 11].

The applications of rhizobacteria in biotechnology highlight their diverse potential and their role in addressing various challenges in agriculture, environmental remediation, and beyond. Ongoing research and innovation in this field continue to uncover new possibilities and optimize the utilization of rhizobacteria for sustainable and beneficial biotechnological applications.

2.7 Rhizobacteria and plant stress tolerance

Plants are constantly exposed to various environmental stresses, such as drought, salinity, temperature extremes, and heavy metal toxicity, which can significantly impact their growth, development, and productivity. Rhizobacteria, or plant growth-promoting rhizobacteria (PGPR), have emerged as valuable allies in enhancing plant stress tolerance [12, 20, 33, 34]. Through a range of mechanisms, rhizobacteria can help plants withstand and overcome adverse environmental conditions. Here are the key ways in which rhizobacteria contribute to plant stress tolerance:

1. Osmotic adjustment: rhizobacteria assist plants in osmotic adjustment, which is essential for overcoming water-related stresses such as drought and salinity [35]. These bacteria produce osmoprotectants, such as proline, betaine, and sugars, which help maintain cellular water potential and protect plant cells from dehydration [35]. Osmoprotectants act as compatible solutes, balancing the osmotic potential inside the cells and allowing plants to maintain turgor pressure under water-deficient conditions [34].

2. Antioxidant enzymes and reactive oxygen species (ROS) scavenging: environmental stresses often lead to the accumulation of reactive oxygen species (ROS), which can cause cellular damage and oxidative stress [36]. Rhizobacteria produce antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), that scavenge ROS and protect plant cells from oxidative damage [37]. By enhancing the activity of these enzymes, rhizobacteria help plants cope with oxidative stress and maintain cellular integrity under adverse conditions [37].

3. Nutrient acquisition and mobilization: rhizobacteria enhance plant nutrient acquisition and mobilization, which is crucial for plant growth and stress tolerance [3]. These bacteria solubilize and mineralize nutrients, such as phosphorus, potassium, and iron, making them more available to plants [3]. Improved nutrient availability allows plants to maintain essential physiological processes, withstand stress, and exhibit better overall performance [3].

4. Phytohormone production: rhizobacteria produce phytohormones, including auxins, cytokinins, and gibberellins, which play important roles in plant growth and stress responses [20]. These phytohormones influence various physiological processes, such as root elongation, lateral root formation, and stress signaling pathways [20]. By producing phytohormones, rhizobacteria can stimulate root growth, enhance nutrient uptake, and improve plant stress tolerance [20].

5. Induced systemic resistance (ISR): rhizobacteria can induce systemic resistance in plants, priming them for enhanced defense against pathogens and pests [12]. When plants perceive the presence of rhizobacteria or their elicitors, they trigger signaling pathways that activate defense mechanisms [12]. This systemic response includes the production of defense-related compounds, such as pathogenesis-related (PR) proteins and antimicrobial compounds, which contribute to improved stress tolerance and protection against diseases and pests [12].

6. Enhanced soil structure and nutrient cycling: rhizobacteria contribute to the improvement of soil structure and nutrient cycling, which are essential for plant health and stress tolerance [13]. They secrete enzymes that degrade organic matter, releasing nutrients trapped in complex compounds [16]. This nutrient solubilization and mineralization enhance nutrient availability in the rhizosphere, benefiting the plant nutrition [13]. Moreover, rhizobacteria can produce substances that promote the aggregation of soil particles, improving soil structure, water infiltration, and root growth [13, 16].

By harnessing the capabilities of rhizobacteria, researchers and farmers can enhance plant stress tolerance and improve crop performance under challenging environmental conditions. These bacteria offer a sustainable and environmentally friendly approach to mitigating the impact of abiotic stresses on plant growth and productivity. Continued research and innovation in this field hold great promise for optimizing the use of rhizobacteria in agriculture and facilitating the development of stress-tolerant crop varieties.

3. Conclusion

In conclusion, rhizobacteria are essential microorganisms that reside in the rhizosphere and interact with plant roots, playing a crucial role in promoting plant growth, health, and stress tolerance. Their diverse mechanisms of action include nitrogen fixation, nutrient solubilization and mineralization, phytohormone production, pathogen suppression, induced systemic resistance, stress tolerance enhancement, and root colonization. These mechanisms collectively contribute to improved nutrient availability, disease suppression, and enhanced plant vigor. Harnessing the potential of rhizobacteria offers promising opportunities for sustainable agriculture, reduced chemical inputs, improved crop productivity, and ecological restoration. Understanding the complex and dynamic interactions between rhizobacteria and plants provides a foundation for developing environmentally friendly agricultural practices and addressing global challenges in food production and environmental sustainability.

4. Future perspectives and challenges concerning rhizobacteria

Rhizobacteria, or plant growth-promoting rhizobacteria (PGPR), have demonstrated significant potential in various applications, including agriculture, environmental remediation, and biotechnology. As research in this field progresses, several future perspectives and challenges arise that warrant attention. Here are some key aspects to consider:

1. Understanding rhizobacteria-plant interactions: further research is needed to unravel the intricate mechanisms underlying rhizobacteria-plant interactions. Understanding the signaling pathways, genetic regulation, and molecular cross-talk involved in these interactions will enable scientists to optimize the use of rhizobacteria for specific applications. In-depth knowledge of the molecular basis of beneficial plant-microbe associations will enhance our ability to engineer plants with improved traits and develop more targeted approaches for sustainable agriculture and environmental remediation.

2. Expanding rhizobacteria diversity and functionality: exploring the vast diversity of rhizobacteria is crucial for discovering new strains with unique functionalities. By studying different rhizobacteria species and their interactions with various plants, we can uncover novel capabilities that can be harnessed for specific purposes. Additionally, identifying key functional genes and metabolic pathways within rhizobacteria will facilitate the development of synthetic microbial consortia with enhanced beneficial traits.

3. Integration with modern biotechnological tools: the integration of modern biotechnological tools, such as genomics, metagenomics, transcriptomics, and synthetic biology, holds promise for advancing our understanding and utilization of rhizobacteria. These tools enable the identification of key genetic elements, metabolic pathways, and microbial communities associated with rhizobacteria. Additionally, synthetic biology approaches allow for the engineering of rhizobacteria and plants to enhance desired traits, stress tolerance, and beneficial interactions.

4. Field-scale application and commercialization: one of the challenges in the practical application of rhizobacteria is scaling up from laboratory studies to field conditions. Field trials and long-term monitoring are necessary to assess the efficacy and consistency of rhizobacteria-based interventions. Additionally, the development of cost-effective production methods, formulation strategies, and delivery systems is crucial for commercializing rhizobacteria products and making them accessible to farmers on a larger scale.

5. Regulatory and public perception: as with any novel agricultural technology, the regulatory framework and public perception of rhizobacteria-based products need to be addressed. Ensuring safety, efficacy, and environmental sustainability are important considerations. Clear guidelines and regulations should be in place to evaluate and approve rhizobacteria products, while public education and awareness campaigns can foster acceptance and understanding of their benefits.

6. Sustainability and integration with other practices: rhizobacteria-based approaches should be integrated with other sustainable agricultural practices, such as or-

ganic farming, conservation agriculture, and precision farming. Combining these practices can optimize resource use efficiency, reduce environmental impacts, and enhance the overall sustainability of agricultural systems. The integration of rhizobacteria with other beneficial microorganisms, such as mycorrhizal fungi, can also maximize the benefits of plant-microbe interactions.

7. Climate change adaptation: climate change poses significant challenges to agricultural systems worldwide. Developing rhizobacteria-based strategies that enhance plant stress tolerance, water-use efficiency, and nutrient utilization under changing climatic conditions is crucial. Rhizobacteria can play a role in improving crop resilience and adaptation to heat stress, drought, and other climate-related challenges.

In conclusion, the future of rhizobacteria research and applications holds great promise but also requires addressing various challenges. Advancements in understanding rhizobacteria-plant interactions, exploring microbial diversity, integrating modern biotechnological tools, scaling up field applications, addressing regulatory aspects, and promoting sustainability are key areas for future research and development. By overcoming these challenges, rhizobacteria-based interventions can make substantial contributions to sustainable agriculture, environmental remediation, and biotechnology, ultimately benefiting both human well-being and the planet.

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References

[1] Mehmood U et al. A brief review on plant growth promoting rhizobacteria (PGPR): A key role in plant growth promotion. Plant Protection. 2018;**2**(2):77-82

[2] Goswami D, Thakker JN, Dhandhukia PC. Portraying mechanics of plant growth promoting rhizobacteria (PGPR): A review. Cogent Food & Agriculture. 2016;**2**(1):1127500

[3] Jha CK, Saraf M. Plant growth promoting rhizobacteria (PGPR): A review. Journal of Agricultural Research and Development. 2015;**5**(2):108-119

[4] Kumar A et al. Does a plant growth-promoting rhizobacteria enhance agricultural sustainability. Journal of Pure and Applied Microbiology. 2015;**9**(1):715-724

[5] de Andrade LA et al. Plant growth-promoting rhizobacteria for sustainable agricultural production. Microorganisms. 2023;**11**(4):1088

[6] Gupta G et al. Plant growth promoting rhizobacteria (PGPR): Current and future prospects for development of sustainable agriculture. Journal of Microbial and Biochemical Technology. 2015;**7**(2):096-102

[7] Santoyo G et al. Rhizosphere colonization determinants by plant growth-promoting rhizobacteria (PGPR). Biology. 2021;**10**(6):475

[8] Bhattacharyya PN, Jha DK. Plant growth-promoting rhizobacteria (PGPR): Emergence in agriculture. World Journal of Microbiology and Biotechnology. 2012;**28**:1327-1350

[9] Meena M et al. PGPR-mediated induction of systemic resistance and physiochemical alterations in plants against the pathogens: Current perspectives. Journal of Basic Microbiology. 2020;**60**(10):828-861

[10] Morcillo RJ, Manzanera M. The effects of plant-associated bacterial exopolysaccharides on plant abiotic stress tolerance. Metabolites. 2021;**11**(6):337

[11] Jain A, Chakraborty J, Das S. Underlying mechanism of plant–microbe crosstalk in shaping microbial ecology of the rhizosphere. Acta Physiologiae Plantarum. 2020;**42**:1-13

[12] Choudhary DK, Prakash A, Johri B. Induced systemic resistance (ISR) in plants: Mechanism of action. Indian Journal of Microbiology. 2007;**47**:289-297

[13] Pii Y et al. Microbial interactions in the rhizosphere: Beneficial influences of plant growth-promoting rhizobacteria on nutrient acquisition process. A review. Biology and Fertility of Soils. 2015;**51**:403-415

[14] Rohrbacher F, St-Arnaud M. Root exudation: The ecological driver of hydrocarbon rhizoremediation. Agronomy. 2016;**6**(1):19

[15] Dodd I et al. Rhizobacterial mediation of plant hormone status. Annals of Applied Biology. 2010;**157**(3):361-379

[16] Pereira S et al. Plant growth-promoting rhizobacteria (PGPR) improve the growth and nutrient use efficiency in maize (*Zea mays* L.) under water deficit conditions. Heliyon. 2020;**6**(10):e05106

[17] Nadeem SM et al. The role of mycorrhizae and plant growth promoting

rhizobacteria (PGPR) in improving crop productivity under stressful environments. Biotechnology Advances. 2014;**32**(2):429-448

[18] Pangesti N et al. Jasmonic acid and ethylene signaling pathways regulate glucosinolate levels in plants during rhizobacteria-induced systemic resistance against a leaf-chewing herbivore. Journal of Chemical Ecology. 2016;**42**:1212-1225

[19] Li X et al. Chemical proteomics reveals an ISR-like response elicited by salicylic acid in Arabidopsis. New Phytologist. 2023;**237**(5):1486-1489

[20] Fahad S et al. Potential role of phytohormones and plant growth-promoting rhizobacteria in abiotic stresses: Consequences for changing environment. Environmental Science and Pollution Research. 2015;**22**:4907-4921

[21] Sun J-Q, Jiang H-L, Li C-Y. Systemin/jasmonate-mediated systemic defense signaling in tomato. Molecular Plant. 2011;**4**(4):607-615

[22] Abdul Malik NA, Kumar IS, Nadarajah K. Elicitor and receptor molecules: orchestrators of plant defense and immunity. International Journal of Molecular Sciences. 2020;**21**(3):963

[23] El-Garhy HA et al. Comparative analyses of four chemicals used to control black mold disease in tomato and its effects on defense signaling pathways, productivity and quality traits. Plants. 2020;**9**(7):808

[24] Rebello S et al. Bioengineered microbes for soil health restoration: Present status and future. Bioengineered. 2021;**12**(2):12839-12853

[25] Pirttilä AM et al. Biofertilizers and biocontrol agents for agriculture: How to identify and develop new potent microbial strains and traits. Microorganisms. 2021;**9**(4):817

[26] Joutey NT et al. Biodegradation: Involved microorganisms and genetically engineered microorganisms. Biodegradation-Life of Science. 2013;**1**:289-320

[27] Saeed Q et al. Rhizosphere bacteria in plant growth promotion, biocontrol, and bioremediation of contaminated sites: A comprehensive review of effects and mechanisms. International Journal of Molecular Sciences. 2021;**22**(19):10529

[28] Ma Y et al. Plant growth promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils. Biotechnology Advances. 2011;**29**(2):248-258

[29] Melini F et al. Effect of microbial plant biostimulants on fruit and vegetable quality: Current research lines and future perspectives. Frontiers in Plant Science. 2023;**14**:1251544

[30] Shahid M, Khan MS, Singh UB. Pesticide-tolerant microbial consortia: Potential candidates for remediation/clean-up of pesticide-contaminated agricultural soil. Environmental Research. 2023;**236**(Pt 1):116724

[31] Bhuyan B, Kotoky R, Pandey P. Impacts of rhizoremediation and biostimulation on soil microbial community, for enhanced degradation of petroleum hydrocarbons in crude oil-contaminated agricultural soils. Environmental Science and Pollution Research. 2023;**30**(41):94649-94668

[32] Bhanse P et al. Role of plant growth-promoting rhizobacteria in boosting the phytoremediation of stressed soils: Opportunities, challenges, and prospects. Chemosphere. 2022;**303**:134954

[33] Vaghela N, Gohel S. Medicinal plant-associated rhizobacteria enhance the production of pharmaceutically important bioactive compounds under abiotic stress conditions. Journal of Basic Microbiology. 2023;**63**(3-4):308-325

[34] Khan N et al. Water conservation and plant survival strategies of rhizobacteria under drought stress. Agronomy. 2020;**10**(11):1683

[35] Jain T et al. Molecular basis of biotic and abiotic stress management attributes of plant growth promoting rhizobacteria. Journal of Postharvest Technology. 2023;**11**(3):29-55

[36] Zandi P, Schnug E. Reactive oxygen species, antioxidant responses and implications from a microbial modulation perspective. Biology. 2022;**11**(2):155

[37] Kisiel A, Miller T. Oxidative status of *Medicago truncatula* seedlings after inoculation with rhizobacteria of the genus pseudomonas, Paenibacillus and Sinorhizobium. International Journal of Molecular Sciences. 2023;**24**(5):4781

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Updates on Rhizobacteria captures in a single volume the wealth of information about the presence, appearance, function, and utilization of rhizobacteria. Rhizobacteria play critical roles in nutrient acquisition and assimilation, improvement of soil texture, and the secretion and modulation of extracellular molecules such as hormones, secondary metabolites, antibiotics, and various signal compounds, all of which lead to enhanced plant growth and development. Rhizobacteria are also integral to plant–microbe interactions and for modulating plant stress responses. *Updates on Rhizobacteria* is a useful resource for instructors and students in advanced undergraduate and graduate courses on rhizobacteria. It is also valuable for researchers seeking new relationships between biological processes that are linked by rhizobacteria and the environment.

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