Published by The College of Science & Information Technology (COSIT), TASFUED, Vol. 19, No. 2, pp. 108-128.

COSIT, TASFUED
Journal of Science and Information Technology (JOSIT)

Lessons from the Genomes: Rhizobacterial Ecology and Genomics

Ade-Ogunnowo, F. E.

Department of Biological Sciences, Tai Solarin Federal University of Education, Ijagun, Nigeria.
Corresponding Author: adeogunnowo@tasued.edu.ng

Abstract

The rhizosphere is a dynamic microbial hotspot where plant roots interact with diverse microorganisms, including plant-growth-promoting rhizobacteria (PGPR). Recent advances in genomic technologies, particularly genome sequencing, functional genomics, and metagenomics, have enhanced understanding of rhizobacterial ecology by revealing genetic traits associated with root colonisation, nutrient acquisition, and stress resilience under environmentally variable conditions. This review synthesises studies published over the past decade to examine the genetic basis of plant–microbe interactions. It evaluates the roles of horizontal gene transfer (HGT), niche adaptation, and the interplay between core and accessory genomes in structuring rhizobacterial communities. The review further highlights that gene presence does not consistently translate to in situ activity, reflecting context-dependent gene expression and environmental modulation of microbial functions. Key gaps include limited functional validation of candidate genes, constraints in linking genomic potential to ecological performance, and challenges associated with extrapolating laboratory-based findings to complex field environments. These insights inform ongoing efforts to develop genomics-guided microbial inoculants for sustainable agricultural systems.
.

Keywords: Rhizobacteria, PGPR, rhizosphere, plant-microbe interactions, genomics, horizontal gene transfer

INTRODUCTION

Rhizobacterial ecology examines the complex interactions between soil microorganisms and the biotic and abiotic components of their environment. These interactions arise from the interplay between microbial genetic potential and environmental conditions, including soil physicochemical properties, plant root exudates, and interspecies competition. The rhizosphere microbiome contributes significantly to key processes such as plant health, nutrient cycling, and soil fertility (Sindhu *et al.*, 2022). Notably, plant-growth-promoting rhizobacteria (PGPR), including *Pseudomonas fluorescens*, *Bacillus subtilis*, and various *Rhizobium* species, facilitate these processes through mechanisms such as nitrogen fixation, phosphate solubilisation, suppression of phytopathogens via antibiotic production, competition for ecological niches and nutrients, and induction of systemic resistance (Lugtenberg & Kamilova, 2009). Recent advances in high-throughput sequencing technologies have enhanced understanding of rhizobacterial ecology by providing genome-scale insights into their structural and functional diversity, evolutionary adaptations, and ecological strategies. However, linking genomic potential to realize ecological function remains challenging, as gene expression and microbial activity are strongly modulated by environmental context. This paper explores the contributions of genomics to rhizobacterial ecology while considering the dynamic interactions between genetic traits and environmental factors, and highlights emerging directions that may further advance the field.

Traditional culture-dependent approaches have increasingly been supplanted by genome-scale investigations made possible through high-throughput sequencing and computational biology. These modern techniques allow researchers to decipher the genetic blueprints

Cite as:

Ade-Ogunnowo F. E. (2025). Lessons from the Genomes: Rhizobacterial Ecology and Genomics. *Journal of Science and Information Technology (JOSIT)*, Vol. 19 No. 2, pp. 108-128.

©JOSIT Vol. 19, No. 2, November 2025.

underpinning key microbial traits such as root colonisation, biofilm formation, and secondary metabolite biosynthesis (Seneviratne *et al.*, 2020). Whole-genome sequencing of model organisms including *Pseudomonas fluorescens*, *Bacillus subtilis*, and various Rhizobium species has generated comprehensive datasets facilitating three complementary approaches: (1) structural genomics, which provides detailed information about protein-coding genes and their architectures; (2) functional genomics, aimed at elucidating links between genomic content and phenotype; and (3) comparative genomics, which identifies conserved and strain-specific features critical for rhizosphere competence.

In parallel, metagenomic approaches have transformed the study of rhizobacterial communities by enabling culture-independent characterisation of the microbiome directly from rhizosphere samples. This has revealed taxonomic and functional profiles across diverse plant hosts and environmental conditions, identifying both core microbiomes and dynamically shifting populations influenced by abiotic stressors, nutrient fluctuations, and plant developmental stages (Bulgarelli *et al.*, 2013; Mendes *et al.*, 2013; Wani *et al.*, 2024). Moreover, the application of metagenome-assembled genomes (MAGs) has further expanded our view by reconstructing partial to near-complete genomes of uncultured microbes, shedding light on the "dark microbial world" within the rhizosphere (Setubal, 2021). A particularly powerful genomic approach is pangenome analysis, which distinguishes between a species' conserved core genome and its variable accessory genome. The core genome typically encodes essential housekeeping functions, while the accessory genome harbours genes involved in environmental adaptation, such as antibiotic resistance, stress tolerance, and specialized metabolism (Medini *et al.*, 2005). In rhizobacteria, this genomic plasticity underpins rapid adaptation to fluctuating rhizosphere conditions, host specificity, and inter-microbial competition, frequently facilitated by horizontal gene transfer (HGT) and mobile genetic elements (Cotta *et al.*, 2024).

To reflect the genomic complexity of rhizobacteria, this review is structured into two main sections: one focused on insights from structural and functional genomics, and another covering comparative genomics, metagenomics, and pangenomics. While these approaches have advanced understanding of rhizobacterial diversity and functional potential, important challenges remain, including limited functional validation of predicted genes, difficulties in linking genomic data to in situ ecological activity, and the context-dependence of microbial functions in the rhizosphere. The review concludes by examining how genome-based technologies can be leveraged to address these gaps and advance rhizobacterial ecology, particularly through informing the rational design of next-generation microbial inoculants for sustainable agricultural systems.

STRUCTURAL GENOMICS

Structural genomics is a high-throughput approach aimed at determining the three-dimensional structures of proteins encoded within a genome (Burley *et al.*, 2019). Unlike traditional structural biology, which focuses on individual proteins, structural genomics integrates techniques such as X-ray crystallography, nuclear magnetic resonance (NMR) spectroscopy, and cryo-electron microscopy (cryo-EM) to systematically analyse protein structures at scale (Grabowski *et al.*, 2016). In plant-growth-promoting rhizobacteria (PGPR), this approach provides insights into protein function, molecular interactions, and evolutionary adaptations that underpin plant–microbe associations (Levy *et al.*, 2018).

Advances in computational biology have further accelerated this field. High-accuracy structure prediction tools such as SWISS-MODEL and AlphaFold2 complement experimental approaches, enabling functional inference for previously uncharacterized proteins (Waterhouse *et al.*, 2018; Jumper *et al.*, 2021). For example, X-ray crystallography has resolved the structure of nitrogenase, a key enzyme in biological nitrogen fixation (Hoffman *et al.*, 2014), while cryo-EM has enabled visualization of large protein complexes such as the Type III secretion system in *Pseudomonas* (Hu *et al.*, 2015). Structural studies have also elucidated the functional mechanisms of proteins involved in rhizobacterial activity, including glucose dehydrogenase in phosphate solubilisation, non-ribosomal peptide synthetases in siderophore biosynthesis, and osmoprotectant

transporters that mediate stress tolerance (Rodríguez *et al.*, 2006; Crosa & Walsh, 2002; Ziegler *et al.*, 2010). In addition, structural analyses of signaling molecules, such as Nod factors and ACC deaminase, have clarified their roles in symbiosis and plant stress mitigation (Radutoiu *et al.*, 2003; Glick, 2014).

Genome Architecture and Organisation in Rhizobacteria

In contrast to protein structural studies, genome architecture refers to the organisation, composition, and distribution of genetic elements within the genome. Rhizobacterial genomes exhibit distinct architectural features that reflect ecological adaptation (Ercole *et al.*, 2021). Typically ranging from 3 to 7 megabases, these genomes are relatively compact, with high gene density and fewer non-coding regions compared to many free-living soil bacteria (Mitter *et al.*, 2017). A key feature of rhizobacterial genome organisation is the presence of mobile genetic elements, including plasmids and transposons, which facilitate horizontal gene transfer (HGT) and contribute to rapid adaptation in the rhizosphere (Van Elsas *et al.*, 2003; Ku *et al.*, 2021). The genome can also be partitioned into a core genome, comprising conserved housekeeping genes, and an accessory genome that encodes niche-specific traits such as nitrogen fixation, siderophore production, and stress tolerance (Tettelin *et al.*, 2008). Operon structures further enable coordinated gene expression, allowing efficient regulation of processes such as phosphate solubilisation and environmental stress responses (Dos Santos *et al.*, 2012).

Comparative genomic studies of agriculturally important rhizobacteria, including *Pseudomonas fluorescens*, *Bacillus subtilis*, and *Rhizobium* species, highlight how genome organisation underpins ecological versatility. For instance, *P. fluorescens* possesses a large accessory genome enriched with genomic islands and mobile elements associated with antibiotic production, siderophore biosynthesis, and biofilm formation (Mavrodi *et al.*, 2009; Loper *et al.*, 2012). In *B. subtilis*, genes involved in sporulation are arranged in tightly regulated cascades, enabling adaptation to environmental stress (Kunst *et al.*, 1997; Sonenshein *et al.*, 2002). Similarly, *Rhizobium leguminosarum* and *Rhizobium etli* exhibit multipartite genomes with duplicated metabolic pathways, reflecting functional redundancy and ecological flexibility (Young *et al.*, 2006).

FUNCTIONAL GENOMICS

The advent of functional genomics has significantly advanced understanding of how rhizobacteria interact with plants at the molecular level. By moving beyond static genome sequences to examine gene expression patterns, protein interactions, and metabolic networks, researchers have gained deeper insights into mechanisms underlying plant growth promotion and stress tolerance (Levy *et al.*, 2018). This integrative approach combines high-throughput technologies with computational biology to link genes to functions in agriculturally important microorganisms (Loper *et al.*, 2012). However, each methodological layer introduces distinct limitations that influence data interpretation and biological inference.

Transcriptomic studies using RNA sequencing (RNA-seq) have revealed how rhizobacteria reprogram gene expression in response to plant root environments. In *Pseudomonas fluorescens*, exposure to root exudates induces genes involved in motility, chemotaxis, and iron acquisition, which are important for rhizosphere colonisation (Mark *et al.*, 2005; Mavrodi *et al.*, 2021). Similarly, *Bacillus subtilis* shows coordinated induction of biofilm formation genes and secondary metabolite pathways during root surface colonisation (Allard-Massicotte *et al.*, 2016; Arnaouteli *et al.*, 2021), while rhizobia exhibit tightly regulated temporal activation of nodulation genes during symbiosis (Gibson *et al.*, 2008). Despite these insights, transcriptomic data are limited by the fact that mRNA abundance does not always correlate with protein levels or enzymatic activity. In addition, RNA extraction from rhizosphere samples can introduce plant-derived contamination and bias, while snapshot sampling may miss transient or spatially restricted expression dynamics.

The power of functional genomics is strengthened when integrated with proteomic and metabolomic approaches. Mass spectrometry-based proteomics has identified post-translational modifications regulating rhizobacterial enzymes, including phosphorylation events influencing nitrogenase

activity in nitrogen-fixing bacteria (Hernández *et al.*, 2009; Rodríguez-Vázquez & Mesa-Marín, 2023). It has also characterised secreted effector proteins such as type III secretion system effectors that suppress plant immune responses (Preston *et al.*, 2001). However, proteomic analyses are constrained by incomplete protein coverage, detection bias toward abundant and soluble proteins, and challenges in capturing membrane-associated or low-abundance regulatory proteins.

Metabolomic profiling using LC-MS and GC-MS has provided insights into chemical signaling in plant–microbe interactions, including phytohormones such as indole-3-acetic acid and quorum-sensing molecules like N-acyl homoserine lactones (Hartmann *et al.*, 2008; Patel *et al.*, 2013). Nevertheless, metabolomic datasets are often difficult to interpret due to metabolite instability, environmental variability, and limited annotation of microbial secondary metabolites, which restricts functional assignment of detected compounds.

Reverse genetics approaches have been essential for validating functional genomic predictions. Transposon mutagenesis in *Pseudomonas* species has identified genes required for rhizosphere competence that are not predictable from sequence data alone (Rainey, 1999), while CRISPR-Cas9 editing has enabled precise functional dissection of secondary metabolite gene clusters in *Bacillus*, revealing roles in biocontrol activity (Chen *et al.*, 2020). Similarly, CRISPR interference (CRISPRi) and mutagenesis approaches have been used to demonstrate the functional significance of siderophore biosynthesis pathways in iron acquisition and ecological competition (Cornelis & Dingemans, 2013). However, reverse genetics methods can introduce off-target effects, polar mutations, or compensatory responses, and may not fully reflect gene function under natural rhizosphere conditions.

The true power of functional genomics emerges when these diverse datasets are integrated through systems biology approaches. Protein–protein interaction networks have mapped how signaling pathways coordinate the complex process of root colonisation (Wang *et al.*, 2018), while metabolic flux analyses predict how rhizobacteria utilise carbon and nitrogen resources in the rhizosphere environment (Pini *et al.*, 2017). Interestingly, dual RNA-seq approaches, which simultaneously capture bacterial and plant gene expression, have uncovered intricate dialogue between partners. For example, *Bradyrhizobium japonicum* and soybean roots coordinate metabolic shifts during nitrogen fixation, with bacteria inducing (nitrogen fixation (*nif*) genes while roots adjust carbon allocation (Tsukada *et al.*, 2009). Particularly promising is the application of machine learning algorithms trained on multi-omics datasets to predict the plant growth-promoting potential of uncharacterized bacterial isolates (Xu *et al.*, 2021). These functional genomic advances are translating into tangible agricultural applications, where molecular markers derived from gene expression studies facilitate the rapid identification of elite PGPR strains (Compant *et al.*, 2019). Furthermore, genetic engineering targets elucidated through functional genomics are being exploited to develop enhanced microbial inoculants (Bashan *et al.*, 2014). Most significantly, this knowledge underpins precision microbiome management strategies with the potential to substantially reduce dependence on synthetic fertilizers and pesticides (Busby *et al.*, 2017).

Genomic traits linked to rhizosphere colonisation

Rhizosphere colonisation by plant-growth-promoting rhizobacteria (PGPR) is a highly competitive and selective process that depends on coordinated functional traits enabling survival and persistence in the root-associated soil environment. These traits operate as integrated ecological strategies rather than isolated gene functions. A central component of successful colonisation is chemotaxis and motility, which determine the ability of bacteria to detect and move toward root exudates. Genes involved in flagellar assembly and function, such as *fliC* and *motA*, contribute to the development of functional flagella and effective bacterial movement, while methyl-accepting chemotaxis proteins encoded by *mcp* genes enable sensing of chemical gradients derived from plant root exudates (Miller *et al.*, 2001; de Weert *et al.*, 2002). Functionally, this gene set enhances bacterial migration toward nutrient-rich root zones, increasing colonisation efficiency and improving access to plant-derived carbon sources. Following initial root attachment, biofilm formation genes support

stable surface colonisation by promoting microbial aggregation and extracellular matrix production. This phenotype enhances bacterial persistence under environmental stress (e.g., desiccation and nutrient limitation) and improves sustained interaction with plant roots, thereby increasing the likelihood of long-term plant growth promotion. Quorum sensing systems, typically encoded by *luxI/luxR*-type regulators, coordinate population-level responses by regulating gene expression in a density-dependent manner (Jung *et al.*, 2020). This regulatory mechanism enhances ecological fitness by synchronising biofilm development, secondary metabolite production, and competitive interactions, ultimately improving rhizosphere establishment and exclusion of competing microbes. Collectively, these gene-regulated traits translate into improved rhizosphere colonisation efficiency, enhanced microbial persistence, and increased plant growth promotion through more stable and functionally active plant–microbe interactions.

Another set of crucial genomic features includes genes for nutrient acquisition, which are essential for survival in the nutrient-limited rhizosphere. Siderophore biosynthesis genes, including the *pvd* and *ent* clusters, enable PGPR to scavenge iron from the environment, often outcompeting pathogenic microbes (Cornelis, 2010). Another key trait is phosphate solubilisation, driven by genes like *gcd* and *pqq*, which enable the mineralization of inorganic phosphate and enhance its availability to plants (Rawat *et al.*, 2021). Nitrogen fixation, carried out by symbiotic or associative PGPR like Azospirillum and Rhizobium, is governed by *nif* and *fix* genes that enable conversion of atmospheric nitrogen into plant-usable forms. These nutrient acquisition traits not only benefit the plant but also promote stable microbial colonisation by providing bacteria with access to essential growth factors (Pahari et al, 2021).

Stress adaptation genes also contribute significantly to rhizosphere colonisation. The rhizosphere presents fluctuating conditions in terms of moisture, pH, and the presence of antimicrobial compounds secreted by plants or competing microbes. PGPR often harbour genes involved in oxidative stress resistance (e.g., katG, sodA), osmoprotection (e.g., proU operon), and antibiotic resistance, which enhance their resilience in unfavourable environments (Timmusk *et al.*, 2014). Additionally, efflux pumps and multidrug resistance genes enable bacteria to survive chemical challenges in the rhizosphere. Together, these genomic traits not only support the physical establishment of PGPR on plant roots but also underpin their functional roles in promoting plant health and resilience. Advances in functional and comparative genomics continue to uncover the complex genetic networks that facilitate successful rhizosphere colonisation, providing valuable insights for engineering more effective microbial inoculants.

Characterising rhizobacterial responses to environmental change

To date, genome-wide gene expression profiling has been most effectively applied to studying microbial responses under controlled laboratory conditions with well-defined environmental parameters. RNA-seq analyses have demonstrated that environmental cues such as temperature fluctuation or oxidative stress can induce global transcriptional reprogramming. For example, exposure of *Pseudomonas putida* to soil desiccation triggers activation of genes involved in oxidative stress response, DNA repair, and efflux pump systems (Matilla *et al.*, 2007). Under drought conditions, many rhizobacteria, including *Bacillus* and *Pseudomonas* species, upregulate genes associated with osmoprotectant synthesis (e.g., trehalose and glycine betaine), oxidative stress defence, and cell envelope modification to maintain cellular integrity (Timmusk *et al.*, 2014; Singh *et al.*, 2014). Proteomic analyses support these transcriptional patterns by showing coordinated increases in stress-response proteins, molecular chaperones, and enzymes involved in reactive oxygen species (ROS) detoxification (Egamberdieva *et al.*, 2017). In *Rhizobium etli*, integrated transcriptomic and metabolomic analyses have shown that phosphate starvation triggers coordinated shifts in gene expression and metabolite production, prioritising phosphate scavenging, membrane remodelling, and stress adaptation (Flores-Tinoco *et al.*, 2016). These responses enhance microbial survival and indirectly contribute to plant stress tolerance through improved nutrient mobilization and induced systemic resistance.

However, extrapolating these laboratory-based findings to natural rhizosphere systems remains challenging due to the high spatial, temporal, and physicochemical variability of soils. Unlike controlled experiments, field rhizospheres are heterogeneous environments characterised by fluctuating nutrient availability, micro-scale oxygen gradients, variable moisture regimes, and complex plant–microbe–microbe interactions. These factors generate dynamic selective pressures that cannot be fully replicated under laboratory conditions, potentially altering both the magnitude and direction of gene expression responses observed in vitro.

Environmental stress in natural systems also shapes microbial community structure at multiple ecological scales. Changes in soil moisture, pH, and temperature can lead to selective enrichment of taxa possessing stress-tolerant traits. For instance, arid soils often favour the proliferation of *Bacillus*, Actinobacteria, and other endospore-forming bacteria due to their resilience to desiccation and thermal stress (Berg & Smalla, 2009). High-throughput 16S rRNA sequencing studies further indicate that microbial diversity often declines under extreme environmental conditions, although functional redundancy may partially buffer ecosystem processes against collapse (Naylor *et al.*, 2017). Importantly, these community shifts differ across climatic zones and soil types, highlighting strong context dependency in rhizobacterial assembly.

In addition, environmental variability influences not only microbial populations but also plant root physiology and exudation patterns, which vary with soil type, nutrient status, and stress exposure. This, in turn, alters microbial gene expression and behaviour in a feedback-dependent manner. Functional genomic studies have shown that flavonoids released by legume roots under nutrient stress induce nodulation (nod) gene expression in *Rhizobium* species, initiating symbiotic nitrogen fixation (Oldroyd *et al.*, 2011). However, the strength and specificity of such signalling responses can vary significantly across soil environments and host genotypes. Comparative functional genomic analyses have further identified rhizobacterial gene clusters that respond differentially to distinct root exudate compositions, demonstrating context-dependent gene regulation across plant species and environmental conditions (Zhalnina *et al.*, 2018). Moreover, integrative omics studies have revealed quorum sensing networks and hormone-mimicking pathways that operate within feedback-regulated systems, allowing rhizobacteria to adjust behaviour dynamically in response to changing environmental and host-derived signals (Spaepen & Vanderleyden, 2011). These findings underscore both the potential and the limitations of laboratory-based functional genomics in fully capturing the complexity of field rhizosphere interactions.

4. COMPARATIVE GENOMICS

The emergence of comparative genomics has enabled a shift from species-level description to evolutionary and ecological interpretation of rhizobacterial diversity. Rather than merely cataloguing gene content, this approach allows the identification of lineage-specific strategies that underpin adaptation to the rhizosphere, a highly heterogeneous and competitive ecological niche (Tettelin *et al.*, 2008; Philippot *et al.*, 2013). Comparative analyses of *Pseudomonas fluorescens*, *Bacillus subtilis*, and *Rhizobium* spp. reveal fundamentally different evolutionary solutions to similar ecological challenges, particularly in terms of genome plasticity, regulatory complexity, and horizontal gene acquisition. These differences reflect contrasting evolutionary trajectories shaped by habitat specificity, host association, and gene flow intensity, rather than simple taxonomic divergence.

Core–Accessory Genome Architecture

Across rhizobacterial lineages, the balance between core and accessory genomes reflects distinct adaptive strategies rather than static genomic partitions. While the core genome encodes essential cellular processes, the accessory genome functions as a flexible evolutionary reservoir that enables rapid ecological response under fluctuating environmental conditions (Lugtenberg & Kamilova, 2009; Mitter *et al.*, 2017). Importantly, comparative studies show that the ecological success of rhizobacteria is not determined by core genome conservation alone, but by the *rate, diversity, and functional relevance* of accessory gene acquisition.

In *Pseudomonas fluorescens*, an open pan-genome structure indicates ongoing gene flux and extensive ecological diversification, particularly in traits linked to secondary

metabolism, competitive exclusion, and stress tolerance (Loper *et al.*, 2012). This genomic openness reflects a generalist survival strategy, enabling rapid functional innovation in response to heterogeneous soil microenvironments. In contrast, *Bacillus subtilis* exhibits a more constrained pan-genome with stronger core genome conservation, suggesting evolutionary selection for metabolic stability and stress endurance rather than continuous niche expansion (Zhang *et al.*, 2015). *Rhizobium* spp. represent a distinct evolutionary strategy in which essential symbiotic functions are modularized on plasmids or symbiosis islands, effectively decoupling core cellular fitness from host-specific adaptation (Young *et al.*, 2006). This modularity provides a high degree of ecological flexibility, allowing rapid host range shifts without restructuring the chromosomal genome.

These contrasting architectures highlight a key evolutionary trade-off in rhizobacteria: genome openness enhances adaptability but may increase metabolic burden, whereas genomic stability enhances efficiency but limits ecological plasticity. Thus, rhizobacterial success is shaped not by a single optimal genome structure, but by lineage-specific balances between stability and innovation.

Horizontal Gene Transfer

Horizontal gene transfer (HGT) represents a central evolutionary mechanism shaping both functional convergence and ecological divergence among rhizobacteria. Rather than acting uniformly, HGT operates at different intensities and via different mechanisms across taxa, producing distinct evolutionary outcomes in rhizosphere colonization strategies (Che *et al.*, 2021).

In *Rhizobium* spp., HGT is strongly linked to ecological specialization through the acquisition and dissemination of symbiosis islands and plasmids carrying nodulation and nitrogen fixation genes (Sullivan & Ronson, 1998; Kaneko *et al.*, 2000). This results in rapid evolutionary transitions from free-living to symbiotic lifestyles, illustrating how HGT drives functional innovation at the ecosystem level. Importantly, this represents ecological *conversion* rather than gradual adaptation, enabling non-symbiotic strains to rapidly enter plant-associated niches (Sullivan *et al.*, 2002).

In contrast, *Pseudomonas fluorescens* exhibits HGT-driven ecological diversification rather than host specialization. Genomic islands acquired through mobile elements encode traits such as antibiotic production, siderophore biosynthesis, and xenobiotic degradation, enabling competitive dominance in complex soil microbial communities (Silby *et al.*, 2009; Loper *et al.*, 2012). This reflects a strategy of ecological generalism, where gene acquisition expands functional breadth rather than narrowing host dependence. Consequently, HGT in *Pseudomonas* promotes niche partitioning and competitive exclusion rather than stable symbiosis.

Although *Bacillus subtilis* exhibits comparatively lower rates of HGT, its evolutionary flexibility is maintained through natural competence and stress-induced DNA uptake, enabling episodic rather than continuous genomic innovation (Dubnau, 1991; Claverys *et al.*, 2006). This results in a punctuated evolutionary model in which genetic innovation is activated under environmental stress, allowing selective acquisition of adaptive traits without extensive genomic restructuring. Compared to *Pseudomonas* and *Rhizobium*, this represents a conservative but resilient evolutionary strategy, prioritizing survival stability over ecological expansion.

These patterns indicate that HGT does not simply increase genetic diversity but actively shapes contrasting ecological strategies: symbiotic specialization in *Rhizobium*, competitive generalism in *Pseudomonas*, and stress-buffered conservatism in *Bacillus*. These lineage-specific outcomes underscore the role of gene flow as a driver of both ecological divergence and functional convergence within rhizobacterial communities.

Evolutionary insights and applications

The evolutionary adaptations revealed by comparative genomics provide a direct foundation for translating rhizobacterial diversity into agricultural biotechnology. In *Rhizobium* species, for instance, symbiotic islands containing nodulation (*nod*) and nitrogen fixation (*nif/fix*) genes reflect long-term co-evolution with legume hosts (Dos Santos *et al.*, 2012). These conserved gene clusters are now routinely used as **functional biomarkers** for identifying and selecting highly efficient nitrogen-fixing strains for

biofertiliser development. Similarly, plant-growth-promoting *Pseudomonas* strains exhibit expanded genomic repertoires associated with rhizosphere competence, including chemotaxis systems and type III secretion machinery that enhance root colonisation efficiency and microbial persistence in competitive soil environments (Ofek-Lalzar *et al.*, 2014; Guzmán-Guzmán & Santoyo, 2022). These traits are increasingly used as **genomic screening criteria** in the selection of commercial PGPR inoculants.

Metabolic specialization driven by gene gain and loss further provides exploitable traits for agricultural applications. Expanded carbohydrate metabolism pathways in many rhizobacteria enable efficient utilization of diverse root exudates, supporting strain persistence across different plant hosts and soil types (Berendsen *et al.*, 2012). In applied settings, this has informed the selection of broad-spectrum inoculant strains capable of maintaining functionality across variable crop systems. Similarly, stress-adaptive gene clusters associated with osmolyte biosynthesis and ion transporters underpin drought and salinity tolerance in rhizobacteria, and are now being used to identify strains suitable for deployment in climate-vulnerable agroecosystems (Ziegler *et al.*, 2010; Jacob *et al.*, 2017).

These evolutionary insights are not only descriptive but increasingly predictive in biotechnology pipelines. Comparative genomics now enables the identification of conserved and accessory gene signatures associated with plant-beneficial traits, which are being used as molecular selection tools for high-throughput screening of PGPR candidates prior to field evaluation (Glick, 2014). In modern microbial biotechnology programmes, genome-informed selection is replacing phenotype-only screening, improving the efficiency and reliability of inoculant development (Compant *et al.*, 2019).

Beyond strain selection, comparative genomic data are now directly informing synthetic biology and microbial engineering strategies. CRISPR-based genome editing guided by naturally occurring genomic variation is being used to enhance beneficial traits such as nutrient acquisition, stress tolerance, and plant colonisation efficiency in engineered rhizobacterial strains (Thankappan *et al.*, 2024). In parallel, comparative metabolic reconstructions are enabling the rational design of synthetic microbial consortia, where complementary metabolic capabilities are assembled to enhance nutrient cycling and ecological stability in the rhizosphere (Bashan *et al.*, 2014). These developments illustrate a shift from descriptive evolutionary genomics toward predictive and design-oriented microbial biotechnology, where evolutionary history directly informs applied agricultural innovation.

Insights into phylogeny

Phylogeny is the evolutionary history and relationships among individuals or groups of organisms (e.g., species, populations). It is typically represented as a branching diagram called a phylogenetic tree, where common ancestors appear at branch points and the length of branches may represent evolutionary time or genetic change. Phylogeny is important because HGT can disrupt the vertical (parent-to-offspring) pattern of inheritance that phylogeny traditionally assumes, leading to evolutionary relationships that differ between individual genes and whole organisms. The soil beneath our feet teems with microbial life, particularly in the rhizosphere where plant roots interact with complex bacterial communities (see Figure 1). Among these, three genera stand out as model organisms for studying plant-microbe interactions: the versatile Pseudomonas, the resilient Bacillus, and the specialised Rhizobium. The structural differences of these genera underpin their diverse modes of adaptation. Through the lens of comparative genomics, evolution has shaped these bacteria into masterful colonizers of the plant root environment, each employing distinct genomic strategies to thrive within this competitive ecological niche.

Pseudomonas species represent quintessential adaptable colonists of the rhizosphere. Their genomes, typically 6–7 megabases in size, read like manuals of ecological versatility, encoding an impressive arsenal of tools for rhizosphere success: sophisticated systems for detecting and responding to plant exudates, numerous pathways for secondary metabolite production, and elaborate mechanisms for microbial competition. What makes Pseudomonas particularly fascinating is their “open” pan-genome with each new environmental isolate often contributes novel genetic elements,

explaining their remarkable capacity to colonise varied plant species and persist under heterogeneous soil conditions (Silby *et al.*, 2011). This genomic plasticity comes at the cost of maintaining larger genomes but provides unmatched flexibility in an ever-changing rhizosphere.

By contrast, Bacillus species embody evolutionary economy. Their more compact genomes (4–5 Mb) reflect a survival strategy based on conservation rather than innovation. These spore-formers maintain a highly stable core genome, with most strains sharing over 80% of their genetic complement (Alcaraz *et al.*, 2010). What they lack in genomic novelty they compensate with specialised biochemical factories, which are clusters of genes dedicated to producing antimicrobial compounds that aid competition in the rhizosphere. Their ability to form spores represents a supreme genomic adaptation to environmental stress, enabling survival through adverse conditions. Comparative studies reveal Bacillus species favour vertical gene inheritance over horizontal acquisition, resulting in more predictable genomic architectures across strains and environments.

The Rhizobium genome exemplifies symbiotic specialisation. These nitrogen-fixing bacteria have undergone dramatic genomic rearrangements to perfect their relationship with legume hosts. Their genomes are often multipartite, with symbiotic capabilities frequently located on transferable mega-plasmids (Galibert *et al.*, 2001). This modular organisation facilitates efficient exchange of symbiotic genes while maintaining core cellular functions. Intriguingly, comparative genomics reveals rhizobia have sacrificed some metabolic capabilities present in free-living relatives, instead focusing genomic resources on refining plant-host communication. Their genomes encode elaborate molecular signaling systems, dedicating up to 10% of genes to regulation – indicating a testament to the complexity of sustaining this delicate symbiosis (Gibson *et al.*, 2008).

Comparative genomics of these model rhizobacteria reveals a fundamental truth about evolution in the rhizosphere: multiple solutions exist for the challenge of plant colonisation. Pseudomonas thrives through genomic flexibility and innovation; Bacillus through conservation and resilience; Rhizobium through symbiotic specialisation. Yet, despite different strategies, all three have converged on successful means to benefit from plant associations. These genomic insights satisfy scientific curiosity and provide blueprints for engineering beneficial plant-microbe relationships for sustainable agriculture. As sequencing technologies continue to advance, even deeper understanding of these bacterial genomes - shaped by millions of years of evolution - will illuminate the complex interplay of life in the rhizosphere.

METAGENOMICS

The rhizosphere represents one of Earth's most dynamic biological interfaces, where plant roots interact with complex microbial communities that strongly influence plant health and ecosystem productivity. Metagenomics has significantly advanced the study of these communities by enabling culture-independent assessment of microbial diversity and functional potential (Bulgarelli *et al.*, 2013). By sequencing total DNA extracted from rhizosphere soils, researchers can infer community composition and reconstruct partial to near-complete microbial genomes, providing insights into plant-driven microbiome assembly processes (Lundberg *et al.*, 2012). However, these reconstructions are inherently influenced by sequencing depth, assembly algorithms, and reference database completeness, which can bias both taxonomic assignment and functional inference.

Across diverse plant species and soil types, metagenomic studies consistently show that plants selectively enrich specific bacterial taxa from bulk soil, particularly members of the Proteobacteria, Actinobacteria, and Firmicutes phyla (Peiffer *et al.*, 2013). This selective enrichment is largely driven by root exudates, which supply chemically diverse carbon substrates that shape microbial community structure and function (Zhalnina *et al.*, 2018). However, linking exudate composition directly to microbial assembly remains challenging due to spatial heterogeneity in the rhizosphere and the limited resolution of bulk soil sampling approaches, which mask microscale variation in microbial distribution and activity.

Functional profiling of rhizosphere metagenomes indicates enrichment of genes associated with nutrient cycling (e.g., nitrogen fixation and phosphate solubilisation), abiotic stress tolerance, and plant growth promotion

(Sessitsch *et al.*, 2012). While these functional predictions provide valuable ecological hypotheses, they often represent *genetic potential rather than realised activity*, and may overestimate functional redundancy due to incomplete genome recovery and uneven gene annotation across taxa.

A major conceptual advance in rhizosphere research is the identification of core microbiomes—taxonomically and functionally conserved microbial assemblages associated with specific plant species across environmental contexts (Lemanceau *et al.*, 2017). For example, wheat cultivars consistently associate with *Pseudomonas* and *Bacillus* species across geographically distinct soils, suggesting host-driven selection pressures (Donn *et al.*, 2015). However, caution is required in interpreting "core" membership, as taxonomic resolution limitations in 16S rRNA and shotgun metagenomics can obscure strain-level variation that may be ecologically significant.

Metagenome-assembled genomes (MAGs) have improved resolution of rhizosphere functional potential by enabling reconstruction of draft genomes from environmental samples. These analyses suggest that distinct microbial lineages acquire genes associated with root adhesion, exudate utilization, and immune modulation, supporting niche adaptation in the rhizosphere (Ofek-Lalzar *et al.*, 2014). Nevertheless, MAG recovery is affected by assembly bias, differential coverage of abundant versus rare taxa, and fragmentation of genomes in complex communities. As a result, MAGs often represent incomplete genomic contexts, limiting accurate inference of operon structure, regulatory networks, and horizontal gene transfer events.

Integration of metagenomics with meta-transcriptomics and metabolomics has provided a more dynamic view of rhizosphere function, revealing that community composition alone is a poor predictor of metabolic activity (Williams *et al.*, 2021). Many rhizosphere microorganisms exist in metabolically inactive or low-activity states and are only activated in response to specific root-derived signals. This highlights a key limitation of DNA-based metagenomics: it captures *potential function*, not *functional execution*. Consequently, ecological interpretation requires caution when linking gene abundance to ecosystem processes.

Thus, while metagenomics has transformed understanding of rhizosphere microbiomes, its interpretative power is constrained by methodological biases (assembly artefacts, incomplete MAG recovery, and limited taxonomic resolution) and conceptual limitations (distinguishing potential from realized function). A critical synthesis therefore suggests that future progress will depend on tighter integration of multi-omics approaches, improved genome-resolved metagenomics, and spatially resolved sampling strategies capable of capturing microscale heterogeneity in plant–microbe interactions.

Impact of horizontal gene transfer on adaptation and niche specialisation

Rhizospheres are widely recognized as environments with elevated rates of horizontal gene transfer (HGT), largely due to high microbial density, intense interspecies interactions, and continuous input of plant-derived organic substrates (McDaniel *et al.*, 2010; van Elsas & Bailey, 2002). However, beyond simply increasing gene flow, HGT fundamentally reshapes rhizosphere evolution by accelerating the redistribution of adaptive traits across phylogenetically distant taxa, thereby decoupling ecological function from evolutionary lineage. This results in rapid functional convergence among unrelated bacteria occupying similar rhizosphere niches.

From an evolutionary perspective, one of the most significant consequences of HGT is the collapse of strict genotype–function boundaries, where key ecological traits become horizontally mobile rather than vertically inherited. The acquisition of nitrogen fixation capability illustrates this process. In several soil-associated lineages, including *Bradyrhizobium* spp., symbiotic islands and plasmids carrying nif operons enable rapid transitions from free-living to plant-associated lifestyles (Gyaneshwar *et al.*, 2011). Rather than representing gradual adaptation, this reflects episodic evolutionary innovation, where complex mutualistic capabilities can emerge in a single gene transfer event. At the ecosystem level, this promotes functional redundancy in nitrogen cycling, stabilizing plant nutrient acquisition even under fluctuating microbial community composition.

HGT also drives ecological niche partitioning through metabolic redistribution,

rather than simple trait acquisition. Comparative genomic evidence from *Pseudomonas* and *Bacillus* spp. shows that catabolic flexibility and transporter diversity are frequently linked to horizontally acquired gene clusters (Loper *et al.*, 2012). This does not merely expand substrate use; it restructures competitive interactions by allowing coexisting taxa to specialize on distinct components of root exudate mixtures. As a result, HGT contributes to the emergence of metabolic networks in which resource partitioning reduces direct competition and promotes microbial coexistence, enhancing overall community stability in the rhizosphere.

A further evolutionary consequence of HGT is the intensification of microbial competition mediated by mobile defensive traits, particularly antibiotic resistance determinants. The strAB streptomycin resistance cluster, located on plasmids in *Pseudomonas* spp., exemplifies how HGT can rapidly shift competitive balance in microbial communities exposed to antibiotic-producing neighbours (Ramette *et al.*, 2011). Importantly, such traits do not simply confer survival advantages; they actively restructure microbial interaction networks by altering susceptibility hierarchies and competitive exclusion dynamics. This highlights HGT as a driver of community reorganization under both natural and anthropogenically influenced selection pressures.

At the ecosystem scale, metagenomic and MAG-based studies increasingly suggest that HGT is functionally biased rather than random, with mobile genetic elements disproportionately carrying genes linked to rhizosphere fitness traits such as nutrient acquisition, stress tolerance, and host interaction (Soucy *et al.*, 2015). This indicates that ecological selection acts not only on organisms but also on mobile genes themselves, effectively treating genes as adaptive units subject to environmental filtering. Consequently, the rhizosphere can be viewed as an evolutionary "gene marketplace," where successful traits are continuously exchanged, retained, or lost depending on their contribution to local fitness. Thus, HGT in the rhizosphere should not be interpreted solely as a mechanism of genetic exchange, but as a major evolutionary force shaping ecological networks, functional redundancy, and community-level adaptation, ultimately influencing both microbial coexistence and plant-associated ecosystem stability.

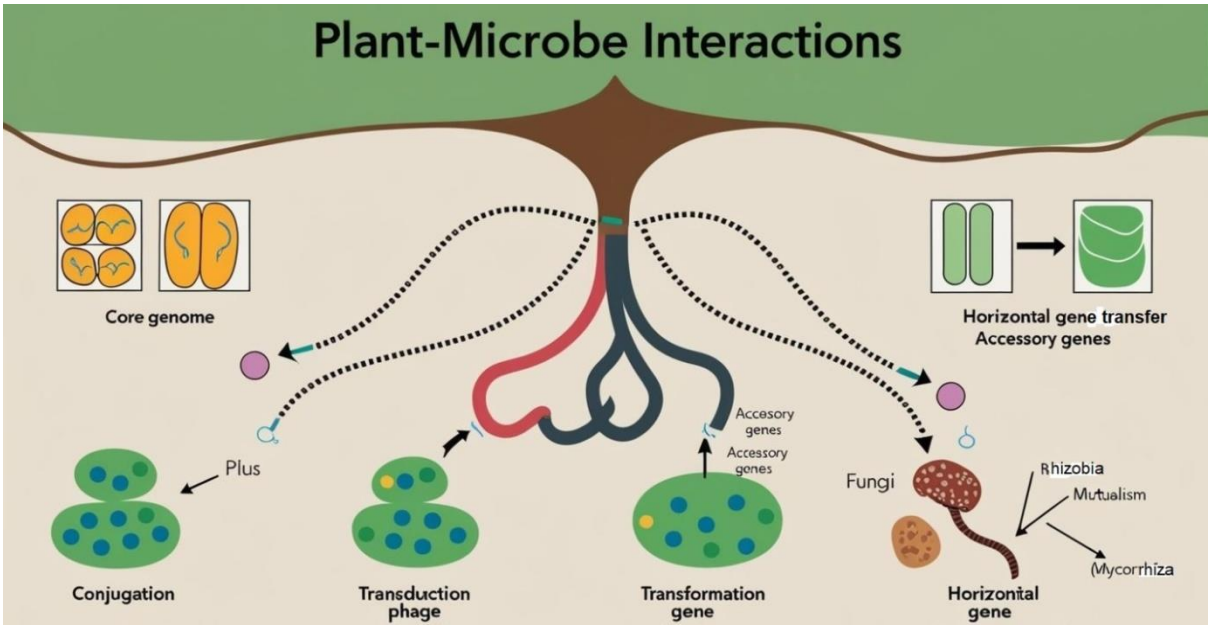

Figure 1. Mechanisms of horizontal gene transfer (HGT) and plant-microbe interactions in the rhizosphere. This diagram illustrates major processes underlying microbial gene exchange and symbiotic interactions in the rhizosphere. The central plant root system is shown engaging with diverse soil microbes that contribute to plant health and environmental adaptation through multiple pathways of HGT and mutualistic interactions. On the left, the core genome - comprising essential, conserved genes shared among all members of a microbial species - is contrasted with accessory genes, which are more variable and often acquired through HGT. Three primary mechanisms of HGT are depicted: (1) conjugation, involving the direct transfer of plasmids between bacterial

cells via a conjugative pilus (Plus in the drawing); (2) transduction, wherein bacteriophages mediate gene transfer between bacterial hosts; and (3) transformation, where bacteria uptake and incorporate free DNA from the surrounding environment. On the right, microbial partners, including fungi and bacteria (e.g., *Rhizobia*) are shown contributing accessory genes to the rhizosphere microbial pool. These genes can enhance microbial fitness and influence traits like nutrient acquisition, stress tolerance, and colonization capacity. *Rhizobia* are highlighted for their role in nitrogen-fixing symbioses with legumes, while mycorrhizal fungi form mutualistic associations that improve plant nutrient uptake. The image emphasises how gene exchange and symbiosis influence the organisation and activity of rhizosphere microbial assemblages, facilitating adaptive plant-microbe interactions in complex soil environments.

PANGENOMICS

The concept of pangenomes has transformed our understanding of microbial diversity in the rhizosphere, revealing a genetic reservoir far more extensive than previously imagined. A pangenome refers to the collective genetic repertoire of a microbial species, comprising both the core genome (shared by all strains) and the accessory genome (variable between strains) (Tettelin *et al.*, 2008). This framework has proven particularly valuable for studying rhizosphere bacteria, where genomic plasticity enables rapid adaptation to plant hosts and environmental fluctuations (Levy *et al.*, 2018). Recent pangenome analyses of key rhizobacteria such as Pseudomonas, Bacillus, and Rhizobium species have uncovered remarkable patterns of genomic variation. The Pseudomonas pangenome, for instance, remains "open", meaning each newly sequenced strain contributes novel genes to the overall gene pool (Silby *et al.*, 2011). This reflects extraordinary genomic flexibility, explaining Pseudomonas's success in diverse rhizosphere environments. In contrast, Bacillus species tend to have more "closed" pangenomes, with a higher proportion of core genes and a limited accessory genome (Alcaraz *et al.*, 2010). Rhizobium species exhibit yet another pattern, whereby symbiotic capabilities are often encoded on accessory genomic elements such as plasmids, facilitating the horizontal transfer of plant-beneficial traits (Haskett *et al.*, 2022).

Pangenome studies have significantly advanced our understanding of how rhizobacteria adapt to the complex and competitive rhizosphere environment. One prominent feature revealed by these analyses is the expanded metabolic repertoire found in many rhizobacterial strains. Specifically, there is an enrichment of accessory genes linked to carbohydrate utilization and secondary metabolite synthesis. These genes enable rhizobacteria to utilise a wide array of plant-derived compounds, contributing to their ability to thrive in diverse soil microhabitats (Levy *et al.*, 2018). Another critical insight from pangenomic investigations is the prevalence and ecological role of mobile genetic elements such as plasmids and transposons. These elements often carry genes conferring plant-beneficial traits, including nutrient acquisition, phytohormone production, and stress resistance. Their mobility enables rapid horizontal gene transfer, accelerating adaptive evolution in rhizobacterial populations and allowing them to respond flexibly to environmental changes (Van Elsas *et al.*, 2003).

Furthermore, pangenome analyses have uncovered evidence of host-specific genetic adaptations. Certain gene clusters are strongly associated with specific plant hosts, suggesting co-evolutionary relationships and fine-tuned symbiotic capabilities. For example, some rhizobacterial strains harbour specialised gene sets that are selectively expressed in response to particular root exudates, enhancing colonisation efficiency and mutualistic outcomes (Ofek-Lalzar *et al.*, 2014). These findings highlight the dynamic interplay between microbial genome plasticity and ecological specialisation in the rhizosphere. Thus, the integration of pangenomics with metagenomic approaches has greatly enhanced the study of rhizosphere microbial communities. This combined strategy enables researchers to track the distribution of core and accessory genes across complex microbial assemblages, identify potentially valuable plant-growth-promoting genes in uncultured microbes, and understand how different agricultural practices impact microbial genomic diversity and ecosystem functioning (Bashan *et al.*, 2014).

GENOMICS-GUIDED MICROBIAL INOCULANT DEVELOPMENT

The development of effective PGPR inoculants depends on a multifactorial selection process guided increasingly by genomic insights. Key criteria include genetic stability, functional versatility, environmental adaptability, and a demonstrated effectiveness in promoting plant health *in situ*. An ideal inoculant strain must possess genes involved in critical plant-beneficial traits, such as nitrogen fixation (*nifH, fixABCX*), phosphate solubilization (*gcd, pqq*), phytohormone biosynthesis (e.g., *ipdC* for indole acetic acid production), and stress tolerance mechanisms (e.g., *katG*, *proU*). Importantly, whole-genome sequencing enables the identification of accessory genes that confer niche-specific advantages such as colonisation ability, antimicrobial resistance, and metabolic flexibility (Compant *et al.*, 2019). Beyond individual traits, comparative genomics helps evaluate the presence of biosynthetic gene clusters (BGCs) for secondary metabolites like antibiotics and siderophores, which can suppress phytopathogens and enhance competitiveness. Additionally, inoculant candidates should lack genes associated with pathogenicity or toxin production, ensuring biosafety for both plants and ecosystems.

Several successful examples illustrate how genomics has revolutionized PGPR-based bioinoculant design. For instance, *Pseudomonas fluorescens* F113 was selected for its well-characterized genome, which includes genes related to antifungal compound production (e.g., *phl* gene cluster for 2,4-diacetylphloroglucinol) and stress adaptation, making it an effective biocontrol agent (Barret *et al.*, 2011). Another case involves *Bacillus amyloliquefaciens* FZB42, whose genome revealed multiple gene clusters for lipopeptides like surfactin and bacillomycin D, contributing to its antagonism against pathogens and enhancement of systemic resistance in plants (Chen *et al.*, 2007). Advances in metagenomics have also enabled the identification of candidate strains directly from rhizosphere samples, followed by functional annotation and synthetic biology approaches to improve or combine beneficial traits. In some projects, genome mining has been coupled with CRISPR-Cas systems to knock out deleterious genes or enhance stress-related pathways, creating custom-designed strains with optimized performance under diverse environmental conditions.

Future innovations in bioinoculant design will rely on the combined insights of genomics, transcriptomics, proteomics, and metabolomics to create highly adaptive and context-specific microbial solutions. Precision agriculture will increasingly rely on customized microbial consortia tailored to crop genotype, soil type, and climatic conditions. Genome editing tools such as CRISPR/Cas9 and synthetic biology will allow for the fine-tuning of intricate regulatory networks and interconnected metabolic pathways, expanding the scope of functional traits that can be engineered into PGPR strains (Arora *et al.*, 2017). Furthermore, machine learning and bioinformatics tools are being developed to predict plant-microbe compatibility and simulate ecological outcomes of inoculant introduction. Regulatory frameworks will also need to evolve to accommodate genetically engineered microbes, ensuring ecological safety and efficacy. Importantly, there is a growing emphasis on translating lab-scale genomic advancements into field-ready products through multidisciplinary collaboration and farmer-centered deployment strategies. As we move toward climate-resilient agriculture, genomics-guided bioinoculants hold the potential to significantly reduce chemical inputs while enhancing productivity and sustainability.

IMPLICATIONS FOR SUSTAINABLE AGRICULTURE

Plant-growth-promoting rhizobacteria (PGPR) are emerging as vital components of sustainable agriculture due to their ability to enhance crop yields and improve plant resilience to biotic and abiotic stresses. By facilitating nutrient uptake, modulating phytohormones, and inducing systemic resistance, PGPR contribute significantly to increased plant productivity under both optimal and stress-prone conditions (Backer *et al.*, 2018). For instance, nitrogen-fixing bacteria like *Azospirillum brasilense* and *Rhizobium* species improve nitrogen availability, species like *Pseudomonas fluorescens* and *Bacillus subtilis*, noted for their phosphate-solubilizing capacity, enhance phosphorus uptake, both of which are essential for robust plant growth (Adesemoye *et al.*, 2009). Furthermore, PGPR strains producing ACC deaminase reduce ethylene levels in plants, mitigating stress effects from drought, salinity, and heavy metals (Glick, 2014). Genomics-

driven selection and engineering of PGPR with targeted traits offer new avenues for developing bioinoculants that are not only efficient in colonizing the rhizosphere but also responsive to specific crop and soil conditions, thereby enhancing the resilience of crop yields amidst increasing climatic variability.

One of the most significant environmental benefits of PGPR lies in their capacity to reduce the widespread use of synthetic fertilizers and pesticides, which has been linked to adverse environmental outcomes including soil quality decline, waterway contamination, and increased greenhouse gas emissions. Biofertilizers derived from PGPR can supplement or even replace chemical fertilizers by naturally fixing atmospheric nitrogen, solubilizing unavailable phosphorus, and mobilizing potassium and micronutrients (Vessey, 2003). Additionally, the biocontrol potential of PGPR, through production of antibiotics, lytic enzymes, and competition for niche and nutrients, offers an eco-friendly alternative to chemical pesticides (Lugtenberg & Kamilova, 2009). The use of PGPR-based formulations has shown consistent promise in integrated pest management (IPM) systems, contributing to sustainable intensification of agriculture with minimal environmental footprint. With advances in genomics and microbial consortia design, the formulation of multi-strain inoculants tailored to specific crops and agroecosystems is becoming increasingly feasible, further enhancing their effectiveness and scalability (Arora *et al.*, 2020).

Despite their proven benefits, widespread adoption of PGPR-based bioinoculants faces several regulatory and practical challenges. Regulatory frameworks for microbial inoculants vary across regions and often lag behind the pace of scientific advancement, particularly in relation to genetically modified strains or synthetic biology applications (Compant *et al.*, 2019). Clear guidelines on efficacy testing, biosafety assessments, and quality control standards are essential to foster trust among stakeholders and ensure responsible use. Additionally, farmer education, extension services, and demonstration trials are critical for promoting adoption, particularly in low-input and smallholder farming systems. Practical challenges such as shelf life, formulation stability, and delivery methods also need to be addressed through advances in encapsulation technologies and carrier materials. Collaborative efforts between researchers, policymakers, private sectors, and farming communities are necessary to translate the promise of genomics-guided PGPR development into field-level impact, thereby aligning agricultural productivity with environmental sustainability goals.

ESTABLISHED ADVANCES IN PGPR RESEARCH

Recent progress in plant-growth-promoting rhizobacteria (PGPR) research has been driven by the integration of multi-omics approaches, combining genomics, transcriptomics, proteomics, and metabolomics to provide a more comprehensive understanding of plant–microbe interactions. These integrated datasets have already demonstrated that PGPR functions are regulated by coordinated genetic, metabolic, and environmental signals rather than single-gene effects, enabling improved resolution of microbial functional potential across environmental gradients (Berendsen *et al.*, 2018). In particular, multi-omics studies have improved the identification of gene–trait associations linked to nutrient acquisition, stress tolerance, and plant colonisation, while also revealing strong context dependency in gene expression across soil and host environments.

Similarly, advances in genome editing, particularly CRISPR/Cas-based systems, have already been applied to functional validation of key PGPR genes through targeted knockouts and regulatory modifications (Wang *et al.*, 2019). These studies have confirmed the roles of specific genes in traits such as biocontrol activity, nutrient mobilization, and stress response. In parallel, early-stage synthetic biology applications have demonstrated the feasibility of constructing engineered microbial strains and simplified consortia with complementary metabolic functions, although most applications remain confined to controlled or semi-controlled environments.

Machine learning approaches have also begun to show utility in analysing large-scale genomic and environmental datasets. Existing models have successfully identified genetic predictors of plant-associated fitness traits and improved classification of microbial functional potential under defined environmental conditions (Sharma *et al.*, 2020). These developments collectively indicate that data-driven prediction of plant–microbe interactions is feasible, although still limited by incomplete training datasets and environmental variability.

EMERGING DIRECTIONS AND FUTURE PROSPECTS

Building on these established advances, the next phase of PGPR research is expected to focus on improving predictive accuracy, ecological realism, and translational scalability. One key direction is the deeper integration of time-resolved and spatially resolved multi-omics data to better capture dynamic microbial responses in the rhizosphere. While initial studies have demonstrated temporal shifts in gene expression and metabolite profiles, future work will need to extend these approaches to field conditions where environmental heterogeneity is substantially greater. This is likely to improve understanding of how microbial functions fluctuate across root zones and environmental gradients.

In genome editing and synthetic biology, current evidence from laboratory and greenhouse studies suggests that targeted genetic modifications can enhance specific PGPR traits. However, future applications will need to address the stability and ecological performance of engineered strains under field conditions, particularly their interaction with native microbial communities. The development of synthetic microbial consortia is similarly grounded in existing evidence that microbial interactions can be complementary, but their predictable performance across diverse soils remains an open challenge.

In parallel, machine learning and artificial intelligence approaches are expected to expand in predictive capability as more high-quality multi-omics and environmental datasets become available. At present, their utility is strongest in controlled datasets, but future improvements in model generalization will depend on incorporating ecological variability, host genotype diversity, and longitudinal field data. This will be essential for moving from correlation-based predictions toward robust, field-applicable decision-support tools.

FIELD APPLICATION AND IMPLEMENTATION CHALLENGES

Translational research will continue to play a central role in bridging laboratory findings with agricultural application. Existing field trials demonstrate that genomics-informed inoculants can improve plant growth and stress resilience under specific conditions, but performance remains variable across geographies, soil types, and management practices. This variability highlights the need for expanded multi-location field validation and standardized evaluation frameworks. Successful deployment will also depend on practical considerations such as formulation stability, microbial viability during storage, and delivery efficiency under real farming conditions (Vargas-García *et al.*, 2021). Engagement with agricultural stakeholders, including farmers and extension services, is already recognised as critical for adoption, but future progress will require stronger integration of socio-economic factors into product design and testing pipelines.

REGULATORY AND POLICY CONSIDERATIONS

Regulatory frameworks for microbial inoculants are evolving alongside technological advances. Existing biosafety and efficacy assessment guidelines provide a foundation for commercial deployment, but future regulatory systems will need to accommodate engineered strains and synthetic consortia. Harmonised international standards will likely become increasingly important as PGPR technologies move across borders and agricultural systems.

CONCLUSION

Genomic and multi-omics approaches have substantially advanced understanding of rhizobacterial ecology by revealing the genetic and functional basis of plant–microbe interactions. Key insights include the importance of core and accessory genome dynamics in shaping ecological adaptation, the role of horizontal gene transfer in accelerating functional innovation, and the contribution of genome plasticity to niche differentiation and symbiotic competence. Comparative genomics has further demonstrated that distinct rhizobacterial lineages adopt divergent evolutionary strategies ranging from symbiotic specialisation in *Rhizobium* spp. to metabolic generalism in *Pseudomonas* spp. and stress-resilient conservatism in *Bacillus* spp., thereby highlighting the diversity of genomic solutions to rhizosphere survival.

Despite these advances, important limitations remain. A major challenge is the persistent gap between genomic potential and realized function, as gene presence does not always translate into in situ expression or ecological activity. In addition, methodological constraints such as assembly

bias, incomplete metagenome-assembled genomes (MAGs), uneven taxonomic resolution, and limited functional annotation continue to restrict the accuracy of community-level inference. Furthermore, laboratory-based omics studies often fail to fully capture the spatial heterogeneity, temporal dynamics, and complex multi-species interactions characteristic of natural rhizosphere environments, limiting direct extrapolation to field conditions.

From an applied perspective, genomic insights are increasingly informing the development of next-generation microbial inoculants and synthetic communities for sustainable agriculture. However, translation into practice requires careful consideration of ecological context, strain stability, and performance variability across soils, climates, and crop systems. Integrating genomics with field-scale validation, ecological modelling, and stakeholder engagement will therefore be essential for ensuring reliable and scalable applications. Ultimately, the continued convergence of genomics, systems biology, and agricultural biotechnology offers a strong foundation for rational design of microbial solutions, provided that current methodological and ecological limitations are explicitly addressed.

REFERENCES

Adesemoye, A. O., Torbert, H. A., & Kloepper, J. W. (2009). Plant growth-promoting rhizobacteria allow reduced application rates of chemical fertilizers. *Microbial Ecology,* 58 (4), 921-929.

Allard-Massicotte, R., Tessier, L., Lécuyer, F., Lakshmanan, V., Lucier, J.F., Garneau, D., & Vlamakis, H. (2016). *Bacillus subtilis* early colonization of *Arabidopsis thaliana* roots involves multiple chemotaxis receptors. *mBio*, 7 (6), e01664-16.

Alcaraz, L. D., Moreno-Hagelsieb, G., Eguiarte, L. E., Souza, V., Herrera-Estrella, L., & Olmedo, G. (2010). Understanding the evolutionary relationships and major traits of *Bacillus* through comparative genomics. *BMC Genomics*, *11* (1), 332.

Arnaouteli, S., Bamford, N. C., Stanley-Wall, N. R., & Kovács, Á. T. (2021). Bacillus subtilis biofilm formation and social interactions. *Nature Reviews Microbiology*, *19*(9), 600–614.

Arora, N.K., Verma, M., Mishra, J. (2017). Rhizobial Bioformulations: Past, Present and Future. In: Mehnaz, S. (eds) Rhizotrophs: Plant Growth Promotion to Bioremediation. Microorganisms for Sustainability, vol 2. Springer, Singapore.

Astapati, A. D., & Nath, S. (2023). The complex interplay between plant-microbe and virus interactions in sustainable agriculture: Harnessing phytomicrobiomes for enhanced soil health, designer plants, resource use efficiency, and food security. *Crop Design*, *2*(1), 100028.

Backer, R., Rokem, J. S., Ilangumaran, G., Lamont, J., Praslickova, D., Ricci, E., ... & Smith, D. L. (2018). Plant growth-promoting rhizobacteria: Context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. *Frontiers in Plant Science*, *9*, 1473.

Barbe, V., Cruveiller, S., Kunst, F., Lenoble, P., Meurice, G., Sekowska, A., ... & Danchin, A. (2009). From a consortium sequence to a unified sequence: The *Bacillus subtilis* 168 reference genome a decade later. *Microbiology*, *155* (6), 1758-1775.

Barret, M., Morrissey, J. P., & O'Gara, F. (2011). Functional genomics analysis of plant growth-promoting rhizobacterial traits involved in rhizosphere competence. *Biology and Fertility of Soils*, *47* (7), 729-743.

Bashan, Y., de-Bashan, L. E., Prabhu, S. R., & Hernandez, J.-P. (2014). Advances in plant growth-promoting bacterial inoculant technology: Formulations and practical perspectives (1998–2013). *Plant and Soil*, 378 (1-2), 1-33.

Berg, G., & Smalla, K. (2009). Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS Microbiology Ecology*, 68 (1), 1-13.

Berendsen, R. L., Pieterse, C. M., & Bakker, P. A. (2012). The rhizosphere microbiome and plant health. *Trends in Plant Science*, 17 (8), 478-486.

Bulgarelli, D., Schlaeppi, K., Spaepen, S., van Themaat, E. V. L., & Schulze-Lefert, P. (2013). Structure and functions of the bacterial microbiota of plants. *Annual Review of Plant Biology*, 64 , 807-838.

Burley, S.K., Berman, H.M., Kleywegt, G.J., Markley, J.L., Nakamura, H., Velankar, S. (2017). Protein Data Bank (PDB): The Single Global Macromolecular Structure Archive. In: Wlodawer, A., Dauter, Z., Jaskolski, M. (eds) Protein Crystallography. Methods in Molecular Biology, vol 1607. Humana Press, New York, NY.

Busby, P. E., Soman, C., Wagner, M. R., Friesen, M. L., Kremer, J., Bennett, A., ... & Dangl, J. L. (2017). Research priorities for harnessing plant microbiomes in sustainable agriculture. *PLoS Biology*, 15 (3), e2001793.

Che, Y., Yang, Y., Xu, X., Břinda, K., Polz, M. F., Hanage, W. P., & Zhang, T. (2021). Conjugative plasmids interact with insertion sequences to shape the horizontal transfer of antimicrobial resistance genes. *Proceedings of the National Academy of Sciences*, *118*(6), e2008731118.

Chen, X. H., Koumoutsi, A., Scholz, R., Eisenreich, A., Schneider, K., Heinemeyer, I., ... & Borriss, R. (2007). Comparative analysis of the complete genome sequence of the plant growth–promoting bacterium *Bacillus amyloliquefaciens* FZB42. *Nature Biotechnology*, *25* (9), 1007-1014.

Compant, S., Samad, A., Faist, H., & Sessitsch, A. (2019). A review on the plant microbiome: Ecology, functions, and emerging trends in microbial application. *Journal of Advanced Research, 19* , 29-37.

Cornelis, P. (2010). Iron uptake and metabolism in pseudomonads. *Applied Microbiology and Biotechnology*, *86* (6), 1637-1645.

Cotta, S. R., Dias, A. C. F., Mendes, R., & Andreote, F. D. (2024). Role of horizontal gene transfer and cooperation in rhizosphere microbiome assembly. *Brazilian Journal of Microbiology*, 1-12.

Crosa, J. H., & Walsh, C. T. (2002). Genetics and assembly line enzymology of siderophore biosynthesis in bacteria. *Microbiology and Molecular Biology Reviews*, 66 (2), 223-249.

Dos Santos, P. C., Fang, Z., Mason, S. W., Setubal, J. C., & Dixon, R. (2012). Distribution of nitrogen fixation and nitrogenase-like sequences amongst microbial genomes. *BMC Genomics*, 13 (1), 162.

Egamberdieva, D., Wirth, S., Bellingrath-Kimura, S. D., Mishra, J., & Arora, N. K. (2019). Salt-tolerant plant growth promoting rhizobacteria for enhancing crop productivity of saline soils. *Frontiers in Microbiology*, 10 , 2791.

Ercole, T. G., Savi, D. C., Adamoski, D., Kava, V. M., Hungria, M., & Galli-Terasawa, L. V. (2021). Diversity of maize (Zea mays L.) rhizobacteria with potential to promote plant growth. *Brazilian Journal of Microbiology*, *52*(4), 1807-1823.

Galibert, F., Finan, T. M., Long, S. R., Pühler, A., Abola, P., Ampe, F., ... & Batut, J. (2001). The composite genome of the legume symbiont *Sinorhizobium meliloti*. *Science*, 293 (5530), 668-672.

Gibson, K. E., Kobayashi, H., & Walker, G. C. (2008). Molecular determinants of a symbiotic chronic infection. *Annual Review of Genetics*, 42 , 413-441.

Glick, B. R. (2014). Bacteria with ACC deaminase can promote plant growth and help to feed the world. *Microbiological Research*, 169 (1), 30-39.

Grabowski, M., Langner, K. M., Cymborowski, M., Porebski, P. J., Sroka, P., Zheng, H., ... & Minor, W. (2016). A public database of macromolecular diffraction experiments. *Acta Crystallographica Section D: Structural Biology*, 72 (11), 1181-1193.

Guzmán-Guzmán, P., & Santoyo, G. (2022). Action mechanisms, biodiversity, and omics approaches in biocontrol and plant growth-promoting Pseudomonas: an updated review. *Biocontrol Science and Technology*, *32*(5), 527-550.

Gyaneshwar, P., Hirsch, A. M., Moulin, L., Chen, W. M., Elliott, G. N., Bontemps, C., ... & Young, J. P. (2011). Legume-nodulating betaproteobacteria: Diversity, host range, and future prospects. *Molecular Plant-Microbe Interactions*, 24 (11), 1276-1288.

Hartmann, A., Rothballer, M., & Schmid, M. (2008). Lorenz Hiltner, a pioneer in rhizosphere microbial ecology and soil bacteriology research. *Plant and Soil*, 312 (1-2), 7-14.

Hoffman, B. M., Lukoyanov, D., Yang, Z. Y., Dean, D. R., & Seefeldt, L. C. (2014). Mechanism of nitrogen fixation by nitrogenase: The next stage. *Chemical Reviews*, *114* (8), 4041-4062.

Hu, B., Morado, D. R., Margolin, W., Rohde, J. R., Arizmendi, O., Picking, W. L., ... & Liu, J. (2015). Visualization of the type III secretion sorting platform of Shigella flexneri. *Proceedings of the National Academy of Sciences*, *112*(4), 1047-1052.

Jumper, J., Evans, R., Pritzel, A., Green, T., Figurnov, M., Ronneberger, O., ... & Hassabis, D. (2021). Highly accurate protein structure prediction with AlphaFold. *Nature*, *596* (7873), 583-589.

Jung, B. K., Ibal, J. C., Pham, H. Q., Kim, M. C., Park, G. S., Hong, S. J., ... & Shin, J. H. (2020). Quorum sensing system affects the plant growth promotion traits of Serratia fonticola GS2. *Frontiers in Microbiology*, *11*, 536865.

Kaneko, T., Nakamura, Y., Sato, S., Asamizu, E., Kato, T., Sasamoto, S., ... & Tabata, S. (2000). Complete genome structure of the nitrogen-fixing symbiotic bacterium *Mesorhizobium loti*. *DNA Research*, *7* (6), 331-338.

Khan, M. S., Gao, J., Chen, X., Zhang, M., Yang, F., Du, Y., ... & Zhang, X. (2020). Isolation and characterization of plant growth-promoting endophytic bacteria *Paenibacillus polymyxa* SK1 from *Lilium lancifolium*. *BioMed Research International*, *2020*, 8650957.

Ku, Y. S., Wang, Z., Duan, S., & Lam, H. M. (2021). Rhizospheric communication through mobile genetic element transfers for the regulation of microbe–plant interactions. *Biology*, *10*(6), 477.

Kumar, A., & Verma, J. P. (2018). Does plant—microbe interaction confer stress tolerance in plants: a review?. *Microbiological research*, *207*, 41-52.

Kunst, F. J., Ogasawara, N., Moszer, I., Albertini, A. M., Alloni, G., Azevedo, V., ... & Yoshikawa, H. (1997). The complete genome sequence of the gram-positive bacterium Bacillus subtilis. *Nature*, *390*(6657), 249-256.

Lemanceau, P., Blouin, M., Muller, D., & Moënne-Loccoz, Y. (2017). Let the core microbiota be functional. *Trends in Plant Science*, *22* (7), 583-595.

Levy, A., Salas Gonzalez, I., Mittelviefhaus, M., Clingenpeel, S., Herrera Paredes, S., Miao, J., ... & Dangl, J. L. (2018). Genomic features of bacterial adaptation to plants. *Nature Genetics*, 50 (1), 138-150.

Loper, J. E., Hassan, K. A., Mavrodi, D. V., Davis, E. W., Lim, C. K., Shaffer, B. T., ... & Paulsen, I. T. (2012). Comparative genomics of plant-associated *Pseudomonas* spp.: Insights into diversity and inheritance of traits involved in multitrophic interactions. *PLoS Genetics*, 8 (7), e1002784.

Lugtenberg, B., & Kamilova, F. (2009). Plant-growth-promoting rhizobacteria. *Annual Review of Microbiology*, *63* , 541-556.

Mark, G. L., Dow, J. M., Kiely, P. D., Higgins, H., Haynes, J., Baysse, C., ... & Morrissey, J. P. (2005). Transcriptome profiling of bacterial responses to root exudates identifies genes involved in microbe-plant interactions. *Proceedings of the National Academy of Sciences*, *102* (48), 17454-17459.

Matilla, M. A., Espinosa-Urgel, M., Rodríguez-Herva, J. J., Ramos, J. L., & Ramos-González, M. I. (2007). Genomic analysis reveals the major driving forces of bacterial life in the rhizosphere. *Genome Biology*, *8* (9), R179.

Mavrodi, O. V., McWilliams, J. R., Peter, J. O., Berim, A., Hassan, K. A., Elbourne, L. D., ... & Mavrodi, D. V. (2021). Root exudates alter the expression of diverse metabolic, transport, regulatory, and stress response genes in rhizosphere Pseudomonas. *Frontiers in Microbiology*, *12*, 651282.

Mavrodi, D. V., Peever, T. L., Mavrodi, O. V., Parejko, J. A., Raaijmakers, J. M., Lemanceau, P., ... & Weller, D. M. (2009). Diversity and evolution of the phenazine biosynthesis pathway. *Applied and Environmental Microbiology*, *76* (3), 866-879.

McDaniel, L. D., Young, E., Delaney, J., Ruhnau, F., Ritchie, K. B., & Paul, J. H. (2010). High frequency of horizontal gene transfer in the oceans. *Science*, *330* (6000), 50.

Medini, D., Donati, C., Tettelin, H., Masignani, V., & Rappuoli, R. (2005). The microbial pan-genome. *Current Opinion in Genetics & Development*, *15* (6), 589-594.

Mendes, R., Garbeva, P., & Raaijmakers, J. M. (2013). The rhizosphere microbiome: Significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS Microbiology Reviews*, *37* (5), 634-663.

Mitter, B., Pfaffenbichler, N., Flavell, R., Compant, S., Antonielli, L., Petric, A., ... & Sessitsch, A. (2017). A new approach to modify plant microbiomes and traits by introducing beneficial bacteria at flowering into progeny seeds. *Frontiers in Microbiology*, *8*, 11.

Naylor, D., DeGraaf, S., Purdom, E., & Coleman-Derr, D. (2017). Drought and host selection influence bacterial community dynamics in the grass root microbiome. *The ISME Journal*, *11* (12), 2691-2704.

Ofek-Lalzar, M., Sela, N., Goldman-Voronov, M., Green, S. J., Hadar, Y., & Minz, D. (2014). Niche and host-associated functional signatures of the root surface microbiome. *Nature Communications*, *5* (1), 4950.

Oldroyd, G. E., Murray, J. D., Poole, P. S., & Downie, J. A. (2011). The rules of engagement in the legume-rhizobial symbiosis. *Annual Review of Genetics*, *45* , 119-144.

Pahari, A., Nayak, S. K., Banik, A., Lakra, P. B., & Mishra, B. B. (2021). Biological nitrogen fixation mechanism and applications. In Agriculturally Important Microorganisms (pp. 137-151). CRC Press.

Patel, H. K., Suárez-Moreno, Z. R., Degrassi, G., Subramoni, S., González, J. F., & Venturi, V. (2013). Bacterial LuxR solos have evolved to respond to different molecules including signals from plants. *Frontiers in Plant Science*, *4*, 447.

Philippot, L., Raaijmakers, J. M., Lemanceau, P., & van der Putten, W. H. (2013). Going back to the roots: The microbial ecology of the rhizosphere. *Nature Reviews Microbiology*, 11 (11), 789-799.

Radutoiu, S., Madsen, L. H., Madsen, E. B., Felle, H. H., Umehara, Y., Grønlund, M., ... & Stougaard, J. (2003). Plant recognition of symbiotic bacteria requires two LysM receptor-like kinases. *Nature*, *425* (6958), 585-592.

Ramette, A., Frapolli, M., Fischer-Le Saux, M., Gruffaz, C., Meyer, J. M., Défago, G., ... & Moënne-Loccoz, Y. (2011). *Pseudomonas protegens* sp. nov., widespread plant-protecting bacteria producing the biocontrol compounds 2,4-diacetylphloroglucinol and pyoluteorin. *Systematic and Applied Microbiology*, *34* (3), 180-188.

Rawat, P., Das, S., Shankhdhar, D., & Shankhdhar, S. C. (2021). Phosphate-solubilizing microorganisms: mechanism and their role in phosphate solubilization and uptake. *Journal of Soil Science and Plant Nutrition*, *21*(1), 49-68.

Rodríguez, H., Fraga, R., Gonzalez, T., & Bashan, Y. (2006). Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. *Plant and Soil*, *287* (1-2), 15-21.

Rohrbacher, F., & St-Arnaud, M. (2016). Root exudation: the ecological driver of hydrocarbon rhizoremediation. *Agronomy*, 6(1), 19.

Seneviratne, C. J., Suriyanarayanan, T., Widyarman, A. S., Lee, L. S., Lau, M., Ching, J., ... & Ramage, G. (2020). Multi-omics tools for studying microbial biofilms: current perspectives and future directions. *Critical Reviews in Microbiology*, *46*(6), 759-778.

Setubal, J. C. (2021). Metagenome-assembled genomes: concepts, analogies, and challenges. *Biophysical reviews*, *13*(6), 905-909.

Silby, M. W., Winstanley, C., Godfrey, S. A., Levy, S. B., & Jackson, R. W. (2011). *Pseudomonas* genomes: Diverse and adaptable. *FEMS Microbiology Reviews*, *35* (4), 652-680.

Sindhu, S. S., Sehrawat, A., Phour, M., & Kumar, R. (2022). Nutrient acquisition and soil fertility: Contribution of rhizosphere microbiomes in sustainable agriculture. In Microbial BioTechnology for Sustainable Agriculture Volume 1 (pp. 1-41). Singapore: Springer Nature Singapore.

Singh, M., Kumar, J., Singh, S., Singh, V. P., & Prasad, S. M. (2015). Roles of osmoprotectants in improving salinity and drought tolerance in plants: a review. *Reviews in environmental science and bio/technology*, *14*, 407-426.

Sonenshein, A.L., Hoch, J.A., Losick R (2002). Bacillus subtilis: From Cells to Genes and from Genes to Cells. In AL Sonenshein, JA Hoch, R Losick (Eds.), Bacillus subtilis and Its Closest Relatives (pp. 3–5).

Soucy, S. M., Huang, J., & Gogarten, J. P. (2015). Horizontal gene transfer: Building the web of life. *Nature Reviews Genetics*, *16* (8), 472-482.

Sullivan, J. T., & Ronson, C. W. (1998). Evolution of rhizobia by acquisition of a 500-kb symbiosis island that integrates into a phe-tRNA gene. *Proceedings of the National Academy of Sciences*, *95* (9), 5145-5149.

Tettelin, H., Riley, D., Cattuto, C., & Medini, D. (2008). Comparative genomics: The bacterial pan-genome. *Current Opinion in Microbiology*, *11* (5), 472-477.

Thankappan, S., Binodh, A. K., Kumar, P. R., Kurien, S., Narayanasamy, S., Prabina, J. B., & Uthandi, S. (2024). Genome Editing of Plant Growth-Promoting Microbes (PGPM) Towards Developing Smart Bio-Formulations for Sustainable Agriculture: Current Trends and Perspectives. *Genome Editing in Bacteria (Part 2)*, 106-149.

Timmusk, S., El-Daim, I. A. A., Copolovici, L., Tanilas, T., Kännaste, A., Behers, L., ... & Niinemets, Ü. (2014). Drought-tolerance of wheat improved by rhizosphere bacteria from harsh environments: Enhanced biomass production and reduced emissions of stress volatiles. *PLoS ONE*, *9* (5), e96086.

Van Elsas, J. D., Turner, S., & Bailey, M. J. (2003). Horizontal gene transfer in the phytosphere. *New Phytologist*, *157*(3), 525-537.

Wani, A. K., Rahayu, F., Alkahtani, A. M., Alreshidi, M. A., Yadav, K. K., Fauziah, L., ... & Singh, R. (2024). Metagenomic profiling of rhizosphere microbiota: Unraveling the plant-soil dynamics. *Physiological and Molecular Plant Pathology*, *133*, 102381.

Waterhouse, A., Bertoni, M., Bienert, S., Studer, G., Tauriello, G., Gumienny, R., ... & Schwede, T. (2018). SWISS-MODEL: Homology modelling of protein structures and complexes. *Nucleic Acids Research*, *46* (W1), W296-W303.

Young, J. P., Crossman, L. C., Johnston, A. W., Thomson, N. R., Ghazoui, Z. F., Hull, K. H., ... & Parkhill, J. (2006). The genome of *Rhizobium leguminosarum* has recognizable core and accessory components. *Genome Biology*, *7* (4), R34.

Zhalnina, K., Louie, K. B., Hao, Z., Mansoori, N., da Rocha, U. N., Shi, S., ... & Firestone, M. K. (2018). Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. *Nature Microbiology*, *3* (4), 470-480.

Ziegler, C., Bremer, E., & Krämer, R. (2010). The BCCT family of carriers: From physiology to crystal structure. *Molecular Microbiology*, *78* (1), 13-34.