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REVIEW ARTICLE

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Exploring the intricacies of plant growth promoting rhizobacteria interactions: an omics review

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Abstract

Background Plant growth-promoting rhizobacteria (PGPR) are beneficial microorganisms that inhabit the rhizosphere. PGPR play a role in stimulating plant growth and development and enhancing plant resistance and tolerance to biotic and abiotic stresses. To effectively fulfil their roles, PGPR engage in intricate interactions with one another, a phenomenon that occurs within the rhizosphere.

Mainbody.

This collaborative synergy among PGPR species within the rhizosphere is essential for them to perform their functions optimally. Nonetheless, the precise mechanisms and dynamics of PGPR-PGPR interactions, particularly at the transcriptomic level, remain the subject of ongoing research. Scientists are actively exploring and studying how these microorganisms interact and coordinate their activities within the rhizosphere, shedding light on the molecular processes underpinning their cooperative efforts. In this review, we undertake a thorough examination centred on the communication systems that regulate interactions among PGPR in the rhizosphere. Our examination delves into the mechanisms by which this communication triggers alterations at both the transcriptomic and metabolomic levels. Additionally, we assess the cutting-edge omics technologies currently available to study these intricate processes.

Conclusion Understanding the modes of communication and molecular mechanisms underlying these interactions is crucial for harnessing their full potential, particularly in sustainable agriculture. By exploring transcriptomic and metabolomic alterations driven by these interactions, as well as the integration of advanced omics technologies, researchers can uncover new insights into decoding these complex processes, paving the way for innovative strategies to enhance sustainable agriculture.

Keywords Chemical communication, Metabolomics, Plant Growth-Promoting Rhizobacteria (PGPR), Rhizosphere, Transcriptomics

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Introduction

Plant Growth-Promoting Rhizobacteria (PGPR) are rhizosphere-dwelling plant-beneficial bacteria that have been reported to convey a favourable influence on plant growth and development by a variety of mechanisms and confers defence against pathogens or diseases (Mashabela et al. 2022a; Mhlongo et al. 2020). PGPR promotes plant growth by either direct or indirect mechanisms

(Goswami et al. 2016). Direct modes of PGPR actions include supplying phytonutrients such as fixed nitrogen or solubilised minerals from the soil to improve plant nutrition and regulate phytohormone levels to promote plant growth and development (Kalam et al. 2020). Indirect mechanisms involve the production of specialised secondary metabolites, which can combat plant pathogens through induced systemic resistance (ISR) in the plant (Mashabela et al. 2022a; Meena et al. 2020). This mechanism enhances the plant's resistance to diseases and other harmful pathogens. Highly sophisticated communication networks that are triggered at various levels of interaction, such as inter-species, intra-species, and inter-kingdom interactions, are used to regulate these direct or indirect mechanisms of action (Menezes et al. 2021; Phour et al. 2020). PGPR can establish a beneficial relationship with their host through these interactions by secreting different compounds, such as phytohormones, siderophores, enzymes, and antibiotics. Additionally, PGPR can encourage the production of secondary metabolites in plants that may have significant ramifications for sustainable agriculture and the creation of innovative plant-based products, which can enhance plant growth, improve crop yield, and increase plant resilience to environmental stress (Kousar et al. 2020; Sunita et al. 2020).

For PGPR to be utilized in agriculture in efficient and long-lasting ways, it is crucial to understand the basis of their interactions. PGPR-PGPR interactions can affect the variety and composition of the microbial population in the rhizosphere, which can affect plant development and health and even the microbial community in the area. These interactions may take place through either mutualistic or competitive mechanisms (Hassani et al. 2018). For instance, in the mutualistic mechanism, certain PGPR strains may recruit other PGPR strains in the rhizosphere through a phenomenon termed chemotaxis. The recruited PGPR may have complementary functions, such as nitrogen fixation, establishment of biofilms, or production of antimicrobial compounds, that can enhance the overall growth-promoting and pathogen-fighting abilities of the community resulting in increased plant growth and health (Garbeva et al. 2014; Hagai et al. 2013). In the instance of competitive mechanism, PGPR can secrete siderophores, which are molecules that bind iron tightly. By sequestering iron, these bacteria can outcompete other microorganisms for this critical nutrient, thereby enhancing their own growth and that of the host plant (Hassani et al. 2018; Amaya-Gómez et al. 2020).

Through the understanding of the interactions among various PGPR, researchers can exploit the beneficial cooperation exhibited by these microbes. For example, some PGPR such as *Bacillus* and *Paenibacillus* spp. can produce antimicrobial compounds (Cochrane and Vederas 2014), which can be used to compete with pathogenic microbes for resources, thereby indirectly benefiting neighbouring PGPR by reducing competition or inhibiting growth of the pathogens. By examining how microorganisms interact with each other, researchers can also uncover strategies to enhance crop productivity and improve soil fertility as highlighted by (Mohanty et al. 2021).

Exploring the rhizosphere: an intriguing microbial habitat

PGPR naturally arises in a diverse community of rhizosphere-dwelling soil organisms. It is therefore crucial to understand how the rhizosphere complements the role of PGPR in their interactions with other microorganisms. Understanding the role played by the rhizosphere in microbe-microbe interactions can help better understand how these complex microbial community's function. The rhizosphere is a nutrient-rich environment that is a habitat by a vast array of microbes such as fungi, bacteria, protists, nematodes, and invertebrates, each one exerting positive, negative, or neutral impacts on other microbes and the associated host plants (Venturi and Keel 2016). Positive microbes in the rhizosphere are those that engage in beneficial interactions with plants and other microorganisms, thus contributing to plant health, growth, and resilience, such microbes include PGPR (Mashabela et al. 2022b; Mhlongo et al. 2020). On the contrary, negative microbes are referred to as minor pathogens that generate phyto-toxins, enzymes, or hormones, which may compete with the positive microbes for nutrients and negatively impact plant health, however without directly parasitizing plant tissues (Sureshbabu et al. 2016) as illustrated in Fig. 1. The neutral group of bacteria does not directly impact the health of plants, but forms part of a complex food web that utilizes the large amount of carbon released by plants into the rhizosphere, known as rhizodeposits (Khare et al. 2020).

The rhizosphere, also known as the epicentre of microbe-microbe and plant–microbe interactions, has been categorized into three sub-zones: the endorhizosphere, encompassing the root cortex and endodermis where microorganisms and mineral ions reside between plant cells; the rhizoplane, the middle zone consisting of epidermal root cells and mucilage; and the eco-rhizosphere, the outermost zone extending away from the root (Mcnear 2013). Within the rhizosphere, various factors come into play contributing to the dynamics of microbe-microbe interactions, thus shaping the overall microbial community and the interactions in this environment. For instance, root exudates such as sugars, organic acids, enzymes, and secondary metabolites significantly impact

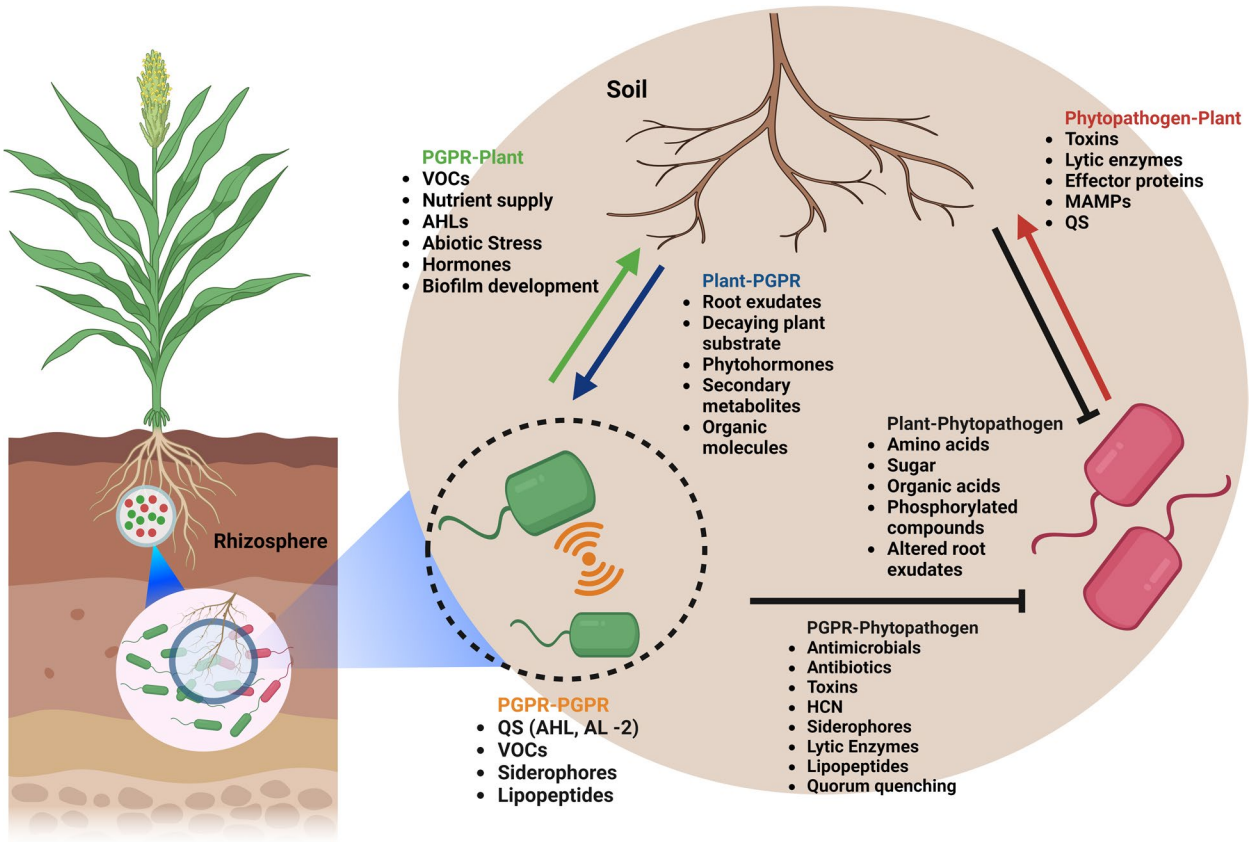

Fig. 1 The intricate tripartite interplay among PGPR, host plants, and pathogenic microorganisms within the rhizosphere. Within this visual representation, plants release phytohormones as a means of attracting PGPR, effectively guiding them toward the root system. Meanwhile, the communication network established among PGPR (gram-positive or negative) functions to suppress the activity of invading pathogens, simultaneously promoting plant growth through the production of phytohormones. In parallel, host plants activate their defense mechanisms, specifically triggering Induced Systemic Resistance (ISR), in response to potential threats posed by pathogenic organisms. Abbreviations: VOCs = Volatile Organic Compounds; AHLs = Acyl-homoserine Lactones; AL-2 = Autoinducer-2; QS = Quorum Sensing; HCN = Hydrogen Cyanide; MAMPs = Microbe-Associated Molecular Patterns

the microbial community's structure and operation in the rhizosphere. This can be observed through the sugars released by plants, which have a clear impact on the growth, proliferation and development of microorganisms. These exudates are known to trigger the activation of genes necessary for the uptake and breakdown of these sugars by microorganisms (Jha and Subramanian 2018).

In addition, *Bacillus subtilis* N11 and *Pseudomonas putida* strain biofilm development and antifungal activity are regulated by organic acids and sugars found in root exudates of bananas and tomatoes (Sun et al. 2017; Zhang et al. 2013). Furthermore, recent studies suggest that specific chemical compounds, such as coumarins, produced by plants in the nutrient-deprived rhizosphere, have been found to stimulate the growth of a distinctive bacterial root microbiota (Harbort et al. 2020; Stringlis et al. 2018; Voges et al. 2019). The results from these studies show that plants can emit coumarins from their roots when there is a lack of iron. Likewise, a study conducted by Koprivova et al. (2019) demonstrated that camalexin biosynthesis, specifically in the roots, governs the beneficial effects of various bacterial strains on plant growth. Nevertheless, this work did not provide evidence of a nutritional component involved in these effects. Overall, the above research indicates that microbes in the rhizosphere play a vital role as a fundamental component of the plant's adaptation to growing in iron-limited soils.

Diverse modes of PGPR communication

Communication is a fundamental process that drives interactions and relationships among organisms. Microbes depend on signalling mechanisms or direct cell-to-cell contact to control various bacterial functions and physiological processes, which encompass motility,

virulence-related factors, population density control, modification of metabolic rates, and regulation of inter and intra-microbial attraction (An et al. 2014; Koua et al. 2020; Luzzatto-Knaan et al. 2019;). These interactions among microbes involve a range of dynamics, including collaboration, competition, and communication, highlighting the complexity of microbial interactions and their impact on various biological processes.

Contact-dependent communication

Microbial communication through physical contact is likely a tactic used by bacteria to regulate the development and actions of neighbouring bacteria, that share a genetic lineage as well as those that compete for resources in close proximity (Kaundal et al. 2016). Frequently, the direct interactions among bacterial cells serve as a means of transportation, facilitating the exchange of various biological substances such as DNA, RNA elements, compounds, nutrients, signal molecules, and metabolites. Importantly, this exchange occurs without the requirement of exposure to the external environment. On the other hand, certain structures like type VI secretion systems can be utilized to deliver inhibitory molecules to nearby hostile bacterial cells (Sgro et al. 2019). This mechanism helps safeguard and promote the growth of specific bacterial populations by defending them against aggressive and undesired neighbouring cells.

For instance, in 2005, Aoki and colleagues introduced the term "Contact Dependent Growth Inhibition (CDI)" to describe a phenomenon in which inhibitory cells rely on direct cell-to-cell contact to impair the growth of target cells. The researchers discovered that specific *Escherichia coli* bacteria, including apathogenic strains, possess a bacterial growth-inhibition system that relied on direct cell-to-cell contact.

In their study, the wild-type *Escherichia coli* isolate EC93 inhibited the growth of *E. coli* K-12 strains, such as MG1655, when both types of bacteria were combined in a shaking liquid culture, they observed that growth inhibition did not occur in shaking liquid cultures unless the two strains were in direct contact, indicating that CDI mechanism relies on this close proximity for effective toxin transfer (Aoki et al. 2005). The CDI system operates through a two-partner secretion mechanism involving CdiA and CdiB proteins, where CdiA is presented on the surface of the inhibiting bacteria and binds to specific receptors on target bacteria. Upon contact, the C-terminal toxin domain (CdiA-CT) is delivered into the neighbouring target cell, leading to growth inhibition (De Gregorio et al. 2019; Feng et al. 2024). This direct transfer of toxins reinforces the idea that CDI is fundamentally a contact-dependent process rather than relying on diffusible signals or chemical induction. Communicating through this network enables the coordination of actions, effective utilization of antimicrobial compounds, establishment of physical barriers, and genetic adaptations. PGPR can form physical barriers to protect plants from pathogen invasion (Li et al. 2021a, b) by creating a denser network of roots and increasing root mass. Also through CDI, they can create biofilms or other protective structures that prevent pathogenic microbes from colonizing plant roots. These combined efforts work together to suppress pathogens, ultimately leading to the preservation of plant health.

Chemical-dependent communication

Chemical-dependent communication refers to a mode of communication between organisms, typically microorganisms, that relies on producing and detecting chemical signals. In this form of communication, organisms release specific chemical compounds, known as signalling molecules or cues, into their environment (Phour et al. 2020). Bacterial chemical communication is crucial in various ecological processes, including biofilm formation, nutrient cycling, and microbial interactions. Studies have shown that chemical communication systems are intricate and involve molecular mechanisms that influence bacterial behaviour and community dynamics (Schmidt et al. 2019; Combarnous and Nguyen 2020). Below, we examine several chemical interactions that may occur during PGPR-PGPR interactions. These interactions encompass a range of biochemical processes through which PGPR communicate, compete, or cooperate within the rhizosphere.

Quorum sensing

Chemical signalling is one of the most common methods of microbial communication. Microbes first send signals to their immediate surroundings to communicate with one another. These signalling molecules can diffuse through the surrounding medium, allowing other microbes in close proximity to detect and respond to these signals (Fig. 2). The ability of microbes to sense and respond to their environment by using signalling molecules is termed “quorum sensing” (QS) (Padder et al. 2018). Phour and colleagues defined QS as the communication process among the microbes in the rhizomicrobiome, that involves the production, release, and recognition of chemical signal molecules (Phour et al. 2020). These signals build up in the local environment and, once they have reached a certain concentration threshold, they bind with receptor proteins, triggering alterations in gene expression and metabolic processes (Abisado et al. 2018). This mechanism enables microbes to adapt to diverse surroundings by regulating genes associated with biofilm formation, virulence factors,

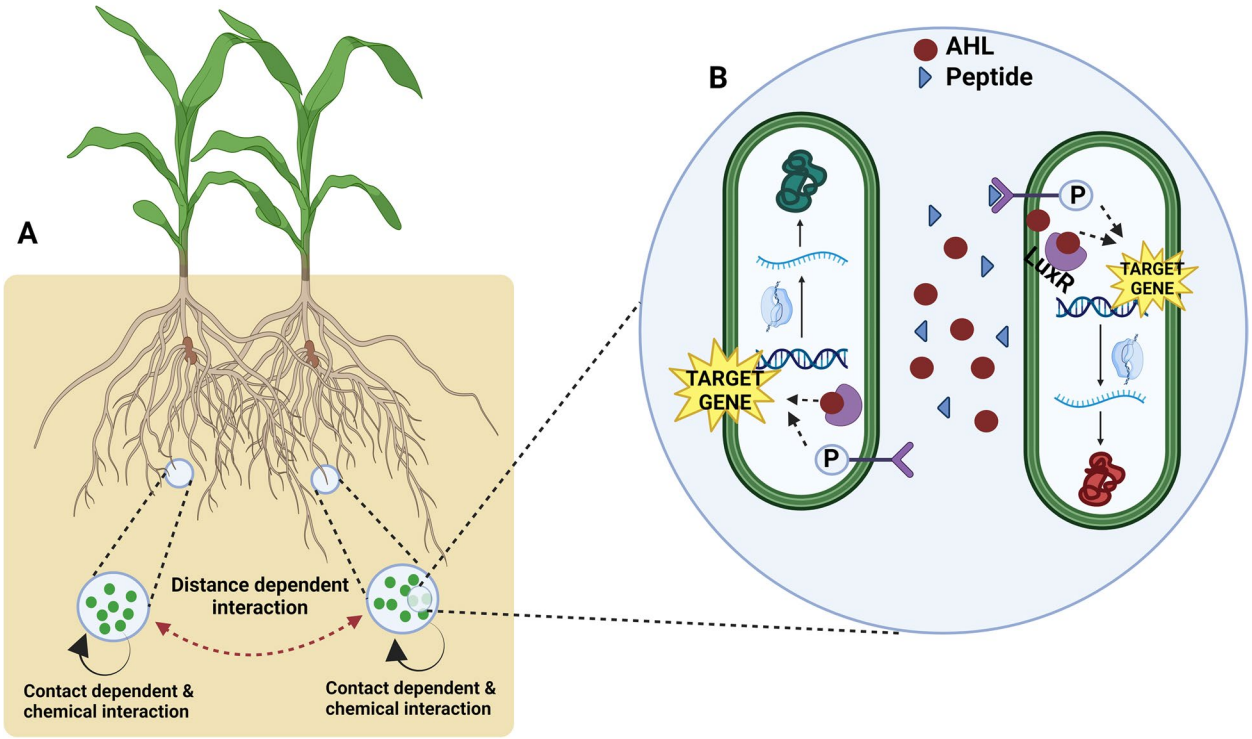

Fig. 2 Illustration of PGPR interaction within the rhizosphere. **A** Contact interaction occurs as PGPR exchanges metabolites while in physical contact, whereas chemical interaction involves the exchange of metabolites and signalling molecules in close proximity. However, long-distance entails the exchange of compounds between PGPR without requiring physical or near proximity. **B** Interaction of PGPR through quorum sensing: AHL and peptide molecules are released into the extracellular environment. Upon reaching a threshold concentration, signifying a quorum, AHL triggers a coordinated response in neighbouring PGPR, activating intracellular LuxR. Meanwhile, peptides bind to membrane-associated receptors, which become phosphorylated initiating a signalling cascade that regulates gene expression and orchestrates collective behaviours of the bacterial cell. Abbreviations: AHL = Acyl-homoserine Lactone

antibiotic production, and the transfer of genetic material through transformation or conjugation (Reuter et al. 2016).

Both gram-negative and gram-positive bacteria use this form of communication (Fig. 2), although the signal molecules utilized vary between the two groups. Gram-negative bacteria primarily utilize N-acyl homoserine lactone (AHL) molecules (referred to as autoinducer-1 or AI-1), whereas gram-positive bacteria predominantly use peptides (known as autoinducer peptides or quorum sensing peptides, AIPs) (Verbeke et al., 2017). AHLs serve as a common mode of cell–cell communication, with a shared chemical structure that exhibits variations in length and composition specifically at the third carbon of the acyl chain (Phour et al. 2020) (Fig. 2). The production of AHLs by a specific species is frequently influenced by the other strain (Ortíz-Castro et al. 2009), meaning that different strains within the same species can generate distinct types of AHLs. Some of the bacteria that produce AHLs include *Acinetobacter, Aeromonas, Agrobacterium, Burkholderia, Erwinia, Enterobacter, Chromobacterium, Methylobacter, Paracoccus, Pseudomonas, Ralstonia, Rhodobacter, Rhizobium, Serratia, Sinorhizobium, Vibrio*, and *Yersinia*, among others (Lade et al. 2014). The cell-to-cell signalling facilitated by AHLs enables these bacteria to coordinate gene expression and control various characteristics, including the formation of biofilms, the secretion of extracellular polymeric substances (EPS), and the production of virulence factors, as previously mentioned (Prabhu and Manerikar, 2019; Vanysacker et al. 2013;).

Furthermore, the quorum sensing system mediated by AHLs is involved in nearly every stage of biofilm development, encompassing initial surface attachment, bacterial proliferation, maturation, and the detachment of mature cells (Subramani and Jayaprakashvel 2019). One instance of the quorum sensing (QS) system being utilized in gram-negative bacteria was observed in *P. aeruginosa*. Within this bacterium, the *lasI* gene encodes an enzyme that synthesizes the AHL signal molecule 3-oxo-dodecanoyl-L-homoserine lactone (OdDHL) (Subramoni et al. 2021). Once OdDHL concentrations reach a critical threshold, the molecule binds to the *lasR* receptor, a transcriptional activator, triggering the expression of genes associated with biofilm development, such as

exopolysaccharides, which are essential for establishing and maintaining biofilms (Subramoni et al. 2021; Zhou et al. 2020). Gram-positive bacteria utilize small molecules named autoinducing peptides (AIPs) as their signalling molecules. These peptides are synthesized, exported, and subsequently detected by other bacteria. Upon reaching a certain concentration, the peptides trigger a response, which can include changes in gene expression or the production of specific compounds (Papenfort and Bassler 2016).

Communication through volatile organic compounds

Volatile Organic Compounds are defined as low-molecular-weight lipophilic biochemicals (100–500 Da) generated by a variety of bacterial and fungal species through distinctive metabolic processes that are genotype-specific (Kanchiswamy et al. 2015). Microbial VOCs (MVOCs) have unique compositions and hold valuable and vital biological information, which plays a significant role in regulating intra- and interspecies interactions, and nearly 1000 microbial species have been documented in the MVOCs 2.0 database (accessible at http://bioinformatics.charite.de/mvoc/), revealing the identification of more than 2000 compounds (Lemfack et al. 2013, 2017).

These volatile compounds primarily consist of alkenes, alcohols, ketones, terpenes, benzenoids, pyrazines, acids, and esters, and they are predominantly considered as byproducts of primary and secondary metabolism, resulting from the oxidation of glucose and its various intermediates (Morath et al. 2012; Schmidt et al. 2015) and they can have direct antagonistic effects against other bacteria. For instance, two rhizospheric bacteria, namely *P. fluorescens* and *Serratia plymuthica*, were found to emit dimethyl disulphide, which exhibited bacteriostatic effects against two plant bacterial pathogens, *Agrobacterium tumefaciens* and *Agrobacterium vitis* (Dandurishvili et al. 2010*)* again, *P. fluorescens* WR-1 generates certain volatile compounds like benzothiazole and 1-methyl naphthalene, which demonstrated bacteriostatic properties against the tomato pathogen *R. solanacearum* (Raza et al. 2016). Interestingly, numerous strains of *Pseudomonas* and *Bacillus*, utilized as biocontrol agents against plant pathogens, have been documented to emit volatile organic compounds (VOCs) that exhibit antibacterial properties (Rajer et al. 2017; Raza et al. 2016; Xie et al. 2018).

Interestingly, the role of VOCs is not limited to antagonism; certain bacteria-produced VOCs can exert beneficial effects on the growth of nearby bacteria in the rhizosphere. For example, VOCs released by *Collimonas pratensis* and *S. plymuthica* can stimulate the growth of *P. fluorescens* Pf0-1 (Garbeva et al. 2014). Moreover, these VOCs lead to the activation of genes associated with motility *in P. fluorescens* Pf0-1, leading to an upsurge in the production of secondary metabolites with antibacterial properties against *Bacillus* (Garbeva et al. 2014). This indicates that *C. pratensis* and *S. plymuthica* might be attracting and fostering the growth of *P. fluorescens* in a cooperative effort to enhance their collective abilities against various bacterial competitors or soil fungal pathogens (Garbeva et al. 2014). The primary role of MVOCs is centered around the interactions between microorganisms, particularly between bacteria and fungi, in a reciprocal manner (Schmidt et al. 2019). These interactions often involve the antagonistic effects of MVOCs with antifungal properties (such as caryophyllene, hydrogen cyanide, 1-undecen, dimethyl disulfide, dimethyl trisulfide, S-methyl thioacetate, benzonitrile, etc.) or antibacterial effects (including γ-butyrolactones, albaflavenone, dihydro-β-agarofuran, 1-undecene, methanthiol, dimethyl disulfide, etc.). However, these compounds may also facilitate beneficial communication, playing a significant role in interactions between microorganisms that are physically separated (Schmidt et al. 2015).

Another form of VOC communication involves the use of Hydrogen cyanide (HCN), a secondary metabolite with phytotoxic properties that serves as an agent to inhibit enzymes during metabolic processes (Ahanger et al. 2014). This substance is produced during the growth and stationary phase and exists as both non-dissociated HCN and cyanide anion within the metabolite solution (Jung & Park 2015). Thus, the cyanide ion undergoes transformation into smaller compounds and is respired as HCN (Kumar Jha and Saraf 2015). PGPR that produce HCN compete with other microorganisms by consuming energy resources and interfering with electron transportation. This disruption also affects enzyme function and cell recovery, ultimately resulting in the disruption and death of the competing cells (Farag et al. 2013).

PGPR have evolved mechanisms to specifically sense and respond to VOCs emitted by other microorganisms. For example, in *E. coli*, the ypdB gene product is responsible for sensing VOCs like 2,3-butanedione and glyoxylic acid, which are emitted by *B. subtilis* (Kim et al. 2013). This interaction triggers significant changes in the bacterium's gene expression, particularly genes involved in motility. These changes suggest that VOC detection is directly linked to adaptive responses that allow the bacteria to adjust to their environment (Kim et al. 2013). Moreover, different PGPR strains produce unique blends of VOCs, which can serve as identifiers for specific species or strains. This specificity enables PGPR to differentiate between themselves and other microorganisms in their environment. For example, a study examining four different PGPR strains found distinct profiles of secreted VOCs (Mhlongo et al. 2022), indicating that

certain VOCs can be used as biomarkers for bacterial classification.

Other forms of interactions and relationships among PGPR

In the rhizosphere, PGPR exhibits a range of interactions with each other, these include synergism, commensalism, and mutualism among others; showcasing their capacity to engage in diverse forms of communication and collaboration. Synergism refers to the cooperative interaction between two or more components resulting in a combined effect greater than the sum of their individual effects. In the context of PGPR, synergism often occurs when multiple strains or species of beneficial bacteria interact with each other. One aspect of this synergistic interaction is the ability of certain PGPR strains to recruit or attract other PGPR to the rhizosphere. This recruitment can occur through various mechanisms, including the secretion of chemical signals. For example, in a study by Luzzatto-Knaan et al., (2019), scientists observed that *B. subtilis* utilizes surfactin, a well-known antimicrobial compound, to attract *P. dentritiformis*. Rather than being inhibited by surfactin, *P. dendritiformis* actively broke it down and accumulated the resulting degradation products (Lipopeptidic products), which acted as territorial markers (Luzzatto-Knaan et al. 2019). This suggests that even if an organism relinquishes energetically demanding characteristics, it can still thrive within compatible neighbouring communities. Therefore, if *P. dentritiformis* were to lose its ability to produce surfactin, it could still thrive in the presence of *B. subtilis*. Through forming microbial partnerships, the growth of particular microbes can also be boosted by specific components with which they are collaborating. In a previous study, Peterson and his team found that the presence of *B.cereus* could facilitate *Cytophaga-Flavobacterium* (CF) bacteria in achieving a high population density by providing peptidoglycan and stimulating the reproduction of CF bacteria (Peterson et al. 2006).

PGPR can also engage in various interactions by occasionally exchanging nutrients through a process known as cross-feeding as illustrated in Fig. 3 below. This involves one microorganism, termed the donor, consuming a primary substrate from the environment and transforming it into a compound released as a beneficial product also termed a "public good" (Canon et al. 2020). This product is then utilized by another interacting partner, known as the receiver. However, these shared resources benefit not only the bacteria that produced them, but also other bacteria within the nearby community or population. Instances of such resources include enzymes that break down antibiotics, surfactants that boost motility, substances forming matrices for biofilms, and many more secondary metabolites. D'Souza et al., (2018) highlighted that microbes engage in this interaction because, in many instances, the donor and the recipient are genetically related. Consequently, by aiding

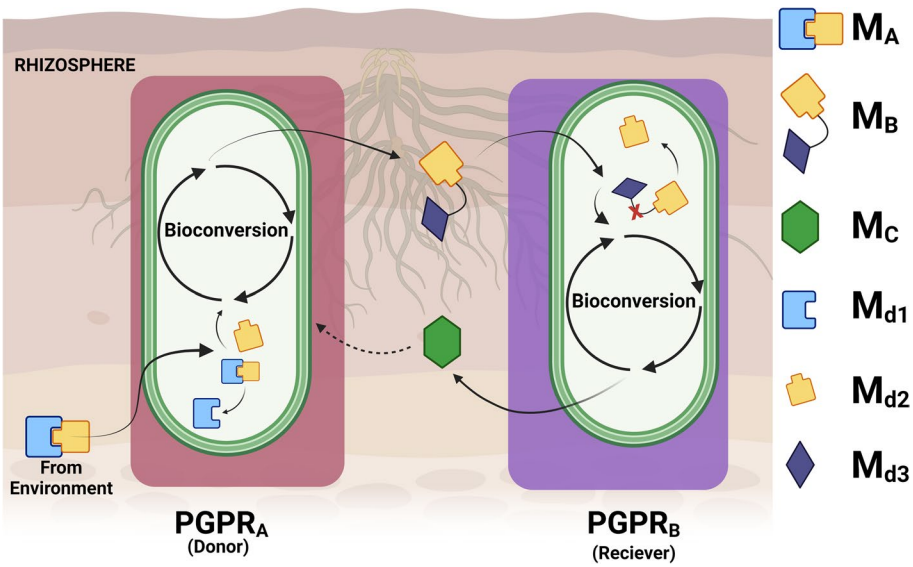

Fig. 3 Schematic representation of cross-feeding between two PGPR species within the rhizosphere: PGPR$_A$ receives metabolite A (M_A) from the surrounding environment. M_A undergoes enzymatic breakdown within PGPR$_A$, yielding two metabolite derivatives (M_{d1} and M_{d2}). M_{d1} is utilized intracellularly by PGPR$_A$ for various biological processes, whereas M_{d2} undergoes bioconversion to generate metabolite B (M_B) which then gets excreted to the environment and serves as a substrate for PGPR$_B$. Within PGPR$_B$, M_B undergoes enzymatic degradation into two derivatives, M_{d2} and M_{d3}. M_{d2} is utilized intracellular for biological processes, while M_{d3} undergoes conversion to form metabolite C (M_C), which can be utilized by PGPR$_A$, completing the cross-feeding cycle between two PGPR species

its relatives, the cooperating individual can enhance the likelihood of indirectly passing on its genes. This concept, known as "kin selection," elucidates altruistic cooperative actions among closely related microbes. Nonetheless, interspecies cross-feeding can also occur. For instance, a prior investigation established a synthetic bacterial coculture to investigate the underlying molecular mechanisms of nutrient cross-feeding. In this study, *E. coli* and *Rhodopseudomonas palustris* exchanged essential metabolites under anaerobic conditions (Fritts et al. 2020). *E. coli* metabolized glucose, a carbon source inaccessible to *R. palustris*, generating ethanol and various organic acids as byproducts. *R. palustris* utilized these organic acids, except formate, as its primary sources. Conversely, *R. palustris* fixed dinitrogen gas via nitrogenase, releasing ammonium, which served as *E. coli's* sole nitrogen source (Fritts et al. 2020). This then means that both species are mutually dependent on each other for survival and growth under experimental conditions created by synthetic coculture. Such cross-feeding dynamics play a crucial role in microbial interaction, facilitating the synergistic utilization of resources and promoting coexistence within the ecosystem.

The communication methods used by PGPR showcase a complexity that lies beneath their interactions, within the rhizosphere. Besides the above-mentioned compounds, PGPR demonstrates a range of communication strategies that enable them to function as a cohesive and efficient microbial community. The exchange of substances, activation of resistance, and interactions related to cycling overall contribute to the coordination of a complex communication symphony of among the PGPR population. These interactions do not only benefit the PGPR themselves, but also, have the potential to significantly influence plant health, nutrient availability, and overall ecosystem resilience. Exploring PGPR communication not only deepens our understanding of ecology but also reveals new possibilities for utilizing their potential in sustainable agriculture and environmental management. As we continue to uncover the intricacies of PGPR communication we discover a tapestry of collaboration that holds promise, for shaping a more environmentally friendly and productive future.

Unlocking insights through advanced multi-omics: investigating transcriptomic and metabolomic perturbations in PGPR-PGPR interactions

From the beginning of its history, the field of chemical communications has relied on bioassay-guided isolation techniques to identify a wide range of chemical signals (Wyatt 2014). Typically, these approaches involved an initial assessment of the crude extract through a biological screening process, such as a behavioural test. This was succeeded by a series of separation stages, leading to the attainment of partially purified or completely pure compounds (Wyatt 2014). Over the past ten years, considerable strides have been made to enhance the analytical tools for measuring mRNA, proteins, and metabolites. These endeavours have resulted in the capability to comprehensively uncover microbial metabolism and its reactions to environmental influences. Hence, achieving an accurate understanding of microbial interaction demands the integration of various informational strata, referred to as the 'multi-omics' strategies. Multi-omics is an all-encompassing strategy that incorporates different omics methods (i.e., genomics, transcriptomics, proteomics, and metabolomics) to investigate and elucidate biological systems at different levels (White et al. 2017). Genomics encompasses the study of an organism's entire set of genes; transcriptomics assesses mRNA transcript levels and gene expression patterns; proteomics quantifies and qualifies protein abundance, while metabolomics identifies the concentrations of small cellular metabolites (Fig. 4). The integration of these technologies offers a comprehensive perspective of a biological system's reaction to chemical signals and associated environmental factors, encompassing all major types of biomolecules, inclusive of carbohydrates, lipids, nucleic acids, and proteins. This accomplishment is a result of advancements in analytical instruments and high-throughput methodologies, computation, and software development, which continually produce extensive datasets.

Exploring PGPR-PGPR interactions with the aid of current advances in transcriptomics

Transcriptomics, an early branch of omics sciences, examines mRNA expression patterns in specific cells over defined periods. This field helps reveal the interactions between genes involved in protein production and cellular processes under a range of environmental and experimental conditions, such as changes in temperature, nutrient availability stress factors (like drought or salinity), and developmental stages (Keagy et al. 2023; Wang at el., 2009). Due to the numerous pathways through which genome expression can be controlled, the content of the transcriptome can undergo significant changes in reaction to environmental stimuli. Consequently, variations or alterations in gene expression levels among different samples can provide insights about the active genes in specific microbial strains. However, traditional methods for studying gene expression were limited by sensitivity and specificity, marking it challenging to detect low abundance transcripts or capture the complexity of gene activity (Dong and Chen 2013; Lowe et al. 2017). These limitations prompted the development of transcriptomics, which has transformed the understanding

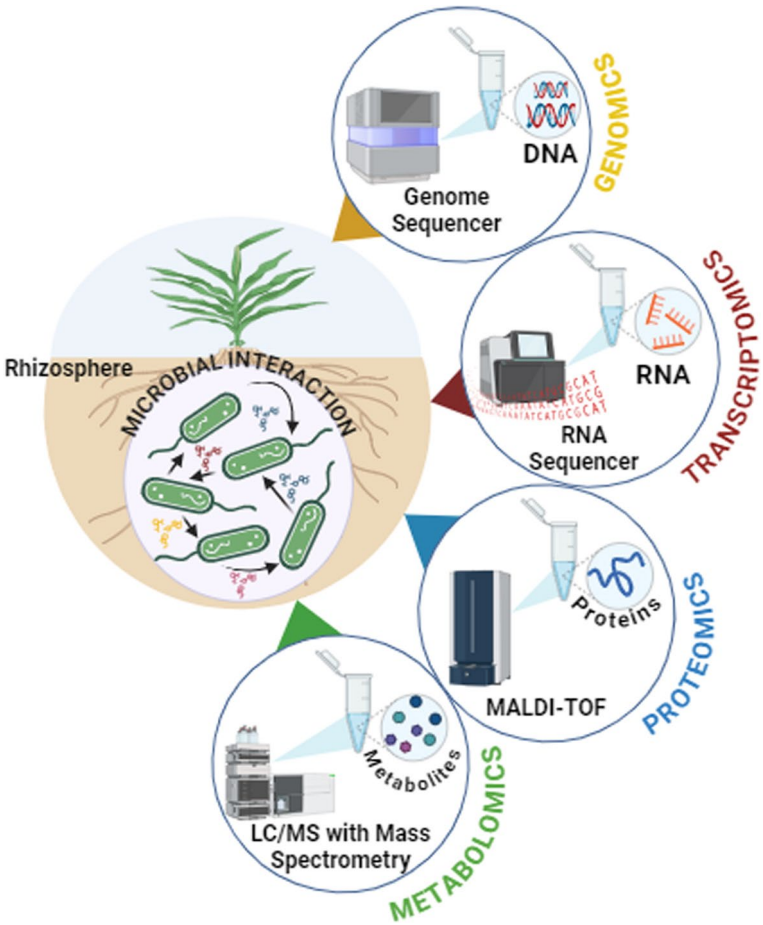

Fig. 4 The schematic diagram illustrates a wide array of 'omics' technologies that are designed to probe different tiers of microbial interaction within the rhizosphere. These technologies encompass genomics, transcriptomics, proteomics, and metabolomics, each with a unique focus on deciphering specific facets of cellular complexity. This holistic approach empowers researchers to explore the intricacies of biological systems, providing valuable insights into the multifaceted layers of cellular data representation

of gene regulation and expression (Lowe et al. 2017). In this work, we explored the technologies used in the past, highlighting their challenges and limitations, as well as their contributions to the foundation of gene expression studies. We also examined how these technologies have advanced, leading to the development of current methodologies that allow for more precise, detailed, and comprehensive analysis of transcriptome, marking a significant leap in the field of molecular biology. Furthermore, we discussed how these cutting-edge technologies can be applied to study PGPR-PGPR interactions, providing valuable insight into the molecular mechanisms underlying microbial interaction.

Past methods for gene expression analysis: serial analysis and microarray

In 1995, serial analysis of gene expression (SAGE) emerged as one of the first sequencing-based transcriptomic methods. SAGE is based on the idea that a short nucleotide sequence tag (usually 10–14 base pairs) can uniquely identify a transcript. (Velculescu et al. 1995). It works by creating short sequence tags from expressed transcripts, linking these tags together, and then sequencing them to determine the frequency of each tag, which corresponds to the expression level of its associated gene. SAGE integrated the application of Sanger sequencing to produce and sequence concise 30-unit tags to assess transcript levels. Advances in the SAGE technique have encompassed methods such as Massively Parallel Signature Sequencing (MPSS) and Cap Analysis of Gene Expression (CAGE, which share the foundational concept of SAGE but focus on identifying 50 transcription start sites). As SAGE was utilized for quantifying gene expression and transcript abundance, it became evident that certain limitations hindered its effectiveness. These limitations included challenges in capturing rare transcripts due to the limited sequencing depth of SAGE libraries (Jongeneel

2005). Additionally, SAGE was not well-suited for detecting alternative splicing events or identifying novel transcripts (Wang et al. 2009). The necessity for many sequencing reads for robust results also posed practical and cost-related constraints. These shortcomings collectively prompted the exploration of alternative technologies like microarrays, which offered higher throughput, greater sensitivity, and the capability to examine a broader range of molecular interactions.

Microarrays were first introduced in 1995 by Schena and colleagues. This technology measures the levels of specific gene transcripts by detecting how they hybridize to matching probes arranged in an array. Microarrays made it possible to analyse many transcripts at once, which greatly reduced the cost and work needed to study each gene. During this timeframe, various microarrays were developed to encompass identified genes in various organisms, either model organisms or those of economic significance. However, microarrays face several limitations that impact their reliability. They are prone to high background noise caused by cross-hybridization, where non-specific binding of samples to probes occurs (Jaksik et al. 2015; Rao et al. 2019). This noise can obscure true signals, making it challenging to differentiate between genuine gene expression changes from artifacts. Additionally, microarrays rely on predefined probes targeting known genetic sequences, limiting their ability to detect novel or rare variants. As a result, critical genetic alterations, particularly those contributing to the interactions but not well-characterized, may go undetected. Furthermore, the specificity of microarray measurements can be low (Jaksik et al. 2015), with inconsistencies in fold-change calculations and variability across platforms, raising concerns about the reproducibility and reliability of data for biological interpretations. Progress in the creation and production of arrays enhanced the precision of probes and enabled the inclusion of a greater number of genes on a single array. Improvements in fluorescence detection heightened the sensitivity and accuracy of measuring transcripts with low abundance levels. Even though there is a limited number of direct publications focused solely on the usage of microarrays for studying interactions between PGPR, it is important to recognize that scientific research often involves building upon existing knowledge and adapting methodologies from related fields. In the case of PGPR-PGPR interactions, researchers have explored microbial interactions, plant–microbe interactions (Alexander et al. 2021; Mavrodi et al. 2021; Sharma et al. 2020), and the broader field of microbiome research, all of which can indirectly provide insights and methodologies for studying PGPR interactions.

Current techniques for gene expression: RNA-seq and nanopore

The advent of microarray technology revolutionized investigations into gene expression; however, it was eventually surpassed by the emergence of RNA sequencing (RNA-Seq). This method of sequencing on a large scale offers notable benefits, including heightened sensitivity and the ability to uncover new transcripts, as well as the analysis of non-coding RNA and alternative splicing. For example, Illumina HiSeq 2500 sequencing was used to investigate how interspecific interactions influence gene expression in *Paenibacillus* sp. AD87, *Serratia plymuthica* PRI-2C, and *Hylemonella gracilis* (Olaf Tyc et al. 2023). RNA-Seq offers a comprehensive and systematic means of delineating an organism's transcriptome, demonstrating minimal bias. This capability holds true across various experimental conditions or cell types (Li et al. 2014), and it bypasses the challenges associated with probe design or cross-hybridization issues. Additionally, RNA-Seq can be repurposed for various analyses, such as differential gene expression, pathway analysis, gene fusion detection, and alternative splicing analysis (Kalam et al. 2017), without additional experiments.

Thus, the development of RNA-Seq has made it possible to simultaneously examine changes brought on by microbial interactions (Olaf Tyc et al. 2023) and it has become an essential tool for thoroughly analysing the transcriptome of several PGPR strains (Fernandes et al. 2017) as well as a wide range of interactions between PGPR and other microorganisms, such as pathogens (Li 2020) and fungi (Neupane et al. 2014). It can has also play a crucial role in understanding how environmental signals impact microbial gene expression and behaviour. For example, the *Lysobacter capsici* (AZ78), a plant-beneficial bacterium was cultured in the presence of rhizosphere bacterial signals, RNA-seq revealed that these signals affected on 21% of AZ78 genes expression. Moreover, indole, a signal that affects motility, development of biofilms, pathogenicity, and antibiotic resistance downregulated a significant number of genes involved in antagonistic behaviour, uptake of iron–siderophore complexes or vitamins (Bejarano et al. 2021),potentially impairing competitiveness and survival. Conversely, compounds like 13-methyltetradecanoic, 2,3-butanedione (BUT), and glyoxylic acid (GLY), upregulated the expression of genes involved in twitching motility and associated with the biogenesis of type IV pili (T4P) T4P (Bejarano et al. 2021), which are important for bacterial adhesion, motility, DNA uptake and biofilm formation (Craig et al. 2019; O'Toole and Wong 2016). RNA-seq can thus provide deep insight into how specific signals regulate bacterial physiology, revealing the molecular mechanisms behind microbial interactions, adaptability and

behaviour in complex environments. Furthermore, RNA-Seq findings are in align with outcomes from microarray analyses (Trost et al. 2015), yet RNA-Seq showcases higher sensitivity, essentially possessing the capacity to identify a notably larger set of differentially expressed genes in comparison to microarrays. Notably, RNA-Seq is unbound by annotations, allowing for the discovery of novel transcripts independent of array design or pre-existing genome annotations.

The changes in sequencing technologies go beyond small improvements, as shown in Fig. 5. These changes are actually big breakthroughs that are changing how biological research is done and used. For example, Oxford Nanopore Technologies created a small sequencing device called the MinION (Loman and Watson 2015). This new technology allows scientists to directly study long pieces of DNA or RNA, and it can be easily scaled up or down. As demonstrated by Haveman and collaborators, this innovative approach serves as a promising means for on-site surveillance of the transcriptional activity within crop microbiomes (Haveman et al. 2021). In their study, they successfully utilized this technology to not only monitor but also contribute to the facilitation and maintenance of plant health, particularly in the context of on-orbit space food production (Haveman et al. 2021). This portable sequencer utilizes a nanopore-based sequencing mechanism, employing motor and nanopore reader proteins embedded within electrically conductive membranes (Wang et al. 2021). However, there are notable limitations to consider, including a relatively high error rate of approximately 10.5% compared to traditional next-generation sequencing methods (Delahaye and Nicolas 2021; Petersen et al. 2019), which can complicate data interpretation and necessitate robust error-correction strategies. Additionally, while the MinION is more affordable than some NGS platforms, the overall costs associated with nanopore sequencing can still be significant, particularly when factoring in bioinformatics support and consumable expenses (Petersen et al. 2019). Furthermore, ongoing improvements are needed in both hardware and software to enhance accuracy and reduce errors, as newer nanopore chemistries aim to improve homopolymer recognition but may require higher input amounts and yield lower outputs. Even though studies exploring PGPR interactions using nanopore sequencing are still developing, some research have already utilized

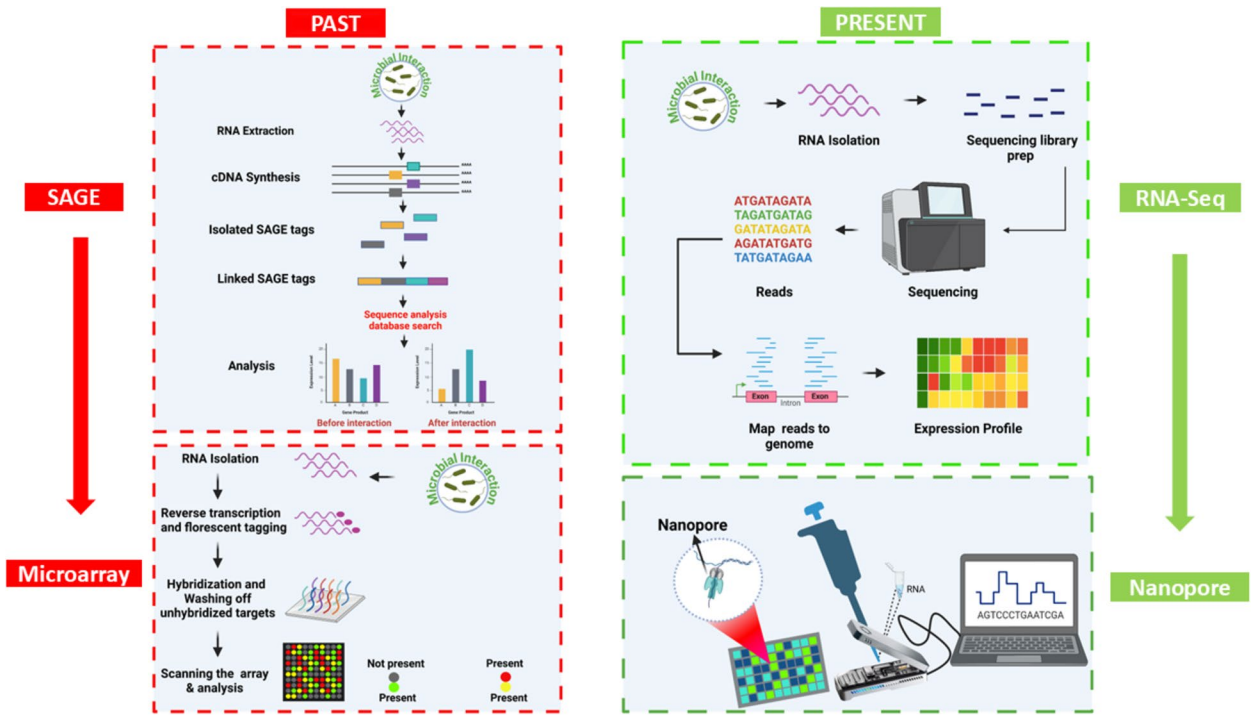

Fig. 5 The advancement of platform technologies for examining interactions between PGPR through transcriptome profiling. In the past, alterations in the entire transcriptome gene expression were characterized using SAGE, followed by the utilization of microarray technology. The constraints associated with these methodologies were addressed through the advent of RNA-Seq, which is unbound by annotations and allows for the discovery of novel transcripts independent of array design or pre-existing genome annotations. Progressing forward, developments in "third generation" sequencing techniques have bolstered the capabilities of transcriptomics strategies. An illustration of this is nanopore. Sequencing, an exceptional and scalable technology that facilitates the direct and real-time examination of extended DNA or RNA fragments

this technology to uncover microbial community dynamics and functional gene expression in the rhizosphere (Chavan et al. 2022; Manter et al. 2024). Enhancements in nanopore sequencing have the potential to enable RNA sequencing directly (eliminating the need for an intermediate cDNA step) without any constraints on read length. This capability allows clearer identification and measurement of transcript isoforms, accurately representing gene expression.

Exploring PGPR-PGPR interactions with the aid of current advances in metabolomics

In addition to investigating and understanding changes in the transcriptome, studying the metabolomes of PGPR is essential to grasp the changes that occur during their interactions. Although the word 'metabolome' had already been in circulation since 1998, the term 'metabolomics' was first introduced in 2001. It involves the comprehensive analysis of all non-genetically encoded substances, encompassing substrates, intermediates, and metabolic products (with a mass of less than 1500 Da) associated with a particular biological system, such as a cell, tissue, or organism, in a specific physiological state (Ernst et al. 2014). Over the past twenty years, this interdisciplinary scientific domain has evolved significantly and has garnered substantial attention, especially within the life sciences fraternity. It has become essential for investigating cellular biochemistry and uncovering the mechanisms behind metabolic shifts in various physiological situations (Tugizimana et al. 2020; Zhang et al. 2020a, b). In the realm of rhizome-microbe interactions, metabolomics has proven effective in a wide range of research endeavours, including investigations into metabolic pathways, relating genotype and biochemical phenotype, PGPR-PGPR interaction (Luzzatto-Knaan et al. 2019), PGPR-fungus interaction (Singh and Lee, 2022) and PGPR-pathogen interaction (Sun et al. 2017). Nevertheless, metabolomics, especially when applied to large-scale studies, faces several impediments. Key challenges include issues related to sample variability, the complexity of biological matrices, difficulties in identifying metabolites due to insufficient databases, and the need for standardized protocols across different laboratories (Tugizimana et al. 2020; Wawrzyniak et al. 2023). Consequently, there has been a heightened emphasis on addressing these challenges by advancing computational tools and enhancing technologies in the field of metabolomics (Tugizimana et al. 2020). In any metabolomic investigation, it is crucial to emphasize that every aspect of the entire process must be meticulously outlined, ranging from sample preparation to analysis of samples. In recent times, there has been a notable implementation of automated sample preparation techniques that have enhanced metabolomics. Tinte and colleagues have outlined several of these automated methods (Tinte et al. 2021). However, in this review, we focus on the contemporary metabolomics technologies employed in studying microbe-microbe interactions and their recent advancements.

Advances in mass spectrometry techniques for microbe-microbe interactions

Mass spectrometry coupled with liquid chromatography (LC–MS and LC–MS/MS) has become increasingly popular in metabolomics due to its superior resolution, specificity, and multiplex capability compared to earlier analytical techniques such as gas chromatography (GC) and nuclear magnetic resonance (NMR) (Chen et al. 2022a, b; Zhou and Zhong 2022). Traditional methods like GC are effective for volatile compounds but often require derivatization for non-volatile metabolites, limiting their applicability. In contrast, LC–MS can analyse a broader range of metabolites, including both polar and non-polar compounds, without extensive sample preparation (Zhou and Zhong 2022). Furthermore, NMR provides structural information but generally has lower sensitivity compared to mass spectrometry (MS), making it less suitable for detecting low-abundance metabolites (Emwas, 2015; Emwas et al. 2019). Despite these advantages, LC–MS/MS is expensive, labour-intensive, and has limited throughput. The specialized nature of this technology, requiring highly trained personnel, has restricted its widespread adoption to large institutions, academic organizations, and reference laboratories (Hétu et al. 2012; Netzel et al. 2014). The key to overcoming these limitations and making it more suitable for routine use lies in advancements in automation.

Spatial metabolomics: mass spectrometry imaging The prevailing metabolomic methods involve extracting metabolites from biological samples and then subjecting them to further detection and quantification of endogenous and exogenous metabolites, frequently using MS (Lu et al. 2017). Although proficient at detecting metabolic changes, this method fails to provide details regarding the spatial distribution of metabolites within the bacterium. Overcoming this limitation requires time-consuming workflows and specialized expertise, making the interpretation of metabolomics data quite challenging (Holzlechner et al. 2019; Alexandrov 2020). The emergence and advancement of spatial metabolomics have facilitated the implementation of in situ metabolomic approaches. This progress allows researchers to analyse metabolites directly within their natural locations, providing a more comprehensive understanding of their spatial distribution within organelles, cells, tissues,

or organs. Mass spectrometry imaging (MSI) stands out as the primary technology in spatial metabolomics, employed to visualize two-dimensional (2D) or three-dimensional (3D) spatial resolutions across organisms, organs, tissues, or cells (Petras et al. 2017). MSI platforms are classified based on their ionization source and mass analyzer, exhibiting differences in speed, sensitivity, and spatial resolution (Swales et al., 2019).

The predominant platform in MSI is matrix-assisted laser desorption/ionization (MALDI), which employs a laser to extract and ionize metabolites simultaneously (Tinte et al. 2021). For example, when *P. fluorescens* SS101 and the protozoan *Naegleria americana* interact, MALDI-MSI was used in combination with live colony (nanospray desorption electrospray ionization) nanoDESI to examine how the lipopeptide massetolide A is dispersed in the spatial context (Cui et al. 2015), providing valuable insights into the dynamics of the interaction between these microorganisms. Another instance where MSI was applied involved examining why *P. dentriformis* is drawn to *B.subtilis* on a chemical level (Luzzatto-Knaan et al. 2019). Scientists discovered that certain signals were present solely in either the *P.dentriformis* colonies or *B.subtilis* monocultures, while others only emerged during their interaction. Notably, ions with m/z values of 248.5 and 659.5 were exclusively detected within the *P. dentritiformis* colonies themselves. Furthermore, the number of ions decreased significantly or even became undetectable when *P.dendritiformis* was cultured alongside *B.subtilis* (Luzzatto-Knaan et al. 2019).

The utilization of such advanced techniques enhances our understanding of the intricate processes and spatial patterns associated with the production and distribution of bioactive compounds during microbial interactions. However, the development and application of MSI technology in spatial metabolomics are being aided by the emergence of additional platforms as desorption electrospray ionization (DESI), laser ablation electrospray ionization (LAESI) which allowed the visualization and the establishment of *P. aeruginosa* and *S. aureus* in a polymicrobial biofilm, as well as the examination of ions after the treatment of the biofilm with the LL-37 antimicrobial peptide (Sn et al. 2015). This highlighted the capability of ambient ionization technique for investigating biofilms and interactions between different species. Furthermore, other techniques, including nanostructure-initiator MS (NIMS), infrared matrix-assisted laser desorption electrospray ionization (IR-MALDESI), and secondary ion MS (SIMS), which utilizes an ion beam, contribute to the expanding toolkit in spatial metabolomics (Alexandrov 2020).

Ion mobility spectrometry Ion mobility spectrometry (IMS) is among the technologies that are emerging in the field of metabolomics, playing a significant role in advancing our analytical capabilities for studying metabolites and their functions within biological systems. By coupling IMS with mass spectrometry (IMS-MS), one may effectively separate, resolve, identify, and characterize the multidimensional structure of analytes (Armenta et al. 2020). IMS encompasses various technologies, classified as either dispersive or selective. Dispersive IMS technologies allow the analysis of all ions, while selective IMS technologies enable the analysis of specific ions (Tinte et al. 2021). Consequently, dispersive IMS technologies like drift tube IMS (DTIMS) and travelling wave IMS (TWIMS) are well-suited for untargeted metabolomics. On the other hand, selective IMS technologies such as field asymmetric IMS (FAIMS) and differential mobility analysers (DMA) are more appropriate for targeted studies, offering improved orthogonality to conventional mass spectrometry data (Tinte et al. 2021). There is a scarcity of research that demonstrates the application of IMS technologies in investigating the PGPR-PGPR interaction. One study conducted by Ratiu et al. 2017 where a portable aspiration-type ion mobility spectrometer (a-IMS) and gas chromatography-mass spectrometry (GC–MS) were utilized to discriminate between different bacteria, namely *E. coli*, *B. subtilis,* and *S. aureus*. This discrimination was achieved through the rapid sensing of bacterial metabolic volatiles, resulting in distinctive metabolic fingerprints. Nevertheless, real-time monitoring of alterations in the headspace or other samples' composition during PGPR-PGPR interaction could be made possible by IMS's quick separation and detection capabilities. This is especially helpful for documenting dynamic events and comprehending the interaction's temporal dimensions. However, while IMS systems can be relatively low-cost compared to other analytical techniques, the need for specialized sample introduction system (SIS) and potential coupling with chromatographic methods can increase overall expenses (Cumeras et al. 2015; Fernández Maestre 2012). Additionally, technology requires a certain level of expertise to operate effectively, as researchers must be well-versed in the principles of ion mobility and mass spectrometry, along with data analysis techniques specific to IMS.

Lab-On-Chip and microfluidic devices: probing microbe-microbe interactions Mass spectrometry (MS) offers excellent sensitivity and efficiency, allowing for the determination of the molecular weight of diverse analytes (Gathungu et al. 2018). While there are various advantages to employing mass spectrometry for analysis, the signal response of the mass spectrometer is susceptible

to interference from the sample matrix (Xu et al. 2021). This vulnerability makes the application of direct MS detection to real complex samples a challenging task. Nevertheless, integrating MS with microfluidic systems offers a solution to overcome these challenges. Microfluidic devices or lab-on-a-chip devices are miniaturized systems that manipulate and control small amounts of fluids, typically at the microliter or nanolitre scale. They are broadly categorized into analogue, droplet-based (DB), and digital microfluidics. Analog and droplet-based systems leverage fluid shear stress between oil and aqueous phases to create continuous fluid streams and isolate droplets in microchannels. These processes involve both passive and active pumping mechanisms (Feng et al. 2020). In contrast, digital microfluidics platforms utilize electrodes coated with a hydrophobic material layer to manipulate and control samples by applying a voltage potential (Pedde et al. 2017). Microfluidic devices incorporate various stages such as sample preparation, preconcentration, separation, and delivery to analytical platforms as part of their integrated processes (Miggiels et al. 2019; Wang et al. 2017). The use of a microfluidic chip for sample pretreatment can reduce preparation time, enhance analysis efficiency, and, when coupled with mass spectrometry for both qualitative and quantitative detection, can deliver precise information about targets while minimizing the occurrence of false positive results (Chen et al. 2021; Wei et al. 2023).

To analyse the interactions within microbial populations across different environments, Hsu and colleagues introduced the Microbial Interaction Network Inference in Microdroplets (MINI-Drop) method (Hsu et al. 2019). The simultaneous cultivation of multiple sub-communities was made possible by randomly encapsulating two or three bacterial strains in droplets. The significant component of this study included merging fluorescence microscopy with an automated computational technique, to quickly ascertain the absolute abundance of every strain in hundreds to thousands of droplets for each condition, making it easier determination of the type and degree of microbial interactions that occurring after co-cultivation (Hsu et al. 2019). In the context of medical research, the integration of an electrospray ionization-quadrupole-time-of-flight-mass spectrometer (ESI-Q-TOF–MS) with a microdevice was used to examine communication between 293 and L-02 cells. Successful detection of epinephrine and glucose was accomplished, and the analysis time was kept under 10 min (Mao et al. 2013). Microfluidic chips have also substituted intricate cultivation processes in traditional bioanalysis by establishing a controllable microenvironment, ensuring precise manipulation of fungi and bacteria on a microscale (He et al. 2022). Typically, samples are partitioned into smaller groups, and in some cases in individual cells, for subsequent immobilization and cultivation. Yu et al. (2022) highlighted several emerging technologies in microbiome research, with micro droplet microfluidics being recognized as the most adopted approach for various applications. This technology is particularly valued for its capability to create millions of segregated microenvironments, allowing for the independent cultivation, detection, and handling of microbial cells that possess distinct biological properties. Additionally, micro droplet microfluidics can be easily integrated with various devices to monitor the phenotypic or genotypic features of captured microbes (Yu et al. 2022). As a result, it facilitates the characterization of microbiomes at a population scale and enables the isolation of specific individuals at the single-cell level, making it a powerful tool in microbiome studies. Additionally, microfluidic devices have also been used as powerful tools for recreating the rhizosphere environment, providing a controlled microenvironment for studying microbe interactions. One notable example is rhizosphere-on-a-chip platform developed by Oak Ridge National Laboratory, which mimics soil conditions to investigate the ecosystem in the rhizosphere. This device allows researchers to observe microbial interactions with root exudates and chemicals in real time, enhancing our understanding of rhizosphere (Aufrecht et al. 2022; Dai et al. 2024). The advancing design of functional structures within microfluidic devices empower the efficient exploration of gene functionalities, morphological attributes, and cell proliferation in a high-throughput manner (He et al. 2022).

Computational tools for signal characterization to unveil microbial interactions Metabolomics studies, particularly untargeted approaches, generate complex and information-rich datasets with high dimensionality, posing challenges in their management and comprehensive information extraction (Goeddel and Patti 2012; Tugizimana et al. 2016). Therefore, the complexity of data generated in MS demands implementing computational tools, particularly for peak detection and deconvolution. The purpose of peak detection and deconvolution is to distinguish and quantify signals associated with the molecules present in a sample, such as metabolites (Yi et al. 2016) while minimizing false positive signals. This step is crucial for subsequent data analysis tasks like profile alignment or biomarker identification, and it can notably simplify the data complexity. Yet, due to the intricate nature of signals and the presence of various sources of noise within data, automatically distinguishing noise from compound signals proves highly challenging (Tugizimana et al. 2016). Determining the threshold

distinguishing noise from a signal is particularly intricate, especially when detecting peaks with low-response values. Therefore, it is typically necessary to adjust several parameters to align with the characteristics of the data derived from MS.

Currently, numerous open-source and commercial software packages have emerged to support peak alignment in metabolomics. Each tool boasts distinct capabilities, offering insights tailored to specific contexts, yet also harbouring limitations (Misra and van der Hooft 2015). For example, XCMS which is widely used bioinformatics software for processing, analyzing and visualizing data from untargeted metabolomics, it was initially proposed for metabolomics peak alignment (Domingo-Almenara and Siuzdak 2020). However, a drawback of the original method was the potential alternative assignment of peaks to adjacent m/z bins. To address this issue, researchers introduced an algorithm named centWave (Tautenhahn et al. 2008). This algorithm identifies regions containing potentially relevant masses in raw data, using continuous wavelet transformation (CWT) and optionally, Gauss-fitting for chromatographic peak resolution. CWT is then used to detect all feasible chromatographic peaks, followed by filtering to eliminate candidate peaks with fewer m/z centroids than specified thresholds. Another software that can be used for peak detection is MarkerLynx™ (Mashabela et al. 2022b; Mhlongo et al. 2020;), this software utilizes the patented *ApexTrack* algorithm for precise peak detection and alignment. Initially, MarkerLynx™ identifies regions of interest in the m/z domain based on mass accuracy, utilizing mass tolerance (Tugizimana et al. 2016). In the process, the *ApexTrack* algorithm governs peak detection using parameters such as peak width, measured at a specified percentage height, and baseline threshold, which determines the ratio of the peak-to-peak baseline. Besides the above-mentioned programs, other computer software tools such as OpenMS, MZmine, Progenesis QI, Skyline, MS-DIAL, and MAVEN (Adams et al. 2020; Chen et al. 2022a, b; Du et al. 2020; Rurik et al. 2020; Zhang et al. 2020a, b), are commonly used for peak picking, alignment and normalization in various analytical techniques, offering advanced algorithms and extensive data processing capabilities.

Following peak-picking, alignment, and normalization, the typical procedures involve statistical analysis to identify dysregulated peaks in a specific phenotype or metabolite annotation. However, there are challenges in confidently identifying metabolites from MS spectra data, particularly in untargeted analysis, due to the diverse nature of metabolites. However, leveraging advanced computational techniques, state-of-the-art mass spectrometry instrumentation, comprehensive knowledge of ion fragmentation, and established databases and libraries have significantly advanced metabolite identification (Yi et al. 2016). Recent advancement has been achieved in accurately identifying unknown microbial metabolites with a level of precision that allows for potential high-throughput analysis. For instance, microbeMASST is a search tool that leverages publicly available MS repository data to determine the microbial origin of both known and unknown metabolites and link them to their respective microbial producers (Zuffa et al. 2024). This advancement has simplified the extraction of microbial data from mass spectrometry-based metabolomics studies, eliminating the requirement for prior knowledge. Other tools, such as DEREPLOCATOR + and VarQuest, have also been developed to identify peptidic natural products and their variants by searching mass spectra against databases (Mohimani et al. 2018). All these advancements aim to simplify the use of old methods by streamlining the process of microbial identification and characterization through MS-based metabolomics.

Unveiling the link between genes and metabolites in PGPR-PGPR interaction through the integration of metabolomics and transcriptomics

The integration analysis of transcriptomics and metabolomics is a powerful approach for establishing connections between genetic components, such as genes and transcripts, and functional components, such as metabolites, within cells. The integration between transcriptomics and metabolomics has been done in many studies for PGPR-plant interaction, PGPR-PGPR interaction, and PGPR-pathogen interaction. For example, Transcriptomics and Metabolomics were used to investigate how *B. velezensis* GS-1's antagonistic properties affect *Magnaporthe oryzae* (Zhang et al. 2022). The analysis of differentially expressed genes (DEGs) and differentially accumulated metabolites (DAMs) demonstrated that the lipopeptide produced by GS-1 led to a decrease in the expression of genes linked to amino acid metabolism, sugar metabolism, oxidative phosphorylation and autophagy, resulting in the inhibition of *M. oryzae* (Zhang et al. 2022). Another study demonstrated the capacity of *P. fluorescens* strain SS101 (Pf.SS101) to bolster Arabidopsis (*Arabidopsis thaliana*) resistance against various bacterial pathogens, including *P. syringae* pv tomato (Pst). By combining transcriptomics and metabolomics, scientists identified approximately 1,910 genes and 50 metabolites that exhibited differential regulation in the roots and leaves of Arabidopsis plants treated with Pf. SS101 when compared to untreated plants (Van de Mortel et al. 2012). Furthermore, multi-omics study

approaches can offer valuable insights into the intricate regulation of metabolomic and stress response pathways during specific metabolomic processes. For instance, the integration of transcriptomics and metabolomics in a study on *E. coli* Nissle 1917 (EcN) examined its metabolic behaviour under anaerobic conditions, revealing that EcN metabolizes galactose, a mucin sugar, at a slow rate (Jungyeon et al. 2024). The analysis showed that galactose metabolism partially redirects carbon flux into the trehalose pathway, resulting in intracellular trehalose accumulation and reduced growth. This approach uncovered 2768 transcripts with significant changes, including a 50-to-100-fold increase in those related to stress resistance metabolism in galactose metabolizing EcN, while transcripts directly linked to trehalose metabolism were upregulated by 2-to-threefold.

The studies above exemplify how the integration of transcriptomics and metabolomics approaches represents a powerful and comprehensive strategy for unravelling intricate biological mechanisms and providing deeper insights into the complex interplay between genetic expression and metabolite profiles in various biological systems, as also illustrated in the flow diagram below (Fig. 6). Despite the advantages of integrating these two omics approaches, researchers face significant challenges when aligning data collected from different time points. These challenges includes discrepancies in data output that can arise due to variations in the number of molecules detected across platforms, complicating comparisons (Cavill et al. 2015; Mohr et al. 2024). Additionally, the inherent differences in the nature of data generated from transcriptomics (gene expression levels) and metabolomics (metabolite concentrations) pose challenges for direct integration, unlike transcriptomics and proteomics where transcripts can often be directly linked to proteins, such associations are not straightforward in transcriptomics and metabolomics (Cavill et al. 2015). Technological constrains also play a role; each omics platform has its limitations that can affect data quality and comparability (Krassowski et al. 2020). Furthermore, navigating post-transcriptional modifications and metabolic transformations adds complexity to data integration efforts (Cavill et al. 2015; Krassowski et al. 2020). Temporal dynamics are another consideration, as biological processes are dynamic and can change over time, influencing both gene expression and metabolite profiles (Yin et al.

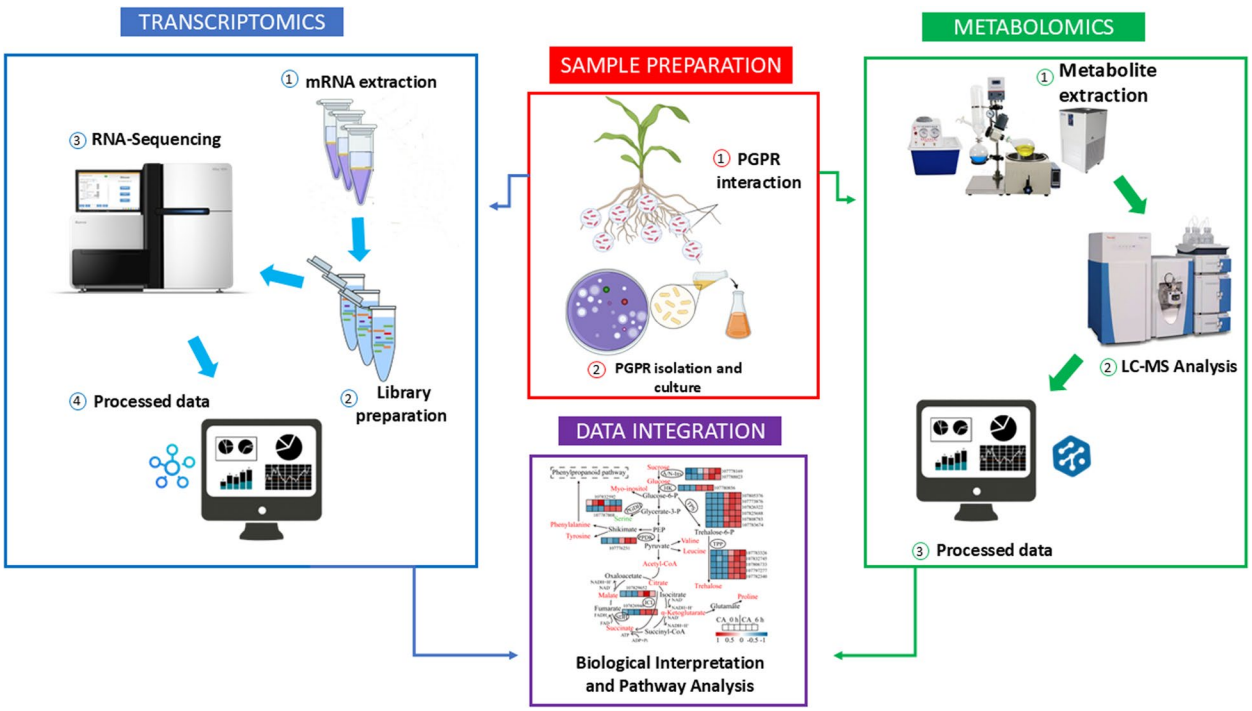

Fig. 6 General overview of transcriptomics and metabolomics integration workflow. It begins with sample preparation where interacting microbes are cultured and processed to ensure the high quality for analysis. In the metabolomics phase, metabolites are extracted and quantified using LC–MS techniques. The data is then processed and subjected to rigorous analysis to identify significant metabolite changes and pathways. Concurrently, in the transcriptomics analysis, mRNA is extracted from the same samples and converted into cDNA libraries, which are then sequenced using next-generation sequencing technologies to generate data that will be processed and allow for the determination of gene expression profiles. The data from metabolomics and transcriptomics analyses are integrated, enabling researchers to comprehensively understand molecular changes within the biological system

2022). Lastly, the analytical complexity of managing large datasets necessitates sophisticated statistical methods for effective data processing and interpretation (Misra et al. 2019; Vitorino 2024). Addressing these challenges is crucial for maximizing the potential of multi-omics approaches in unravelling complex biological interactions. Considering the advantages and cost-effectiveness, starting with metabolomics followed by proteomics, transcriptomics and genomics may be a good alternative to the traditional top-down strategy. This approach is advised since metabolites provide a more accurate representation of phenotype (Pinu et al. 2019). To enhance the analysis, researchers are also developing advanced computational methods for integrating multi-omics data. For example, Godoy et al. (2024) highlighted several innovative methods for integrating omics data, emphasizing their potential to improve the understanding of complex biological systems. These include techniques such as Similarity Network Fusion and Gene-Metabolite Network, which enable the effective correlation of diverse omics layers.

Concluding remarks and perspective

The emergence of PGPR utilization has had a profound and transformative impact on the field of agriculture and plant science, more generally, on PGPR have proven to be valuable allies in promoting plant growth, enhancing crop yields, and mitigating various environmental stressors. This innovative approach has heralded a new era of sustainable and environmentally friendly farming practices.

However, the utilization of PGPR often involves their cooperative behaviour within groups or communities in the rhizosphere, the soil region surrounding plant roots. This group dynamics among PGPR can have significant advantages in promoting plant growth and health. Additionally, PGPR communities enable resource sharing, allowing bacteria to exchange nutrients and metabolites, thus optimizing resource utilization. This collaborative approach not only enhances plant growth but also exemplifies the intricate and mutually beneficial interactions that occur in the rhizosphere, contributing to more sustainable and productive agriculture.

Despite the relatively limited number of published studies that have investigated PGPR-PGPR interactions both at the transcriptomics and integrated transcriptomics and metabolomics level, a significant and growing body of research sheds light on the importance of these interactions. This growing field of research is beginning to provide essential insights into the complex molecular and biochemical processes that drive PGPR-PGPR interactions in the rhizosphere. Furthermore, the use of transcriptomics and metabolomics in the investigation of PGPR-PGPR interactions has the potential to unveil the underlying molecular mechanisms driving these cooperative partnerships. Researchers can learn more about how PGPR interacts and collaborates at the cellular level by analysing changes in gene expression and metabolite profiles. This knowledge is critical for realising the full potential of PGPR-based agricultural techniques, which allows for more focused and effective approaches to increasing crop output while lowering dependency on chemical inputs.

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Authors' contributions
KM managed the literature searches, wrote the first draft of the manuscript, performed the analyses, and interpreted results. NRS, FA, LS, TM, MDM AND MIM proofread, thoroughly reviewed and critiqued the draft. While KM, NRS and MDM revised and rewrote some aspects of the manuscript. MIM is the principal investigator. All authors approved the article for publication.

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