

Review

Bacillus for Plant Growth Promotion and Stress Resilience: What Have We Learned?

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Abstract: The rhizosphere is a thin film of soil that surrounds plant roots and the primary location of nutrient uptake, and is where important physiological, chemical, and biological activities are occurring. Many microbes invade the rhizosphere and have the capacity to promote plant growth and health. *Bacillus* spp. is the most prominent plant growth promoting rhizobacteria due to its ability to form long-lived, stress-tolerant spores. *Bacillus*-plant interactions are driven by chemical languages constructed by a wide spectrum of metabolites and lead to enhanced plant growth and defenses. Thus, this review is a synthesis and a critical assessment of the current literature on the application of *Bacillus* spp. in agriculture, highlighting gaps that remain to be explored to improve and expand on the *Bacillus*-based biostimulants. Furthermore, we suggest that *omics* sciences, with a focus on metabolomics, offer unique opportunities to illuminate the chemical intercommunications between *Bacillus* and plants, to elucidate biochemical and molecular details on modes of action of *Bacillus*-based formulations, to generate more actionable insights on cellular and molecular events that explain the *Bacillus*-induced growth promotion and stress resilience in plants.



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1. Introduction

Edaphic factors and genetics play a pivotal role in the growth and yield of crop plants [1]. Over the past decades, genetic engineering and plant breeding approaches have been employed to develop new cultivars with desired traits, such as high yield and resistance to environmental stresses [2]. However, there is a less commercial success for genetically modified crops due to ethical constraints concerning genetically modified organisms (GMO) [3]. To obtain better crop yield, applications of chemical fertilizers have been the opted strategy. However, over time, studies and empirical evidence have shown that this traditional method—the use of chemical fertilizers—is not sustainable due to the inherent negative effects these products have on the environment. The excessive utilization of chemical fertilizers has shown to lead to toxic build-up of heavy metals, soil acidification and soil crust, thereby reducing the soil content of organic matter and humic substance. Soil acidification reduces crop phosphate intake, raises the concentration of harmful ions in the soil, and inhibits crop growth [4].

The incorporation of biostimulants, such as plant growth-promoting rhizobacteria (PGPR)-based formulations, in cropping systems has increasingly shown to be a promising strategy for sustainable agriculture and global food security, aligning with the United Nations sustainable development goals (SDGs) [5]. A broad array of bacterial species has been reported to possess plant growth-promoting attributes with the prominent species belonging to the genus *Bacillus*. Members of the genus *Bacillus* are ubiquitous, Gram-positive, and aerobic bacteria [6,7]. *Bacillus* species produce a multitude of enzymes, antibiotics, and metabolites which give them prominent applications in various sectors such as pharmaceuticals and agriculture. Furthermore, their uniqueness and popularity arise from their spore

forming ability which enables them to grow in uncondusive environmental conditions [8]. When applied to the plant as dormant cells, these *Bacillus* spores must germinate to form metabolically active cells. Following germination, these bacteria could be attracted by chemotaxis and leading to the root colonization process (which is mechanistically complex) and exerting growth promotion potentials [9]. Upon colonization, *Bacillus* spp. elicit direct (e.g., siderophore production, nitrogen fixation, phytohormone production and nutrient solubilization) and indirect mechanisms (such as production of exo-polysaccharides (EPS), biofilm formation, hydrogen cyanide (HCN) and lytic enzymes) to promote plant growth and yield, under various environmental conditions [7].

Thus, this review is a synthesis and a critical assessment of the current literature on the application of *Bacillus* spp. in agriculture, highlighting gaps that remain to be explored to improve and expand on the *Bacillus*-based biostimulants that are currently on the market. Considering ongoing efforts to understand the chemical intercommunication between rhizobacteria and plants, we herein review the current knowledgebase on key metabolites secreted by both the bacteria and the plant, their role in the communication between the two organisms, and responses to various environmental stresses. We acknowledge the growing literature and reports on emerging studies and insights on molecular mechanisms underlying rhizobacteria–plant interactions. The intent of this review is to provide a synthesis on the belowground chemical lexicon used for these interactions, with a focus on *Bacillus*, briefly summarizing the current state of knowledge in this regard. Furthermore, we discuss and emphasize that *omics* sciences, with a focus on metabolomics, offer unique opportunities to decode the “dark matter” in the chemical intercommunications between *Bacillus* and plants, to elucidate biochemical and molecular details on modes of action of *Bacillus*-based formulations, to generate more actionable insights on cellular and molecular events that explain the *Bacillus*-induced growth promotion and stress resilience in plants.

2. *Bacillus* spp.-Based Biostimulants and Growth Promotion Mechanisms

Various plant-associated *Bacillus* spp. have been commercialized as biostimulants for plant protection and growth promotion [5]; and examples of these formulations are given in Table 1. However, there has been a slow rate of exploitation of these *Bacillus*-based formulations. This is mainly due to a lack of understanding of the chemistry and biochemical mechanisms underlying the modes of action of the *Bacillus* spp. strains and defining the efficacy of these formulations when applied to crop plants under field conditions. The latter are multifactorial, impacting the growth and productivity of the plant. Various studies have illustrated discrepancies in the performance of rhizobacteria applied under well-controlled conditions and field conditions [10]. There is indeed a need for fundamental studies to understand the metabolism and the biochemistry of *Bacillus* strains, to elucidate biochemical and molecular events that define *Bacillus*-plant interactions at different levels and in both systems, i.e., bacteria and host plants.

Table 1. *Bacillus* spp. biostimulant formulations.

Microorganism	Formulation Type	Additives	References
Consortium * of <i>Bacillus licheniformis</i> , <i>Bacillus laterosporus</i> and <i>Bacillus amyloliquefaciens</i>	Liquid	No additives disclosed	[11–13]
<i>Bacillus megaterium</i>	Granules	Lactose monohydrate PVPK-30, sodium alginate	[14]
<i>Bacillus megaterium</i>	Powder	Talc, clay and cellulose; CMC, sodium benzoate, CaCO ₃ , Glucose, sucrose, mannitol, yeast, peptone.	[15]
<i>Bacillus amyloliquefaciens</i>	Powder	Sucrose, powder skimmed milk, MgSO ₄	[16]
<i>Bacillus amyloliquefaciens</i>	Liquid	Sucrose, powder skimmed milk, MgSO ₄	[16]
<i>Bacillus amyloliquefaciens</i>	Powder	MgSO ₄	[17]

Table 1. Cont.

Microorganism	Formulation Type	Additives	References
<i>Bacillus cereus</i>	Powder	Glucose, fructose, D-galactose, sucrose, trehalose, cellobiose, glutamic acid, soluble starch, glycerol, sorbitol, peptone, nonfat skimmed milk.	[18]
<i>Bacillus cereus</i>	Powder	Talc, CMC, CaCO ₃ , Glucose	[19]
<i>Bacillus subtilis</i>	Powder	Soybean flour	[20]
<i>Bacillus subtilis</i>	Liquid	Groundnut oil, Pongamia oil and sunflower oil; glycerol	[21]
<i>Bacillus subtilis</i> and <i>licheniformis</i>	Powder	Natural zeolite Synthetic zeolite	[21]

* This consortium is on the market as a biostimulant product, BACSTIM® 100.

Despite the lack of fundamental understanding of molecular mechanisms that govern modes of action of *Bacillus* spp., the applications of *Bacillus*-based formulations on plants (Table 1) have shown positive impacts such as plant growth promotion. The latter is thought to be due to *Bacillus*-enhanced nutrient uptake and hormonal modulations. Nutrient availability plays a pivotal role in seed germination and plant growth. However, the bioavailable forms of nutrients such as phosphorus and nitrogen are limited in the rhizosphere. Thus, *Bacillus* spp. assists in converting the complex forms of these essential nutrients to simple available forms [2]. *Bacillus* spp. can facilitate the uptake of phosphates by the roots through the secretion of phosphatases and low molecular weight organic acids such as formic acid, acetic acid, lactic acid, glycolic acid, fumaric acid, and succinic acid which acidify the environment to aid the conversion of inorganic phosphates into free phosphate [22]. In addition, *Bacillus* spp. also produces siderophores which help to solubilize iron from minerals and organic compounds in the rhizospheres. Low molecular weight siderophores such as enterobactin, pyochelin, alcaligin, and rhizoferin have iron-chelating ability making it very arduous for other microbes to access iron. The siderophore-iron complexes can be readily absorbed by the plants; however the transport systems involved therein are not fully understood [23]. *Bacillus* also facilitates iron mobility by inducing the upregulation of iron acquisition genes in plants. Additionally, studies have shown that *Bacillus* can increase the concentration of metal ions, which are often a limiting factor for plant growth by breaking them down into nanoparticles thus facilitating their mobilization as illustrated in Figure 1 [24].

Furthermore, upon colonization, *Bacillus* can directly enhance plant growth by the secretion of cytokine hormones and volatile organic compounds (VOCs) that modify plant hormone networks, promoting cell division and growth [25]. Two VOCs, 3-hydroxy-2-butanone and 2,3-butanediol, which are produced by *B. subtilis*, have shown to boost plant growth by altering cytokinin and ethylene homeostasis [26]. In another study by Zhang et al. [27], *B. subtilis* modulated auxin homeostasis by lowering its levels in leaves and inducing optimal growth in the *Arabidopsis* plant. Spermidine, which is also produced by various *Bacillus* spp., was reported to promote plant growth via the induction of expansins, and the reduction of ethylene levels in plants [28]. Despite the proposed models that explain these plant growth promotion events induced by *Bacillus* spp., the chemical language that dictates, mechanistically, *Bacillus*-plant interactions is still poorly understood. Thus, in the following section, we look at the growing literature on chemical communications between plants and PGPR, pointing out key postulations.

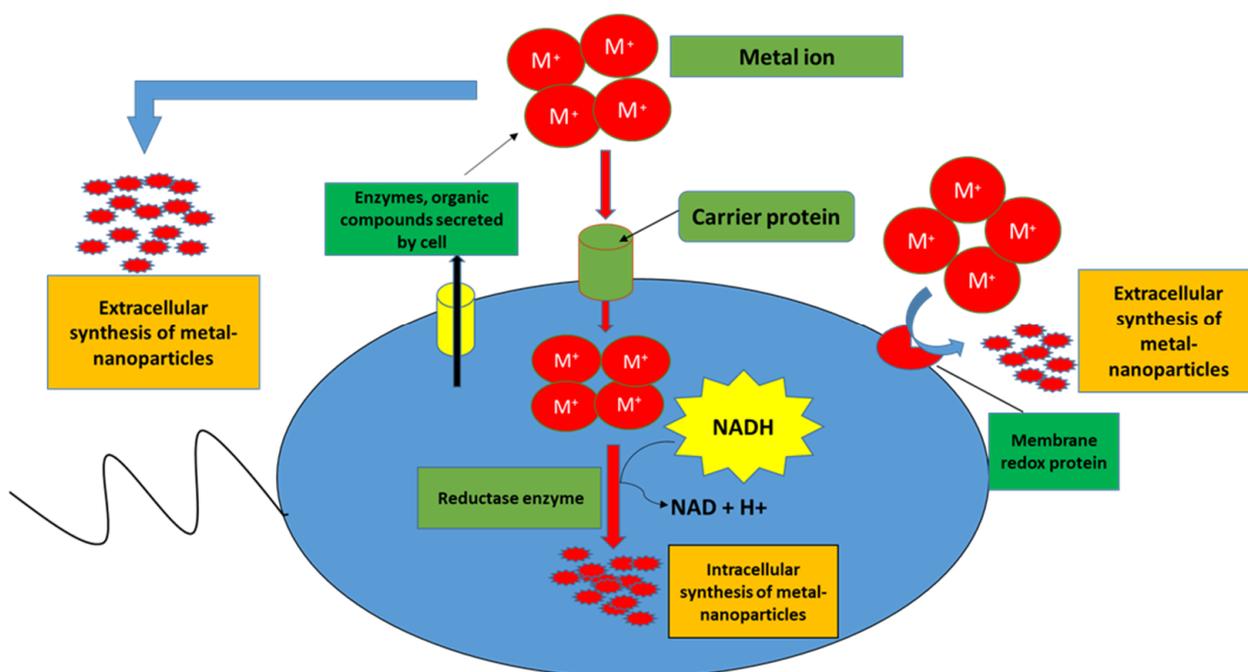


Figure 1. Intra- and extracellular biosynthesis of metal-nanoparticles by *Bacillus* spp. Extracellular biosynthesis of metal-nanoparticles is carried out by trapping metal ions on the cell surface and reducing them in the presence of secreted enzymes or metabolite and/or membrane-bound enzymes. In the intracellular biosynthesis of metal-nanoparticles, after transfer of metal ions into cell cytoplasm, the metal ions are reduced as a result of metabolic reactions with enzymes such as alpha-NADPH-dependent nitrate reductase.

3. Chemical Conversation between Plant and *Bacillus* spp. Which Leads to Plant Growth Promotion

The communication between plant roots and microbes in the rhizosphere is highly organized and regulated through a dynamic range of specialized metabolites and exudates which ultimately result in altered gene expression in one or both of the interacting partners [29]. The compounds produced by *Bacillus* spp. include phytohormones, ACC deaminase, volatile organic compounds, polyamines, lipopeptides, and acyl homoserine lactose. On the other hand, plants produce a range of various low-molecular weight molecules such as phytohormones, organic acids, and flavonoids (Figure 2).

The success of microbial based biostimulants relies on the root colonization ability of the beneficial microbes. As abovementioned, root colonization is a complex process which involves a highly dynamic molecular language (which remains enigmatic) between the microbes and the plant in the rhizosphere. Furthermore, this process is influenced by various parameters such as bacterial communities, root architecture and exudates (which depend on the developmental stages and physiological statuses of the plants), (a)biotic factors, and the physicochemical and biological components of the soil [30]. An elaboration on these belowground interactome associates is beyond the scope of this review; however, it suffices here to highlight that understanding the various factors that define the belowground interactions between the bacteria and plant is essential for better and novel exploration of these beneficial bacteria for agricultural practices.

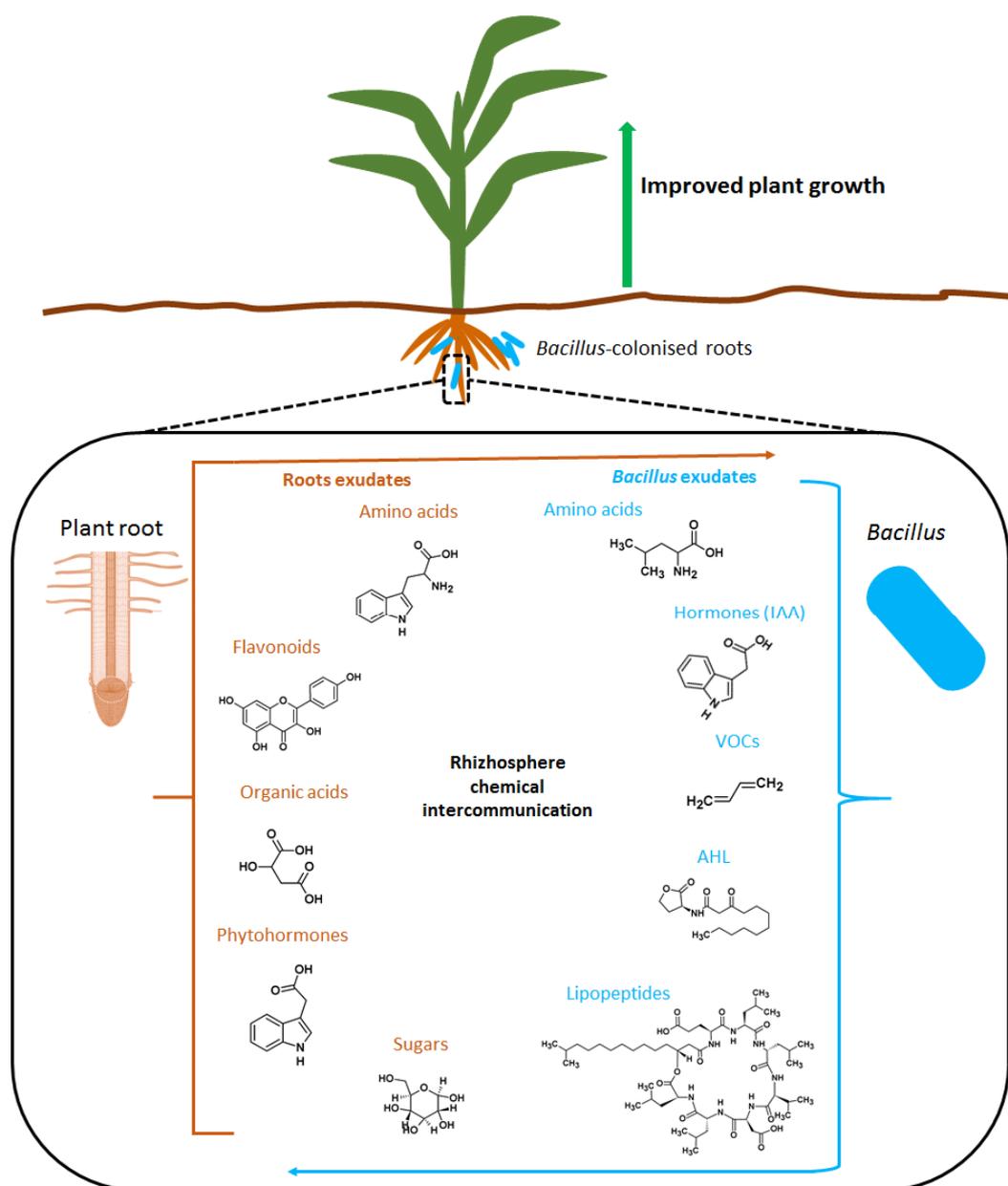


Figure 2. Some molecules involved in *Bacillus*-plant interactions. Plants produce various compounds including the hormones salicylic acid (SA), jasmonic acid (JA), cytokinins (CK), and indole acetic acid. Plants also produce organic acids, flavonoids, amino acids, and 1-aminocyclopropane-1-carboxylate (ACC) as signaling molecules. On the other hand, *Bacillus* produce volatiles such as 2,3 butanediol, lipopeptides such as surfactin, and phytohormones such as gibberellic acid and acyl homoserine lactone (AHL) as signaling molecules.

3.1. The Chemical Lexicon of *Bacillus* spp.-Phytohormones and Organic Compounds

Bacillus spp. are known to produce a wide range of hormones that act as signal molecules in the rhizosphere and involved in the belowground interaction networks (Figure 2). Some of the phytohormones known to be produced by *Bacillus* spp. include indole acetic acid (IAA), cytokinins (CKs), gibberellins (GAs), and abscisic acid (ABA) [31,32]. IAA is a potent signaling molecule vital for plant-microbe interactions and directly improve plant growth by elevating plant auxin pool which leads to cell elongation, vascular tissue development, and apical dominance [32]. Recent metabolomics and molecular studies have enabled the identification of genes involved in IAA biosynthesis in *B. amyloliquefaciens*

showing that it can occur through multiple pathways [33]. Among the various IAA synthesis pathways that have been identified, tryptophan has been confirmed to be the main precursor [32]. Furthermore, studies have pointed out that plant roots secrete tryptophan in the rhizosphere (Figure 2) which is utilized by rhizobacteria as a precursor for IAA biosynthesis [34,35]. This typically points to the belowground chemical interactomes that define *Bacillus*–plant interactions.

Bacillus spp. produce a vast range of cytokinins (CKs) (Figure 2) including zeatin, zeatin riboside, zeatin glycoside, izopentyl adenine, and izopentyl adenosine, which have been reported to be produced by *B. cereus*, *B. megaterium*, and *B. subtilis* [36]. Inoculation of lettuce plants with CK-producing *B. subtilis* increased shoots biomass [25]. Furthermore, the production of zeatin and zeatin riboside by *B. subtilis* can elicit the exudation of amino acids from the roots in wheat, subsequently increasing the diversity and quantity of beneficial microbiota in the rhizosphere [37]. Another group of phytohormones that is produced and secreted by *Bacillus* spp. is gibberellin (Figure 2) which is also involved in different plant developmental processes and the regulation of many physiological processes [38]. In the lettuce plant, the production of gibberellic acid by *Bacillus* spp. has been associated with an increase in nutritional metabolites such as amino acids, macro- and micronutrients, fructose, and carotenoids, thus increasing the quality of the crop [39].

In addition to hormones, rhizobacteria are reported to use volatile organic compounds (VOCs) in the chemical interactions with plants or other microorganisms (Table 2). Studies have shown that various genera of bacteria can regulate plant growth from a distance [40]. Some of these VOCs exuded by bacteria act as growth promoter molecules [41]. VOCs are low molecular weight and high vapor pressure compounds and act as signal molecules over short distances [42]. These features enable VOCs to facilitate intercellular and organismal interactions [43]. VOCs from *Bacillus* spp. are categorized into aldehydes, ketones, alkyls, alcohols, alkenes, esters, acids, ethers, heterocyclic, and phenolic compounds; however, many of these VOCs are still unknown [44,45]. Due to the structural diversity of these microbial VOCs, many natural functions have been inferred as highlighted in Table 2. For example, the production of the VOCs albuterol and 1,3-propanediol by *B. subtilis* induced differential expression of genes involved in the biosynthesis of the phytohormones auxin, gibberellin, cytokinin, and ethylene [42]. This subsequently leads to altered levels of the endogenous content of the related hormones in the roots and leaves, suggesting the involvement of these hormones in signal transduction pathways induced by VOCs which ultimately enhances plant growth. The natural function of these microbial VOCs is not only limited to plant growth but also infer protection against biotic stresses and induce plant tolerance against abiotic stresses. Investigations of these *Bacillus* VOCs-mediated effects on plants have focused on how they influence the signaling of phytohormones such as salicylic acid (SA), jasmonic acid (JA), ethylene, auxin, etc. [40]. Even though the effects of these *Bacillus* VOCs on plants are well documented, the underlying signaling mechanisms are still poorly understood [46].

Table 2. Effects of VOCs produced by *Bacillus*.

VOCs	<i>Bacillus</i> spp.	Plant	Response	References
2,3-Butandiol	<i>B. subtilis</i> ; <i>B. amyloliquenfaciens</i>	<i>Arabidopsis thaliana</i> , <i>Solanum tuberosum</i>	Induced systemic resistance/tolerance, plant growth promotion	[47,48]
Butyrolactone	<i>B. cereus</i> , <i>B. subtilis</i>	<i>Arabidopsis thaliana</i>	Growth promotion, modify root system architecture	[49]
Acetophenone	<i>B. megaterium</i> , <i>B. pumilis</i>	<i>Arabidopsis thaliana</i>	Growth promotion, modify root system architecture	[49]
2-Butanone	<i>B. subtilis</i> ; <i>B. amyloliquenfaciens</i>	<i>Nicotinia tabacum</i>	Growth promotion	[50]
3-Pentanol	<i>B. megaterium</i> , <i>B. pumilis</i>	<i>Capsicum annum</i>	Induced systemic resistance, plant growth promotion	[51]

As abovementioned, the belowground chemical interactome is highly complex and dynamic. One of the factors that define the performance of these PGPR is the efficacy in the root colonization by the bacteria, which is highly influenced by root exudates. The latter depends on the developmental stages and physiology of the plants. Furthermore, the microorganisms in the rhizosphere have diverse roles in supporting plant growth, development, and inhibition of host pathogens. This implies interdependency between the host and microbes in the aboveground and belowground interactions. Thus, understanding rhizosphere colonization mechanisms by PGPR is essential for generating inoculants able to compete and efficiently colonize the rhizosphere of plant crops and having a great impact on crop production and more consistent results.

3.2. Plant Root Exudates in Plant–*Bacillus* Crosstalk

Root exudates are a complex mixture of primary and secondary metabolites released into the rhizosphere by the roots and they maintain constant communication between plants and microbes in the rhizosphere [52]. Root exudates are composed of amino acids, organic acids, phenolics, sugars, and proteins with their highly diversified chemical properties based on different plant species, plant growth stages and the microbiota in the rhizosphere [53]. One of the roles of root exudates is to serve as signals for root colonization by beneficial microbes [54] as shown in Figure 2. PGPR can colonize the roots through chemotaxis, which is the mobility capacity of the bacteria along a chemical signal gradient [55]. Thus, root exudates are the architects of the rhizosphere and congruently the colonization of bacteria on the roots can modulate the metabolites exudation pattern [56].

The bacterial chemotaxis is elicited when a root exudate molecule binds to its cognate receptor [57]. Given the chemical diversity of root exudates, systematic identification of chemo-attractants and chemo-repellents in root exudates and elucidation of how these various root-secreted compounds are detected by multiple chemoreceptors of a PGPR strain can provide a comprehensive understanding of how PGPR colonizes the rhizosphere. In a study by Feng et al. [57], 39 chemo-attractants and 5 chemo-repellents were identified from cucumber root exudates for a well-studied PGPR strain *B. amyloliquefaciens* SQR9. In this study a mutant strain with 8 putative chemoreceptors deleted, lost chemotactic response to all the 44 compounds showing the importance of these chemoreceptors in plant–PGPR interactions and subsequent colonization. Further characterization of these chemo-effectors (root exudates) and chemoreceptors will broaden our insights into *Bacillus*–root interaction and provide valuable information to enhance the rhizosphere colonization ability of *Bacillus* spp., which will promote their application in agricultural production.

Post root colonization by *Bacillus*, the bacteria proliferate by receiving key signaling compounds and nutrients from the root exudates which ultimately lead to biofilm formulation on the root system which is an indication of a successful root colonization [58]. A study by Yuan et al. [58] showed that root exudates of banana containing organic acids induced both chemotaxis and biofilm formation in *B. amyloliquefaciens*. In this study, malic acid showed the greatest chemotactic response whereas fumaric acid significantly induced biofilm formation. Root exudates can be modified by environmental perturbations and thus influence the plant–PGPR interaction as it was illustrated in a study by Cesari et al. [59] where drought conditions modified the pattern of molecules exuded by roots, increasing the exudation of naringenin, oleic, citric, and lactic acids, and stimulating the release of terpenes of known antioxidant and antimicrobial activity. Changes in the molecular profiles of these exudates due to drought allowed for enhanced interaction between the roots and PGPR thus reversing the negative effects of drought condition on plant growth. These findings can assist in the formulation of biostimulant inoculants containing key molecules exuded during stress which can improve plant–PGPR interactions and thus promote plant growth and enhance defenses against various stresses.

4. *Bacillus* spp. Confers Protection to the Plant from Environmental Stresses

Major yield deficiencies, crop damage, and changes in growth rates of plants are caused by abiotic and biotic stresses. Many strategies have been proposed to combat the negative impacts of environmental stresses, and these have included, for instance, the overexpression of single genes encoding enzymes involved in the transportation of ions and scavenging of reactive oxygen species (ROS) being the most popular over the years. The application of this approach is limited due to the resultant pleiotropic effects on the growth of the plant and multiple pathways involved in response to environmental stress [60]. Another strategy is the use of chemical-based fertilizers and pesticides which are increasingly becoming harmful to the environment and some of these xenobiotics have become highly toxic in the agro-food chain. Therefore, nonharmful, environmentally friendly, sustainable, and nature-inspired solutions and strategies are urgently needed, and these include the use of PGPR formulations. The latter can aid in alleviating the effects of environmental stress. Several studies have demonstrated that *Bacillus* spp.-based biostimulants trigger plant protective mechanisms against both abiotic and biotic stresses.

4.1. *Bacillus* spp. against Biotic Stress

Biological control, utilizing beneficial microbes, is an excellent approach to limiting the adverse effect of disease-causing microbes on plant health and productivity. Considerable effort has been placed on identifying microbial biocontrol agents that can repress phytopathogens, especially those that are responsible for soil-borne diseases, and that can enhance agricultural productivity [61,62]. Many strains of *Bacillus* exhibit the ability to act as biocontrol agents against pathogens and thus can be used to suppress diseases [63]. Several mechanisms, both direct and indirect, are responsible for their ability to control pathogenic microbes as shown in Figure 3. These include the production of a wide array of antibiotic compounds (lipopeptides), the ability to form endospores, the ability to form biofilms on root surfaces, and the ability to induce host systemic host resistance, and stimulate plant growth [64].

Biotic stresses often lead to the production of reactive oxygen species (ROS) which lead to oxidative stress and are toxic to the cells. The inoculation of plants with *Bacillus*-based formulations has shown to elicit the production of antioxidant defense enzymes such as superoxide dismutase and peroxidase, which scavenge the ROS [65]. In a study by Zebelo et al. [66], cotton plants inoculated with *Bacillus* spp. demonstrated an increase in gossypol and jasmonic acid levels and secretion reducing larval feeding by *spodoptera exigua*. There was also up-regulation of genes involved in synthesis of allelochemicals and jasmonates in the inoculated plants. Furthermore, *Bacillus* spp. secrete various catabolic enzymes such as proteases, chitinases, and glucanases, as well as peptide antibiotics and secondary metabolites that contribute to pathogen suppression [40]. *Bacillus* spp. secrete cyclic lipopeptides such as iturin and surfactin (Figure 3) which play a role in disease suppression by acting as bi-functional molecules through their antifungal activity and elicitation of induced systemic resistance (ISR). The latter involves pathogen recognition at plant cell surface, stimulation of early cellular immune-related events, systemic signaling through a fine-tuned-hormonal cross talk and activation of defense mechanisms [67]. *Bacillus* spp. can elicit ISR in plants, which switches on pathogenesis related genes, mediated by phytohormone signaling pathways and defense regulatory proteins to pre-condition plants against future pathogen ambush [68].

Bacillus spp. secrete the secondary metabolites, cyclic lipopeptides (CLPs) which are involved in developmental processes such as motility and biofilm formation as well as biocontrol primarily based on their antimicrobial activity [69]. The synthesis of these CLPs is accomplished by multimodular peptide synthetases and depends on a functional phosphopantetheinyl transferase (Sfp) which transfers 4'-phosphopantetheine from coenzyme A to the carrier proteins during non-ribosomal synthesis [70]. Some CLPs secreted by *Bacillus* have emerged as plant immunity elicitors (Figure 3). The best-described *Bacillus* CLP is surfactin. When applied as pure compound on roots, surfactin induced ISR in bean, tomato,

tobacco, against *B. cinerea*, in melon against *Podosphaera fusca*, and peanut, against *Sclerotium rolfsii* [71–74]. The *Bacillus* CLP iturin has shown high bioactivity when applied to leaves as compared to the roots. The biochemical and molecular mechanisms that lead to this contrast are not well understood [75].

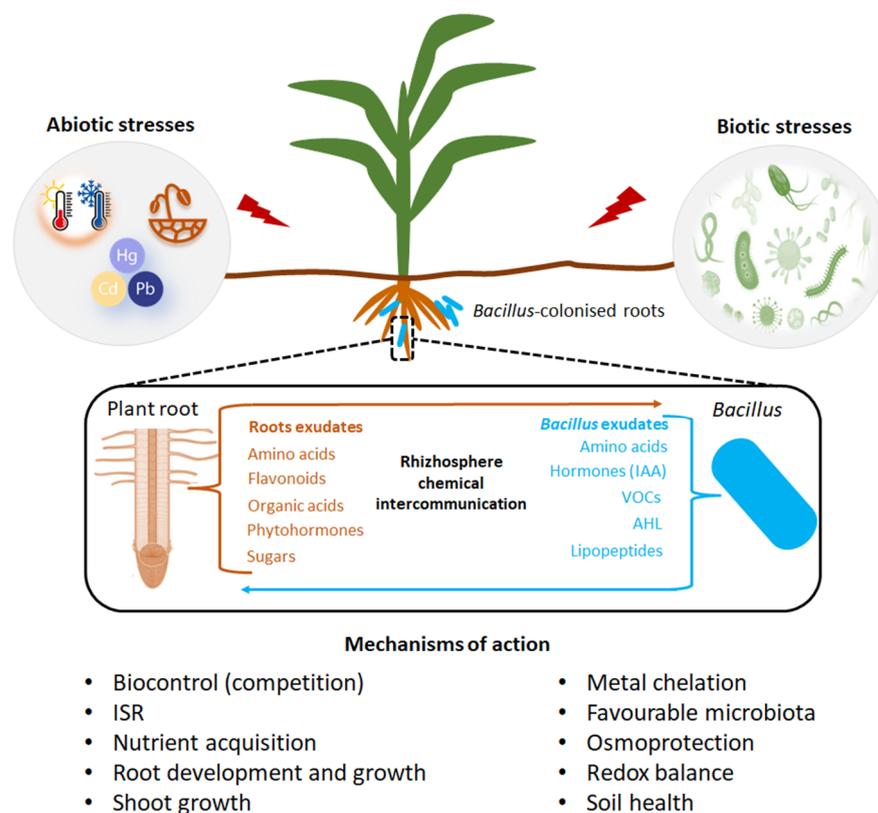


Figure 3. An overview of mechanisms employed by *Bacillus* spp. in the mitigation of biotic and abiotic stresses. *Bacillus* produce cyclic lipopeptides which activate pathways regulated by jasmonic acid and ethylene and thus elicit induced systemic resistance (ISR). They also produce hydrogen cyanide (HCN) and the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase which lower the levels of plant ethylene. Siderophores produced by *Bacillus* chelate iron thus making it unavailable to the pathogen. Several root associated *Bacillus* spp. bacteria produce zeatin, gibberellic acid (GA), indole-3-acetic acid (IAA), salicylic acid (SA), abscisic acid (ABA) as well as volatile organic acids (VOCs) which help plants to withstand stress by enhancing its antioxidant potential, by up-regulation of the antioxidant system and by accumulation of compatible osmolytes thus reducing oxidative stress-induced damage; improving photosynthetic capacity and membrane stability; promoting cell division and stomatal regulation; stimulating growth of root system, and acquisition of water and nutrients.

Bacillus strains also produce volatile organic compounds which act as elicitors of plant immunity. The most characterized VOCs secreted by *Bacillus* are 2,3-butanediol and acetoin (Figure 3) produced from glucose in the central metabolism [76]. In a study by Rudrappa et al. [77], exogenous application of acetoin triggered ISR and protected the maize plant against *Pseudomonas syringae*. What is intriguing in this study is that the expression of acetolactate synthase (an enzyme involved in the synthesis of acetoin) was significantly upregulated in the presence of maize root exudates during the late exponential growth phase, suggesting that root exudates play a role in eliciting acetoin biosynthesis in *Bacillus* [78]. The effects exerted by these secondary metabolites produced by *Bacillus* on plant health still need further elucidation as the response of the plant in the tandem presence of the beneficial *Bacillus* and the pathogen is complex.

4.2. *Bacillus* spp. against Abiotic Stress

Being sessile organisms, plants have to withstand various adverse abiotic stresses such as drought, salinity, heat/or cold, and heavy metal toxicity which pose a major threat to agriculture by negatively impacting plant growth and yield worldwide [79]. These stresses elicit stress responses in plants, including an accumulation of reactive oxygen species (ROS) and reduced photosynthetic activity, which ultimately leads to reduced plant growth and crop yield. PGPR such as *Bacillus* spp. can mediate the induction of abiotic stress responses in plants [80]. These responses to abiotic stresses are attributed to metabolic regulations which often require wide changes in the concentration, composition, and distribution of both primary and secondary metabolites. Biostimulants containing *Bacillus* strains have shown the potential to stimulate abiotic stress tolerance [81]. However, the biochemical and molecular mechanisms governing this *Bacillus*-induced stress resistance and tolerance are still enigmatic. Mechanisms suggested including changes of phytohormone levels through the secretion of phytohormones by *Bacillus* or ACC deaminase activity that decrease ethylene levels [82].

Cytokinins such as zeatin produced by *Bacillus* play a pivotal role in maintaining cellular proliferation and differentiation [83]. A study by Hussain and Hasnain [84] showed that the extracts obtained from cell cultures of *B. licheniformis* and *B. subtilis* are capable of increasing the weight and size of cucumber cotyledons separated from the seedlings, by inducing plant cell division due to the presence of cytokinins zeatin and zeatin ribose thus enhancing tolerance to salt stress. Cytokinin production has also been related to the ability of *Bacillus* species, such as *B. subtilis*, to increase plant tolerance to drought in lettuce [85] and *Platycladus orientalis* [86], or against salinity in wheat, due to the production of Zeatin, promoting plant growth [87]. *Bacillus* also produces auxins such as indole-acetic acid (IAA) which has been reported to confer tolerance against heavy metals [87,88]. For example, the study by Sun et al. [88] demonstrated that the application of IAA-producing *Bacillus altitudinis* alleviates iron stress in *Triticum aestivum* L. seedlings by both bioleaching of iron and up-regulation of genes encoding ferritins. Another study by Ji et al. [87] also showed that wheat treatment with IAA-producing *B. subtilis* can increase plant tolerance under salinity conditions.

Bacillus is also capable of producing gibberellic acids (GAs) which is a group of phytohormones involved in the processes of seed germination, flower initiation, leaf expansion, stem elongation, or flower and fruit development [89]. Various *Bacillus* species have the ability to produce a wide range of different GAs in vitro [90]. In a study by Ji et al. [87], the production of GAs by *B. subtilis* was related to its ability to increase the tolerance of wheat under salinity. Moreover, a study by Kang et al. [91] showed that the production of various GA compounds by *B. tequilensis* was involved in the induction of thermotolerance in soybean plants due to an increase of endogenous jasmonic acid and salicylic acid contents and a downregulation of abscisic acid showing the interaction between the various phytohormones in stress regulation. Abscisic acid (ABA) is considered an essential messenger in the adaptive response of plants to abiotic stress. Under water-deficit conditions, ABA plays a vital role in providing plants the ability to signal to their shoots that they are experiencing stressful conditions around the roots, eventually resulting in water-saving anti-transpirant activity, notably stomatal closure and reduced leaf expansion [92]. ABA upregulates the processes involved in cell turgor maintenance and synthesis of osmoprotectants and antioxidant enzymes conferring desiccation tolerance [93]. Study by Zhang et al. [94] reported a proportional increase in ABA concentration upon exposure of plants to salinity. A study by Pan et al. [95] also reported that the production of ABA by *B. subtilis* resulted in an increased tolerance to the heavy metal cadmium in *Brassica chinensis* plant by reducing cadmium-induced photosynthesis inhibition and the oxidative damage in plant tissues through increased levels of antioxidant compounds.

Although many studies have shown that there is a positive correlation between *Bacillus* synthesized phytohormones and plant growth and development under various abiotic stresses, there is a lack of knowledge of the underlying biochemical mechanisms

involved in the enhanced responses to abiotic stresses. Thus, to decipher all the chemical spheres and pathways involved in the interaction between *Bacillus* phytohormones and the plant under various environmental stresses, there is a need for more systemic biology studies. Apart from phytohormones, *Bacillus* spp. also produce VOCs, which induce abiotic stress tolerance. *Bacillus* VOCs have the potential to simultaneously up-regulate high affinity *HKT1* in shoots and down-regulate it in the roots, thus enhancing *HKT1*-dependent shoot-to-root sodium ion circulation, which is crucial in salinity tolerance. This was observed in a study by Zhang et al. [96] that VOCs such as 2,3-butanediol produced by *B. amyloliquefaciens* GB03 strain reduced accumulation of sodium ions in *Arabidopsis* shoots resulting in enhanced salinity stress tolerance. Zhang et al. [97] reported that exposure of *Arabidopsis* to *Bacillus* VOCs resulted in the accumulation of high levels of choline and glycine betaine, which are vital osmo-protectants that confer drought tolerance in plants [98].

The molecular mechanisms governing *Bacillus*-mediated abiotic stress tolerance are not limited to *Bacillus*' ability to produce phytohormones and VOCs. Other mechanisms associated with enhancing stress tolerance involve triggering biological and physiological processes such as ROS detoxification mechanisms, osmoprotection, stomatal regulation, membrane stability, xylem hydraulic conductance, root zone water, and nutrient availability and metal chelation [99–101]. For example, recent studies by Nephali et al. [11], Lephatsi et al. [13] and Othibeng et al. [102] revealed that the application of *Bacillus* consortium (a commercial biostimulant known as Bacstim® 100) to maize plants improves drought tolerance via mechanism such as enhanced energy production, enhanced expression of drought stress responsive defense genes, osmoregulation, redox homeostasis, strengthening of the plant cell wall and membrane remodeling.

As highlighted herein, there is vast knowledge on the *Bacillus*-plant interactions leading to growth promotion and stress resistance. However, there is still a lack of fundamental research that provides direct mechanisms of action underlying *Bacillus*-plant interactions. Thus, further research on the components and effects of *Bacillus*-based biostimulants on the plant health and growth is required to advance the development of a scientifically based biostimulant industry with the potential to encourage effective exploration and application of *Bacillus*-based biostimulants in agriculture for improved and sustainable food security. Comprehensive study approaches such as “omics” sciences have shown great potential to interrogate the metabolism of plants and *Bacillus* to generate mechanistic models with actionable knowledge and insights on how *Bacillus* promotes plant growth and enhances defense responses.

5. Omics Sciences in Studying *Bacillus* spp. and Its Effects on Plants: Metabolomics Applications

Omics refers to a biological study field where a comprehensive assessment of molecular entities is performed at different levels in the systems biology, such as DNA (genomics), RNA (transcriptomics), proteins (proteomics), and other cellular molecules (metabolomics) ([103]; Figure 4). Advancements in these technologies have proven instrumental in decoding important multilayered biochemical events underpinning the effects of *Bacillus*-based biostimulants on plants [99,104–106]. Metabolomics is a multidisciplinary *omics* science which offers a comprehensive identification and quantitation of low-molecular-weight molecules, namely metabolites. Plant metabolites are considered the “cause of plant phenotype” [107]. As such, the application of metabolomics in investigating the rhizospheric complex and dynamic chemical communications between *Bacillus* and plants would be of great value to agricultural research [108].

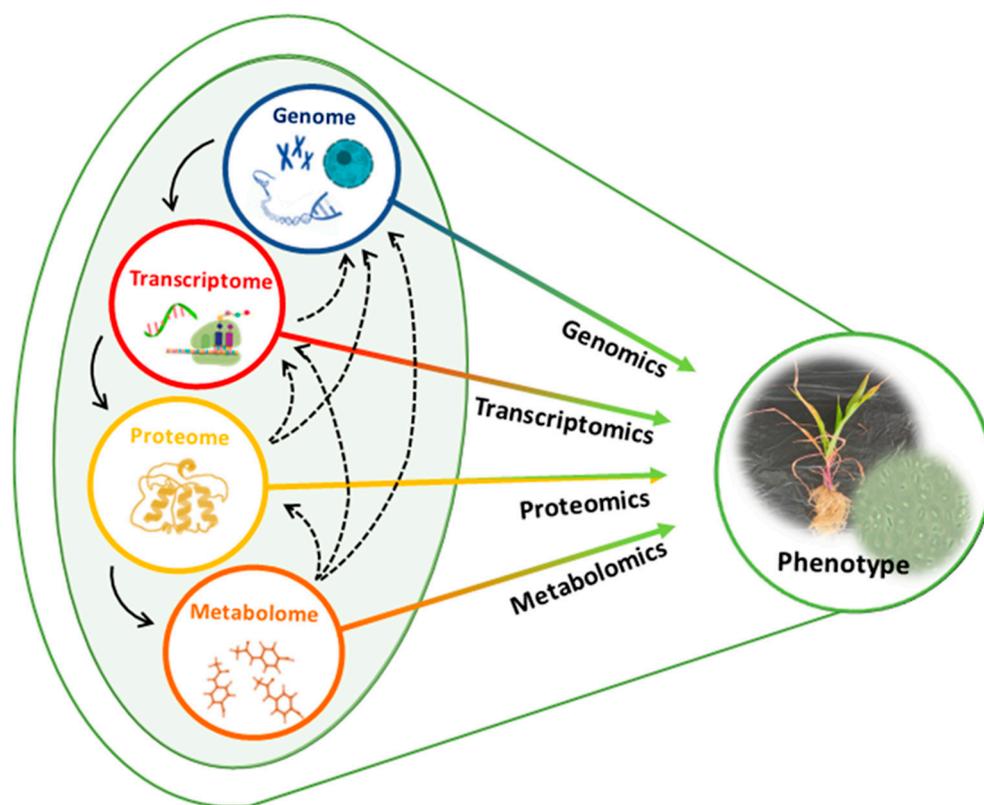


Figure 4. Omics technologies in systems biology. The omics cascade shows the flow of information from gene level to the metabolome with metabolomics being the closest link to the phenotype.

The belowground intercommunication involves inter- and intra-organismal interactions (i.e., plant–*Bacillus*, plant-to-plant, and *Bacillus*–other microbes) [109]. Such multidimensional interactions are facilitated and controlled through the interconnected belowground signaling networks, resulting in altered plant growth and development. Thus far, as highlighted in Section 3, the known signaling molecules and metabolites include root exudates, volatile organic molecules (VOCs), allelochemicals, lytic enzymes, toxins, and quorum sensing molecules (QSM), phytohormones, siderophores, lipopeptides, and lytic enzymes [110,111]. While some studies have made significant contributions to characterizing the composition of rhizobiospheres, there are still considerable gaps in understanding how plants shape their rhizobiospheres and how the bacterial presence and exudates affect the plant and its root microbiota [29,112].

Considering the intricate complexities in the rhizosphere mentioned above, studying these interactions concertedly is challenging and almost impractical. Thus, the existing literature mainly focuses on investigating one or two of the interacting partners to intently extract useful and accurate information that can offer the best description of these dynamic interactions. As such, the following sections discuss metabolomics studies aimed at investigating (i) *Bacillus* intracellular and extracellular chemical space, (ii) interactions between *Bacillus* and other microbes in the rhizosphere, and (iii) *Bacillus* and plant intercommunications.

5.1. Application of Metabolomics in Investigating *Bacillus* Chemical Space

The emergence of omics sciences such as metabolomics allows a comprehensive examination of biological systems at a global level and the exploration of interrelationships and dynamics between the multi-component systems, i.e., holobiont [113]. The untargeted metabolomics studies interrogating the chemical space of *Bacillus* are still very limited; however, most studies employ analytical platforms such as chromatography coupled to mass spectrometry and nuclear magnetic resonance (NMR) spectroscopy (commonly utilized

in metabolomics) to structurally characterize bacterial metabolites (Table 3). For example, the application of LC-MS and NMR spectroscopy revealed the identity of two novel cyclic depsipeptides turnagainolides A and B and that these compounds contain a combination of R and S amino acids and the (E)-3- hydroxy-5-phenylpent-4-enoic acid fragment Hppa, which are hallmarks of nonribosomal peptide biosynthesis [114].

Table 3. Identified metabolites in the intracellular and extracellular chemical space of *Bacillus* spp.

<i>Bacillus</i> Species	Metabolite	Chemical Nature	Bioactivity	References
<i>B. subtilis</i> (unidentified marin starin)	Bacilotetrins	Cyclic-lipotetrapeptides	Antimicrobial	[115]
<i>B. amyloliquefaciens</i> AP183	Bacillusin	Macrocyclic polyene	Antimicrobial	[116]
<i>B. subtilis</i> (unidentified marin starin)	Gageotetrins	Linear lipopeptides	Antimicrobial, anticancer	[117]
<i>B. subtilis</i> DSM 16696	Macrolactin	Macrolides	Antimicrobial	[118]
<i>B. subtilis</i> DSM 16697	Plipastatin A	Lipopeptides	Antifungal	[119]
<i>B. subtilis</i> MTCC 10403	Furanoterpenoids	Polyketide	Antimicrobial	[120]
Unidentified <i>Bacillus</i> strain	Turnagainolides	Depsipeptides	Activation of SHIP1	[114]
<i>B. subterraneus</i> 11593	Bacilsubteramide A	Alkaloid		[121]
<i>B. cereus</i> RKHC-09	Cereusitin A	Cyclic tetrapeptide	Antifungal	[122]
<i>B. amyloliquefaciens</i> HAB-2	Bacillomycin	Cyclic lipopeptide	Antifungal	[123]
<i>Bacillus</i> sp. FS8D	Pseurotin A	Spirocyclic	Anticancer	[124]
<i>B. coagulans</i> 14	Coagulin	Peptide	Antibacterial	[125]
<i>B. thuringensis</i>	Bacthurucin f4	Peptide	Antifungal	[126]
<i>B. cereus</i>	Cerein	Peptide	Antibacterial	[127]
<i>B. megaterium</i>	Megacin	Peptide	Antibacterial	[128]
<i>B. thuringensis</i> S	Thuricin	Peptide		[129]
<i>B. licheniformis</i>	Halobacillin 5b	Hemolytic, cytotoxic		[130]
<i>B. amyloliquefaciens</i> GSB272	Bacilysin 1	Antifungal, antibacterial		[131]
<i>B. subtilis</i> 168	Bacilysocin	Fungicidal, antibacterial		[132]
<i>B. licheniformis</i> 1001, <i>B. licheniformis</i> M017, <i>B. amyloliquefaciens</i>	Lichensins Surfactins	antimicrobials		[12]

In another study, LC-MS and NMR spectroscopy-guided metabolic profiling was employed and two new cyclic-lipotetrapeptides, bacilotetrins A and B were identified in the extracellular milieu of *B. subtilis* [115]. A cyclic lipopeptide plipastatin A1 was identified from the extracellular milieu *B. amyloliquefaciens* SH-B74 using a combination of analytical platforms including LC-MS, GC-MS, and NMR [119]. The cumulative findings of such studies led to wide-ranging secondary metabolites that are known to be present in various *Bacillus* strains (Table 3). Such metabolites include peptides of low molecular weight that are generated ribosomally (bacteriocins) or non-ribosomally; lipopeptides such as surfactins, iturins, and fengycins and polyketides such as macrolactins, difficidins, and oxididifficidins [116]. Interrogating and profiling the intracellular and extracellular metabolome of the *Bacillus* strains will provide insightful science-based information that can be leveraged in designing and formulating novel *Bacillus* biostimulants. Moreover, understanding the intracellular metabolome of *Bacillus* and the metabolites they secrete to the exterior, can enhance the understanding of *Bacillus*-plant intercommunication [12,133].

With innovative developments in analytical technologies (integrating artificial intelligence, AI and machine learning, ML), advancements in chemometrics and statistical methods (big data analytics and management), and the integration of orthogonal biological approaches, untargeted metabolomics is becoming a fundamental fulcrum to creating a “Rosetta stone” for deciphering the encryptions existing in the rhizosphere ecology [134,135]. One of the emerging analytical aspects is ion mobility technologies such as ion mobility spectrometry (IMS). Interfacing IMS with mass spectrometry (IMS-MS) increases the

analytical power, enabling the efficient separation, resolution, identification, and multi-dimensional structural characterization of analytes [136,137]. Currently, there are limited studies demonstrating the application of IMS technologies in investigating the *Bacillus* spp. One of the very few studies include a recent study by Ratiu et al. [138], where a portable aspiration-type ion mobility spectrometer (a-IMS) and gas chromatography-mass spectrometry (GC-MS) were applied to discriminate between different bacteria, *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus*, by rapid sensing of the bacterial metabolic volatiles, which produced differential metabolic fingerprints.

Another analytical tool that has proven instrumental for profiling live microbial colonies is nanoDESI MS [134]. The applications of this technique allowed highly sensitive metabolic profiling directly off living microbial communities, requiring zero sample preparation. Other advantages of this tool include the ability to capture a wide variety of metabolite families within a single mass spectrum directly from a live specimen thus giving comprehensive visuals into the chemical space of bacteria. Such insights are fundamental to describing the microbial chemotype that are more accurate to the phenotype [134]. For example, in this study by Watrous et al. [134], nanoDESI MS and MS/MS molecular networks demonstrated an elevation of metabolite content within *B. subtilis* across the 60 h time growth period, with increased production of structural variants of the cyclic peptides surfactin, plipastatin, and subtilisin steadily increasing over time.

Furthermore, some of the recent advancements in data handling and metabolite annotation include fourth industrial revolution (4IR)-inspired computational tools such as molecular networking (MN) integrated with in silico annotation tools [139–141] are positively impacting the chemical and biological interpretation of untargeted metabolomics studies on *Bacillus* and *Bacillus*–plant interactions. The application of MN in microbial metabolomics enabled detection and visualization of related metabolites in *Bacillus* cells via spectral similarities within and between data sets as chemical families are grouped together [12,134]. For instance, the study by Nephali et al. [12] applied MN tools to profile the intracellular chemical space of PGPR *Bacillus* strains: *B. laterosporus*, *B. amyloliquefaciens*, *B. licheniformis* 1001, and *B. licheniformis* M017 and their consortium—this study showed higher content of surfactins in *Bacillus* consortium and *B. amyloliquefaciens* and higher content of lichenysins *B. licheniformis* strains compared to other strains under study. Additionally, MN proved instrumental to uncovering the temporal dynamics in *B. subtilis* (Watrous et al., 2012) and *B. laterosporus*, *B. amyloliquefaciens*, *B. licheniformis* 1001, and *B. licheniformis* M017 [12]. Time-dependent MN showed an increasing number of lipopeptides over time in *B. amyloliquefaciens*, *B. licheniformis* 1001, *B. licheniformis* M017, and *B. laterosporus* and their consortium, and the stationary phase showed the highest content of lipopeptides in the cells [12]. A recent study by Wang et al. [142] revealed novel compounds Hetiamacin E and F from *B. subtilis* PJS using MS/MS-based MN. Another study by Purves et al. [143] demonstrated the application of metabolomics and MN for microbial secondary metabolite bioprospecting. Decoding metabolic profiles of *Bacillus* strains will contribute toward understanding the chemical space of PGPR, which will subsequently help in decoding the chemical communication underlying PGPR-plant interactions.

Despite the increasing advancements in the metabolomics workflow mentioned above, the chemical space of *Bacillus* is still not fully elucidated. Currently, the metabolites that have been found in *Bacillus* include amino acids, hormones, organic acids, nucleotides, cofactors, and sugar-phosphates [144] and structurally diverse secondary metabolites, such as lipopeptides (e.g., iturins, fengycins, and surfactins), polyketides, polypeptides, macrolactones, lipoamides, fatty acids, isocoumarins [53,145,146], siderophores such as hydroxamates (e.g., schizokinen, pyochelin), catecholates (e.g., bacillibactin, petrobactin), and carboxylates (e.g., rhizobactin) [147,148]. Identification of these metabolites in *Bacillus* cells have contributed toward understanding the biochemistry of *Bacillus* and their interaction with the host plants and other microorganism. For example, metabolites such as lipopeptides, macrolides, and polyketide have been demonstrated to possess antimicrobial and antifungal activities (Table 2), mechanisms that are involved in biocontrol of phy-

topathogens [148]. Moreover, other bacterial metabolites such as hormones (indole acetic acid) and amino acids (tryptophan) have been shown to promote plant growth [148]. However, the current knowledge is barely a tip of the iceberg, as a large percentage of *Bacillus* metabolome remains unexplored [144,149]. Comprehensively annotating and characterizing the metabolome of *Bacillus* will advance our understanding of belowground chemical communications between *Bacillus* and plants. Such accurate models would explain mechanisms of action of *Bacillus*, molecular events that govern *Bacillus*-mediated enhanced plant growth and stress protection. This actionable knowledge is necessary for innovatively designing and implementing *Bacillus*-based formulations for sustainable agriculture.

5.2. Application of Metabolomics in Investigating *Bacillus* and Other Microorganisms' Interactions in the Rhizosphere

The holobiont dynamics (assembly of the phytomicrobiome) involves the chemical intercommunications between the microbe–microbe interactions and plant–microbe interactions [150]. As rhizosphere-dwelling bacteria, *Bacillus* are surrounded and constantly interacting with other microbes via different types of communications [151]. The intercellular interactions between microbes occur via four main mechanisms such as cell–cell signaling, production of secondary metabolites, cell–cell contacts and metabolic interplay [152]. The cell–cell signaling involves the interexchange of diverse chemical signaling molecules such as volatiles, quorum sensing signals, and secondary metabolites to communicate, regulate, and synchronise microbial behaviors. However, this signal network is not well understood—there are knowledge gaps in determining the predominance, diversity, and function of signaling molecules and how such factors drive the communal behavior and population dynamics within phytomicrobiome ecosystem.

As such, metabolomics approaches can be employed to bridge the existing knowledge gaps [108,150,153]. One of the very few is a study by Wen et al. [154], which demonstrated that several bacterial groups such as *Bacillus* and *Chitinophaga* were negatively related to the pathogen abundance. The GC-MS analyses revealed significantly different metabolomes in two groups of rhizosphere soils, i.e., the rhizosphere soil of lower harbored more sugars such as fructose, sucrose and melibiose than that in high pathogen abundance, indicated their potential biocontrol ability. A recent study by Andric et al. [155] applied a highly advanced analytical platform, MALDI-FT-ICR MS imaging, revealing that *Bacillus* mobilizes its cyclic lipopeptide surfactin to improve motility and reduce the toxicity of *Pseudomonas* by acting as a chemical deactivator of *Pseudomonas* lipopeptides, sessilins and tolaasins.

The secretion of lipopeptides surfactins by *Bacillus* displays an antagonist relationship with other microbes and has also been shown to exert a symbiotic relationship with other bacterial species, thus shaping the plant microbiome. For example, the study by Luzzatto-Knaan et al. [133], demonstrated the role of surfactins as an interspecies recruitment factor. In this study by Luzzatto-Knaan et al. [133], mass spectrometry and MN approaches were applied and revealed that *B. subtilis* secretes surfactins to recruit *Paenibacillus dendritiformis* to its ecological niche and that *P. dendritiformis* actively degrades surfactins originating from *B. subtilis* and marks its territory by accumulating the resulting surfactin degradation products. Such studies illustrate the indispensability of metabolomics in revealing the ecological roles of secondary metabolites such as lipopeptides during microbial interspecies interactions as well as the regulation of their expression under naturally competitive soil conditions [133,155]. Moreover, studies like these bring the scientific community much closer to fully understanding the mechanisms of *Bacillus* mechanisms for agricultural applications.

5.3. Application of Metabolomics in Investigating the *Bacillus* and Plant Intercommunications

Various metabolomics studies have illustrated that the *Bacillus*–plant interaction (which may involve the secretion of secondary metabolites from *Bacillus*, Table 2) can confer enhanced growth-promotion and defense priming of the plant through the reprogramming of the plant metabolome [146]. For example, a study by Nephali et al. [11] revealed an increased pool of tricarboxylic acid (TCA) intermediates, reprogramming of

amino acid profiles and differential changes in phenolics and lipids as key metabolomics signatures induced by the application of *Bacillus*-based biostimulants on maize plants. Another metabolomics by Kang et al. [156], showed decreased levels of glucose, fructose, sucrose, and trehalose in *B. simplex*-treated soybean roots compared to the control group—decreased sugar levels was translated to reduced food sources for nematodes. Furthermore, treatment with *B. simplex* led to higher levels of melibiose, gluconic acid, lactic acid, phytosphingosine, and noradrenaline in soybean roots, which promoted nematocidal activity thus, improving disease resistance.

A metabolomics approach was also applied to reveal the underlying mechanisms employed by *Pa. alvei* NAS-6G6 and *B. velezensis* N54 in plant protection against biotic (*Fusarium pseudograminearum* crown rot) and abiotic (drought) stress. In this study, *Pa. alvei* NAS-6G6 was found to induce unique protection capacity against biotic and abiotic stress, and combined stresses by upregulating different defense metabolites *S. bicolor* plants and altering metabolic pathways such as riboflavin metabolism under biotic and drought stress and glutathione metabolism under combined biotic (*Fusarium pseudograminearum* crown rot) and abiotic (drought) stress. *B. velezensis* N54 upregulated arginine and proline metabolism, *Pa. alvei* NAS-6G6 upregulated riboflavin metabolism under biotic stress condition (*Fusarium pseudograminearum* crown rot) [157]. Riboflavin and proline are known to play significant roles in regulating antioxidant mechanisms and osmoprotection [157]. A recent metabolomics study by Shahid et al. [146], profiled extracellular secondary metabolites and hormones from *Bacillus* spp., which positively correlated to plant growth promotion and antifungal properties. The application of metabolomics to investigate the effects of *Bacillus* and *Bacillus*-based formulations is gaining some momentum, thus generating a wealth of knowledge driving toward the complete elucidation of *Bacillus*-mediated plant growth promotion and stress protection mechanisms. Thus, a reader is referred to review papers by Nephali et al. [99] and Lephatsi et al. [13] for more details on applying metabolomics approaches in studying microbe-plant-stress interactions.

Bacillus spp. are emerging as key microorganisms in the biostimulant industry for maintaining sustainable food security. However, as reviewed in this manuscript there are still major knowledge gaps and bottlenecks in the formulation of *Bacillus*-based biostimulants. This includes poor characterization of active components and synergies in the biostimulant composition and undefined mechanisms and modes of action of biostimulant products, at cellular and molecular levels. Moreover, it is difficult to predict the field application efficacy of these biostimulants due to various environmental factors that may affect field crops, such as the soil chemistry, biotic and abiotic stresses. These limitations hinder not only the designing of novel formulations and biostimulant-based agricultural strategies but also the establishment of a standardized legislative framework and regulatory system for the biostimulant industry. Metabolomics, a multidisciplinary *omics* science, offers unique opportunities to predictively decode the mechanisms and modes of action of biostimulants on crop plants, and elucidating signatory markers and metabolic profiles that define the biostimulant action (Table 2). The application of metabolomics in biostimulant research can help us to comprehensively decipher the molecular basis behind biostimulant-induced plant metabolomic reprogramming which leads to improved plant health, growth, and increased yield. Such fundamental and indispensable knowledgebase would improve our understanding of biostimulants, also providing a roadmap for translational applications—designing of novel formulations and devising biostimulant-based precision agricultural programs and modules—for sustainable food security.

6. Conclusions and Future Prospects

In this critical review we narratively revisited the importance of PGPR such as those belonging to the genus *Bacillus* in improving plant growth and enhancing plant protection against adverse environmental factors. Furthermore, the incorporation of biostimulants, such as microbial formulations (e.g., *Bacillus*-based products), in cropping systems has increasingly shown to be a promising strategy for sustainable agriculture and global

food security, aligning with the United Nations sustainable development goals (SDGs). Despite the current wealth of knowledge on *Bacillus* spp. and its applications, molecular mechanisms that govern modes of action of *Bacillus*-based formulations remain poorly understood. Thus, this review suggests that *omics* sciences, with a focus on metabolomics, offer unique opportunities to illuminate the chemical intercommunications between *Bacillus* and plants, and to elucidate biochemical and molecular details on modes of action of *Bacillus*-based formulations.

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