

Root Exudates and Their Molecular Interactions with Rhizospheric Microbes

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Abstract Biologically important plant-microbe interactions are mediated by a wide array of signal compounds rhizodeposited from both plant and microbial species. Root exudates are some of the potentially important low molecular weight compounds secreted from plant roots. They are involved in building a network of biointeractions through several physical, chemical, or biological interactions. Application of bioinoculums has significantly improved growth parameters and yield of many economically valued crops. Root exudates mediate the plant-microbe interactions by colonizing the roots and promoting root growth. Also, root exudates improve chemical and physical characteristics of the rhizospheric soil. Some of the beneficial plant-microbe associations include nitrogen fixation by rhizobium, symbiotic biointeractions with AM (arbuscular mycorrhizal) fungi, and PGPR (plant-growth-promoting *Rhizobacteria*). These interactions improve plant growth and quality, stress tolerance, and plant defense responses. Root exudates constitute a wide variety of secondary metabolite constituents that help plants to guard against microbial infections, insects, or herbivore attack. Root exudates secreted by plants act as antimicrobial agents to curb various harmful rhizospheric pathogens. In this chapter, we provide a summary of literatures on the significance of plant-microbe interactions in the improvement of plant morphological and biochemical features. Further, detailed information on various types of root exudates and their role in mediating plant-microbe interactions and possible exploration of root exudates as a novel antimicrobial compounds are also discussed.

Keywords Soil microbes • PGPR • Mycorrhizae • Signal molecules • Antimicrobials

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1 Introduction

In nature, plants exhibit variety of biotic interactions between rhizospheric soil microbes by means of extremely complex mechanisms mediated by a wide array of signals produced from both plants and microbial species (Badri et al. 2009; Huang et al. 2014). Plants secrete root exudates as key signals into their surroundings to facilitate its better survival by establishing positive interactions with microbial community in the rhizosphere (Haichar et al. 2008; Bonfante and Anca 2009; Xie et al. 2012). However, the complex molecular interactions occurring between the soil microbes and plant roots are mainly modulated by exudates of roots. These exudates are known to build a network of interactions with plant roots and their surrounding rhizospheric microbes through various physical, chemical, or biological interactions (Huang et al. 2014; Haichar et al. 2014). Over past few years, researchers have suggested that the application of plant-growth-promoting rhizobacteria (PGPR) is sustainable in agricultural practices and development of agricultural biotechnology products such as biofertilizers, phytostimulators, biopesticides, and bioremediators. PGPR are extensively utilized all over the world, and their application rate is extremely rising due to numerous advantages. The growth and yield of many agriculturally important crops have been significantly increased by the application of PGPR (Akhtar and Siddiqui 2010; Bhattacharyya and Jha 2012; Ahemad and Kibret 2014).

Some past research studies have clearly stated that the microbial associations are very specific to plant species (Figueiredo et al. 2011; Haichar et al. 2014). Most of the legume plants are well known to associate with bacterial strains of rhizobacteriaceae family to fix the atmospheric nitrogen. Sugiyama and Yazaki (2012) reported that mutual symbiotic association fixes annually about 40–60 million metric tons of atmospheric nitrogen. However, the rhizobial bacterial species are also reported to secrete indole acetic acid. Changes occurring due to these auxin levels are shown to influence root nodule organogenesis and development. Similarly, it is reported that the auxin and cytokinin ratio play a key role in the regulation of nodule development (Figueiredo et al. 2008).

Since, PGPR are not only used as yield stimulators but also as bioprotectors in the management of plant pathogens or diseases (Figueiredo et al. 2008). Similarly, the symbiotic association of arbuscular mycorrhizal (AM) fungi with plants improves the uptake of water and mineral nutrients and also provides resistance toward stresses and pathogens (Akhtar and Siddiqui 2008; Akhtar and Panwar 2011; Akhtar et al. 2015). In this symbiotic association, both fungi and plants benefited each other. For instance, mycorrhizal fungi help the plants in uptake of nutrients from soil and in return provide carbohydrates to plants. However, the molecular mechanisms of nutrient exchange between the host plant and fungi are yet to be understood clearly (Thompson and Cunningham 2002; Bonfante and Anca 2009). The mycorrhizal fungi and their propagules, hyphae, and rhizomorphs form a network or a bridge between plant roots, fungi, and soil through which movement of nutrients is believed to occur (Bonfante and Anca 2009; Akhtar et al. 2011).

This network formation is mainly mediated by numerous signaling incidents involving low molecular weight compounds secreted from both plant and fungi (Paszkowski 2006; Parniske 2008).

Plant and soil fungal association used is agricultural practices to improve the soil fertility known as biofertilizers. This biological approach improved the soil fertility as well as prevents the environment by the hazardous effect of synthetic fertilizers. Also, this emerging practice has totally reduced the application of chemical products for controlling various plant diseases as eco-friendly approach (Figueiredo et al. 2008; Bais et al. 2008), as concluded by several past investigators that the plant roots secrete numerous types of compounds which are believed to facilitate the possible interactions between the plant root and the surrounding environment especially during symbiotic interactions (Bais et al. 2008; Bonfante and Anca 2009; Sugiyama and Yazaki 2012; Rashid et al. 2015). Over the last few years, rhizospheric investigation has witnessed the existence of these interactions between root and root, root and microbe, or root and insects (Badri and Vivanco 2009; Shukla et al. 2013; Haichar et al. 2014). Some of the examples of root exudates include amino acids, sugar molecules, organic acids, mucilage (polysaccharides), various proteins, phenolic acids, and secondary metabolite compounds (Bais et al. 2008; Badri et al. 2013; Haichar et al. 2014; Rashid et al. 2015). The secretion of root compounds is a normal process of plant root rhizodeposition to release major source organic carbon into soil (Nguyen 2003; Badri and Vivanco 2009). However, more research efforts are still required to understand the molecular mechanisms of root secretions. Root exudation mediates the plant-microbe interactions by colonizing the roots and promoting root growth. The rhizosphere soil provides an environment for diverse class of microbial community which is useful as well as harmful to the plants. Some of these microbes associate to form beneficial interaction with the plants. In contrast, plant interactions with pathogenic bacteria can be harmful to the plants. Studies have revealed the existence of rich microbial community around the rhizospheric soil of different plant species.

Hence, plant-microbe interactions might be both positive and negative approaches depending on the other factors of its rhizosphere vicinity (Mougel et al. 2006; Micallef et al. 2009; Sugiyama and Yazaki 2012; Haldar and Sengupta 2015). Extensive research reports accumulated over the past last decade have witnessed the new understanding on these beneficial interactions of plant exudates and microbial flora of rhizospheric soil. These studies have unlocked the possible exploration and application of plant microbial interactions by using various biotechnological tools and techniques for better crop production (Yedidia et al. 2001; Woo et al. 2006). Some of the negative plant-microbe interactions modulated by root exudates are the association with microbial pathogens, parasitic plants (Shukla et al. 2013). Rhizospheric bacterial strains utilize root exudates as nutrient source and mediate in the process of contaminant elimination and also can degrade various ecological pollutants (Bais et al. 2008; Shukla et al. 2013). Few researchers have emphasized on the aspect of understanding the possible functions of root exudates and the competent microbes in the process of phytoremediation and rhizoremediation (Gleba et al. 1999; Shukla et al. 2010, 2013). Also, root exudates act as antimicrobial molecules

to provide tissue-specific resistance against various pathogenic bacterial strains (Bais et al. 2005). Hence, knowledge on the mechanisms of interactions is very crucial in exploring the applications of plant-microbe interactions in many ways in the modern-day agricultural practices. This chapter describes the importance of root exudates, applications, and their role in understanding various mechanisms of interactions.

2 Root Exudates and Their Characteristics

The rhizospheric soil surrounding the plant roots is characterized by many kinds of distinctive biochemical, ecological, and physical interactions that are largely mediated by various chemical compounds released by plant roots into their immediate vicinity. These wide arrays of chemical compounds that are exuded to the rhizosphere by the plant roots are generally known as root exudates (Walker et al. 2004; Huang et al. 2014). The quantity of root exudate secretion depends mainly on plant species, age, cultivar type, plant root metabolic attributes, root system architecture, and environmental conditions that come across during plant growth (Bertin et al. 2003; Haichar et al. 2008; Compant et al. 2010; Haldar and Sengupta 2015). Secretion of plant root exudates into the soil requires large amounts of energy (5–21 % of fixed carbon). Primarily, root exudates are the low molecular weight carbon-containing chemical compounds that are derived mainly from the products of photosynthesis (Bertin et al. 2003). Root exudates function as potent chemical messengers to facilitate rhizobacterial chemotaxis process and mediate biological interactions through wide array of complex molecular networks (Walker et al. 2004; Bais et al. 2006; Glick et al. 2007; Cheng et al. 2009; Xie et al. 2012; Haichar et al. 2014). Root exudates are known to perform various functions such as the regulation of plant-microbe association, encouragement for various symbiotic interactions, prevention from herbivores attack, and inhibition of other competent plant growth in their surroundings (Haldar and Sengupta 2015). Moreover, it also improved the chemical and physical characteristics of the rhizospheric soil (Walker et al. 2003; Haichar et al. 2014; Yadav et al. 2015).

Root exudates encompass a wide array of chemical constituents including primary and secondary metabolites, ions, mucilage, free oxygen molecules, and water molecules (Hejl and Koster 2004; Bais et al. 2006), while other arrays of signal molecules include amino acids (glutamine, arginine, cystine, asparagine, aspartic acid, cysteine), enzymes, peptides, sugars (oligosaccharides, fructose, arabinose, glucose, mannose, maltose), vitamins, nucleotides, organic acids (ascorbic acid, acetic acid, benzoic acid, ferulic acid, malic acid), fungal stimulators, plant inhibitors, chemoattractants, growth regulators, sterols (campesterol, cholesterol, sitosterol, stigmasterol), fatty acids (palmitic, stearic, linoleic, linolenic, oleic), tannins, phenolic compounds, and few other miscellaneous chemicals. Some of the examples of primary root exudates comprise amino acids, enzymes, proteins, organic acids, sugars, lipids, flavonoids, allelochemicals, siderospores, coumarins,

and aliphatic and aromatic chemical metabolites (Bertin et al. 2003; Shukla et al. 2013; Huang et al. 2014; Halder and Sengupta 2015). Among all these root exudates, organic acids play a significant role by serving as energy source for microbial cellular metabolism and also act as intermediate in biogeochemical cyclic reactions in the rhizospheric soil (Shukla et al. 2013).

Legume plants are widely consumed throughout the world, and hence, metabolic profiling studies and other basic research have been mainly focused on the same plant species in order to understand the types, characteristics, and functions of root exudates. Moreover, legume plants exhibit some of the biologically significant property, viz., fixation of atmospheric nitrogen through symbiotic association with rhizobacteria. Some of the commonly explored plants for root exudates and other metabolites include *Medicago sativa*, *Trifolium repens*, *Pisum sativum*, *Lotus japonicus*, *Medicago truncatula*, *Phaseolus vulgaris*, and *Glycine max* (Desbrosses et al. 2005; Farag et al. 2009; Hernandez et al. 2009; Rispaill et al. 2010; Sugiyama and Yazaki 2012). Most of the characteristic properties and primary metabolic activities involved in symbiotic nitrogen fixation have been discovered through various classical methods involving studies on plant biochemistry, genetics, and molecular biology. Use of genetic approaches, transcriptomics, proteomics, and other functional genomics studies has provided better understanding of metabolic activities of nodule formation in some of the model plants such as *Lotus japonicas* and *Medicago truncatula* (Desbrosses et al. 2005; Sugiyama and Yazaki 2012; Xie et al. 2012).

In Arabidopsis plant, many reports have identified numerous root exudates such as sugars, amino acids, fatty acids, and an assortment of proteins (De-la-Pena et al. 2008; Badri et al. 2009; Badri and Vivanco 2009; Chaparro et al. 2013). Flavonoids and other phenolics are the most common compounds looked for in majority of the metabolomic studies (Abdel-Lateif et al. 2012; Badri et al. 2013). Use of GC-MS (gas chromatography-mass spectrometry) profiling has revealed the possible plant metabolites such as asparagine, octadecanoic acid, glutamate, cysteine, putrescine, homoserine, mannitol, gluconic acid, threonic acid, glycerol-3-P, and glyceric acid-3-P to be involved in root nodulation process in legume plants (Desbrosses et al. 2005). Some of the key signal molecules exuded from the legume plants that are involved in the interaction of plant microbes includes isoflavonoids derived from phenylpropanoids, and also they act as defensive compounds. The process of symbioses in legume and rhizobacterial nodulation is mediated by multiple actions of flavonoids which act as a signal molecule (Cooper 2007; Subramanian et al. 2007; Farag et al. 2008; Abdel-Lateif et al. 2012). The intracellular and extracellular secondary metabolome compounds of *M. truncatula* were analyzed by Farag et al. (2008) by using HPLC (high-performance liquid chromatography) analysis coupled with UV (ultraviolet) photodiode array detection method and mass spectrometry. The study revealed three novel methylated isoflavones (6-hydroxy-7,4'-dimethoxyisoflavone, 7-hydroxy-6,4'-dimethoxyisoflavone, and 5,7-dihydroxy-4',6-dimethoxy isoflavone). Their study also highlighted the flexibility involved in the metabolic isoflavonoid biosynthetic pathways which depend on the nature of external stresses or elicitations. It has been reported that strigolactone secreted by plants like *L. japonicus* are involved in facilitating the arbuscular mycorrhizal symbiosis (Steinkellner et al. 2007). Root exu-

dates (vestitol) of *L. japonicus* function as chemical barriers to suppress the invasion of *Striga hermonthica* (a parasitic weed) into its roots (Ueda and Sugimoto 2010).

The complex interaction between rhizobium and roots is because of definite genetic as well as metabolic signals communicating between both symbionts (Geurts et al. 2005; Rispaïl et al. 2010). The signal compounds communicate between both plant host and rhizobia to form symbiosis. Also, rhizobium produces a large number of signaling compounds including Nod factor and many other surface polysaccharides which are involved in mediating the process of host-specific symbioses. Likewise, specific root exudates are secreted by host plant to mediate the preinfection events by triggering Nod factor biosynthetic pathways. The synthesized Nod factors then stimulate the accumulation of flavonoids by inducing flavonoid-encoding gene expressions (Cooper 2007; Haichar et al. 2014). Some of the reports suggest that flavonoids regulate the transport and accumulation of auxins inside the cortical cells to mediate root nodule development (Wasson et al. 2006; Subramanian et al. 2007). In another study by Rispaïl et al. (2010), inoculation of a symbiont *M. loti* to *L. japonicas* induced diverse alterations in the quantity of phenolic compounds secreted by the roots, while the compounds vestitol, sativan, and phytoalexin were not observed in the root zone after inoculation (Rispaïl et al. 2010; Badri et al. 2013). The identified coumestan and two other unidentified flavanones increased after inoculation of *M. loti* are described to be involved in nod gene stimulation. Proteomic and metabolomic approaches are being effectively used to study the targeted root exudate compounds in legume plant species. Plant-microbe interactions such as plant-rhizobia, plant-PGPR, and plant-arbuscular mycorrhizal fungi are described well in legume plants, and these interactions have significantly enhanced plant growth and yield (Sugiyama and Yazaki 2012; Haichar et al. 2014). The exudation of root compounds takes place through different processes such as passive transport, active transport, and transporter-mediated processes (Bais et al. 2006; Badri and Vivanco 2009).

3 Beneficial Plant-Microbe Interactions Mediated by Root Exudates

3.1 Root Exudates and Plant-Rhizobacteria Interactions

Biological interactions between plant and microbes occur through various molecular mechanisms and benefit the plant directly or indirectly. Root exudates modulate positive plant-microbe interactions and thereby regulate the plant growth, development, and yield. Some of these beneficial interactions include fixation of atmospheric nitrogen through root nodule formation by rhizobia in legume plants, providing tolerance against biotic as well as abiotic stresses and interactions with PGPR to improve plant growth and quality (Gray and Smith 2005; Bais et al. 2006; Badri et al. 2013; Huang et al. 2014). Moreover, biofilms, antibiotics, and other metabolites produced by bacteria interact with plants positively to impart

protection against likely pathogens, insects, and herbivores. Few root compounds secreted into rhizospheric soil exhibit allelopathic effect (Bais et al. 2004, 2006; Foley and Moore 2005; Ueda and Sugimoto 2010). Over the past few decades, many research studies on molecular interactions between legumes and *Rhizobium* spp. to form root nodule have been well documented. The root nodules are unique organs occurring in legume plant roots, and it harbors rhizobacteria involved in fixing atmospheric nitrogen. This specialized structure allows plants to utilize fixed nitrogen directly, and bacterium obtains its nutrients for its survival, and thus this is a mutual association benefitting both the species. Rhizospheric root soil favors the increased microbial activity, and plant-microbe symbioses are usually initiated by colonization of these soil-borne microbes. This is due to the fact that plant roots release abundant organic carbon that favors microbial ecology (Hartmann et al. 2009; Haldar and Sengupta 2015). It appears that rhizobacteria are attracted toward plants due to signal compounds and nutrients released by its roots (Bais et al. 2006). These bacteria form networks with plant roots through recognizing signal molecules produced by roots and further induce colonization by producing more signals. These signals are recognized by microbes to initiate symbioses with plants through physical interaction mediated by pili, fimbriae, adhesins, flagella, Type III and Type IV secretion system, and signal proteins (Lugtenberg et al. 2002; de Weert et al. 2002; Bais et al. 2006). Many studies have stated that flavonoids in the exudates act as a major chemical compound to attract rhizobia (Faure et al. 2009; Badri et al. 2013). About 4000 different types of flavonoids have been recognized and characterized in plants. Interestingly, isoflavonoids are observed only legume plants (Bais et al. 2006). Flavonoids are believed to induce several nod genes of the rhizobium spp. to produce nod factors (lipochitoooligosaccharides) that cause curling of root hairs, form infection thread, and finally initiate bacterial colonization to form nodules. Nod factors could be modified with the substitution of acetate, carbamoyl, sulfate groups, and sugars (Bais et al. 2006; Haldar and Sengupta 2015). The familiar nod genes of rhizobia include nod A, nod B, and nod C. Also, there are species-specific nod genes. In addition, lipooligosaccharides released from bacteria were shown to stimulate plant genes responsible for flavonoid biosynthesis. It has been reported that these nod genes are involved in the synthesis of nod factors, and the expression of genes specific to species modifies nod factor structure (Perret et al. 2000; Riely et al. 2004; Badri et al. 2013). Horiuchi et al. (2005) reported that the interaction between *Medicago truncatula* (a legume) and *Sinorhizobium meliloti*, a root exudate, dimethylsulfide, attracts nematodes (*Caenorhabditis elegans*), and these nematodes carry *Sinorhizobium meliloti* to the vicinity of plant roots. Xanthonenes, isovanillin, and vanillin are some of the other non-flavonoid-related molecules which induce the expression of nod D gene clusters. However, they are needed in large quantity compared to flavonoids (Cooper 2007; Badri et al. 2009). The interaction of a rhizobium, *Mesorhizobium tianshanense*, with *Glycyrrhiza uralensis* (licorice) plants revealed the secretion of canavanine, a chemical compound commonly observed in the root exudates and seed coats of many legume plants (Cai et al. 2009). Canavanine was found to be toxic to various soil bacteria but nontoxic to rhizobacterial strains as they

possessed a mechanism to detoxify it. This research supports the exudation of specific antimetabolites by legume plants in order to select right rhizobia for a successful symbiosis. Nod genes of *Bradyrhizobium japonicum* are induced by the exudates such as isoflavonoids, genistein, and daidzein that are released by *Glycine max*. However, these compounds inhibit the expression of nod gene in *Sinorhizobium meliloti*, a nitrogen-fixing bacterium. Luteolin is another common flavonoid which can encourage the expression of nod genes in *S. meliloti* (Bais et al. 2006). Malic acid secreted in *Arabidopsis* plant root has shown to regulate the defensive responses activated by pathogens and effectively recruits *Bacillus subtilis* FB17, a beneficial rhizobacterial strain (Rudrappa et al. 2008). Similarly, Neal et al. (2012) have reported that benzoxazinoids (root exudates), such as 2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one, attract beneficial rhizobacteria (*Pseudomonas putida*) to the site of rhizosphere. Badri et al. (2013) have revealed that *A. thaliana* plant root exudates phenolic compounds which serve as signal compound to attract soil bacteria. The amount of root exudates and their composition differs with the environmental changes and surrounding soil microbial flora. Also, pH of the rhizospheric soil encourages the growth of microbial community in the root surrounding (Bravin et al. 2009; Haldar and Sengupta 2015).

3.2 Root Exudates and Plant-Mycorrhizal Interactions

About 80 % of plant species including terrestrial plants, ferns, angiosperms, woody gymnosperms, and grasses are found to have symbiotic interactions with soil mycorrhizal fungi (ectomycorrhizae, endomycorrhizae, vesicular arbuscular mycorrhizae (AM), ericoid and orchid mycorrhizae). This symbiotic association enhances plant growth by increased uptake of nutrients, while fungi are benefitted with nutrients (carbohydrates and lipids) of the host plant roots (Bais et al. 2008; Haldar and Sengupta 2015). AM fungi associate with plants in a similar way as observed in plant-rhizobia interaction. Both mycorrhizae and rhizobia make use of similar signal molecules and proteins to regulate their associations with plants. Similar to rhizobia, AM fungi also recognize host plant species based on the available root exudates in the soil. Therefore, it is hypothesized that both AM fungi and rhizobia associations share a common origin of plant-microbe interaction and probably originated from a fungi (Nagahashi and Douds 1999, 2003; Levy et al. 2004; Bais et al. 2006; Akhtar et al. 2011). However, the exact mechanism of mycorrhizal association with specific host plant is yet to be recognized. As AM fungi are found in the rhizosphere soil, their propagules such as hyphae, rhizomorphs, and also spores are known to form a network of connections or a bridge between plant roots, fungi, and soil through which movement of nutrients is believed to occur (Simard et al. 1997; Bonfante and Anca 2009). This network formation is mainly mediated by numerous signaling incidents involving low molecular weight compounds secreted from both plant and fungi (Paszkowski 2006; Besserer et al. 2008; Parniske 2008). Flavonoids

present in low quantity are often proposed to stimulate the initial symbiotic association of AM fungi (Vierheilig and Piche 2002; Besserer et al. 2006; Haichar et al. 2014). Nevertheless, it is very well understood that the main signal factor involved in bridging plant-mycorrhizal symbiosis is considered to be strigolactone, a root exudate released from the plant (Akiyama et al. 2005, 2010; Haldar and Sengupta 2015). Strigolactones are carotenoid pathway-derived plant hormones which are generally produced when there is a nutrient deficiency. They also regulate plant growth and developmental processes by inhibiting shoot branching or by modifying plant structure (Akiyama et al. 2010). Strigolactones when released into the soil specify the host plants to symbiotic fungal species or plant parasites and stimulate the branching of hyphae during the symbiotic association between AM and host plant species (Lopez-Raez et al. 2008; Smith 2014; Al-Babili and Bouwmeester 2015). Root exudates of tomato, sorghum, pea, *L. japonicas* contained strigolactones, while they were absent in the root exudates of tobacco, carrot, and alfalfa. This indicates that there are many other signal compounds in the root exudates which are also responsible for the activation of fungal hyphae branching (Garcia-Garrido et al. 2009; Sugiyama and Yazaki 2012). Strigolactones were also observed in the root exudates of non-host plants of AM such as white lupin (*Lupinus albus*) and *Arabidopsis thaliana* (Yoneyama et al. 2008; Goldwasser et al. 2008). *Lupinus albus* was shown to produce pyranisoflavones that inhibit the fungal hyphae growth and development (Akiyama et al. 2010). Similarly, Oba et al. (2002) have reported that root exudates of *Lupinus* species (*L. luteus*, *L. aridus*, and *L. cosentini*) inhibited growth of AM fungal hyphae (*Gigaspora margarita*). This could be a competitive strategy to suppress the possible mycorrhizal associations in other plant species or to strengthen their competing fitness.

Studies on gene expression, RT-PCR (reverse transcriptase polymerase chain reaction), and blotting techniques have indicated that during the initial phase of hyphal penetration, many signal molecules from plants are released to chemoattract mycorrhizal fungi. Several early nodulin genes such as Psam5, PsENOD12A, MsENOD2, MtENOD11, MsENOD40, and leghaemoglobin Vflb29 are shown to get induced during the early development of symbioses (Fruhling et al. 1997; Albrecht et al. 1998; Kosuta et al. 2003). In *Pisum sativum* plants, gene expression findings have suggested that induction of *PsENOD12A* and *Psam5* genes is found during appressorium formation and hyphal penetration into the root cortex (Albrecht et al. 1998; Roussel et al. 2001). In *Oryza sativa* plants, Blilou et al. (2000) have reported that appressorium formation is due to expression of *Ltp* (lipid transferase protein) gene in epidermal cells by using gene-promoter β -glucuronidase (GUS) fusion studies. However, in *Medicago truncatula*, *ENOD11* gene was found to get activated transcriptionally in cortical and epidermal cells where hyphae penetrate during *Gigaspora rosea* infection (Chabaud et al. 2002). In physically separated culture of AM and *Medicago truncatula*, Kosuta et al. (2003) have demonstrated that root signal molecules induce hyphae to secrete fungal factors which further induce the expression of the *MtENOD11* (nod factor-inducible gene). This study was confirmed by using a *pMtENOD11-gusA* reporter gene expression system. The study also reports that in all the tested AM (*Gigaspora rosea*, *Gigaspora margarita*,

Gigaspora gigantean, and *Glomus intraradices*), transgene expression was initially observed at the root cortex and later extended from the root hair emergence region to the matured root hair region. This suggests that though AM infection occurs in cortex zone of roots, its proliferation is restricted mainly to root tissues, and this mechanism is highly regulated by host plant. According to García-Garrido and Ocampo (2002), plant-mycorrhizal symbiotic establishment triggers plants to activate various defensive mechanisms such as degradation of elicitors, control of signal compound concentration, defensive regulation through nutrition and hormone, and regulation of symbiotic genes and pathogen-related gene expression. However, activation and regulation of these defensive responses during symbiosis are yet to be understood. In many cases, host plant defenses are very weak, and it differs from the responses that are usually noticed during plant-pathogen relations. The enzymes such as chalcone synthase and phenylalanine ammonia lyase responsible for flavonoid biosynthesis were induced in *M. truncatula* cells which contained arbuscules. However, the defense-specific enzyme isoflavone reductase was not induced. This suggests that mycorrhizal fungi growth is stimulated by flavonoid biosynthesis and not the phytoalexins (antimicrobials) (Harrison 2005; Bais et al. 2006). The induction of *lpt* (lipid transfer protein) gene was found to regulate the appressoria formation and hyphal penetration of *Glomus mosseae* during colonization with *Oryza sativa* roots (Blilou et al. 2000). In a study by Lanfranco et al. (2005), it was found that when spores of *Gigaspora margarita* (BEG 34) were exposed to *L. japonicus* and *M. truncatula* root exudates, induction level of *GmarCuZnSOD* gene was found to increase. This gives evidence on the involvement of fungal reactive oxygen species-scavenging systems in plant-fungi interactions. Further studies have suggested that reactive oxygen species and SOD produced from *Oidiodendron maius* and *Glomus intraradices* play a pivotal role in mycorrhizal symbiosis (Abba et al. 2009; Gonzalez-Guerrero et al. 2010). A necrotrophic fungus, *Sclerotinia sclerotiorum*, was found to repress defensive mechanisms of host plants such as *Lycopersicon esculentum*, *Nicotiana benthamiana*, or *N. tabacum* (Veluchamy et al. 2012). Though many researchers have identified the role of root exudates in mediating plant-fungal associations, still many chemical communications in the rhizosphere are yet to be documented at the molecular level for better exploitation of symbioses for agricultural benefits.

3.3 Root Exudates and Plant-PGPR Interactions

PGPR are a group of naturally occurring useful rhizobacteria that colonize with a plant root system and exhibit positive synergistic effect by stimulating plant growth, development, and yield. PGPR trigger the production of growth hormones as well as facilitate uptake of nutrients effectively by plants from their surroundings. Moreover, they release inhibitor compounds that guard plants against diseases or other environmental stresses (Jahanian et al. 2012; Ipek et al. 2014). Application of chemically synthesized fertilizers, pesticides, and plant nutrients

has significantly reduced with the application of PGPR in modern agricultural practices (Bhattacharyya and Jha 2012). It is believed that PGPR establish association with plants through plant root signals. However, meager information is available on the involvement of root exudate compounds in mediating the process of plant-PGPR interactions and their regulatory acts. Root exudates of plants containing chemical signals are contemplated to communicate with signal molecules of PGPR during their interactions. de Weert et al. (2002) have reported the chemotactic reaction of *Pseudomonas fluorescens* WCS365 during its root colonization in tomato plants. The major chemoattractive root exudates of tomato for *P. fluorescens* were found to be malic acid and citric acid. All nonmotile mutants of *P. fluorescens* (cheA mutants) showed no chemotactic response. Likewise, other root exudates including amino acids and carbohydrates are also reported to have dominant chemoattractive ability for PGPR population in rhizospheric soil (Somers et al. 2004; Huang et al. 2014). Arabinogalactan proteins are complex plant cell wall proteins unique to plant organs and root exudates. These fascinating sets of macromolecules are also involved in facilitating the interactions of plant roots with rhizobacteria (Nguema-Ona et al. 2013; Huang et al. 2014). Cannesan et al. (2012) have reported that arabinogalactan proteins of *Pisum sativum* and *Brassica napus* roots induced encystment formation and inhibited germination of *Aphanomyces euteiches* zoospores. A study by Vire et al. (2005) suggests the positive role of arabinogalactan proteins in *A. thaliana* root colonization with PGPR. Likewise, Xie et al. (2012) report the chemotactic ability of arabinogalactan proteins to beneficial microbial species. According to them, root exudate of pea, wheat, legumes, and *Arabidopsis* showed a novel mode of arabinogalactan-induced polar attachment with *Rhizobium leguminosarum*. These reports thus suggest that arabinogalactan proteins play a major role in the attachment of rhizobacterial strains to root surfaces. Bacilio-Jiménez et al. (2003) characterized the rice plant root exudates and studied the chemotaxis of *Corynebacterium flavescens*, *Bacillus pumilus*, *Azospirillum brasilense*, and *Bacillus* sp. isolated from the rice rhizosphere. The study revealed the positive chemotactic nature of root exudates for all the rhizobacterial strains tested. The major outer membrane proteins (MOMPs) of rhizobacteria share a homology with bacterial porins. These MOMPs possess cell surface-exposed domains where adhesion process might take place to initiate plant-bacterial interaction (Burdman et al. 2000). In another study by Burdman et al. (2001), MOMPs of *Azospirillum brasilense* were shown to act as an adhesion to assist in bacterial cell aggregation and root attachment in sweet corn, forage corn, sorghum, wheat, tomato, common bean, and chickpeas. Many phytoestimulants (cytokinins, auxins, and gibberellins) are secreted by PGPR to improve plant development. Customarily, plants also release root exudates that serve as nutrients for PGPR around the rhizosphere. Some of the root exudates such as tryptophan also serve as precursors for phytohormone synthesis in plants (Steenhoudt and Vanderleyden 2000; Bais et al. 2006; Lawal and Babalola 2014). PGPR produce 1-aminocyclopropane-1-carboxylate deaminase, a precursor for the biosynthesis of a phytohormone, ethylene which is involved in root growth regulatory mechanisms (Glick et al. 2007). The volatiles (acetoin and 2,3-butanediol) secreted by

Bacillus spp. were shown to improve *Arabidopsis* plant growth. This suggests that plant-rhizobacterial association may not always require physical attachment (Ryu et al. 2003; Doornbos et al. 2012).

4 Root Exudates as Antimicrobials Confer Plant Protection

A diverse class of microbial population prevalent in the rhizosphere soil is mainly influenced by plant root exudates. However, some of these bacterial and fungal strains are pathogens which cause diseases and hence can be detrimental to plants. These damages are managed by plants through defensive responses like suppressing pathogenic microbial strains or recruiting helpful microbial strains. Moreover, plant exudates constitute a wide array of secondary metabolites that help plants to guard against microbial infections, insects, or herbivores attack (Foley and Moore 2005; Doornbos et al. 2012; Haichar et al. 2014). Root exudates secreted by plants act as antimicrobial agents to curb the harmful rhizospheric pathogens. In response to pathogens, plant releases root exudates (defensive proteins, phytoalexins, and other unnoticed chemicals) into their surroundings. Root exudates which act as antimicrobial compounds include indole, benzoxazinone, terpenoids, flavonoids, phenolics, and isoflavonoids. These antimicrobials are observed in plants such as rice, *Arabidopsis*, soybean, corn, and a legume, *Medicago truncatula* (Bais et al. 2004, 2006; Perry et al. 2007). The secretions of *A. thaliana* roots function as antimicrobials provided tissue-specific defensive response to various pathogenic bacterial strains (Bais et al. 2005). But, *Pseudomonas syringae* strain showed resistance to these antimicrobials and infected the plant roots. This resistance ability was proposed to be dependent on the secretory system (type III). Similarly, *P. aeruginosa* forms root colonization, and eventually biofilm is formed which resists antimicrobials secreted from roots (Walker et al. 2004). Hairy roots of soybean induced the biosynthetic pathway of phenylpropanoids, to secrete isoflavones when challenged with a pathogen, *Fusarium solani* (Lozovaya et al. 2004). It is well described that phenylpropanoid pathway is activated in response to pathogenic fungi or other biotic stresses (Lanoue et al. 2010; Miedes et al. 2014). Rosmarinic acid (a caffeic derivative) was produced from hairy root cultures of basil (*Ocimum basilicum*) when challenged with a plant pathogen (*Pythium ultimum*) that causes root rot diseases. Rosmarinic acid was shown to possess a strong antimicrobial property against several rhizospheric microbes (Bais et al. 2002). In a study by Rudrappa et al. (2008), infected *Arabidopsis* plants with *Pseudomonas syringae* (a bacterial leaf pathogen) pv. Tomato DC3000 (Pst) was shown to recruit *Bacillus subtilis* FB17 as a biocontrol agent to infected plant roots. In their study, the rhizospheric strain *B. subtilis* FB17 showed chemotaxis to signal molecule, malic acid secreted by infected plant roots. Plant roots secrete many defense proteins in addition to antimicrobials that also confer root resistance or mediate plant-microbe interactions (De-la-Pena et al. 2008; Denance et al. 2013). Likewise, Lanoue et al. (2010) investigated the likely secretion of defensive root exudates in barley (*Hordeum vulgare*) when

challenged with a fungal pathogen, *Fusarium graminearum*. The results revealed that root exudates inhibited the germination of *F. graminearum* macroconidia. The identified root exudates included *t*-cinnamic, ferulic, *p*-coumaric, vanillic, syringic, 4-hydroxyphenylacetic, indoleacetic, and benzoic acids. In other ways, harmful microbes can be repressed by recruitment of biocontrol bacteria such as *Pseudomonas* spp. that establishes efficient root colonization. Root colonization by *Pseudomonas* spp. can result in suppression of wide range of plant pathogens (Akhtar and Siddiqui 2010; Lanoue et al. 2010). When plants are infected by pathogens, they secrete natural compounds called glucosinolates that are later hydrolysed by an endogenous thioglucosidases enzyme called myrosinases to yield several antimicrobial compounds such as isothiocyanates, thiocyanates, and nitriles (Halkier and Gershenzon 2006). A diterpene compound, rhizathalene A produced by noninfected *A. thaliana* plants, is considered to give defense against herbivorous insect attack (Denance et al. 2013; Haichar et al. 2014). Plant defense responses against pathogens and pests are mainly regulated by signaling networks of the major phytohormones such as jasmonic acid, jasmonates, salicylic acid, and abscisic acid (Robert-Seilaniantz et al. 2011). Researchers have proposed that strigolactone compounds provide plant defenses by regulating jasmonic acid signaling pathway to secrete defense-related hormones (Dor et al. 2011; Denance et al. 2013). Irrespective of numerous advanced studies carried out to understand natural compounds of root exudates and their role as antimicrobials or defensive molecules, their significance in the rhizosphere is yet to be established completely. Hence, these findings could pave a way for future scientists to focus on the direction to discover novel new lead molecules as antimicrobials from root exudates.

5 Conclusion and Future Prospects

We described an overview of research information on the importance of biologically important plant-microbe interactions. Also, a wide range of root exudates and their significant role in mediating various plant-microbe interactions are discussed in detail. This chapter mainly focuses on the symbioses of plants with rhizobia, AM fungi, and PGPR as they are widely considered for developing agricultural biotechnology products such as biofertilizers, phytostimulators, biopesticides, and bioremediators. Moreover, these beneficial microbial inoculums are used in the sustainable agricultural practices worldwide. Volatile compounds of root exudates play a significant biological role in establishing communications between plant roots and the rhizospheric microbial flora. Through these biointeractions, plants are benefited through increased nutrient uptake from soil and better defensive responses against unfriendly surroundings. However, these biointeractions mediated by root exudates are yet to be understood clearly due to the fact that all biological interactions occur below ground. Hence, there is a need to establish new methodologies to explore their interactions under lab conditions. Literature survey has witnessed that root exudates function as signal molecules during plant-microbe interactions. Many study reports have

identified several genes and their regulatory expressions to produce root exudates for establishing biointeractions. Yet, more research efforts are needed to understand these interactions in detail at molecular level. Understanding about other root exudate genes, regulatory aspects of these genes and their expression under different environments, gene manipulation studies to modify root exudate products, alterations in the biosynthetic pathways of root exudates, and factors effecting root exudation are some of the research areas for the coming years. Progress in these research areas could be beneficial in developing economically valued crop plants with a capacity to produce higher useful root exudates. As plant interaction studies are mainly restricted to only few rhizosphere microbes, future research should focus on the understanding of other possible plant-microbe interactions in the complex rhizosphere environment. Also, chemical characterization of these rhizodeposits will pave a way in the discovery of novel metabolites with antimicrobial activity.

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