Rhizosphere Biology

Udai B. Singh
Jai P. Rai
Anil K. Sharma *Editors*

Re-visiting the Rhizosphere Eco-system for Agricultural Sustainability

Springer

Rhizosphere Biology

Series Editor

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Udai B. Singh • Jai P. Rai • Anil K. Sharma
Editors

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Preface

Plants create a dynamic micro-biosphere in the soil, around the roots, called as "rhizosphere," which harbors a number of microorganisms for sustaining their growth and development. It renders a small zone around the roots and is one of the most energy-rich habitats on Earth. Its micro-environment is contrastingly different from that of a non-rhizosphere soil. Consequently, rhizosphere is the central player in a complex food web in which numerous members of flora and fauna profusely take advantage of the plant's resources. Soils with diverse and multi-trait microbial communities are considered healthy and are crucial for enhancing crop productivity. The rhizosphere, one of the most dynamic interfaces on Earth, contains up to 10^{11} microbial cells per gram of soil representing over $\sim$30,000 bacterial species. In the last decades, rhizosphere biology has gained attention due to unraveling of new mechanisms, processes, and molecules in the rhizosphere that contribute to the enhancement of plant productivity. The rhizospheric microbes and associated processes are being utilized for harnessing the potential of soils for their effective and sustainable functioning in the agro-ecosystems. This zone contains sloughed-off root, root exudates, mucilage, and gases. The gases released from roots in the soil dissipate to comparatively longer distances leading to the extended size of the rhizospheric zone up to many centimeters. The rhizosphere is of paramount importance to ecosystem services, namely carbon and water cycling, nutrient trapping/cycling or mobilization, carbon uptake and storage, etc. Plant rhizosphere is the battlefield for a variety of beneficial and harmful organisms. Besides insect herbivores and plant pathogens in the rhizosphere, plants also nurture a vast community of commensal and mutualistic flora and fauna that provide the plant with indispensable services like nitrogen fixation, protection from pathogens, enhanced mineral uptake, and growth promotion. The significance of plant–microbe interactions in the rhizosphere ecosystem is enormous for agricultural sustainability. These interactions may be positive such as the interaction of the plants with the beneficial soil microorganisms for inducing the plant growth, conferring abiotic and biotic stress tolerance, and modulating several pathways of the plants for the proper establishment and revitalization of degraded and contaminated soils or negative likes the host–pathogen interactions leading to disease development in the plants. Moreover, below-ground communication among the plant, soil insects, and microbes plays an important role in the rhizosphere ecosystem functioning and modulates the physio-biochemical pathways leading to better plant growth and productivity under biotic and abiotic stresses. Further, plant secretome shapes the rhizospheric microbial community by recruiting the specific microflora around the root system and interacting with them. However, rhizospheric interactions are quite complex and dynamic and are rather difficult to elucidate as they take place under different circumstances and at different interfaces in and around the rhizosphere. It is evident that recent technological advancements play a crucial role not only in the elucidation of but also in large-scale exploitation of the rhizospheric interactions for enhancing the agro-ecosystems' resilience to abiotic and biotic stresses and thus maximizing the sustainable food production under such adverse conditions. In this context, the proposed book *Re-visiting the Rhizosphere Ecosystem for Agricultural Sustainability* is an opportune contribution to the topical information on plant–microbe interactions offering a great scope for harnessing the beneficial interactions for agricultural sustainability.

This book encompasses and addresses various issues of plant and soil–microbe interrelationship that are to be modulated either by resident microbes or by their external application. The book discusses rhizospheric microbes and their role in modulating functions of soil and crop plants in detail. It also provides information on microbiomes in the rhizosphere, cross-talk among microorganisms and plants, functions of soil microflora, regulations relating to biofertilizers and biostimulants, and products and technologies of microbes in the soil. It also covers conventional and modern aspects of rhizosphere biology such as rhizosphere microbes as biofertilizers, biostimulators and biofortifiers, microbial signaling in the rhizosphere, and recent tools in deciphering rhizomicrobiome. The book provides the latest understanding of rhizosphere microorganisms for enhanced soil and plant functions, thereby improving agricultural sustainability and food and nutritional security. The aim of the book is to compile high-quality reviews and research articles offering new insight into rhizosphere interactions, ecology and function of the rhizosphere, harnessing plant–microbe interactions for biotic and abiotic stress tolerance, etc. By bringing all these areas together within the ambit of this special book, we hope to build cohesion between conventional and most modern approaches of science to design the future path for *Agricultural Sustainability*. This book addresses various issues of phytobiome and rhizomicrobiome in detail. The book covers (1) the composition, structure, and function of the rhizosphere; (2) recruitment of microorganisms in the rhizosphere; (3) rhizo-deposits and their role in rhizosphere interactions among the plant, microbe, and other ecological components; (4) understanding the below-ground communication in the rhizosphere for better plant growth; (5) omics approaches to unravel the rhizosphere interactions and functions; (6) rhizosphere engineering for ecosystem restoration and sustainable crop production in degraded lands; (7) rhizosphere engineering for systemic resistance/tolerance to biotic and abiotic stresses; and (8) microbial inventerization for sustainable crop production. We expect that the book would be useful for students, agricultural scientists, biotechnologists, plant pathologists, mycologists, and microbiologists, the farming community, scientists of R&D organizations, as well as the teaching community, researchers, and policymakers to understand the roles of rhizospheric microorganisms and associated ecosystem in sustainable agriculture and provide directions for the future course of action.

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Evolution of the Knowledge and Practice of Endophytic Microorganisms for Enhanced Agricultural Benefit and Environmental Sustainability

1

Gary E. Harman

Abstract

Microorganisms have been known for more than a century to provide multiple benefits to plants. A key to understanding the benefits imparted is knowledge of the specific kinds of interactions that occur continuously between plants and microorganisms. This chapter reviews how knowledge of these has evolved over time, what the resulting benefits are, and how microorganisms can be used to benefit plant agriculture and the natural environment.

1.1 Introduction

Fundamental to any discussion of benefits is the concept of holobionts. Plants like any other "higher" organism do not exist as independent entities; rather they are the visible part of an association of the plant and the microorganisms that colonize it, becoming part of an ecological whole (Margulis and Fester 1991). Most of these associations are beneficial, especially those that colonize the interior spaces of plants. Such organisms are termed endophytes, meaning that they live within plants. Plant–microbial entities where the microbes are endophytic and highly beneficial, when selected microorganisms have been purposefully introduced to enrich and enhance the plant's microbiome, we have described as Enhanced Plant Holobionts (EPHs) (Harman and Uphoff 2019).

There have been a number of advances in knowledge and understanding regarding such associations that justify a different paradigm for conceiving and practicing modern agriculture, one that does not treat plants as isolated entities, to be improved

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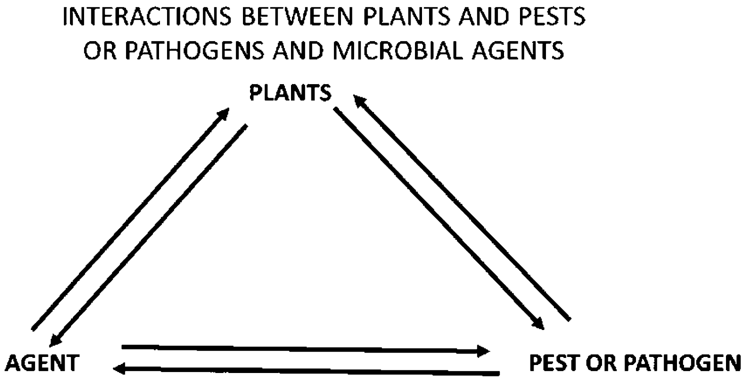

Fig. 1.1 Types of interactions between plants, microbial agents, and other microorganisms. Except for Rhizobia and mycorrhizal fungi, early views of the interactions centered on the interactions between agents and other microorganisms. As understanding evolved, the plant–agent interaction was appreciated more. We now know that many benefits to plants, including disease and pest control, alleviation of abiotic stresses, nutritional benefits, and increased photosynthesis, occur as a consequence of systemic responses of plants due to the plant–agent interaction

genetically and provided with external inputs to improve plant growth and protection. Important steps in the development of this alternative understanding of how to advance agriculture are provided in bullet form below.

- An understanding that microorganisms can be highly beneficial to plants.
- A more sophisticated view of the interactions among pathogens or pests, the plant, and beneficial microbial agents. These relationships are shown diagrammatically in Fig. 1.1.
- Appreciation that some microorganisms are endophytic, residing not just around or on plants, but actually within plant organs, tissues, and cells.
- Seeing that many microorganisms are multifunctional vis-à-vis plants and impart many diverse advantages to their hosts.
- Recognizing that there can be many advantages from this association for plant agriculture and beyond this for the natural environment, including abatement of climate change.

This review is divided into sections that describe the history and evolution of these interactions, considering also how they affect social and agricultural sustainability.

1.2 History and Concepts of Microbial–Plant Interactions

Some organisms have been known for more than a century to be endophytic. Mycorrhizal fungi, which live symbiotically rather than parasitically within plant roots, were first named and described in 1882 (Kamieński 1882). *Rhizobium*, a genus of bacteria that benefit leguminous and other plants, was first described in 1889 (Young et al. 2001). The endophytic nature of many other organisms that favor plant growth and health has been reported later. For example, *Trichoderma* were first described as rhizosphere-competent in 1988 (Ahmad and Baker 1988) after some, but by no means all, were shown to be endophytic (Chao et al. 1986). Difference in the abilities of some strains but not others of the same species is an important concept that will be emphasized throughout this review.

Many other organisms contain strains that are endophytic, including both fungi and bacteria. Specific genera are *Piriformospora indica* (Gill et al. 2016), *Pseudomonas* (Pieterse et al. 2014), and *Gliocladium rosea* (Sutton and Peng 1993). Many of these organisms colonize only the roots of plants and are restricted to them; but *G. rosea*, for example, also colonizes above-ground plant parts. Some *Trichoderma* strains also colonize above-ground parts of plants. For example, some of these strains colonize the stems and leaves of cacao, while these same strains colonize only roots of pepper plants (Bae et al. 2011).

1.3 Benefits to Plants

Several aspects of the benefits that microorganisms provide to plants are not a consequence of the endophytic nature of the microorganisms involved, but are a consequence of other mechanisms. These include mycoparasitism, by which certain microorganisms protect plants by infesting pathogens, which is well described in Chet (1987). Various types of competition between beneficial microorganisms and plant pathogens occur, whereby the latter are curbed by competition for space and/or for nutrients. This can be difficult to prove, but it undoubtedly occurs. Competition for iron nutrients in the soil is a special case, well documented, as described in Hubbard et al. (1983) and Trapet et al. (2016). Since these are well described elsewhere, they will not be covered in this chapter. Various mechanisms whereby microorganisms benefit plants have been reviewed in Harman and Uphoff (2019) and are mentioned only in passing here.

1.3.1 Control of Plant Diseases and Pests, and Alleviation of Abiotic Stress

Control of pests and diseases and of abiotic stresses is considered together since similar systems and mechanisms are activated for both kinds of stresses. Both happen via microbes inducing modifications in plant gene expression and impacts on the proteins encoded from the plant's genome. Endophytic microorganisms can induce systemic responses throughout the plant that are evoked by elicitor molecules; such responses are elicited by many endophytes. These elicitors have been summarized and reviewed, for example, in Chi et al. (2005), Waller et al. (2005), Lorito et al. (2010), Pieterse et al. (2014), Mo et al. (2016), Fukami et al. (2018), and Harman and Uphoff (2019). These references cover *Rhizobium*, *Azospirillum*, *Piriformospora indica*, *Trichoderma*, *Pseudomonas*, and mycorrhizal fungi, demonstrating the widespread occurrence of similar systems. The references include induced plant resistance to both biotic and abiotic stresses.

An important aspect of all of these mechanisms is control of reactive oxygen species (ROS). ROS are strongly active molecules that damage almost all of the plant's physiological systems, including photosynthetic elements, proteins, and nucleic acids. The plant's production of ROS is induced by both biotic and abiotic stresses, and even by the process of photosynthesis if high light levels result in overexcitement of electron flows beyond the capacity of the plant's photosystems to absorb them (Nath et al. 2016). Plants minimize the damaging effects of ROS by producing reducing compounds and enzymes that detoxify ROS, converting it to less damaging molecules (Mittler 2002). All of the endophytes mentioned above have this capability in common, also helping to control ROS (Waller et al. 2005; Mastouri et al. 2010; Pieterse et al. 2014; Mo et al. 2016).

1.3.2 Improvements in Photosynthesis

Photosynthesis converts sunlight into energy by fixing carbon. Both energy and carbon are essential to all life on Earth. Increased photosynthesis is essential if we are to meet increasing demand for food and fiber by doubling plant productivity (Ort et al. 2015). This need is particularly acute because increases in crop productivity have stalled at the global level (Foyer et al. 2017). Efforts to increase the photosynthetic capacity of plants by making genetic modifications have resulted in more frustration than success (Foyer et al. 2017). Engaging endophytic microorganisms in enhancing photosynthesis is the only evident method currently available to increase essential process.

Many or perhaps most bacterial or fungal endophytes have the capacity to improve photosynthesis. Pertinent references include Mo et al. (2016) for mycorrhizal fungi and Chi et al. (2005) for *Rhizobium* in cereals. A complete description of events occurring with *Trichoderma* is provided in Harman et al. (2019). Like resistance to biotic and abiotic stresses, these fungi induce systemic responses that result in maintenance of and an increase in photosynthetic elements, including photoactive pigments such as chlorophyll and carotenoids and the proteins involved in synthesis of most of the necessary machinery for photosynthesis. An important factor in this improvement is an increased level of gene expression and of the proteins they encode. The gene transcripts involved are identified in Doni et al. (2019) and Harman et al. (2019).

Another aspect of the increased photosynthetic capacity of plants is the maintenance of their existing capabilities. The previous section described the harmful effects of ROS on most physiological systems in plants. Curbing these effects is an important part of the contribution that endophytes make to enhance photosynthetic capacity of plants that they have colonized.

1.3.3 Enhanced Nutrition

Plant growth is frequently enhanced by the activities of endophytic microorganisms. One reason for this enhancement results from improvements in nutrient uptake and acquisition. The Rhizobiaceae–legume interaction results in fixation of atmospheric nitrogen in nodules on roots and alleviates much of the need for added nitrogen from fertilizers. This has tremendous environmental and economic impacts, since the production of nitrogen fertilizers is expensive, requires substantial amounts of fossil fuels to produce, and runoff of excess nitrates into waterways results in the growth of undesirable algae that can result in fish kills and other environmental impacts (Anonymous 2020). Some endophytic microorganisms can increase plant-available N levels on a per-area basis because they contribute to increased total biomass (Harman and Uphoff 2019).

Some endophytes solubilize nutrients in soil and transfer these to plants, thereby improving the nutrient status of plants. Mycorrhizae are well known for their ability to raise the phosphorus status of plants as well as that of other nutrients (Parniske 2008). Other endophytic organisms, such as *Trichoderma*, also solubilize important plant nutrients, making them available to plants (Altomare et al. 1999).

1.3.4 Enhanced Plant Growth and Yield

As a consequence of all of these mechanisms by which endophytes improve plant growth, plants that have enhanced, larger, more diverse, and more active microbiomes frequently are larger and yield more. The literature contains numerous references to this, reviewed in Harman and Uphoff (2019). Greater yields provide more food to a growing world population and are therefore essential for both agricultural and societal sustainability.

1.4 Improvements in Soil Health and Sustainability

Endophytic microorganisms provide marked improvements to soil health, which contributes to agricultural sustainability. Enhanced photosynthesis is required for this and for the other benefits described in this review. All of these require energy and fixed carbon compounds, and these must come from photosynthesis (Shoresh and Harman 2008). Soil health benefits include higher levels of organic compounds in the soil that enhance microbial diversity (Li et al. 2020).

In various studies, a combination of factors including minimal tillage and cover cropping has resulted in an increase in soil organic matter (SOM). Greater SOM improves yields and offers a potential for reducing greenhouse gases, especially CO_2 (Paustian et al. 2016). However, a systems approach is required, not just introducing a single change. If the farming system is not modified, carbon put into the soil may be released as quickly as it is delivered due to enhanced microbial respiration at least in the absence of cover cropping (Anonymous 2014). So, depending on the farming systems used, carbon sequestration may or may not occur.

None the less, increased fixed carbon added to the soil usually results in improved soil health. The combination of cover crops plus reduced tillage can result in improvements in water infiltration, erosion control, and increased formation of soil aggregates (Schmidt et al. 2018, 2019). Soil aggregates are particularly important because these aggregates protect organic matter from soil microbes, thereby minimizing respiratory loss of fixed carbon (Six et al. 1998; Olchin et al. 2008). Thus, while carbon sequestration can occur, a systems approach needs to be followed to insure that the storage of carbon in the soil results (King 2011).

1.5 Conclusion

This review demonstrates the evolution of knowledge and practice to enhance agricultural benefits and environmental sustainability. Rhizobia and mycorrhizae have been known for about a century to colonize interior spaces of roots. However, studies on other microorganisms have considered primarily the interactions between different microbes and focused primarily on the control of diseases.

Beginning in the 1990s, an appreciation was generated for the interactions between plants and microorganisms and within the diverse communities of microorganisms around, on, and within plants. Over this same period, some microorganisms, including fungi and bacteria, were demonstrated to be endophytic and to function as true symbionts.

A very important component of this understanding was the knowledge that these organisms cause systemic reactions in plants and that these effects provide large benefits to plants and to plant agriculture. These interactions result in increased resistance to biotic and abiotic stresses, improve the nutrient status of plants, and increase their photosynthetic capabilities. These are becoming increasingly important for improving food production for a growing world and for creating environmental sustainability.

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Mycorrhizosphere Revisited: Multitrophic Interactions

T. Muthukumar, C. S. Sumathi, V. Rajeshkannan, and D. J. Bagyaraj

Abstract

The soil and plant roots provide natural habitats for a diverse assemblage of microorganisms that play an important role in nutrient cycling and other ecosystem processes. The services provided by these microbial processes are important for maintaining the diversity and functioning of ecosystems worldwide. In this chapter, we examine the information available on the plant–mycorrhizal fungal interactions in soil with a focus on the mycorrhizosphere. Available evidences do indicate that the microbial populations in the mycorrhizosphere could substantially differ from those of the rhizosphere and surrounding bulk soil. Further, the various microbial interactions in the mycorrhizosphere are important for the development and health of plants both in the natural and anthropogenic ecosystems. The microorganisms solubilize or decompose the complex macromolecules in the mycorrhizosphere and make the nutrients available for plants and other microorganisms. Some of the bacteria residing in the mycorrhizosphere could also act as mycorrhiza helper bacteria and plant

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growth-promoting rhizobacteria with numerous functions. The carbon exuded by the plants directly into the rhizosphere and the fungi in the hyphosphere play a pivotal role in maintaining the diversity and activities of microorganisms in the mycorrhizosphere. At present we are only looking at a glimpse of the complex intricate interactions that are occurring in the mycorrhizosphere. However, evidences available so far suggest that multitrophic interactions in the mycorrhizosphere play a significant role in maintaining the health and productivity of plants and soils in sustainable natural and agroecosystems.

2.1 Introduction

The plant root is necessary for maintaining soil integrity and fertility besides anchoring and obtaining soil resources. The development of roots unleashes a number of physicochemical and biological activities in the soil. These changes are brought about either by the removal or addition of substances to the soil by plant roots. One of the most striking changes that happen in soil biology in response to root development is the alterations in the population and activities of microorganisms. Plant roots are frequently associated with archaea, bacteria, and fungi along with a myriad of other organisms. The microorganisms influenced by plant roots play a key role in making available the various nutrients required for the normal growth and development of plants and also are important for maintaining the fertility of the soil. Plants thus rely on microorganisms in spite of having several physiological and anatomical adaptations of their own. Among the microbes interacting with plant roots, some colonize the roots and substantially modify the morphology and nature of the root system. The microorganisms colonizing root tissues are placed better to influence various plant processes than those that are present over or surrounding the roots (Hassani et al. 2018). Therefore, plant roots are not individual entities but are composite organs that act as niches for diverse microbes. This ability of plants to establish symbiosis with different types of organisms enabled them to colonize and thrive in stressful terrestrial habitats (Morris et al. 2018).

Among the different types of microorganisms establishing symbiosis with plant roots, the most common and widespread are the mycorrhizal fungi. The fungi belonging to diverse fungal groups like the Ascomycota, Basidiomycota, and Mucoromycota can associate with more than 90% of plant species (Spatafora et al. 2016; Brundrett and Tedersoo 2018). The symbiosis established between mycorrhizal fungi and plant roots greatly varies in their structure and there are seven types of mycorrhiza recognized namely the ecto, arbuscular, orchid, arbutoid, monotropoid, ericoid, and ectendomycorrhiza. Although some types of mycorrhizal association are restricted to certain plant families (ericoid, arbutoid, monotropoid, orchid) or to specific plant growth forms (ecto, ectendo), arbuscular mycorrhiza is more common as it occurs in more than 72% of the extant plant taxa and establishes an association with diverse plant families and growth habits (Brundrett and Tedersoo 2018).

Though the fungus is obligately dependent on the host plant for carbon (e.g., arbuscular mycorrhiza), but some mycorrhizal types can also exist as saprophytes in others. The nature of the relationship between plants and mycorrhizal fungi is mutual, but can also tilt in favor of the plant or the fungus depending on the environmental conditions and plant growth stage (Newton et al. 2010; Ryan and Graham 2018; Field et al. 2020).

The mycorrhizal fungi benefit plants by improving their nutrient and water uptake and protect plants from different types of abiotic and biotic stresses (Smith and Read 2008; Lakshmipathy et al. 2019). Also, mycorrhizal fungi can have an important role in assisting interplant nutrient and signal transfer among plants in vegetation and in improving soil structure and quality through aggregation. In addition to their direct interaction with plants and soil, the mycorrhizal fungi also establish an intricate relation with a wide range of microorganisms in the soil (Hestrin et al. 2019).

An understanding of the functionality of mycorrhizal symbiosis, their interaction with other microorganisms, and ecosystem processes is essential for the efficient use of the symbiosis for plant and habitat restoration and conservation (Pickles et al. 2020). The interaction between the mycorrhizal fungi and the host plant is diphasic with a part of the fungi within the root and the other in the soil. The part of the fungi that is present in the soil grows indefinitely beyond the nutrient depletion zone surrounding the roots exploring the soil for resources. These extraradical hyphae act as bridges in connecting the roots and the external soil environment. The intraradical phase of the mycorrhizal fungi transfers the nutrients taken up and translocated by the extraradical hyphae and also acquires and distributes the host-derived sugars and lipids to the soil hyphae. However, these functions and processes could be modified by the impact of other factors and modifying conditions. In this chapter, we first discuss the two major types of mycorrhizal symbiosis (arbuscular and ecto), and then the rhizosphere, hyphosphere, and the mycorrhizosphere. The reason for concentrating on arbuscular and ectomycorrhizal (EcM) types is that these mycorrhizal types are abundant in many natural and manmade ecosystems worldwide, occurring in 73% of the examined plant species (Brundrett and Tedersoo 2018). Moreover, these two mycorrhizal groups are well studied than others and are of application significance than the other mycorrhizal types. Furthermore, some ectomycorrhizal (EcM) trees like *Picea abies* and *Pinus sylvestris* are arbuscular mycorrhizal (AM) when they are young and later shift to EcM symbiosis (Kothe and Turnau 2018) while others like the eucalyptus, *Alnus*, and casuarinas are AM and/or EcM depending on environmental conditions.

2.2 Major Mycorrhizal Types

2.2.1 Arbuscular Mycorrhiza

Arbuscular mycorrhizal symbiosis is formed by fungi belonging to Glomeromycotina and Mucoromycotina of the phylum Mucoromycota (Spatafora et al. 2016). AM association is characterized by the presence of short-lived arbuscules that are formed in the root cortical cells and act as transit points for the transfer of nutrients to the plants. The AM symbiosis formed by taxa in Mucoromycotina is termed as fine root endophyte (FRE) owing to the fine nature of the fungal structures compared to those formed by members of Glomeromycotina. The fungus enters the roots directly or after forming an appressorium on the root surface. The penetrating hyphae coils in the first few cells and then ramifies the root cortex inter- and/or intracellularly. The intraradical hyphae can be either linear or coiled and different types of AM morphologies are recognized based on the type and distribution of the fungal structures within roots. Though *Arum* and *Paris* type of AM colonization were recognized in many plant species, a closer look at the AM colonization pattern in roots of several plant species revealed a large number of variants within them and is termed as intermediate types (Dickson 2004; Dickson et al. 2007). The diameter of the intraradical hyphae can vary with the associating fungi. For example, FRE produces much finer hyphae (<2 μm) compared to the more coarse glomeromycotean fungi (>3 μm). In addition to these intraradical structures, fungal storage structures that are rich in lipids called vesicles also occur in the colonized roots. Nevertheless, intraradical vesicles are not formed by fungi belonging to Gigasporaceae of Diversisporales and these fungi form vesicle-like structures called auxiliary cells in the soil. In FRE, terminal or intercalary hyphal swellings resembling vesicles are formed on the intraradical hyphae. The intraradical mycelium can extend into the soil forming an extensive network increasing the root surface area and connecting roots of coexisting plants in a plant community. The extraradical mycelium can reach 25 m/g of soil or more and exhibit great plasticity in exploiting the soil resources that are inaccessible to plant roots and act as a niche for diverse microorganisms (Lebrón et al. 2012; Jansa et al. 2013; Leyva-Morales et al. 2019). The extraradical mycelium of AM fungi can bear spores that act as chief perennating structures in seasonal vegetations. But, some species of AM fungi can also produce spores within plant roots.

2.2.2 Ectomycorrhiza

In EcM symbiosis, more than 20,000 fungi belonging to Ascomycetes, Basidiomycetes, and Zygomycetes establish symbiosis with approximately 6000 woody gymnospermous and angiospermous tree species (Kumar and Atri 2018). This symbiosis has a significant influence on the functioning of forest ecosystems, especially in the subtropical, boreal, and temperate regions where most of the component trees are colonized by EcM fungi (Mello and Balestrini 2018). In these ecosystems, the fungal mycelium of different EcM fungi can constitute up to 30% of the soil microbial biomass and interconnects a wide range of plant root systems, and these extramatrical networks are popularly known as the wood-wide web. The EcM association is defined by the occurrence of a fungal mantle that sheaths the host root and a Hartig net that envelops the rhizodermis and the cortical cells. The Hartig net presents a large surface area for the exchange of resources between the symbionts. The hormonal interaction between the plant and EcM fungi substantially alters the root architecture of the host plant that includes the development of short roots and inhibition of root hair formation (Kumar and Atri 2018). The EcM fungal hyphae may sometimes combine to form macroscopic structures termed as rhizomorphs that connect the sporocarps and aid in the acquisition of water (Johnson and Gehring 2007). The extraradical mycelial network of EcM fungi is much more extensive when compared to AM fungi and constitutes around 200 m of hyphae/gram of soil with a growth rate of 2–8 mm/day (Read and Boyd 1986; Ekblad et al. 2013).

Like AM fungi, EcM fungi also occupy two niches: the host root and the soil. The EcM fungi access the soil for minerals, effectively take them through their extraradical hyphal network, and partly transfer it to the host roots. Although saprophytic, EcM fungi have lost a significant part of the capacity to decompose organic matter (OM) rich in lignocellulose (Martin et al. 2016). The adaptation of the EcM fungi to a symbiotic lifestyle though resulted in the loss of certain functions, but still, the fungus has acquired some mechanisms adopted by the biotrophic phytopathogens to colonize host roots and obtain sugars. However, the EcM symbiosis lacks specific morphological structures like the haustorium of phytopathogens to acquire host resources (Kumar and Atri 2018).

2.3 Rhizosphere

2.3.1 Soil as a Natural Support for All Living Organisms

Soil is a heterogeneous enriched medium that shelters many organisms and acts as solid physical support for plants (Farley and Fitter 1999). Microorganisms are important in regulating the functioning of soil nutrient cycling, structure formation, and plant interactions, both positively and negatively. Moreover, in both managed and natural soils, microorganisms participate in the critical processing of decomposition of OM, toxin removal, and the cycling of carbon, nitrogen (N), phosphorus (P), and sulfur (Garcia and Kao-Kniffin 2018).

Soil aggregation is a biogeochemical and physical process in cultivated and bulk soils. The penetration and existence of fungal mycelial networks in plant roots and their interactions with soil enrich the soil ecosystem. Such long-term interactions allow the mycorrhizal mycelium to deposit a special type of protein called glomalin in the soil. Glomalin is an insoluble glycoprotein that glues the nearby soil particles and forms soil aggregates. The recommended method of extracting this glomalin as well as glomalin-related soil protein (GRSP) is done at 121 °C in citrate buffer and quantified by Bradford assay as described by Rillig (2004). There are two fractions of glomalin, i.e., total glomalin and easily extractable glomalin (EEG). The occurrence of glomalin is strongly correlated with improved soil aeration, proper water drainage, enhancing soil carbon sequestration, and promoting microbial activity (Lovelock et al. 2004; Rillig 2004). Sumathi (2010) quantified influence of inoculation of *Curcuma longa* with *Trichoderma* sp., and other plant growth-promoting rhizobacteria (PGPR) on AM fungal colonization and EEG in soils. The results of the study indicated that inoculation with PGPR like *Azospirillum*, *Pseudomonas* sp., and phosphate-solubilizing bacteria (PSB) enhanced the production of EEG fractions in the rhizosphere and AM fungal colonization in turmeric roots. This indicates that microbial inoculum in the soil significantly supports the biochemical activities of AM fungi and its contribution to nutrient cycling and plant nutrition. Glomalin is a form of OM that could engage in the sequestration of nitrogen and phosphate. This is evidenced by the existence of a strong correlation between plant protein concentration and EEG (Sumathi 2010). This relation between plant protein and EEG may be due to the translocation of phosphate and carbon that are necessary for the synthesis of proteins by the AM fungal hyphae (Miller and Jastrow 2000). These were also evidenced in a later study where the application of PGPR increased the AM fungal colonization levels in turmeric roots and OM content of the soil (Sumathi et al. 2013).

The AM fungal hyphae that are trapped in the soil aggregates undergo a slow decomposition process, having an estimated residence time of 6–42 years (Rillig 2004). Glomalin production significantly reduces the turnover of the AM fungal hyphae. The soil aggregates represent more the 5% of total soil carbon, significantly contributing to long-term soil carbon sequestration (Wright and Upadhyaya 1998; Rillig et al. 2001). Although the actual factors that control glomalin production are still unknown, factors like soil type, climate, AM fungal species involved, host plant, and their productivity are presumed to contribute to the concentrations of glomalin in the soil (Sumathi et al. 2013).

2.3.2 Difference Between Rhizosphere and Non-Rhizosphere

Hiltner (1904) defined the rhizosphere region as the soil in the root-zone area with interactions of microorganisms. The rhizosphere region supports a large group of living organisms than bulk soils. Diverse microorganisms ranging from unicellular to multicellular and macroscopic organisms survive in the rhizosphere environment. The colonization of microorganisms in the rhizosphere is induced by the activity of plant roots. Many microorganisms are attracted toward the nutrients released by the plant roots. The exudates from the plant roots called “rhizodeposits” contain a variety of low-molecular-weight molecules like small amino acids, glucose, fatty acids, and organic acids and high-molecular-weight molecules like polysaccharides and polygalactic acids. Some molecules present in the exudates may act as signal molecules to initiate colonization of roots by microorganisms.

Root exudation activates “rhizosphere priming,” which is an increased rate of microbial-mediated decomposition of the OM in the soil (Kuzyakov 2002). The liberation of low-molecular-weight carbon compounds into the rhizosphere by plant roots induces the saprophytic microbes in the soil to produce extracellular enzymes that mediate the breakdown of high-molecular-weight organic compounds. This results in the liberation of labile inorganic and organic nutrients available to both plants and soil microorganisms.

Plant and microbe interaction in the rhizosphere takes place following various principles and mechanisms that may be either beneficial or harmful. Plants attract root colonizing microorganisms through a chemical signaling process known as chemotaxis. Further, most of the plants behave as obligate mycotrophs as they have the ability to induce germination of mycorrhizal spores present in the soil and to attract the germinating hyphae (Vierheilig et al. 1998).

Non-rhizosphere soils are not influenced by plant roots and therefore are characterized by low fertility as well as the diversity and abundance of microorganisms (Li et al. 2016; Olahan et al. 2016). However, non-rhizosphere soil is essential for the soil aggregate stability and resistance of the soil to nutrient leaching and soil erosion (Elbl and Záhora 2014). Long-term monoculture of black pepper is known to considerably increase the relative abundance of *Acidobacteria* in the non-rhizosphere, and contrarily the abundance of *Bacteroidetes* and *Firmicutes* tended to decrease (Li et al. 2016). Nevertheless, populations of certain microorganisms appear to be similar in both rhizosphere and non-rhizosphere regions. For example, populations of the pathogenic fungus *Fusarium* has been shown to increase both in the rhizosphere and non-rhizosphere soils with time thereby increasing the incidence of wilt in both the soil regions (Li et al. 2016). Cropping patterns could also substantially affect the microbial diversity in the non-rhizosphere soils. Continuous cropping of ginseng both in the understory wild and farmlands decreased the operational taxonomic unit (OTU) richness of fungal communities in the non-rhizosphere soils (Bao et al. 2020). In addition, changes in microbial communities in response to the season also tend to vary in the rhizosphere and non-rhizosphere regions. An analysis of the seasonal changes in bacteria and fungi in the rhizosphere and non-rhizosphere regions of *Camellia yuhsienensis* over a one-year period indicated different responses in the soil regions. The abundance and changes in OTUs were higher in the rhizosphere region than in the non-rhizosphere region for both fungi and bacteria (Li et al. 2020).

2.3.3 Microbial Interactions

Apart from the mycorrhizal symbiosis, other microorganisms also form mutual relationships not only with plants but also with the associating fungi forming multitrophic interactions (Bonfante and Anca 2009). For instance, a non-culturable Gram-negative bacterium *Glomeribacter gigasporum*, a taxon of Burkholderiaceae, lives as an endosymbiont in the spore vacuoles, intraradical mycelium, and hyphae of AM fungi *Gigaspora margarita*. Similarly, some rhizobacteria belonging to *Bacillus*, *Paenibacillus*, and *Pseudomonas* frequently associate with AM fungi.

The PGPR in the soil ecosystem also form symbiotic associations with plant roots. These rhizobacteria stimulate the development of AM fungal mycelium and spore germination as well as plant root colonization (Barea et al. 1998). Microorganisms associated with plants improve nutrient acquisition by roots and stimulates hormone production. The PGPR perform various beneficial activities, in particular nitrogen fixation, phosphate solubilization, and suppression of plant pathogens (Martínez-Viveros et al. 2010). The well-studied plant growth supporting bacterial genera are *Rhizobium*, *Azospirillum*, *Bacillus*, *Pseudomonas*, and *Serratia*.

Plant-associated bacteria and fungi confer direct support for plant growth simultaneously inhibiting plant pathogens (Thakore 2006).

The mycorrhizal colonization brings diverse changes in the plant root architecture and physiology, as well as enriches the quality and quantity of root exudates. The changes in root exudation pattern occur when plant roots are colonized by AM fungi. The rhizosphere is extended by the extraradical mycelium of the AM fungi that explores the bulk soil for resources. The fungal hyphae also exude carbon and the region of the soil influenced by the fungal hyphae is called hyphosphere. This soil region also supports special microbial communities like the rhizosphere. The activation of plant defense mechanisms against specific target pathogens is an important criterion to use AM fungi as biofertilizer. The interaction between plant-parasitic nematodes and AM fungi is reported to be opportunistic. The biocontrol activity of AM fungi is also an added advantage of AM fungi–plant symbiosis, which supports increased plant growth and deters or decreases the damages caused by plant pathogens.

2.4 Mycorrhizosphere

The soil region encompassing the rhizosphere and the hyphosphere is called the mycorrhizosphere (Jeffries and Barca 2012) (Fig. 2.1). The influence of AM fungi and the plant root systems on the microbial communities and nutrients in the soil is known as the mycorrhizosphere effect. Angelo Rambelli in 1973 shaped the concept of mycorrhizosphere to indicate soil microhabitat that was influenced by the metabolites exudated by the roots and its associated mycobiont (Rambelli 1973). The intimate interaction of the associating fungi with the plant root system renders the mycorrhizosphere more complex than the root-influenced rhizosphere (Voronina 2009; Voronina and Sidorova 2017). Therefore, this region is not only influenced by the roots but also by the associated symbiont. Previous studies have explored only the interaction and benefits between the plants and their associated symbiotic fungi. However, the role of mycorrhizosphere microbiota was later perceived with the evolution of the multitrophic concept in mycorrhization and great advancements in research techniques (Voronina et al. 2011; Voronina and Sidorova 2017). In addition

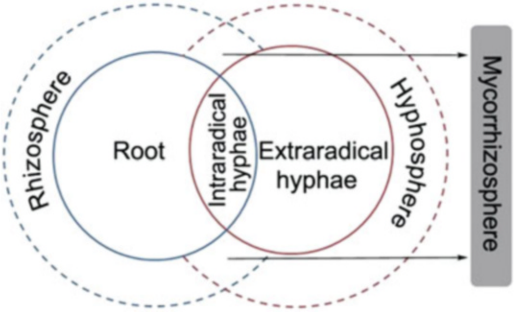

Fig. 2.1 Diagrammatic representation of the mycorrhizosphere concept

to the extraradical hyphal networks in the soil, the intraradical phase of the mycorrhizal fungi can also shape the microbiota in the mycorrhizosphere. This stems from the fact that colonization of roots by mycorrhizal fungi has been shown to modify the quality and quantity of root exudates (Vives-Peris et al. 2020).

The establishment of the functional mycorrhizosphere is dependent on the successful establishment of the symbiosis between the plants and the associating fungi. The primary step in this process is the maintenance, growth, and survival of AM fungal spores in the soil and stimulation of mycelium development for pre-symbiotic growth. In addition to the plant signals, bacteria present in the mycorrhizosphere are also known to play a pivotal role in the establishment of symbiosis both by AM and EcM fungi. Roesti et al. (2005) showed that the bacterial communities associated with the spores *Funneliformis geosporus* (=*Glomus geosporum)* and *Septoglomus constrictum* (=*Glomus constrictum)* are more dependent on fungal identity than the host plant identity. Moreover, it was also suggested that the composition of the bacterial communities associated with spores of the AM fungi could be greatly influenced by the spore wall composition or the exudate of the fungi rather than by the root exudates of the host plant. A scanning electron microscopic observation of the *F. geosporus* spores showed that the bacteria colonizing the spore surface was presumably feeding on the outer layer of the spore wall and it was speculated that the activity of the spore surface bacteria could benefit the process of spore maturation and its eventual germination (Roesti et al. 2005). Moreover, in plants, the associating bacteria also increase the susceptibility of roots for mycorrhizal colonization and simultaneously induce root to recognize mycelium. Finally, it also modifies the physicochemical properties of the soil that would benefit mycorrhiza formation.

2.4.1 Mycorrhiza Helper Bacteria

The group of soil bacteria supporting the formation of mycorrhizal symbiosis with plants is called “helper bacteria” or “mycorrhiza helper bacteria” (MHB). The first association of MHB with endomycorrhizal fungi was reported by Mosse (1962). Both Gram-positive and Gram-negative bacteria act as MHB. The bacterial genera reported functioning as MHB includes *Agrobacterium*, *Azospirillum*, *Azotobacter*, *Pseudomonas*, *Bacillus*, *Burkholderia*, *Bradyrhizobium*, *Enterobacter*, *Klebsiella*, *Rhizobium*, and others of which *Pseudomonas* tends to be the dominant genus. The Actinomycetes *Rhodococcus*, *Streptomyces*, and *Arthrobacter* can also act as MHB. The fungus *Trichoderma harzianum* was also found to enhance AM fungal colonization and hence Jayanthi et al. (2003) suggested the term to be changed as “mycorrhiza helper organisms” to accommodate bacteria, fungi, or any other enhancing mycorrhizal symbiosis, which was not followed by many later workers. The constructive effect of MHB on mycorrhizal–host plant symbiosis depends on root sensitivity and stimulation of spore germination (Xavier and Germida 2003; Frey Klett et al. 2007). A direct beneficial aspect of MHB involves the stimulation of plant growth and indirect benefit includes inhibition of pathogen growth or a reduction in the adverse effects of phytopathogens on plants (Hernandez Montiel et al. 2013; Deveau and Labbé 2016). The abundance of the bacteria can vary in the hyphosphere. Some bacteria attach to the fungal hyphae and stimulate the production of hyphal exudates while others mediate the adhesion of other bacteria or some of them can form biofilm on the hyphoplane (Haq et al. 2014).

MHB benefits plant nutrition by being an N-fixing rhizobacteria (Paul and Lade 2014). Additionally, they interfere directly with the immune response of the plant by activating several pathways like jasmonic acid and salicylic acid (Cameron et al. 2013; Kurth et al. 2013). Some species of MHB are known to produce hormones like auxins and ethylene that modulate root development (Vacheron et al. 2013). The bacterial gene operons containing Type III Secretion System (T3SS) are enriched at the surrounding area of mycorrhizal fungi. T3SS functions as injecting systems of bacteria to deliver small effector molecules into plant root nuclei to induce some functions. T3SS is related to formation of both symbiotic and pathogenic associations. Still, the mechanisms of injections by bacteria into eukaryotic cells are not clearly understood (Warmink and van Elsas 2008; Viollet et al. 2011).

2.4.2 Interactions of Mycorrhizal Fungi with Beneficial Microflora

The microorganisms existing in the mycorrhizosphere region exert valuable effects on plants as well as other symbiotic microorganisms. When such microorganisms positively influence the growth of plants, they are designated as plant growth-promoting microorganisms (PGPM). The bacteria surviving in the roots' rhizospheric region are called rhizobacteria and those supporting plants' development or metabolism are considered PGPR. The term PGPR was coined by Kloepper and Schroth (1978). The PGPR stimulates plant growth either through direct or indirect mechanisms (Bashan and Holguin 1998). In the rhizosphere, the presence of *Funneliformis mosseae* enhances the population load of *Pseudomonas fluorescens*, which exhibits plant growth-promoting effect (Edwards et al. 1998).

Nitrogen is the most important nutrient necessary for plant growth. The molecular nitrogen (N_2) available in the atmosphere (78%) is in the gaseous form and should be converted to the chemical form of ammonia (NH_3) that can be utilized by the plants. This simple reduction process is carried out by bacteria capable of producing the enzyme "nitrogenase" (Olivares et al. 2013; de Bruijn 2015). Nitrogen fixation can be possible either by artificial or natural means; the natural way is quite cost-effective. This particular process is carried out by free-living and symbiotic bacteria. The symbiotic bacteria include *Rhizobium* and *Frankia*. For instance, the bacterial genus *Azospirillum*, which is known to fix atmospheric N, forms an associative association with plant roots. The free-living bacteria *Azotobacter*, *Klebsiella*, and *Bacillus* are known to fix nitrogen in the soil.

The soil bacteria and fungi participate in the breakdown of organic substrates and release nutrients into the soil environment. This process is known as mineralization. Phosphorus mineralization releases orthophosphates from organic phosphates into the soil (Marschner 2008; Richardson et al. 2009; Bagyaraj et al. 2015). For instance, bacteria and fungi belonging to *Bacillus*, *Pseudomonas*, *Aspergillus*, and *Penicillium* carry P mineralization in the soil. Mineralization is carried out by the non-specific activity of phosphatase enzymes (including acid and alkaline phosphatases). These phytase enzymes act specifically on phytates to release orthophosphates. P release by microbial activity enhances plant growth. If mycorrhizal fungal hyphae are present in this habitat, it effectively absorbs the P nutrients and transfers them to plant roots (Richardson et al. 2009). The evidence for this symbiotic association is given by Koide and Mosse (2004).

Certain studies have shown that the EcM can modulate the composition of the bacterial communities associated with the mycorrhizospheres. Shirakawa et al. (2019) examined the influence of EcM fungal species associated with *Pinus densiflora* on cultural bacterial communities in the mycorrhizosphere. The results of the study indicated that Gram-positive bacteria are excluded from the mycorrhizosphere of *P. densiflora* due to the antibacterial activity of the EcM fungi. Moreover, increased diversity of plant genotypes has been shown to alter the root competition and the levels of EcM and endophyte fungal colonization in the mycorrhizosphere (Baum et al. 2018). Several studies have shown that EcM roots can significantly influence the archaea and bacterial community composition in forest soils (Fransson and Rosling 2014; Rinta-Kanto and Timonen 2020). An investigation on the impact of the EcM fungi *Tuber panzhihuanense* in symbioses with *Corylus avellana* suggested a high diversity in microbial communities during the initial stages of symbiosis development (Yang et al. 2019). Moreover, the structure of the bacterial and fungal communities associated with *C. avellana* was completely different in the presence and absence of the EcM symbiosis. Certain bacteria like *Herbiconiux*, *Pedomicrobium*, and *Rhizobium*, and the fungal taxa *Monographella* were more prolific in the presence of EcM symbiosis (Yang et al. 2019). A culture-independent analysis of the bacterial and archaeal populations associated with the mycorrhizospheres of *Pinus sylvestris–Suillus bovinus* in a boreal forest of Finland indicated that archaea dominated by Thaumarchaeota taxa were more abundant than the bacterial taxa dominated by Acidobacteria, Actinobacteria, and Proteobacteria (Rinta-Kanto and Timonen 2020). Further, a higher prokaryotic population was associated with the soil hyphae of *S. bovinus* than in the roots. Some of the factors that contribute to the variation in the prokaryotic population in EcM could be root age, mycorrhizal, and soil hyphal abundance. Though bacterial communities associated with EcM roots tend to stabilize with time, significant variability in bacterial communities has been noted during the early stages of colonization and symbiosis establishment (Marupakula et al. 2016). In a recent study, Gorka et al. (2019) showed that the EcM hyphae could accelerate the transfer of host-derived carbon to bacterial communities far away from roots and this carbon transfer was associated with the changes in the soil nutrients. These results clearly indicate that the EcM symbiosis could modify the microbial communities associated with their host plant in addition to their influence on soil factors.

2.4.3 Interactions of Mycorrhizae with Pathogenic Microorganisms

Mycorrhizae are involved in effective disease control mechanisms. The popular AM fungus *F. mosseae* increased the population of PGPR particularly *P. fluorescens* in the rhizosphere soil (Edwards et al. 1998). Among bacteria, *Pseudomonas* and *Bacillus* are the potent candidates for biocontrol agents. A combination of mycorrhizae and rhizobacteria associated with plants develops resistance against fungal pathogens (Paulitz and Linderman 1991). Pathogens make entry into the host cells and induce disease symptoms. Further, the symptoms are regulated by the colonization of AM fungi in the host plant and induce the immune response (Induced Systemic Resistance—ISR) against plant pathogenic microorganisms (Liasu and Shosanya 2007). Various compounds are found associated with ISR including enzymes like superoxide dismutase (SOD), chitinases, chitosanases, and peroxidases and specific proteins like pathogenesis-related type-1 proteins (PR-1 proteins). The accumulation of molecules like phenolics and jasmonic acid indicates the disease resistance nature of plants. The effectiveness of endomycorrhizae to control soil-borne fungal pathogens is due to their efficiency to induce rhizobacteria against suitable factors supporting pathogen in the mycorrhizosphere. AM fungi confer biocontrol activity against pathogens inducing systemic and local resistance mechanisms (Toussaint et al. 2008). ISR increases the density of the plant cell wall and thus restricts the phytopathogens to the outer root cortex (Benhamou et al. 1998).

Inoculation of AM fungi prevents the attack of pathogens and confers protection for the plants (Singh et al. 2010; Bagyaraj 2018). Mycorrhizosphere creates a safe environment by decreasing the growth of nematodes and reducing the incidence of conidial formation by *Fusarium oxysporum f. sp. chrysanthemi.* The colonization of tomato plant roots with *Rhizophagus intraradices* (=*Glomus intraradices*) reduces the growth and root rot symptoms of *Fusarium solani f. sp. phaseoli* (Akköprü and Demir 2005). The biocontrol potential of AM fungi resides in the competitive environment with the soil fungal pathogens for space and nutrients. The combined inoculation of *R. intraradices* and bacteria *Pseudomonas striata* and *Rhizobium* sp. significantly reduced the gall formation and population development of nematode in chickpea (Akhtar and Siddiqui 2008).

Like AM fungi, ectomycorrhizal association also protects its host plants from various diseases. Osaki-Oka et al. (2019) showed that the volatile extracts of the EcM fungi *Russula* aff. *anthracina*, *Russula chloroides*, and *Russula senecis* inhibited the conidial germination of the phytopathogenic fungus *Alternaria brassicicola*. Similarly, inoculation of *Pinus tabulaeformis* seedlings with seven EcM fungi (*Suillus lactifluus, Suillus bovinus, Suillus tomentosus, Handkea utriformis, Amanita vaginata, Suillus laricinus*, and *Schizophyllum* sp.) reduced the mortality and improved the seedling growth and root architecture against the pine wilt disease caused by the nematode *Bursaphelenchus xylophilus* (Chu et al. 2019). In a recent study, Chartier-Fitz Gerald et al. (2020) showed that the EcM fungal (*Lactarius quieticolor*, *Suillus granulatus*, and a *Suillus* sp.) inoculation improved the seedling growth and afforded protection against the fungal pathogen

Fusarium circinatum. Studies have also shown that the EcM can modulate the susceptibility of the host plants variedly against different pathogens. For instance, association with the EcM fungus *Suillus luteus* decreased the susceptibility of *P. sylvestris* to the fungal pathogen *Heterobasidion irregulare*, but not against *Heterobasidion annosum* (Gonthier et al. 2019).

2.4.4 Interactions of Mycorrhizae with the Stressed Soil Environment

Soil acts as a substrate for growth and reproduction of microorganisms and other small living communities. These organisms actively participate in the conversion of complex organic molecules into simpler substances that can be easily absorbed by plant roots and other organisms. Different types of pollutants are introduced into soils due to various anthropogenic activities and many of these soil pollutants rarely undergo the degradation process. One of the most common and widespread pollutant added to the soil are the heavy metals. These pollutants are brought into the soil environment by human activities like industries, metal plating, electronic wastes, and petroleum products. Moreover, agricultural activities like the use of pesticides, agrochemicals, phosphatic fertilizers, and sewage sludges for irrigation also add a higher concentration of heavy metals into the soil environment. Heavy metal pollution causes drastic health defects in plants, animals, and humans. Microorganisms are capable of detoxifying the toxic heavy metal pollutants. Among different types of microorganisms, mycorrhizal fungi are efficient candidates for detoxifying soil pollutants and protecting plants from these toxic chemicals (Sumathi 2010). Nevertheless, heavy metals can influence the formation and functioning of AM fungi. An excessive concentration of heavy metals can reduce the spore number and germination, hyphal length, and percentage of colonization of AM fungi (Krishnamoorthy et al. 2019). In addition, heavy metals can have a harsh impact on the ecology and diversity of AM fungi. Several mechanisms are associated with AM fungi in imparting tolerance to heavy metals in plants, which have been reviewed in detail by Riaz et al. (2020). Release of the glomalin protein by mycorrhizal hyphae into the soil is one of the most important mechanisms for the amelioration of heavy metal toxicity. Other processes involved in heavy metal tolerance involve extracellular chelation, binding, and accumulation of heavy metals in cell walls of spores and hyphae (Krishnamoorthy et al. 2019). The vesicles and spores of AM fungi act as storage structures of heavy metals. This reduces the availability of heavy metals in the rhizosphere or mycorrhizosphere. The AM fungi also absorb and transfer the metal ions from the soil to plant roots that are then translocated to the aerial parts of plants. The glomalin protein eases the uptake of water, and improves the soil structure by increasing the water holding capacity, gaseous exchange, and availability of water and nutrient both in stressed and normal soils. Glomalin also reduces the bioavailability of metal ions and participates in indirect mechanisms for increased water and nutrient uptake, particularly P acquisition through hyphae, mediating the growing of plants in heavy metal stressed soils (Garg and Bhandari 2012; Garg and Chandel 2015).

The influence of an AM fungus *Rhizophagus irregularis* and an EcM fungus *Sphaerosporella brunnea* inoculation either individually or in combination on growth and trace element extraction was examined in *Salix miyabeana* (clone SX67) on an industrial landfill that was decommissioned several decades back in Canada (Dagher et al. 2020). *Salix* plants inoculated with *S. brunnea* accumulated the highest biomass after two growing seasons. Contrarily, the inoculation of *R. irregularis* either alone or along with EcM failed to significantly influence the plant biomass and the trace element extraction. Inoculation of *S. brunnea* also significantly decreased the concentrations of copper, lead, and tin in the soil (Dagher et al. 2020). The MHB also accompanies the mycorrhizal fungi in detoxifying the toxic compounds present in the soil (Duponnois and Garbaye 1990). Remediation of xenobiotic compounds using the rhizosphere processes associated with plants is also called phytostimulation. The xenobiotic compounds are detoxified by the multitrophic activities in the mycorrhizosphere that acts as a remediation unit in the ecosystem (Bhawana and Fulekar 2011).

2.5 Perspectives of Mycorrhizosphere for Sustainable Agriculture

Managing mycorrhizosphere interactions (mycorrhizosphere tailoring), involving co-inoculation with selected AM fungi and other beneficial microorganisms like N-fixers, P-solubilizers, and PGPR, is recognized as a possible biotechnological tool to improve the growth and productivity of crop plants in sustainable agriculture. It is fundamental that the establishment of mycorrhizosphere-tailored plants increases soil nutrient content and improves other physicochemical properties, which define soil quality promoting plant growth. Some studies also brought out that tailored mycorrhizosphere improved plants' survival, growth, and out-planting performance under biotic and abiotic stress conditions (Azcon-Aguilar and Barea 2015; Karthikeyan et al. 2016). Further, few studies revealed that mycorrhizosphere-tailored plants act as an inoculum resource for other crops/surrounding vegetation grown under inter/mixed cropping systems. Most of the mycorrhizosphere studies have been done under pot culture conditions. Evaluation of such inoculation studies under field conditions is rather limited. Studies on mycorrhizosphere tailoring, improving growth, nutrition, and yield of crop plants, and also the possibility of reducing chemical fertilizer input, thus upholding the theme of sustainable agriculture, are discussed briefly hereunder.

2.5.1 Mycorrhizosphere Tailoring with AM Fungi and N-Fixers

Janse (1896) was the first to describe the interaction between AM fungi and rhizobia in the legume *Pithecellobium montanum*. The early field experiments conducted with *Medicago sativa* by Azcón-Aguilar et al. (1979) and soybean by Bagyaraj et al. (1979) under field conditions clearly brought out that dual inoculation with AM fungi and rhizobia shows a synergistic effect in improving nodulation and AM fungi colonization consequently enhancing growth, nutrition, and yield compared to single inoculation and uninoculated plants. This was followed by similar reports in other legumes. Several studies on the interaction between AM fungi and symbiotic N-fixing organisms suggest that the interaction is synergistic, improved nodulation and N-fixation by N-fixers, and N-fixers enhanced colonization and P uptake by AM fungi; thus dual inoculation improves plant growth much better than single inoculation with either of them (Meena et al. 2018). It is well known that N-fixation has a high phosphate requirement, which is provided by AM fungi. Extracellular polysaccharides and dehydrins produced by rhizobia enhancing colonization by AM fungi have been observed. The response of cowpea and pigeon pea to dual inoculation with AM fungi *Rhizophagus fasciculatus* (=*Glomus fasciculatum*) and *Rhizobium* sp. with and without added P (22 kg P ha^{-1}) was studied in a P-deficient soil. Plants inoculated with both the organisms and supplemented with P recorded the highest shoot dry weight, and N and P contents, indicating the need for the addition of a small amount of P to derive maximum benefit from dual inoculation with AM fungi + rhizobia (Manjunath and Bagyaraj 1984). Almost similar observations were also reported in the tree legume *Leucaena leucocephala* inoculated with *R. fasciculatus* and rhizobia (Manjunath et al. 1984). Another co-inoculation study with AM fungi + rhizobia done with soybean genotypes brought out that deep-rooted genotype benefitted more from co-inoculation than shallow-rooted genotype. The improvement in plant growth parameters was more prominent in low fertile soil (Wang et al. 2011). Another study demonstrated that combined inoculation of chickpea with *F. mosseae* + *Rhizobium ciceri* with a split application of N fertilizer significantly increased the yield and quality of the grain compared to inoculation with any one of the inoculant (Malekian et al. 2019). Meng et al. (2015) investigated inoculation with *F. mosseae* and *Rhizobium japonicum* in maize/soybean intercropping system. Inoculation improved the N-fixation efficiency of soybean and promoted N transfer from soybean to maize, resulting in improvement of yield in both the crops. Raklami et al. (2019) studying the interaction among AM fungi + rhizobia + PGPR in *Vicia faba* under field conditions reported synergistic interaction between the three organisms, resulting in improved growth, nutrition, and yield of the crop. Certain conclusions that can be drawn from the experiments done so far are, to screen and select the best AM fungi and rhizobia for the target legume, and then study the interaction between the two selected symbionts under polyhouse and finally under field conditions. Another conclusion drawn is that the tripartite symbiosis is not effective in soils with high levels of N and P (Barea et al. 2014). Clearly, mycorrhizosphere interactions in legume plants have a relevant significance on N and P cycling in the biosphere to benefit sustainable agriculture.

Synergistic interaction between AM fungi and free-living N-fixing *Azotobacter* was reported for the first time by Bagyaraj and Menge (1978). This was confirmed by later studies by many workers as outlined in the review by Karthikeyan et al. (2016). Amirnia et al. (2019) carried out field experiments with *R. intraradices* and *Azotobacter* sp. inoculation to lentil under rainfed and irrigated conditions. Under both conditions, dual inoculation increased the biomass, yield, and seed protein concentration. The benefit of inoculation was much higher under irrigated conditions. In another study conducted at Desert Research Centre, Egypt, with AM fungi and *Azotobacter* inoculation under four water regimes brought out that dual inoculation significantly enhanced yield attributes and water use efficiency of barley compared to single inoculation with either of them (Abdelhameid and Kenawey 2019). Kumar et al. (2016) evaluated the effect of AM fungi consortia and *Azotobacter* sp. on *Jatropha curcas* under field conditions in a semi-arid region. A significant improvement in plant growth and fruit yield was evident when AM fungi and *Azotobacter* were co-inoculated.

2.5.2 Mycorrhizosphere Tailoring with AM Fungi and P-Solubilizers

Studies on dual inoculation with AM fungi and phosphate-solubilizing microorganisms (PSM) brought out increased plant growth and yield compared to single inoculation with either of them. This is because P solubilizers solubilize and release H_2PO_4 ions from unavailable forms of P and AM fungi help in the uptake of H_2PO_4 ions from soil (Aliasgharzad et al. 2009; Bagyaraj et al. 2015). Some workers found that PSM inoculated onto seeds or seedlings maintained high populations, longer in the rhizosphere of mycorrhizal than non-mycorrhizal roots. Combined inoculation of PSM with AM fungi along with rock phosphate could improve crop yield in nutrient-deficient soils (Sabannavar and Lakshman 2009). Synergistic interactions between AM fungi and PSM with consequential benefit on plant growth have been demonstrated not only in crop plants but also in forest trees (Adesemoye et al. 2008). Interaction between the AM fungus *Rhizoglomus irregulare* and PSM *Pseudomonas putida* was studied in an intensive maize agricultural system. The results clearly showed that dual inoculation increased maize productivity and at the same time improved P use efficiency (Pacheco et al. 2020). In calcareous soils low availability of P is a major problem because of precipitation of P by calcium, thus making it immobile. Maize crop was inoculated with AM fungi + PSM and treated with rock phosphate in the first season. In the next season, wheat crop was taken as a subsequent crop to check the residual effect of inoculation. The results of both the field trials indicated the beneficial effect of dual inoculation in increasing grain yield and P uptake, suggesting the replacement of expensive P fertilizer in P-deficient calcareous soils (Wahid et al. 2020).

Field studies have shown that inoculation with efficient AM fungi not only increases the growth and yield of crop plants but also reduces the application of phosphatic fertilizer by nearly 50%, especially in marginal soils deficient in nutrients. Though the rock phosphates available in India are of low grade and not fit for the manufacture of phosphatic fertilizer, they can be used with PSM plus AM fungi as a potential source of P for crop plants, thus bringing down the import of P fertilizers/rock phosphate in our country. Some studies also revealed that the total cost of cultivation and the gross income, net profit per hectare, and the cost–benefit ratio are also high when AM fungi and PSM are inoculated together with rock phosphate under field conditions (Ajimuddin 2002).

2.5.3 Mycorrhizosphere Tailoring with AM Fungi and PGPR

Interaction studies carried out with AM fungi and PGPR also revealed enhanced plant growth and yield. The synergistic effect of PGPR and AM fungi on plant growth promotion is well documented (Bagyaraj 2014; Desai et al. 2016). The combined application of *P. fluorescens* and *F. mosseae* resulted in improved growth of chickpea compared to the application of the two bioinoculants separately and also reduced the galling and multiplication of the nematode pathogen *Meloidogyne javanica* (Siddiqui and Mahmood 2001). In combined inoculation of PGPR and AM fungi, PGPR stimulating spore germination and colonization of AM fungi and in turn better plant growth has also been reported (Desai et al. 2016). Synergistic interaction between *Glomus bagyarajii* and *Trichoderma harzianum* promoting growth and yield of *Piper longum* (Ulfath Jaiba et al. 2006) and that of chilli with *F. mosseae* and *Bacillus sonorensis* inoculation (Thilagar et al. 2014) has been reported. Another study brought out that co-inoculation of pigeon pea with *Ambispora leptoticha* (=*Glomus leptotichum*) and *Pseudomonas*, and finger millet with *R. fasciculatus* and *Pseudomonas* as intercrop benefitted both the crops under field conditions in different agro-ecological locations (Mathimaran et al. 2018). Inoculation of neem with *F. mosseae* + *Paenibacillus polymyxa* improving the growth and nutrition of seedlings in the nursery and when planted in the field has been reported recently (Nikhil et al. 2020). In medicinal and aromatic plants, dual inoculation with AM fungi and PGPR enhanced not only growth and yield but also the secondary metabolite concentration of medicinal value, which has been reported by many workers. An increase in eugenol concentration in *Ocimum sanctum* (Jyothi and Bagyaraj 2017), with anolide concentration in *Withania somnifera* (Anuroopa and Bagyaraj 2017), and essential oil content in *Ocimum basilicum* and *Satureja hortensis* (Khalediyan et al. 2020), has been brought out by earlier workers. Field studies conducted with selected AM fungi and PGPR combination revealed that in addition to improved growth and yield of crop plant, application of chemical fertilizer can be reduced. Inoculation with *F. mosseae* + *Trichoderma harzianum* not only improved growth, P nutrition, and yield of *Andrographis paniculata*, but also helped in saving 25% of P fertilizer application (Arpana and Bagyaraj 2007). Application of *Acaulospora laevis* + *Bacillus licheniformis* enhanced the growth and yield of *Withania somnifera* and also reduced the application of NPK—fertilizers by 25% (Anuroopa et al. 2017). In *Ocimum tenuiflorum* treatment with *Funneliformis* *monosporus* (=*Glomus monosporum*) + *Pantoea dispersa* and in chilli with *F. mosseae* + *Bacillus sonorensis* resulted in 50% saving of NPK fertilizer application (Thilagar et al. 2018; Jyothi and Bagyaraj 2018). Another study brought out that dual inoculation with the AM fungus *R. fasciculatus* and PGPR *P. fluorescens* was very effective in ameliorating the root rot and wilt disease of the medicinal plant *Coleus forskohlii* caused by *Fusarium oxysporum* (Rakshapal Singh et al. 2009). The production of good-quality seedlings that will establish better in the field is very much essential for crop productivity. Growing seedlings in pro trays is an innovative technique adopted in nurseries, including India, where seedlings are grown in soilless media under polyhouse conditions (Singh et al. 2017). Inoculating the substrate/seeds with selected AM fungi + PGPR has helped in the production of healthy vigorously growing seedlings that will perform better when planted in the field. Recent studies on inoculation with *F. mosseae* + *Bacillus sonorensis* resulted in vigorously growing seedlings of vegetable crops like tomato and capsicum (Abhaya et al. 2019; Desai et al. 2020), and flowering plants like zinnia and balsam (Sukeerthi et al. 2020).

2.5.4 Mycorrhizosphere Tailoring with Microbial Consortia

Studies have shown that inoculation with microbial consortia consisting of efficient AM fungi together with a N-fixer, P-solubilizer, and PGPR, carefully screened and selected for a particular crop or forest tree species, is more beneficial than AM fungi alone in improving the growth, biomass, and yield. Thus, the recent trend is to use microbial consortia of more than two microorganisms for inoculating crop plants and forest tree species, the consortia mostly consisting of one selected AM fungi with several selected PGPR. These studies have brought out clearly that inoculation with such microbial consortia is far superior to inoculating singly with any AM fungi or PGPR alone, or a combination of a single AM fungi + single PGPR. Few other studies brought out that such mycorrhizosphere tailoring with selected microbial consortia not only improves the growth and yield of crop plants but also saves the use of chemical fertilizers.

Microbial consortia consisting of *F. mosseae* + *Paenibacillus polymyxa* + *Pantoea agglomerans* increased the growth and yield of French beans and also saved the application of NPK fertilizers by 50% (Hemlata and Bagyaraj 2015). Microbial consortia consisting of AM fungi *Claroideoglomus etunicatum* (=*Glomus etunicatum*) + PGPR *Azotobacter chroococcum* + *Trichoderma harzianum* + *Burkholderia cepacia* in addition to increasing the yield and essential oil content of the aromatic crop *Pogostemon cablin* reduced the application of N and P fertilizer by 50% (Arpana et al. 2010). The performance of a selected microbial consortia *Scutellospora calospora* + *Azotobacter chroococcum* + *Bacillus coagulans* + *T. harzianum* on *Acacia auriculiformis* was evaluated through large-scale nursery trials. The results showed a 31% increase in the dry biomass of inoculated seedlings compared to uninoculated seedlings. These seedlings when planted in the wasteland and monitored for six years showed a 52% higher biovolume index than that of the uninoculated trees (Raghu et al. 2020a). Similar results were obtained with *Tectona grandis* inoculated with microbial consortia *Ambispora leptoticha* + *Azotobacter chroococcum* + *T. harzianum* (Raghu et al. 2020b).

Therefore, the need of the hour is to develop microbial consortia for inoculating different crop plants and the promotion of these consortia in the ecofriendly sustainable production of crop plants for the benefit of mankind. More field inoculation trials to exploit the benefit of tailored mycorrhizosphere enhancing crop productivity should be a component in sustainable agricultural strategies in the future, particularly in a world of depleting non-renewable resources. The mycorrhizosphere tailoring is likely to become even more importunate due to the agro-ecological threats of agrochemicals, which are urgently required to be reduced to increase food quality, sustainable food production, and environmental protection.

2.6 Conclusion

Generally, plants interact with a wide range of microorganisms either directly or indirectly. Some of the organisms like the mycorrhizal fungi that associate with roots benefit plants in various ways. This positive influence of mycorrhizal symbiosis is modulated by other organisms that are present in the rhizosphere and hyphosphere. Studies conducted so far clearly suggest that the mycorrhizosphere encompassing the spheres of the root and the associating fungal hyphae could influence the diversity of microorganisms and the soil characteristics. Therefore, this region of the soil is of great relevance in improving plant growth and health. Moreover, whatever evidences that are available on the various processes in the mycorrhizosphere are only the tip of an iceberg. There are several aspects like the factors that determine the discrimination of the rhizosphere, hyphosphere, and mycorrhizosphere as indicated by Voronina and Sidorova (2017). Furthermore, the exchange of signals between the different components of the mycorrhizosphere, stability, and succession of various microbial groups during the establishment and functioning of the mycorrhizosphere are yet to be ascertained. In addition, understanding the role of plant diversity on the mycorrhizosphere is important as plant roots in vegetation are connected through the common mycelial network. Therefore, untangling the complexity and dynamism of the mycorrhizosphere would greatly enable to increase the efficiency of the mycorrhizal symbiosis in sustainable crop production systems. This suggests the need for intensifying research on mycorrhizosphere tailoring for improving plant productivity under different agro-ecological conditions in the years to come.

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Conservation Strategies for Rhizobiome in Sustainable Agriculture

3

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Abstract

The use of chemicals in agriculture to achieve the highest yield was trending in farming practices, which led to the depletion and shifting of microbial community over the years. In sustainable agriculture, the use of organic products was the innovative approach along with the incorporation of microbial products to enhance the growth and productivity of the crop with minimizing the various biotic and abiotic stresses. Instead of adding the microbial population to the soil, conservation of microbial population or microbiome by adopting the various

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strategies was the most prominent approach in sustainable agriculture, which enhanced the income of farmers as well as improved the growth and development of the crop. This chapter highlights the importance of microbial community, keeping the rhizosphere parameters in view and utilizing their activity to develop enrichment strategies for the growth and development of plant system.

3.1 Introduction

Agriculture and the environment have a 'hand in hand' relationship that is justified through the footprints of agriculture on the environment and vice versa. Both entities have a very significant role in influencing the functional aspects of each other. Agriculture and related practices have huge contributions to climate change, water degradation, land degradation, and many more processes. Simultaneously, the environment also has a huge part to play in agricultural systems (Rockström et al. 2017). The conflict between the agricultural community to increase the production and/or productivity to meet the demands of the growing population and environmentalists to save or exert the least negative impacts on the environment gave rise to the term 'sustainable agriculture' (Gold 1999). Thus, for achieving sustainability in agriculture, we have to incorporate alternate input options in the package of practices instead of the chemical alternatives such as synthetic fertilizers, pesticides and insecticides. One such alternative is the utilization of beneficial microbes that can provide nutrients to crop plants and protect them against various abiotic and biotic stresses. Plant microbiome is known to bring different changes in the surrounding and the host plant itself according to the varying set of environmental conditions. These changes support the survival of plant and hence are believed to help achieve the goal of sustainable agriculture. Plant microbiome is defined as the total of all microbes that are associated with a host plant. It is further divided into two groups, namely phyllosphere microbiome and rhizospheric microbiome, depending on the part of the plant they inhabit (Berendsen et al. 2012; Turner et al. 2013; Schlaeppi and Bulgarelli 2015; Rodriguez et al. 2019).

Among the two, roots are the primary microhabitat for all the plant–microbe interactions and the microbe–microbe interactions and also the site for inhabitation of a more diverse range of microbes than phyllosphere (Jones et al. 2009; Singh et al. 2019). Besides, the rhizospheric microbiome or rhizobiome has been proven to embellish the growth and yield of crops that are regulated by certain environmental factors like the plant genotype, climate, soil, and human activities (Igiehon and Babalola 2018). Rhizobiome of a plant is present in three root sites, namely: endorhizosphere, rhizoplane, and rhizosphere. The area inside the root within cells and between the cells is known as endorhizosphere, the surface of root is known as rhizoplane and the region of soil surrounding the roots of plant is known as rhizosphere (Reinhold-Hurek et al. 2015). Rhizobiome is also considered as a plant's second genome (Berendsen et al. 2012). The microbes constituting the rhizobiome carry out different ecological processes and biogeochemical cycles (Philippot et al. 2009). The rhizobiome is the largest prevalent ecosystem on this planet owing to the innumerable microbes, countless interactions, and immense energy flux (Barriuso et al. 2008). Hence, it is very necessary to have comprehensive knowledge about the structure and function of rhizobiome. A detailed understanding even about the general structure and function of rhizobiome can be utilized in improving crop health and yield (Vukanti 2020). There is a need for the development of novel techniques that can help in the conservation of this rhizobiome of crop plants and thereby aid in achieving the goal of sustainable agriculture.

Soil is the medium that provides the base as well as the materials essential for plant growth and survival. The rhizobiome is also a part of the soil and its composition has an intimate relationship with the plant's health (Yang et al. 2019). With advent of the Green Revolution, there has been overuse of synthetic fertilizers, pesticides, monocropping, and other input-intensive practices that have caused a serious disbalance in the ecological systems. Since rhizobiome is a fundamental part of the ecological system, their diversity and population also got affected (Du et al. 2020). The rhizobiome is involved in many of the processes in the soil environment, which include conversion of energy, cycling of nutrients, and health of plants (Zhang et al. 2019). Some of the microbes of the rhizobiome also possess the capacity to degrade the contaminants and pollutants from the soil and thus fulfil the purpose of bioremediation (Dubey et al. 2020). Many microbes of rhizobiome by virtue of their metabolic processes supply materials to the plants and also reduce the risk of infection by phytopathogenic microbes (Leghari et al. 2016; Lu et al. 2019; Zilli et al. 2020; Peng et al. 2020). Additionally, the rhizobiome also has microbes that are deleterious to plant health and development, but their population and action are kept in check by the beneficial microbes of the community. The composition and stability of rhizobiome have an intimate relation with plant health and development (Xia et al. 2016). It is proved time and again that the proportions of beneficial and harmful microbes can be modified to obtain a steady state of rhizobiome so as to embellish the health of a plant and strengthen its resistance to different stresses, which in turn will increase the production and productivity sustainably (Du et al. 2020). In this chapter, we present the possible path for achieving the goal of sustainable agriculture through the conservation of rhizobiome.

3.2 Importance of Rhizobiome in Agriculture

The importance of rhizobiome in agriculture can be emphasized from the fact that the crop plants and the rhizospheric microbes have an intimate relationship that is beneficial, pathogenic, or non-effectual, based on the plants and microbes involved. The relationship between plant and rhizobiome decides the survivability of the host plant (Olanrewaju et al. 2019). The communication between them is bi-directional since the plant regulates the composition and dynamics of its rhizobiome through root exudates as well. Our attempts to enhance plant productivity by whatever means have put soil under extreme pressure. The microbes inhabiting the soil are an indispensable part of the soil system and in most cases aid in the growth and development of plants. Exploiting these beneficial effects of microbes on a plant in achieving the target of higher yields and sustainable agriculture can come in handy while simultaneously relieving pressure from the soil. Talking about the beneficial effects, the rhizobiome can increase the growth of a plant by enhancing its resistance to phytopathogens, water retention capacity, and nutrient uptake (Evelin et al. 2009; Berendsen et al. 2018). The beneficial relationship between plant and rhizobiome has been studied comprehensively for biological control, promotion of plant growth, and activities in biogeochemical cycles. All these three play an essential role in maintaining the good health of a plant. Some of the microbes in rhizobiome have been proven to be highly useful for plants especially during unfavourable and harsh growing conditions (Meena et al. 2017). Thus, there is an increasing importance of rhizobiome conservation in agriculture as a potential method for achieving sustainability along with higher productions (Igiehon and Babalola 2018).

The importance of rhizobiome in agriculture can be demonstrated through their two sets of activities. First set of activities consists of functions and processes that change the dynamics of a plant's surroundings, and edaphic and climatic conditions, bringing indirect effects, and the second set of activities consists of functions and processes carried out by the microbes of rhizobiome that have a direct effect on plant systems. Many microbes present in the rhizosphere are capable of degrading soil contaminants and are often used as bioremediators (Dubey et al. 2020). Different metabolic activities of different microbes of rhizobiome increase the availability of nutrients such as nitrogen, phosphorous, and potassium. These two actions of rhizobiome microbes aid in increasing the soil fertility and productivity and ultimately the growth and health of a plant (Lu et al. 2019). One of the prime examples of rhizospheric microbe, which increases the availability of nutrients in the soil, is *Rhizobium*. They fix the inert nitrogen present in the atmosphere to the soil and convert them to ionic forms that are readily available to the plants for uptake. This is achieved by the microbe through a series of chemical processes that subsequently provide nitrogen to plants in an immobilized form (Leghari et al. 2016). Its importance in the sustainable approach of making nitrogen available to crop plants is very well established (Zilli et al. 2020). Many microbes also convert unavailable phosphorus to available forms through the actions of organic acids and enzymes. In addition to this, the beneficial microbes of rhizobiome also eliminate and reduce the population of phytopathogenic microbes (Wang et al. 2015; Lu et al. 2019; Peng et al. 2020). This is achieved through their antagonistic actions against the phytopathogenic microbes, which comprise antibiosis, parasitism, and competition for space and resources (Cabot et al. 2018; El-Sharkawy et al. 2018).

Direct effects on crop plants by the beneficial microbes of rhizobiome are through the production of phytohormones, enzymes, siderophores, and induction of defence responses in plants. Beneficial microbes like *Trichoderma*, which is a part of many plant's rhizobiome, is known to produce auxin, gibberellins, and 1-aminocyclopropane-1-carboxylate (ACC) deaminase that embellish the growth of a plant (Jaroszuk-Ściseł et al. 2019). *Trichoderma* spp. have also been known to induce production of peroxidases, polyphenol oxidases, chitinases, and β-1,3-glucanases in crop plants, which help in defending them against the phytopathogens (Baiyee et al. 2019). Some of the microbes also induce the expression of pathogenesis-related genes in crop plants, which also helps in the same (Sun et al. 2018). The endophytic constituents of rhizobiome form a symbiotic association with the host plant and directly provide nutrients and water to plants (Spagnoletti et al. 2017; Stürmer et al. 2018). The beneficial bacterial population of the rhizobiome also acts as plant-growth promoters through many actions that have direct effects on plants (Jogaiah et al. 2016; Patil et al. 2016; Misra et al. 2017). Many of the plant-growth-promoting rhizobacteria (PGPR) also increase the content of alkaloids in plants, which helps in warding off the ill-effects of abiotic stresses and simultaneously increasing the nutritional status (Rajasekar and Elango 2011; Ghorbanpour 2013). Talking on the front of the phytopathogenic component of rhizobiome, regulation and manipulation of their presence and population can bring undoubted improvement in plant health.

Since a myriad of microbes form the rhizobiome of a plant and have a significant effect on the plants as well as the soil, a deep understanding and deciphering of the underlying mechanism would certainly help in improving the production in agriculture along with achieving sustainability. This would open many avenues for ensuring global food security as well (Xie et al. 2019). Framing of crop plant breeding programmes for breeding a genotype that can recruit or change the dynamics of its rhizobiome according to the available environmental conditions is a booming prospect (Wei and Jousset 2017). This would help in achieving an environmental adapting genotype of crop plants through recruitment of rhizospheric microbes. Soil amendments and seed treatment with beneficial microbes is also another approach that can lead to a changed structure of rhizobiome of a crop plant and that has been proven to be effective in increasing the yield and adaptation to several abiotic and biotic stresses. These augmentation techniques bring beneficial changes in plant–microbe interactions and lead to the establishment of an associative and symbiotic relationship. The rhizospheric microbes that act as bioremediators will certainly help in the alleviation of degraded agricultural lands. Additionally, many unproductive and unfertile lands can be turned into productive and fertile agricultural fields by the application of beneficial microbes present in the plant's rhizobiome. They can either work in association with their host plant (generally) or alone (exceptions) in making the soil fertile and productive. The rhizospheric microbes with biological control activities can be utilized as a potential tool for the management of various phytopathogenic diseases in crop plants and to reduce the dependence of the farming community on synthetic pesticides.

3.3 Understanding the Rhizosphere Ecology, Biology, and Microzone

Total microorganisms present in a community has been considered as microbiome (del Carmen Orozco-Mosqueda et al. 2018). Similarly, all the microorganisms present in the rhizosphere constitute the rhizosphere microbiome. The complex community present in the rhizosphere can be treated as plant's secondary genome, which contains microorganisms as well as other genetic elements affecting plant health (Berendsen et al. 2012). The functioning of rhizobiome for plant growth promotion and defence is not well explored due to the unavailability of suitable tools and techniques. Several efforts have been made to understand the role of rhizobiome, such as the use of the latest sequencing techniques for the metagenomics of the rhizospheric soil (Turner et al. 2013; Schlaeppi and Bulgarelli 2015). Sequencing techniques along with metabolomics, metagenomics, proteomics, metatranscriptomics, and transcriptomics can be helpful to understand the microbial diversity of the rhizosphere. Furthermore, these techniques can also unravel the relationship between plants and microbes present in the rhizosphere for plant health and plant protection.

Microorganisms present in the rhizobiome can be divided into two groups: either beneficial or non-beneficial. Human pathogenic bacteria can make an inter-kingdom jump due to the presence of the microsites on plants (Holden et al. 2009). The genotype of the plant, species of the plant, and the constituent of the soil determine the recruitment of specific microbes in the rhizosphere (Bakker et al. 2012). A few reports (Mendes et al. 2013; Lakshmanan et al. 2014) suggested the role of malic acid in the stimulation of *Bacillus subtilis* in the rhizosphere. Rhizobiome can influence the plants in several ways as there is the existence of both beneficial and non-beneficial or pathogenic microorganisms (Liu et al. 2015). Bever et al. (2012) suggested the existence of both positive and negative associations between plant and rhizospheric microorganisms.

3.4 Assessing the Rhizobiome in Different Ecosystems

Several microorganisms are associated with the rhizosphere of the plant, collectively known as rhizobiome. These microorganisms are involved in nutrient acquisition for plants or acquiring nutrients from the plant, impacting the plant health positively as well as negatively. Several factors such as plant genotype, species of plant, and rhizosphere environment are affecting the recruitment of these microbes. Several reports (Gottel et al. 2011; Shakya et al. 2013; Cregger et al. 2018) suggested the use of next-generation sequencing for the study of rhizobiome. The presence of fungi and bacteria in the rhizosphere is influenced by the host species (Bonito et al. 2014). The genotype of the plant more prominently govern the recruitment of the microbes (Bálint et al. 2013; Cregger et al. 2018) in the above-ground part of the plant tissues, including leaves and stem.

Soil is the major factor governing the composition of *Populus* rhizospheric microorganisms such as fungus and bacteria (Bonito et al. 2014; Cregger et al. 2018). Major diversity of cellulolytic and antagonistic organisms including *Trichoderma viride* and *Humicola grisea* was found around the plant rhizosphere. Several findings (Kaushal et al. 2018; Ahmed 2018) reported the role of *Trichoderma* species in the biological control in the natural agricultural systems. The presence of *Trichoderma* species in the rhizospheric soil reduces the soil-borne pathogens and strengthens the plant against the pathogens present in the foliar region (Oancea et al. 2017). The reports are suggesting the role of these beneficial microorganisms in the improvement of soil texture, which ultimately improves soil aeration as well as strengthens the plant resistance against biotic and abiotic stresses. Fungi have the ability to mobilize the nutrient as well as to help in the nutrient uptake due to the production of the enormous hyphal network around the plant roots (Garay-Serrano et al. 2018).

Several microbes found in the region of Babadag plateau in Rendzic Leptosols were reported having the ability to help in soil formation by nutrient recycling, decomposition of the cellulose as well as humus formation. The secondary metabolites secreted by the microbes in the rhizosphere help in the aggregation of soil and make the soil environment favourable for the plant growth. Few of the reports suggested the role of beneficial microbes for selective suppression of the pathogenic microbes in the rhizospheric soil (Alabouvette and Steinberg 2006). Different modes of interactions are present in the rhizosphere with microbes including symbiosis, predation, and competition. The role of edaphic microorganisms was also suggested (Wieder et al. 2013) for carbon sequestration and controlled emission of CO_2. Microbial secondary metabolites contain antimicrobial actions as well as several enzymes that can be used commercially for biotechnological purposes (Marco et al. 2003; Mapari et al. 2005). Beneficial microbes can be used for the biocontrol purpose, which could ultimately reduce the use of hazardous biochemical (Hanson and Howell 2004; Namdeo 2007; Matei and Metei 2008).

We can talk about the study on seagrass, which are marine flowering plants found in the intertidal and shallow subtidal zones, to know about the microbial structure of this ecosystem. These plants are mainly responsible for the coastal ecosystems by serving as a food source, space for the animals, stabilizing sediments, and nutrient recycling. The root zone of the plant contains both the beneficial and non-beneficial microbes. Different species of this plant contain different kinds of microbial diversity in their respective rhizospheres and it was suggested that the rhizobiome includes mainly bacteria related to the sulphur cycle and is affected mainly by the plant metabolism. The rhizospheric ecosystem of solanaceous plants is one of the important ecosystems for the agricultural system. Two plants including tomato (*Solanum lycopersicum*) and chilli pepper (*Capsicum annuum*) are of major economic importance. These plants are known to exhibit different microbes in their rhizosphere, involved in governing the host plant health and its productivity.

The assembly of rhizobiome has been proposed to take place in two steps. First, the rhizosphere is colonized by a subgroup of the soil microbial community inhabiting the bulk soil, and then the rhizoplane and the endosphere is colonized by the subgroup of the earlier colonized rhizospheric microbial community. All the microbes that get associated with the plant form a core microbial community of the rhizobiome. This core microbial community has both fungal and bacterial species that are common to many species of plant (Sasse et al. 2018; Olanrewaju et al. 2019; Ávila et al. 2019). The accumulation of microbes from the bulk soil community is due to the richness of nutrients in the rhizospheric region by the accumulation of exudates secreted by plants, which consist of sugars and amino acids. These sugars and amino acids serve as the source of carbon and nitrogen for the microbes due to which they get attracted to the rhizosphere. According to the ‘cry for help’ hypothesis, plants also secrete certain unique metabolites or some general metabolites in large concentration to recruit the soil microbes that help them in the alleviation of stress faced by plants in a particular time and place (Rolfe et al. 2019). The microbes of rhizobiome which settle on the root surface of the plant or the rhizoplane generally mobilize the inorganic nutrients from their immobilized forms and also fix those present in the atmospheric region which indirectly helps in plant growth promotion (Lopez et al. 2011). The microbes of the rhizobiome that enter the intercellular space of root tissues in a plant without harming the plant are known as endophytes. They induce resistance to phytopathogens by modulating the defence genes of plants (Passari et al. 2016; Sasse et al. 2018).

In the earlier microbiome studies pertaining to agriculture, the main focus was to find the influence of different packages of practices on the soil microbial complex and generally excluded the factors related to the host genotype and those related to the interaction of agricultural practices and host genotype (Gomez-Montano et al. 2013; Hartmann et al. 2015; van der Heijden and Hartmann 2016; Poudel et al. 2019). In advance studies, the effects of host genotypes were also included and it was observed that there is a significant variation in the structure and composition of rhizospheric microbial complex depending on the genetic background of a plant in many crops like maize, rice and *Arabidopsis* (Bulgarelli et al. 2012; Lundberg et al. 2012; Peiffer et al. 2013; Edwards et al. 2015; Lebeis et al. 2015; Mendes et al. 2018). These variations in the rhizospheric microbial complex can be explained physiologically due to the varying nature of root exudates and rhizodeposits of a plant species, which is regulated by the genetic constituents (Nasholm et al. 2000; Uren 2007; Reeve et al. 2008). Through this varying nature of root exudates and rhizodeposits, plants provide specific cues that recruit selected microbes from the bulk soil microbial community (Zhalnina et al. 2018; Beattie 2018). The root exudates and rhizodeposits also contain certain molecules that deter away the pathogenic or non-beneficial microbes (Hassan and Mathesius 2012). The findings from these advanced studies suggested that there is a host-specific microbiome filter that functions actively in the selection and recruitment of specific microbes in its rhizobiome. The rhizobiome, thus, varies from plant to plant and genotypes to genotypes (Lundberg et al. 2012; Edwards et al. 2015; Panke-Buisse et al. 2015; Fonseca-García et al. 2016; Wang et al. 2017; Cregger et al. 2018).

3.5 Effect of External Parameters on the Rhizobiome

Rhizobiome determines the overall wellbeing and productivity of the plants (Lakshmanan et al. 2014). It prompts various functioning expressions in plants like productivity, robustness, and fecundity. Inspecting plant rhizobiome assists in knowledge of the interaction between plant and rhizobiome along with genetic and functioning of the host and key metabolic and physiological facets of rhizobiome complex (Rout and Southworth 2013). The interconnection between the plant and rhizobiome needs a bridge that connects them like root exudates and soil parameters. This assists plants in interacting with the rhizobiome affecting the plants significantly (Bais et al. 2006). The rhizobiome reacts to the root exudates acting as a signalling molecule. Exudates comprise of various components like mannose, arabinose, glucose, amino acids, fatty acid, nucleotides, auxin, enzymes, alkaloids, flavonoids, and vitamins (Gunina and Kuzyakov 2015; Hayat et al. 2017). Besides these compounds, other key carbon compounds produced from plant roots are carbon specified by photosynthesis (Bamji and Corbitt 2017). The microbes release consequential compounds modifying the mechanism of plant and signals aid in acquiring nutrition (Brazelton et al. 2008; Kim et al. 2011).

The symbiotic association between host plant and the microbes of the rhizobiome has been studied; their interaction, progression, and ecology of symbioses have not been studied extensively. The rhizobiome plays elementary role in nutrition of the plants. The root-associated fauna plays important role in advancement of plants and the associated biosphere (Fitzpatrick et al. 2018).

3.5.1 Overview of Rhizobiome Structure in Agricultural Fields

The investigation of the rhizobiome will magnify the understanding of the growth and development of the plants (Mendes et al. 2013). For instance, methanogens are usually existing in the geographical areas where paddy is cultivated. The knowledge of this fact might enhance the possibilities involved in the methane cycle. Other than this, it also gives knowledge about other correlated microbes of paddy along with other flora present (Edwards et al. 2015). It was noticed that the diversity of the microbes in the paddy field has a remarkable impact on the field. The rhizospheric studies reveal that rhizobiome is immensely affected by the site of cultivation. It is evident that with different plant attributes the microbes in the vicinity of the root system also differ, i.e. attributes differ among endosphere and rhizosphere systems. The rhizospheric variegation was found emphatically linked to the productive capacity of the host plant and obstructively related to the stretch of the rootstock whereas the diversification of the endospheric complex was found related to the density of root hair (Fitzpatrick et al. 2018). The kind of soil along with the environment affects the structure of the rhizobiome. Other than this, the genotype of the host and root exudates impact the rhizospheric complex (Haney et al. 2015). The microflora of the rhizobiome harmonize with the plants in the ecosystem, so they might have coexisted in agreement to the requirements of one another for being compatible in the ecological system (Philippot et al. 2013).

By modifying the constituents of the exudates of the plant, roots stimulating the liberation of an increased quantity of beneficial exudates through re-programming of plant microbes can enrich the rhizobiome (Přikryl et al. 1985; Bulgarelli et al. 2012). Hence, the antimicrobial and secondary metabolites play important role in establishment and competition for space within the rhizobiome. To achieve better communication among rhizobiome, plant and environmental conditions, competition for supremacy and establishment is important. Paddy is grown in submerged soil. The oxic areas are developed in the rhizosphere. It is adjoined by anoxic soil due to the release of O_2 by the means of aerenchymatous cells present in the root system of rice plants (Yuan et al. 2016; Zhao et al. 2019). The oxic and anoxic complexes thus developed influence the microbial community (Revsbech et al. 1999; Li and Wang 2013). The studies on *Brachypodium* and maize revealed that the type of microbial community differs with the type of root system (Kawasaki et al. 2016; Yu et al. 2018). Even microbial complex may differ with various tissue of the plant.

3.5.2 Factors Related to the Development of Rhizobiome

Soil, surrounding environment and host plant influence the formation of the rhizobiome; for example, the microbes including fungi and bacteria differ with the type of soil and root system in *Populus*. The microorganisms of a particular taxon are not linked to the genotype of *Populus*. The studies have revealed that microbes are from the order Burkholderiales, Rhizobiales, Chitinophagales, and Cytophagales (Bonito et al. 2019). The experimental studies on the rhizobiome of *Populus* dissociate the genotype of the plant under the impact of structure and properties of soil affecting the microbial communities (Bonito et al. 2019). It was also evident that the texture of the soil, geographical area and extent of phosphorus are positively related to the microbial communities. This reveals that the taxon of the microbes is not the same all around the landscape and the rhizobiome is affected by soil and ecological conditions. It became obvious from different studies that variability between rhizospheric and endospheric communities of *Populus deltoides* was because of variations in topographic, climatic, and habitat conditions. Such results were also found in *Pinus*, *Arabidopsis,* and *Agave* (Lundberg et al. 2012; Talbot et al. 2014; Fonseca-García et al. 2016). It was also noticed that the rhizomicrobiome of a plant is affected by the genotype and species of the host (Bonito et al. 2014). The above- and below-ground parts have different impacts on the microbiome (Bálint et al. 2013; Cregger et al. 2018).

3.5.2.1 Effect of Vermicompost on Rhizobiome

The organic matter available in the soil comprises of microbes. The microorganisms present in the soil react to functions of the surrounding environment and soil like nutrient cycle, putrefaction, and suppressing of pathogens (Doran and Zeiss 2000). The amount of organic content checks the activity of the microbes, including more amounts of nitrogenous compounds in soil. Applying vermicompost enhances biomass and diversification of the microbes in the soil. Vermicompost along with earthworm casts provides a magnificent channel to harbour bacteria for nitrogen fixation in the soil. There was a significant increase in Gram-negative bacteria following application of increased dosage of vermicompost (Lazcano and Dominguez 2011). A generous amount of vermicompost enhances the community of microorganisms like *Bacillus stearothermophilus*, *Azotobacter chroococcum*, and *Pseudomonas putida*. The microbial activity activated by vermicompost intercepts leaching of the nitrogen.

3.5.2.2 Effect of Biochar on Rhizobiome

Soil biome is essential for proper processing of ecosystem functions. Biochar addition affects the biome of the rhizosphere by enhancing the structure and functions of the microbial population (Steiner et al. 2008; Hammes and Schmidt 2009; Hongyan 2010; Liang et al. 2010; Chintala et al. 2014). An increased amount of colonies of *Trichoderma* and *Bacillus* was observed upon application of biochar (Graber 2009). The application of biochar makes the soil pH appropriate for the microbial communities (Wuddivira et al. 2009). It provides a satisfactory domain for colonization, reproduction, and growth ability for rhizobiome (Thies and Rillig 2009).

3.5.2.3 Effect of Chemical Pesticides on Rhizobiome

The application of pesticides might change diversification of the microbes in soil resulting in deterioration of soil fertility (Lo 2010). The application of pesticides contaminates the soil making it noxious to rhizobiome (Aktar et al. 2009). Arbuscular mycorrhiza boosts doorway for water and minerals for the plant, enhancing its drought sufferance etc. Application of herbicides, however, turns down the establishment of arbuscular mycorrhiza (Druille et al. 2013). The soil biome is damaged by the application of herbicides (Nicolas et al. 2016). The pertinacious ability of herbicides in soil is hazardous to the biome of rhizosphere, which is advantageous to crop productivity (Thiour-Mauprivez et al. 2019). These noxious chemicals contaminate the soil and water resources (Noshadi and Homaee 2018). A reduction in community of microbes was observed after applying herbicide (Silambarasan et al. 2017) (Table 3.1).

3.6 Conservation Strategies for Enriching the Soil with Useful Rhizobiome

Rhizobiome that has been reported to increase plant growth, development, and yield is influenced by various factors such as climate change, plant cultivar, anthropogenic activities, and soil types. Even the age of the plant also has an influence on the rhizospheric microbiome (Chaparro et al. 2014). In anthropogenic activity, we use chemical fertilizers for the fulfilment of nitrogen, which is directly linked with environmental degradation greatly affecting the plant rhizobiome. We can replace/reduce the chemical nitrogen-based fertilizers by using the nitrogen-fixing free-living and endophytic rhizobacteria that can help secure the rhizospheric microbial communities. Different species of *Rhizobium*, *Bacillus,* and *Azospirillum* have been reported to enhance the biomass of plants in case of both above and below ground, which ultimately results in a positive impact on the crop for sustainable agricultural production (Igiehon and Babalola 2018).

The rhizobiome of a plant is less diverse than that of the surrounding soil and the microbial diversity mainly leads a narrow bacterial lineage. A study of rhizobiome associated with dicots and monocots, viz. *Arabidopsis* (Bulgarelli et al. 2012; Lundberg et al. 2012), sugarcane (Yeoh et al. 2016), maize (Peiffer et al. 2013),

Table 3.1 Chemical effect on different rhizospheric microorganism

S/N	Chemical type	Name of chemical input	Effects	References
1	Herbicide	Glyphosate	Toxic to soil fungus *Aspergillus nidulans*	Nicolas et al. (2016)
2	Herbicide	Glyphosate	Reduces the spore viability of arbuscular mycorrhizal fungi (AMF)	Druille et al. (2013)
3	Herbicide	Glyphosate	Increased frequency of the soil-borne fungus *Fusarium solani*	Sanogo et al. (2000)
4	Fungicide	Mancozeb	Total fungi, actinomycetes and *Pseudomonas* bacteria were significantly reduced	Magarey and Bull (2003)
5	Herbicide	Atrazine, pendimethalin	Lower microbial population	Silambarasan et al. (2017)
6	Herbicide	Pendimethalin	Significant reduction of soil microbe population	Nalini et al. (2013)
7	Herbicide	Triclopyr	Inhibits soil bacteria that transform ammonia into nitrite	Pell et al. (1998)
8	Insecticide	Methamidophos	Significantly decreases microbial biomass by 41–83%	Wang et al. (2006)
9	Herbicides	Glyphosate	Reduces growth and activity of free-living nitrogen-fixing bacteria in soil	Santos and Flores (1995)
10	Herbicide	2,4-D	Inhibits the transformation of ammonia into nitrates by soil bacteria	Martens and Bremner (1993)
11	Fungicide	Butachlor	Reduced population of *Azospirillum* and aerobic nitrogen fixers in non-flooded soil	Lo (2010)
12	Insecticide	Fenamiphos	Detrimental to nitrification bacteria	Lo (2010)

rice (Edwards et al. 2015), oak (Uroz et al. 2010), lettuce (Schreiter et al. 2014), and barley (Bulgarelli et al. 2015), reveals the presence of dominant phyla (especially proteobacteria and actinobacteria) and certain bacterial lineages found consistently more in the rhizobiome. The soil type is a key factor in determining the rhizobiome because of the plant recruiting the rhizobiome first and foremost from the soils they reside (Bulgarelli et al. 2013; Sarma et al. 2015). The molecular survey of culture-independent rhizobiome reveals the influence of soil type on microbial community being stronger than that of the host plant. However, such types of research have mainly been performed on model crops (Yeoh et al. 2017). Further, the secondary factor other than the soil type is plant host phylogeny that influences rhizobiome, but the effect is particularly less than soil type (Bulgarelli et al. 2012; Lundberg et al. 2012). Similarly, the comparison of rhizobiome in maize, wheat and sorghum (monocots) (Bouffaud et al. 2014) and *Arabidopsis* and *Cardamine hirsuta* (eudicots) (Schlaeppi et al. 2014) reveals the major variation in rhizospheric microbial communities of the two outlying plant species. Hence, it is clear that the host phylogeny of plants also has greater influences on rhizobiome composition. Overall, the research exhibits the establishment of core rhizobiome before the evolution of various plant lineages and these microbial communities evolved themselves with their host plants, and the functions of rhizobiome are also found conserved. In another study, the independent root and endospheric metagenome showed similar functionality such as bacterial motility, protein secretion system, iron acquisition, and nitrogen metabolism in rice, wheat, and cucumber (Sessitsch et al. 2012; Ofek-Lalzar et al. 2014). In the findings of these studies, we get a list of bacterial lineages for further research in their plant–microbe interactions accompanying rhizobiome recruitment, function, persistence, turnover etc., and the knowledge about these things might be helpful to enhance crop production (Yeoh et al. 2017). Hence, the knowledge that the interaction of plant-phylogeny-related rhizobiome and soil type influenced rhizobiome becomes more significant to conserve rhizobiome, so that the benefits in crop productivity can be enhanced (Sarma et al. 2015).

The conservation practices for a particular plant and related rhizobiome would be helpful to enhance plant health and production. Among them, certain practices are linked with plant processes that influence their growth. Further, the plants can be manipulated to change the pH of rhizosphere, release compounds to improve nutrient availability and enhance the growth of rhizobiome by protecting them from various abiotic and biotic stresses. Similarly, the rhizobacteria present in rhizobiome that are beneficial in plant growth can be engineered to regulate the synthesis of plant hormones that are helpful in plant growth and production of various antibiotics and enzymes to cope up with soil-borne diseases. Rhizobiome conservation is also assisted by the plant because plant can modify the beneficial microbial communities to enhance the proliferation of certain antibiotic-producing strains that make soil suppressive to soil-borne diseases. The fitness and richness of rhizobiome can be improved by soil reclamation that permits the selection of microbial consortium against the various soil-borne pathogens. The amendment in the plant through genetic engineering also plays important role in influencing the rhizobiome, and the best example is quorum quenching mechanism that inhibits the virulence in *Pectobacterium*.

3.6.1 Conservation Through Manipulation of Rhizospheric pH

The rhizospheric pH plays an important role in the conservation/richness of the rhizobiome. The pH of rhizospheric soil is greatly influenced by the exudates secreted from plant roots. The pH is a negative logarithm of H^+ concentration; that means a higher concentration of H^+ in the rhizosphere lowers the pH and vice versa. The proton efflux from the plant roots is governed by H^+-ATPase protein family that utilizes energy from adenosine triphosphate (ATP) to pump H^+ via plasma membrane against the electrochemical gradient. The H^+ efflux also contributes to the nutrient acquisition by solubilizing certain elements in the rhizosphere due to a lowering in pH (Hinsinger et al. 2003). Lowering pH creates local acidifications that lead to the enhanced availability of phosphorus and iron, which are generally fixed in the insoluble complex form in the soil. In dicots and monocots other than Gramineae family, the iron uptake involves the secretion of iron-chelating compounds that reduce the Fe^{3+} to Fe^{2+} before the uptake. There are various microbes present in rhizosphere that enhance the reduction process and make iron available in a soluble form that can be taken by plants. In contrast, grasses release the phytosiderophores for the same process and make iron available to the root surface for absorption (Neumann and Römheld 2007). Similarly, the release of organic acids, viz. malate and citrate, along with certain enzymes like phosphatases and phytases promote the growth of certain microbes to access hardly soluble inorganic and organic phosphorus (Dinkelaker et al. 1995; Vance et al. 2003; Ryan et al. 2009). These organic compounds' efflux is also responsible to protect certain crops and microbes from Al^{3+} toxicity by chelating Al^{3+} ions in acidic soil and thus preventing the damage at root apical meristem (Ryan et al. 2009). Hence, the release of elements and compounds from plant roots helps in plant growth by providing nutrients. The organic acids secreted from roots also help in rhizodeposition. These root exudates, thus enhance nutrient acquisition, tolerance to mineral stresses and stimulate the growth of beneficial microbes by conserving the rhizobiome.

3.6.2 In Situ Conservation of Rhizobiome

In situ conservation of rhizobiome is the conservation of microbial diversity present in rhizobiome protected in their natural environment, i.e. in various geographical locations that are defined as hotspots. The deep-water rice, copper mine wasteland, Prairie plants and agronomic crops, pea cultivar in field conditions, grass ecosystem and Polar regions are the examples. Conservation, in natural areas, of populations of high-valued microbes is the basic condition for conservation of the rhizobiome. The strategies for conservation of certain endophytic species of plant genus *Zea* were explored and it was found that the domestication of its wild ancestor (teosinte) to current maize (corn) evolved from Mexico to Canada. The study revealed the presence of core microbiota conserved in *Zea* seeds during the evolution (Johnston-Monje and Raizada 2011). In another molecular study in *Silene paradoxa*, the bacterial communities of seeds showed the transfer of endophytic bacterial community into the next generation of the plant. Hence, this study sets an example that specific plants from a particular location might be helpful in in situ conservation of endophytic microbial community (Mocali et al. 2017). In recent past, in situ conservation provides the opportunity to reveal greater diversity of microbes in the environment, where the total and culturable rhizobiome of wheat plant is conserved in microwell chambers (MWCs) (Acuña et al. 2020). Therefore, in situ conservation can be an appropriate means for the conservation of rhizobiome.

3.6.3 Ex Situ Conservation of Rhizobiome

Ex situ conservation of microbial diversity means the conservation of microbes and their factors outside their natural environment. The factors mean the gene banks, man-made wild field banks of microbes with plants, artificial propagation of plants with their rhizobiome, botanical gardens and microbial culture collections. Ex situ conservation is involved in the conservation of genetic resources of microbial communities, and protects them from extinction.

The establishment of microbial culture collection was started in the late nineteenth century and microbes were isolated on various growth media in pure culture and maintained in the laboratory with the mandate distribution of microbial strains for the purpose of research (Smith 2003). The idea for establishing the culture collections is valuable due to the availability of microbes for basic research and agricultural purposes. The repository or culture bank is useful in exploring the evolutionary and metabolic patterns of important microbes that play a major role in industry, agriculture, and health divisions (Sharma et al. 2017).

3.7 Impact of Rhizobiome on Soil Enrichment Along with Plant Growth and Development

The microbial diversity present in the rhizosphere of a plant is considered as rhizobiome, which normally helps in better growth of the host plant. The rhizobiome present in the rhizospheric region of plants can be influenced by various signalling mechanisms between the plants and microbes as per the requirement. The rhizospheric microbial community enhances nutrient absorption in plant roots and also increases the availability of nutrients from distant sources directly and indirectly (Vessey 2003). Various rhizospheric microbes oxidize manganese in the rhizosphere to reduce the toxicity of manganese in the plant grown in less oxygen conditions in saturated soil. However, few of them also improve manganese availability in aerated soil with high calcium carbonate content (Babalola 2010). Rhizospheric microbes are the major part of PGPRs, which colonize the plant roots and promote plant growth by nutrient absorption and reduction of plant pathogens (Babalola 2010). PGPRs also act as biofertilizers other than by enhancing the indigenous microbial diversity without any negative effects or contamination in soil, unlike conventional agriculture that uses chemical fertilizers (Conway and Pretty 2013; Rascovan et al. 2016). However, each PGPR does not necessarily act as a biofertilizer, e.g. those enhancing plant growth indirectly such as by reducing pathogens and pests cannot be considered biofertilizers as such. However, certain PGPRs act as the agents for both, increasing the plant growth directly as well as indirectly through suppression of phytopathogenic microbial community. For example, *Burkholderia cepacia* reduces growth of the phytopathogen *Fusarium* spp. and also enhances maize growth in iron-deficient conditions by siderophore production (Bevivino et al. 1998). Certain arbuscular mycorrhizal fungi (AMF) act as pesticides (Babikova et al. 2014). The rhizobiome thus influences the growth of above- and below-ground biomass of plants (Uma et al. 2013).

Soils under different environmental conditions vary in their structure, pH, nutrient status, organic matter, and texture. The soil type is also responsible for various activities of rhizobiome present in the plant rhizosphere. The soil pH and availability of organic carbon greatly influence the growth of various plant pathogenic nematodes, fungi, bacteria, and beneficial microbes (Rotenberg et al. 2005; Toljander et al. 2008; Dumbrell et al. 2010). The beneficial microbes help the plant by making a symbiotic relationship and support acquisition of nutrients like nitrogen and phosphorus. Other than N and P, iron is an additional nutrient that can be provided to plants by symbiotic relationship with rhizobacteria. However, iron (III) is present in more quantity in soil but plants absorb iron (II) in its reduced form (Salisbury and Ross 1992). Microbes produce siderophores that chelate iron (III) and make it available at the root surface where it is reduced to iron (II) and absorbed immediately (von Wirén et al. 2000). The rhizobiome plays a crucial role in the activation of plant defence against various invading pathogens by stimulating phytohormonal signalling. The microbial communities belonging to bacteria (*Burkholderia*, *Pseudomonas*, *Bacillus,* and *Erwinia*) and fungi (*Trichoderma*, *Aspergillus,* and AMF) are reported to solubilize and mobilize P, Zn, and K in the form that can be easily absorbed by plants (Sudhakar et al. 2000). The P absorption plays an important role in the proper functioning and development of plants from the reservoir of non-soluble form in the soil. The rhizobiome solubilize them and makes them available to the plant (Hameeda et al. 2008; Richardson et al. 2009).

Apart from nutrient enrichment and protection from pathogens, the rhizobiome also plays a significant role in the reduction of various abiotic stresses of the plant. Salt, drought, cold, high temperature, and heavy metal toxicity are the most important abiotic stresses that greatly influence plant growth, development, and yield. The study reveals that the activity of various microbes from the rhizobiome helps in stimulating the plant response that is effective against these abiotic stresses. In recent years, the use of microbes is more focused on such types of rhizobiome that assist plant growth and development under stress conditions. This emerging strategy of application of rhizobiome that promotes plant health and copes up with abiotic stresses is gaining global attention. Thus, the rhizobiome of a particular plant not only enriches the soil with nutrients but also protects plants from various abiotic and biotic stresses and promotes better development and yield of the crop.

3.8 Future Aspects

Modernization of agriculture practices poses a severe threat to the microbial community along with some anthropogenic activities including climate change. The role, importance and ecology of the microbiome are emphasized in this chapter along with conservation strategies, highlighting the need to educate the farmers to incorporate such practices for the sake of making their farming sustainable. These microbes can play important roles in biogeochemical cycling by providing the atmospheric N, unavailable phosphorus, facilitating uptake of water and nutrients, making the otherwise unavailable micronutrients available, improving the crop health by reducing the impacts of the biotic and abiotic stresses, and thus enhancing the plant growth and yield of the crop. Ultimately, conservation will help in sustainable farming practices, which is an approach of tremendous importance in enhancing gains through better crop health, and achieving food security and conserving the microbiota simultaneously to ensure sustainability in the business of farming.

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Exploring the Rhizosphere Microbiome for Sustainable Agriculture Production

4

Anamika Dubey and Ashwani Kumar

Abstract

Most published research shows that the vast variety of microorganisms present below ground affects the above-ground biodiversity. The rhizosphere is considered the most complicated microbial habitat present on the earth's surface, often referred to as the secondary genome of the plant. Recent evidence also supported that soil microbial diversity plays a key role in determining terrestrial ecosystems' evolutionary responses and ecology to ongoing environmental changes. Recent advances in next-generation sequencing have improved the discipline of metagenomics, allowing researchers to get insight into the lives of previously uncultivable microbes. Metagenomics techniques are crucial for elucidating their taxonomic and functional potential and evaluating plant–microbe interactions to improve plant performance in the face of various stressors and to ensure long-term crop production. This chapter highlights key knowledge gaps in rhizosphere biology, summarizes current plant–microbiome engineering researches, and suggests future research directions. Additionally, this chapter emphasizes the need of using metagenomics and bioinformatics methods to get a better understanding of the plant microbiome.

4.1 Introduction

The rhizosphere is the first plant-influenced habitat encountered by the groups of microbes present inside the soil, and therefore it is a narrow zone of contact between the plant roots and soil particles (Dessaux et al. 2016; Kumar and Dubey 2020).

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Majorly, every part of the plant is colonized by varieties of microbes like bacteria, fungi, and viruses, collectively referred to as phytomicrobiome or plant microbiome. Therefore, the rhizosphere microbiome plays a pivotal role in maintaining plant health, providing defense against pests and diseases, facilitating nutrition acquisition, and helping the plant to withstand various stresses (Soni et al. 2017; Kumar and Dubey 2020). The relationship between microorganisms and plants is intimate in so that the microbial population that inhabits and colonizes the rhizosphere, rhizoplane, endosphere, and phyllosphere of plants is regarded as the secondary genome of the plant (Berg et al. 2014). The rhizosphere microbiome contains a huge diversity of microbes that interact with each other in positive and negative ways, just as gut and skin microbial populations impact human health, rhizosphere microbial communities impact plant health. The plant and its microbiota are called as holobionts (Lakshmanan et al. 2014; Dessaux et al. 2016). The positive interactions constitute a symbiotic association between roots and mycorrhizal fungi, epiphytes, and plant growth-promoting rhizobacteria (PGPR). Negative interactions include parasitism by groups of bacteria and fungi and competition created by a variety of herbivores.

Evidence suggests that only a small percentage of microbes on the planet (approximately 1%) can be cultured; this illustrates our limited understanding of the structural and functional diversity of the microbial world, as well as the vast emporium of potential medicines and useful industrial products that exist virtually intact in the microbial world (Soni et al. 2017). The root is another essential part of the plant that forms the interface between the plant and the soil environment. Roots release various exudates that interact and cooperate with microorganisms inhabiting soil (Reinhold-Hurek et al. 2015). Important questions need to be answered about these components that contribute to the soil-selective microbial enrichment of the area around the roots or the rhizospheric area (Bais et al. 2006; Doornbos et al. 2012). Previously, carbohydrates were recognized as the general chemical determinants in the rhizosphere, but now amino acids are also considered as chemical determinants present in the rhizosphere (Moe 2013; Soni et al. 2017). Various flavonoids and other secondary metabolites produced by plants were identified as key drivers for the successive formation of host-specific microbial communities in the rhizospheric zone (Weston et al. 2015; López-Ráez et al. 2017). Therefore, the promising avenue for engineering the rhizosphere microbiome is to take the meta-organism into account and try to optimize the whole system instead of optimizing each part separately. The studies conducted by different workers help us to understand the role of these PGPR or plant growth-promoting bacteria (PGPB) isolated from the rhizosphere of the plants for improving health and performance under different types of stress conditions (Ahmad et al. 2012; Kumar et al. 2013, 2015, 2016).

Recently, next-generation sequencing (NGS) technologies have made it feasible to study the immense microbial diversity in the rhizosphere in detail. Metagenomics or culture-independent method is a powerful approach for directly analyzing the total genomic content of microbial diversity from the environmental samples (Allan 2014). It is typically based on two approaches: (i) sequence-based approach, which involves sequencing of the entire DNA sample, after which the sample is assembled and annotated with a more targeted approach (e.g., 16S rDNA); and (ii) functional approach, which includes the construction of metagenomics libraries in a heterologous host, which are then screened for a particular function. Integration and analysis of the information obtained from both sequence-based and functional metagenomics approaches enable a more inclusive examination of the structural and functional analysis of microbial diversity than ever before.

The recent knowledge of the rhizosphere microbiome, its functions, and their promising biotechnological potential was summarized by several workers (Mendes et al. 2013; Berg et al. 2014). Many studies have shown the importance of metagenomics to the survey of uncultivable microbial communities in different parts of humans, but taxonomic and functional studies related to plants are still limited and rarely emphasized in detail. The main goal of this chapter is to mention the role of the cultivable and uncultivable microbial communities in the maintenance of plant growth, health, and productivity, including the induction of the concept of engineering of plant microbiome for sustainable crop production.

4.2 Modulators of the Rhizosphere Microbiome

Different parameters such as abiotic and biotic factors modulate the diversity of microbes and their composition in the rhizosphere. Hence, the levels to which these factors influence microbial communities are not entirely understood. The abiotic component include climate change, which is caused due to various anthropogenic activities, and biotic components include soil type and variety of pathogens that help in determining the composition of rhizosphere microbiome (Berendsen et al. 2012; Chaparro et al. 2012; Philippot et al. 2013; Spence and Bais 2013). Studies conducted by Caruso (2018) revealed that the soil helps in shaping the mycorrhizal communities in the rhizosphere. The type of root exudates helps to determine the structure of microbial communities and the physicochemical properties of the soil influencing plant health. Climate change causes different abiotic stresses like drought, salinity, and temperature (Prudent et al. 2015; Kashyap et al. 2017). It has been well documented that species of *Rhizobium* can tolerate dry environments, but their diversity is significantly reduced. However, the scarcity of water affects the nitrogen-fixing ability of rhizobium and the growth and development of leguminous plants (Kunert et al. 2016; Dubey et al. 2019a). Rapid economic and industrial growth has increased anthropogenic activities, a significant cause of pollution and degradation of the ecosystem that significantly affects the microbial community present in the soil. The structure of the indigenous communities of bacteria present in the soil gets homogenized due to the transformation of the forest into farmlands (Rodrigues et al. 2013). Hence, to overcome these harmful effects of climate change, we need to manipulate the rhizosphere microbiome of the plant so that it can withstand extremely harsh conditions (Dubey et al. 2019b).

4.3 Root-Associated Microbiome

Numerous researches were performed previously on plant–microbe interactions with several pathogens. In nature, plants are exposed to several pathogens and severely invaded by many pests and pathogens, leading to great economic loss in agriculture. To protect it, plants can trigger a sophisticated immune response. Furthermore, they recruit more microbes in their rhizosphere that positively affect a plant to improve its growth and boost its immune responses. Unraveling many vital issues regarding how the plant immune system symphonies interact with microbes at the biochemical and molecular levels will need more investigation into these plant–microbe interactions. Several previously performed experiments assessed the microbial diversity associated with different compartments of the plant. Still, only a few microbes are pathogenic though; most of the microbes inhabiting plant-related niches have positive interactions and promote plant survival and fitness (Mendes et al. 2013; Philippot et al. 2013). Outcomes of these researches would help develop imperishable approaches for developing disease resistance in next-generation crops that will help obtain enhanced yields with minimum use of fertilizers or pesticides (Malla et al. 2022). For the first time, scientists unraveled that microbes present in the soil impose physiological constraints on soil pathogens and suppress plant disease (Mendes et al. 2011). Impartially, the microbes present in the soil also communicate a certain degree of hostility to the system across "invaders," thereby relating the microbial diversity to its innate ability to inhibit or to restrict the survival of exotic organisms (Van Elsas et al. 2012).

Plants usually interact with microbes primarily with the help of their roots. The most intense interactions between plants and microbes occur at the rhizosphere, the interface between the plant's roots and the soil. Plant roots provide various compartments to the microbes at the root–soil interface, such as rhizosphere, endosphere, and rhizoplane, and at this interface, physical and chemical properties of soil change, which influences microbial population (Nihorimbere et al. 2011).

However, more habitats are colonized by various microorganisms, and many workers have characterized their activity in association with roots. In the rhizosphere, microbes play key roles in several vital ecosystem processes, such as sequestration of carbon (C) and cycling of nutrients (Singh and Cameotra 2004). Most terrestrial land plants form a close and intimate association with highly complex microbiota, including rhizosphere, phyllosphere, and endosphere.

Rhizodeposition fuels an initial substrate-driven community shift in the rhizospheric region, which has the greatest effect on the microorganisms there. These microorganisms connect the genotype of host-dependent fine-tuning of microbial profiles in the selection of endophytes and colonizing different root assemblages (Dubey et al 2019a). The substrate-driven selection also underlies microbial communities at the phyllosphere but exclusively at the immediate leaf surface. Microorganisms colonizing both roots and leaf surface areas contain microbes that allocate indirect protection against pathogens. However, microorganisms present in rhizospheric areas or roots appear to serve as additional host functions through the nutrients acquisition from the soil for the growth of the plant. Roots of the plants secrete 5–21% of carbon, which is photosynthetically fixed in the form of soluble sugars, vitamins, purines, inorganic ions, organic acid, amino acids, and some secondary metabolites; the bulk of compounds, like phytosiderophores and nucleosides; and the polysaccharide mucilage produced by root cap cell. Rhizodeposition accounts for about 11% of net photosynthetically fixed carbon, approximately 27% of carbon allocated to roots, and 10–16% of total plant nitrogen. However, these values vary greatly depending upon the species of plants and plant age (Jones et al. 2009).

4.4 Mycorrhiza and Plant Growth-Promoting Rhizobacteria (PGPR)

The association between arbuscular mycorrhizal fungi (AMF) with the roots of the plants constitutes more than 80% in terrestrial plants, different from those selective associations between leguminous plants and between *Rhizobium* species (Kumar et al. 2015). Phosphorus (P) and nitrogen (N) are the hindering factors for the growth and development of plants. Mycorrhizal symbiosis is the most prime and ubiquitous example of this type of interaction distinguished by the exchange of water and phosphorus for the plant in commerce of carbon for the fungus (Kumar et al. 2010, 2013, 2016). Hence, phosphorus is usually found in the form of strengite and variscite, often inaccessible to the plants even though it is found abundantly in the soil, while calcium is found in apatite in alkaline or acid conditions. Plants absorb phosphate in a soluble form, such as in the form of $H_2PO_4^-$ or HPO_4^-. Some bacterial species release organic acids that chelate the cations bound to phosphate into the soil (Vassilev et al. 2006).

It is estimated that more than 90% of plant species interact with arbuscular mycorrhizal fungi (Bouwmeester et al. 2007; Kumar et al. 2016). The mycorrhizal fungi consume approximately 20% of the carbohydrate products synthesized from plants. Arbuscular mycorrhizae (AM) thrive in the soil in the form of spores till they recognize a specific host plant. Therefore, in exploration for particular roots of the host plants, these spores germinate and spread hyphae through the soil; hyphal branching is stimulated in response to strigolactones present in plants (Kumar et al. 2013, 2016). Fungi form the appressoria after coming in contact with the specific host plant, and with the help of these appressoria, they improve passage to the spaces present between the root cells by using lipo-chito-oligosaccharides (Maillet et al. 2011). Conclusively, inside cortical cells, the fungi form arbuscules. Arbuscular mycorrhizal fungi have recently gathered in greater numbers, due to a combination of genetics and cell biology, as well as the availability of genomic sequences from both mycorrhizal fungus and plants, which has led to increased monitoring. In this context, the advent of new techniques such as omics methods ranging from phylogenomics to metabolomics reveals the communication and variety of symbiotic relationships while also elucidating the role played by various partners in the connection (Kaushal et al. 2020).

Bacteria that enhance plant growth and provide multiple benefits to the plant are collectively called plant growth-promoting rhizobacteria (PGPR). These bacteria colonize the rhizospheric area and can enhance plant growth and productivity. They are present in significant numbers and positively influence the plant's growth under specific soil and environmental conditions (Spaepen and Vanderleyden 2011). This can be achieved through direct or indirect interactions with the plant roots. Mechanisms involved in direct plant growth promotion include phytohormone production, atmospheric nitrogen fixation, solubilization of phosphate, and siderophore production (Etesami and Maheshwari 2018), whereas indirect mechanisms of plant growth promotion are mainly related to suppressing soil-borne pathogen and deleterious microorganisms by exclusion and antagonism. These mechanisms are attributed more to general plant health than to plant growth promotion by the production of siderophores or antibiotics by the bacteria (Etesami and Maheshwari 2018).

Compost and chemical fertilizers formed with the help of PGPR significantly affect the growth and yield of different crop plants. However, with the help of some novel approaches using composted material and blending it with PGPR that can either directly or indirectly facilitate rooting, it is converted into a value-added product such as an effective biofertilizer (Kumar et al. 2016).

4.5 Key Mechanisms Adopted by Host for Recruiting Microbial Diversity

Microbial communities that colonize the host plant benefit from resources derived from the plant and taxonomically create uniform community patterns. Principally, two different, albeit not mutually exclusive, mechanisms might produce such microbiota structures. Plants growing in the soil provide unoccupied niches for intruding microbial strains capable of exploiting the available resources, thus, resulting in stochastic colonization events. On the other hand, plant–microbe co-evolution might provide the basis for a plant-driven selection process, resulting in the active recruitment of microbiota members or at least keystone species that provide functions to the plant host. Root exudates released by the plants are considered the key drivers for establishing plant host-specific microbial diversity in the rhizospheric zone (Marschner et al. 2004). With various chemicals secreted by different parts of the roots into the soil, these chemo-attractants are broadly referred to as root exudates. The importance of root exudates as below-ground defense substances has been underestimated for a long time. These are released behind the root tips, primarily in the elongation zone. Root exudates include thousands of different substances, and a major portion of the carbon (C) is stored as carbohydrates, proteins, sterols, amino acids, and a variety of organic acids; these substances are referred to as water-soluble substances. The differences based on the quantity and availability of carbon in various zones present in the root are specific for different rhizospheric community structures (Yang and Crowley 2000). Consequently, plants secrete a blend of molecules, not just one substance, in response to environmental stimuli. This mixture of exudates that are released by roots relies on exterior aspects, such as the height of the plant, age of the plant, soil parameters, and photosynthetic activity of the soil, and these properties vary with species to genus level and also are specific at species or even genotypic level (Semchenko et al. 2014).

Microorganisms are recognized as the primary colonizers as the root tip grows toward the soil and penetrates into the deep layers of soil; as continuous root growth occurs, the elongation zone is rarely colonized by microbes present in the soil. Therefore, microbial communities increase a few centimeters rapidly behind the root tips due to volatile root exudates, which act as chemo-attractants for soil microorganisms and are essential for the growth and development of microbial communities. On the other hand, older parts of the root also release some exudates for growth and establishment of microbes that include cellulosic and other recalcitrant materials that form cell wall; these chemicals are sloughed from the tissues present in the root cortex. Differences in the distribution and quantities of these substances, including secondary metabolites and alkaloids, terpenes, flavonoids, and their relative importance in influencing rhizosphere community structure have been observed. Many secrets of the life of microbes and their habitation in the rhizospheric area were recently disclosed due to the latest advancement in microscopic and molecular tools.

There are many different components liable for shaping rhizosphere microbiome, but for the variations in community structure, the plant exerts a highly selective pressure as great as that of the soil; hence, microbial communities are actively engaged in various key processes such as the formation of soil, decaying of organic matter, eradication of toxins, and recycling of nutrients like nitrogen (N), carbon (C), phosphorus (P), and sulfur (S). Therefore, these microbes inhabiting the soil find it difficult to maintain the function of soil in both natural and artificially managed agricultural ecosystems. Also, the rhizosphere plays a centrally important role in conducting significant processes that include plant growth, root health, and nutrient recycling. Hence, the microorganism is the key driver for various processes like sequestration of carbon, the functioning of the ecosystem, and cycling of nutrients in terrestrial ecosystems (Liu et al. 2018). These microbes are actively engaged in eliminating various soil-borne plant diseases and promoting plant growth and vegetation changes. Even though the microbiota that resides inside the plant as endophytes can be unraveled into groups of organisms that can be studied in isolation; these distinguishable microorganisms reciprocate in an allied way to the drivers providing support for their functioning and composition found in the rhizosphere. The functioning of the plant-associated microbial community helps plants to ameliorate various diseases and plant growth stimulation by assortment and transportation of various nutrients (Yang et al. 2009; Lundberg et al. 2013).

Root exudates play a vital role in plant–microbe interactions. Roots of plants secrete various phytochemical compounds that can mediate different types of interactions, including plant–faunal, plant–microbe, and plant–plant interactions (Huang et al. 2014). Plants use various transport mechanisms to export and release aggregates into the rhizosphere (Badri et al. 2009; Weston et al. 2012). In general, a plant root secretes root exudates either as diffusates by passive mechanisms or as secretions by active mechanisms. Most of the organic compounds with low molecular weight are generally secreted by the plants via a passive process, whereas uncharged and polar molecules are transferred directly by passive diffusion. Arbuscular mycorrhizal fungi (AMF) are among the most abundant organisms present on the earth; hence, they represent approximately 5–10% of total global soil microorganism biomass (Lanfranco and Young 2012).

Besides these symbiotic interactions, root exudates released by plants are also involved in the commencement of plant and PGPR interactions. The plant growth-promoting rhizobacteria or PGPR enhance the growth of plants by direct and indirect mechanisms. Plant roots release various clues like root exudates that magnetize the diversity of PGPR (Haichar et al. 2008). The carbohydrates and different types of amino acids act principally as chemo-attractants, hence providing strong accuracy, and these root exudates have great potential to convey signals to neighbors. As a result, roots employ a biochemical process to transform signals received from other plants, roots, and soil microorganisms. Although this mechanism is primarily unknown, a recent ecological study has demonstrated that root exudates are crucial for soil interactions (Hinsinger et al. 2009; Sugiyama 2019). Numerous root exudates are known to affect nutrient accessibility, and also, they have the potential to negotiate resource competition. For example, the plant-available concentration of ions, such as phosphorus (P) and zinc (Zn), increases with the exudation of acid phosphatases, protons, and carboxylates (Hawkes et al. 2005; Hinsinger et al. 2009; Sugiyama 2019).

4.6 Mechanism of Action of PGPR

One of the alternatives and evolving strategies used to solve this problem is naturally occurring plant growth-promoting bacteria (PGPB). This eco-friendly microbial community is equally active in stimulating disease management and crop productivity under normal and stressful situations. This approach can be among the most effective approaches for reducing the usage of chemicals, which can undesirably impact human health directly and indirectly (Glick 2014; Dubey et al., 2022a; b; 2021). Several reports show the effectiveness of PGPB for improving plant growth and health. These microbes are well known for their potential to improve plant growth through direct and indirect mechanisms (Fig. 4.1).

4.7 The Direct Mechanism of Action

Direct mechanisms involve the microbial synthesis of phytohormones, such as indole-3-acetic acid (IAA), cytokinins, and gibberellins, by these microbes. Also, these microbes can fix atmospheric nitrogen, and solubilize iron (Fe) and phosphorus (Rosenblueth and Martínez-Romero 2007). Moreover, these microbes have the potential to produce 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which

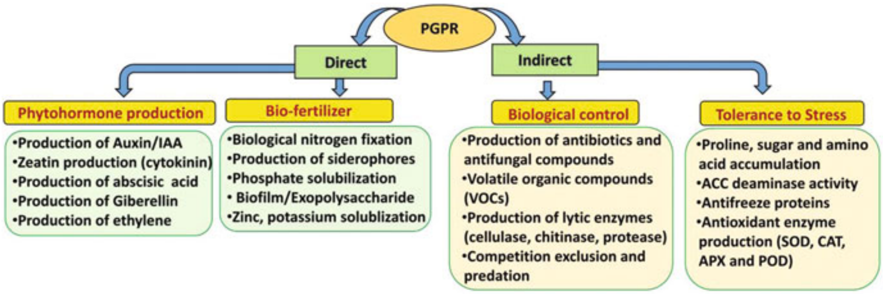

Fig. 4.1 Mechanism of action of PGPR

lowers plant ethylene levels because ACC is a precursor for ethylene production (Santoyo et al. 2016).

4.7.1 Fixation of Atmospheric Nitrogen

Nitrogen (N) is one of the essential nutrients present in the atmosphere. It has a key role in enhancing the growth and productivity of the plant. However, about 78% of nitrogen is inaccessible to the plants from the atmosphere. Biological nitrogen fixation (BNF) is the process of fixing atmospheric nitrogen into a soluble form that plants can utilize by a variety of N_2 microorganisms present on the earth. BNF is the process to reduce gaseous dinitrogen to ammonia by the nitrogenase enzyme complex ($N_2 + 8H^+ + 16\ ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16Pi$) and it is well known in rhizobia–legume symbiosis (accounting for up to 460 kg fixed N hectare^{-1} year^{-1}). Approximately, two-thirds or 66% of the nitrogen is fixed globally by the BNF process, while the remaining nitrogen is synthesized by the Haber–Bosch process (Jiménez-Vicente et al. 2015).

4.7.2 Phosphorus Solubilization

Different types of organic acids may act as metal chelators present in the rhizosphere of the plant. Still, these metal chelators have a profound effect on phosphorus availability compared to the availability of micronutrients. Like iron, phosphorus is another element found abundantly in soil but cannot be absorbed directly from the soil (Mehta and Nautiyal 2001). Strategies to improve phosphorus availability/uptake can contribute significantly to plant growth because less than 5% of the phosphorus content of soils is bioavailable to plants. Microorganisms with the capacity to solubilize mineral phosphorus are abundant in most of the soils (up to 40% of the culturable population) and can be easily determined by plating on a solidified medium with the incorporation of an insoluble phosphorus form (e.g., hydroxyapatite). Halo formation around colonies indicates the phosphorus solubilization capacity of these strains. Well-known bacterial isolates found in these types of soil belong to *Bacillus*, *Pseudomonas*, or *Penicillium* genera (Kumar et al. 2012).

4.7.3 Siderophore Production

Some metal chelators present in the rhizospheric zones of the plants will increase the availability of metals inside the soil. Some micronutrients constituting iron (Fe), copper (Cu), zinc (Zn), and manganese (Mn) are vital to the growth of the plants. These metal chelators establish different types of complexes with soil metals, thus releasing metals that are bounded with soil particles, and hence increasing the mobility and solubility of the metals (Miethke and Marahiel 2007). Iron is often an abundant element present in the soil, but it is not accessible to plants owing to the low solubility of Fe^{3+} oxides, and plants acquire it with the help of microbes present in the soil. Phytosiderophores have been recognized as including non-proteinogenic amino acids such as avenic and mugineic acids. A mass of bacteria that have plant growth-promotion activities sequesters the Fe^{3+} present in insoluble form from the rhizospheric soil with the help of siderophores (Miethke and Marahiel 2007; Hider and Kong 2010). Plants uptake iron bound by bacterial siderophores, even though they secrete these siderophores and have a lower affinity for binding iron. This acquisition of iron via microbial siderophores reduces iron availability in the rhizosphere, leading to slower growth of other microorganisms (especially fungi) that may be parasitic to the plant (Loaces et al. 2011).

4.7.4 ACC Deaminase Activity

Ethylene was the first phytohormone described as a fruit-ripening hormone but is now known to have a much broader role in various processes, like stress response in plants, senescence, abscission, and pathogen-defense signaling (Danish et al. 2020). The synthesis of ethylene is highly susceptible to environmental stimulants, including temperature, sunlight, and other plant hormones, in response to various abiotic and biotic stresses (Gowtham et al. 2020). Hence, the production of the ethylene level in plants is also elevated in response to stress. Numerous groups of bacteria evolved with the skill to produce (ACC) deaminase or 1-aminocyclopropane-1-carboxylate; for example, *Burkholderia* spp. can degrade excess amounts of 1-aminocyclopropane-1-carboxylate (Glick 2014; Danish et al. 2020). It is the direct precursor of ethylene in the ethylene biosynthesis pathway. Hence, this can be achieved due to the production of nitrogen and energy as a by-product and the reduction in response to various stresses, which ultimately leads to plant growth promotion. The efficiency of bacteria increases near the plant cells in which biosynthesis of ethylene takes place (Hardoim et al. 2008). Bacteria with ACC deaminase activity frequently provide many benefits and are considered the significant forerunners in sustainable agricultural systems (Glick 2014).

4.7.5 Signaling of Phytohormones

The phytohormones play a crucial role in the growth and development of the plant, combining both non-remittance developmental pathways and dynamic responses to the environment (Kumar and Dubey 2020). Therefore, phytohormones are considered a key constituent of plant–microbe interactions. Microbial communities produce a variety of phytohormones. Plant responses toward abiotic and biotic stress tolerance, modulation, and regulation of signals exchange (Hause and Schaarschmidt 2009) are involved in the signaling pathway against a pathogen with necrotrophic effects (Stein et al. 2008). Crosstalk mediated by salicylic acid, jasmonic acid, and ethylene activates plant systemic acquired resistance (SAR) and response or induced systemic resistance (ISR); hence phytotoxic microbial communities are reduced. When ethylene is present in a lower concentration, plants can respond to a wide range of environmental stresses. Still, when ethylene concentrations are high, this can lead to plant growth inhibition and even the death of the plant (Kumar and Dubey 2020).

Moreover, strigolactones and brassinosteroids are the other compounds identified with hormonal activity (Kumar and Dubey 2020). In the growth medium of many plant-associated bacteria and soil bacteria, phytohormone production is frequently observed; in many cases, the single strain is used to produce different compounds. It has been speculated that phytohormones can be used as signaling molecules between host and bacteria, and the existing crosstalk between auxin (IAA or indole acetic acid) and production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity or biosynthesis pathway of ethylene is blocked (Penrose and Glick 2003; Barnawal et al. 2019).

Moreover, many bacterial species can also regulate and influence the production of plant hormones. Inoculation of seedlings of *Miscanthus* plant with a temperate grass endophyte, *Herbaspirillum frisingense* (GSF30T), stimulates shoot and root growth. Transcriptome analyses revealed that there is regulation of jasmonic acid and ethylene signaling pathway, indicating that the phytohormone activity promotes and modulates plant growth (Straub et al. 2013). A different group of bacterial endophytes were cultured from sweet potato; the cuttings were inoculated with strains of endophytic bacteria that produce auxin and indole acetic acid (IAA), and the cuttings gave rise to roots more rapidly than cuttings that were not inoculated (Doty et al. 2009). It was demonstrated that GSF30T *Herbaspirillum frisingense* produces IAA in the culture (Rothballer et al. 2008), and it was also concluded that the growth of wheat seedlings increases when inoculated with *B. subtilis* (due to the production of auxin). *Azospirillum* spp. is also known to enhance and stimulate plant growth by producing auxin and nitrogen fixation. These bacterial strains can be applied in agriculture fields for sustainable agricultural production. Strain B510 of *Azospirillum* sp. isolated from surface-sterilized stems of rice significantly increases the yield of rice plants when seedlings are re-inoculated; however, three strains of *Pseudomonas* enhance the growth and spike length of wheat plants in the field as well as in laboratory conditions (Jiménez-Vicente et al. 2015).

4.8 Indirect Mechanism of Action

Indirect mechanisms include suppression of pathogen infection via antifungal or antibacterial agents. The other indirect mechanisms include assisting plants in acquiring nutrients via phosphate solubilization, nitrogen fixation, and siderophore production. Besides these mechanisms, plant-associated microorganisms improve nutrient acquisition by supplying minerals and other micro/macronutrients from the soil (Hassan 2017). Therefore, isolation and characterization of endophytic bacteria with various properties from unexplored hosts will have many applications to improve plant growth promotion (Santoyo et al. 2016).

4.8.1 Induced Systemic Resistance

Induced systemic resistance has emerged as an essential mechanism by which selected PGPR and fungi in the rhizosphere prime the whole plant body for enhanced defense against a broad range of pathogens, insects, and herbivores (De Vleesschauwer and Höfte 2009). *Induced systemic resistance* is a generic term for the induced state of resistance in plants triggered by biological or chemical inducers, protecting non-exposed parts against future attacks by pathogenic microbes and herbivorous insects. Plants are in constant contact with a variety of pathogenic microbes (Doornbos et al. 2012). The plants must recognize the invaders and activate fast and effective defense mechanisms against the pathogens to avert these pathogens. Inducible defense responses depend upon the bifurcate innate immune system. One is PAMP-triggered immunity (PTI), which identifies and responds to molecules common to different groups of microorganisms, termed pathogen-associated molecular patterns (PAMPs). The term “PAMP” has been criticized because most microbes are not just pathogenic; therefore, the term microbe-associated molecular pattern (MAMP) has been proposed. MAMPs are sustained among non-pathogenic microorganisms, including endophytes. They are often identified by various toll-like receptors (TLRs) and other pattern-recognition receptors (PRRs) present in plants and animals. Microbe-associated molecular patterns (MAMPs) act as elicitors recognized by plants and trigger MAMP-triggered immunity (MTI). MAMP-triggered immunity, or MTI responses, constitutes the fabrication of the molecules like reactive oxygen species (ROS) and nitrogen that act as antimicrobial compounds and help in signaling (Newman et al. 2014).

These molecules activate innate immune responses and protect the specific host from the diseases by determining some conserved non-self-molecules. Bacterial lipopolysaccharides and endotoxins include flagellin, glycoproteins, elongation factor, chitin, and lipoteichoic acid from Gram-positive bacteria, and damage caused by pathogenic infection gives rise to endogenous signals derived from the plant, called damage-associated molecular patterns (DAMPs; Tanaka et al. 2014). Plants generally recognize MAMPs and DAMPs with pattern-recognition receptors (Pieterse et al. 2009). As MAMPs are also conserved in plant-beneficial microbes, they may evoke responsive defense such as induced systemic resistance (ISR; Doornbos et al. 2012; Kusajima et al. 2018).

Effector-triggered immunity (ETI) is the second branch of plant defense. They respond to virulent pathogenic components secreted directly into the host cell cytoplasm to surpass the protective response and secrete effector protein molecules into the apoplast. PTI and ETI are similar; hence, they involve the procreation of signaling molecules such as salicylic acid, jasmonic acid, ethylene, and/or reactive oxygen species (ROS) and inducing defense-related genes and underpinning of cell walls. Plant cells can recognize and proceed to molecular components of bacteria by using defense-related responses; therefore, the immune system of plants plays a vital role in shaping the framework of the microbiome. Despite the innate immune responses, endophytes can establish themselves inside the tissues of plants in high numbers.

Inoculating plants with non-pathogenic bacteria can induce resistance against a broad spectrum of pathogenic organisms below- and above-ground parts. This ISR depends mainly on jasmonic acid and ethylene signaling (Stein et al. 2008). In this way, plants are primed to react more quickly and strongly to a pathogen attack. ISR has been detected for several microbes and their cellular derivative determinants (so-called MAMPs), such as cell envelope elements, flagella, and siderophores (Zamioudis and Pieterse 2011; De Vleesschauwer and Höfte 2009). Well-characterized ISR-inducing microbes include several *Pseudomonas*, *Bacillus*, and *Serratia* species and *Trichoderma harzianum*. Most plant responses have been studied in *Arabidopsis thaliana*, but ISR has also shown that a partial plant growth promotion can be observed upon inoculation (Pieterse et al. 2014).

4.8.2 Biological Control and Plant Protection

Biological control, or biocontrol, is the process of suppressing harmful or pathogenic living organisms by using other living organisms. Biocontrol has been extensively studied under laboratory conditions and in field situations, leading to several commercial products. Most products are based on *Bacillus* and *Trichoderma* strains owing to seed formulation issues, although *Pseudomonas*-based products have also been commercialized in recent years (Berg and Smalla 2009). Production of Rosmarinic acid (an antimicrobial compound produced by rhizospheric and endophytic bacteria) provides an alternative mechanism for plant protection (Dubey et al. 2020b). Rosmarinic acid provides a dynamic antimicrobial activity against a wide range of microbial communities colonizing the rhizospheric area of the soil, and it was induced in the exudates produced by the hairy root cultures of sweet basil following a challenge by *Pythium ultimum* (Martinez-Klimova et al. 2017; Naik et al. 2019). Endophytic bacteria inhibit pathogenic quorum-sensing by producing specific antimicrobial products, thereby inhibiting communication, the formation of biofilm, and virulence, without suppressing the growth of bacteria. Endophytic bacteria can also degrade quorum-sensing molecules and suppress biofilm formation in *Pseudomonas aeruginosa* PAO1 by producing cell-free lysates (Rajesh and

Ravishankar Rai 2014). Thus, bacterial endophytes can protect the host against harmful pathogens that develop resistance to the plant defenses. Although this quorum-sensing does not impel selective pressure for developing antibiotic resistance, it is another anti-virulence approach for cross-examining drug-resistant bacteria (Kusari 2014).

4.9 Quorum-Sensing and Biofilm Formation

Quorum-sensing is a process of cell to cell communication or regulated expression of genes in response to changes in density and cell population (Kasim et al. 2016). Quorum-sensing has resulted in the regulation of many bacterial genes to limit collaborative performance, such as those involved in biofilm formation, virulence, pathogenesis, and swarming (Choudhary and Johri 2009). Biofilms comprise the complex extracellular matrix of exopolysaccharides and proteins adhering to a solid surface and containing a multicellular assembly of bacteria. The production of these biofilms enables bacterial species to adhere to plant tissues and is an intrinsic element of plant–microbe interactions (Ramey et al. 2005).

Transposon mutagenesis of plant-associated *Bacillus amyloliquefaciens* sp. *plantarum* FZB42 genes are identified, which are required for the formation of biofilm, bacterial swarming, colonization of roots, and enhanced plant growth in aseptic conditions (Budiharjo et al. 2014). Bacteria present within biofilms are more tolerant toward antimicrobial compounds and are physiologically and phenotypically different from those free-living bacteria (Ramey et al. 2005). Some mutants of *Bacillus subtilis* that cannot form biofilm cannot protect *Arabidopsis* from infection caused by bacteria *Pseudomonas syringae* (Bais et al. 2004). *Bacillus subtilis* strain 6051 is mutated, resulting in a mutant strain that is susceptible to surfactin synthesis. Surfactin is a lipopeptide with antimicrobial activity. Whereas *Bacillus subtilis* strain 6051 forms biofilm and releases surfactin and is estimated to be homicidal for *P. syringae*, the mutant was incompetent as a biocontrol and could not form durable biofilms (Bais et al. 2004). The formation of biofilms can also alter the fate of other compounds present in their proximity due to their physiological response during the absorption of water and organic or inorganic solutes.

The plant-associated biofilms are proficient in providing defense from exterior stress, decreasing microbial competition, and providing a protection to the host plant by supporting growth and yield (Auger et al. 2006; Sandhya et al. 2009; Dubey et al. 2020a). Biofilm-producing bacteria also play an imperative role in improving soil fertility and bioremediation (Auger et al. 2006). The study conducted by Wang et al. (2019) used biofilm-forming *Bacillus amyloliquefaciens* 54, which significantly enhanced drought tolerance by increasing survival rate, relative water content, and root vigor. Furthermore, the biofilm-forming ability of *Bacillus amyloliquefaciens* improves salt stress tolerance in barley (Kasim et al. 2016), and *Bacillus subtilis* protects against tomato wilt disease by the formation of biofilm (Chen et al. 2013). Although some studies have been published, there is little direct evidence illustrating the role of biofilm formation in protection against abiotic and biotic stresses, especially drought stress (Auger et al. 2006; Chen et al. 2013; Kasim et al. 2016).

4.10 Metagenomics Tools for Plant Microbiome Analysis

High-throughput or next-generation sequencing technology is expeditiously upgraded in speed, cost, and quality. Therefore, it is extensively used to analyze whole prokaryotic communities colonizing different niches (Malla et al. 2019). Here, we have discussed this technology for determining the microbial community's taxonomic composition and functional role, which occupied different compartments of the plant body. We briefly discussed other high-throughput sequencing platforms and recently developed and commonly used bioinformatics tools applied to metagenomics analysis.

The advances in next-generation sequencing (NGS) or high-throughput sequencing have remolded the field of microbial ecology. This type of cutting-edge technology has led to the establishment of a relatively new area, that is, "metagenomics," which is described as the genomic analysis of genetic material directly recovered from the environmental sample. In this way, it avoids the need for isolation and lab culturing of individual microbial species that act as a significant obstacle to culture-dependent methods. Most of the bacterial species colonizing the rhizospheric area are recently unable to be cultured in the labs, and the cultivation-dependent methods are often insufficient for quality analysis of the rhizosphere microbiome (Bell et al. 2014). Many metagenomics computational/statistical tools and databases have evolved in the last decades, and some have been presented in Table 4.1. The conventional approaches were based on isolation and culturing of microorganisms present in the soil, which account for less than 1% of total microbial populations (Torsvik and Ovreås 2002). However, polymerase chain reaction (PCR) amplification of specific genes of interest is widely used in research; this method is known as "shotgun metagenomics" (using 16S rRNA as a marker gene) or "marker gene amplification". Such strategies allow a much rapid and elaborative generation of genomic profiles of samples isolated from the environment at a very moderate price. Full shotgun metagenomics will enable researchers to sequence the whole genome present within an environmental sample comprehensively. This also provides a means to study uncultivable microbial communities that are otherwise impossible or difficult to inspect. Marker gene metagenomics is a fast and straightforward way to procure a taxonomic or community distribution profile or fingerprint using PCR amplification and sequencing of the 16S rRNA gene, an intact marker gene.

The 16S rRNA gene sequencing technique is extensively used to expose various bacterial communities in the biological sample and to construct phylogenetic associations. All bacterial cells possess these genes, which are highly conserved regions that help us know the evolutionary relationships and act as a valuable target for pyrosequencing analyses and PCR amplification of microbial diversity. A thorough inspection of a metagenomics sample requires specific consecutive bioinformatics venture that comprises (i) quality control, (ii) assembly, (iii) detection of

Table 4.1 List of bioinformatics software for metagenomics data analysis

S. N.	Software	Access	Applications	Website address	Reference
1.	MicrobiomeAnalyst	Web-based	Comprehensive statistical, functional, and meta-analysis of microbiome data	*MicrobiomeAnalyst*	Dhariwal et al. (2017), Chong et al. (2020)
2.	BiomMiner	Web-based	Comprehensive analysis of microbiome data	*BiomMiner readme – Microbiome Analysis Center (gmu.edu)*	Shamsaddini et al. (2020)
3.	PICRUSt2	Web-based	Functional potential of a community based on marker gene-sequencing profiles	https://github.com/pic rust/picrust2	Douglas et al. (2019)
4.	FastQC	Web-based	Annotation	http://www.bioinformatics.babraham.ac.uk/projects/fastqc/	Andrews (2017)
5.	EBI	Web-based	To compare functional analyses of sequences	https://www.ebi.ac.uk/metagenomics/	Mitchell et al. (2016)
6.	KEGG	Local	Biological interpretation of genome sequences	http://www.kegg.jp/blastkoala/	Kanehisa et al. (2016)
7.	GraPhlAn	Local/web-based	Produces high-quality visualizations of microbial genomes and metagenomes	http://segatalab.cibio.unitn.it/tools/graphlan	Asnicar et al. (2015)
	MetaBAT	Local	Binning millions of contigs from thousands of samples	https://bitbucket.org/berkeleyLab/metabar	Kang et al. (2015)
	deFUME	Web-based	Processing, annotation, and visualization of functional metagenomics sequencing data	https://github.com/EvdH0/deFUME	Van Der Helm et al. (2015)
8.	MetagenomeSeq	Web-based	Analysis of differential abundance of 16S rRNA gene in meta-profiling data	http://bioconductor.org/packages/release/bioc/html/metagenomeSeq.html	Paulson et al. (2013)
9.	MetaPath	Web-based	Identification of metabolic pathways differentially abundant among metagenomics samples	http://metapath.cbcb.umd.edu/	Liu and Pop (2011)
10.	IMG/M	Web-based		https://img.jgi.doe.gov/cgi-bin/m/main.cgi	Markowitz et al. (2012)

			Functional annotation, the phylogenetic distribution of genes, and comparative metagenomics analysis		
11.	BioMaS	Web-based	Taxonomic studies of environmental microbial communities	http://galaxy.cloud.ba.infn.it:8080	Fosso et al. (2011)
12.	QIIME	Local	Data trimming and filtering, diversity analysis, and visualization	http://qiime.org/	Caporaso et al. (2010)
13.	Galaxy portal	Web-based	Web repository of computational tools that can be run without informatics expertise	https://usegalaxy.org/	Goecks et al. (2010)
14.	mothur	Local	Data trimming and filtering, diversity analysis, and visualization	http://www.mothur.org/	Schloss et al. (2009)
15.	RDP	Web-based	Data trimming and filtering, and diversity analysis	http://rdp.cme.msu.edu/	Cole et al. (2007)
16.	MG-RAST	Web-based	Processing, analyzing, sharing, and disseminating metagenomics datasets	http://metagenomics.anl.gov/	Meyer et al. (2008)
18.	MEGAN	Local	Diversity analysis and visualization (needs similarity alignments as input)	http://ab.inf.uni-tuebingen.de/software/megan	Huson et al. (2007)

gene, (iv) annotation of the gene, (v) taxonomic study, and (vi) comparative study while depositing the sequenced results in the database structured computational repository, which enables advanced management of data, processing of data, meta-mining, and mining capabilities (Ladoukakis et al. 2014).

4.11 Future Perspectives and Concluding Remarks

The significant societal challenges to produce more food with less fertilizer and agrochemical inputs in crop protection have dramatically increased the awareness of the importance of the root microbiome in plant health for current agriculture. However, with the increasing researches and literature available about plant microbiome, the composition and their prominent roles are advanced in research. Many components will undoubtedly contribute to the rapid expansion of this approach in the future. With the increasing quality of sampling procedure, extraction of DNA, targeted gene amplification isolated from different microbial communities, and others, representing methodological biases will eventually decline in the future. Hence, the broad access of sequence-based analyses allowed by recent development in DNA sequencing techniques makes sequencing of nucleic acids (DNA or RNA) from great numbers of samples possible. Therefore, sequencing cost has declined due to the generation of the massive amount of data vital for the characterization of complicated communities of microbes, which supports the emergence of studies related to the microbiome. This is also dependent on the development of mathematical modeling and advancement in bioinformatics. These advancements in technologies provide most of the robust description of the composition of taxonomic community and phylogeny of microbes, therefore expanding the information available in public databases. By exogenous inoculation of particular microbes and in controlled environmental conditions, it is possible to alter the structure of the microbial community to benefit particular sets of these microbiomes, which leads to increased resistance in plants or harnessing efficiency in the uptake of specific nutrients. In this regard, the development of so-called “microbiome-driven cropping systems” might result in the next revolution in agriculture, resulting in a more sustainable system for plant production.

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From Rhizosphere to Endosphere: Bacterial-Plant Symbiosis and Its Impact on Sustainable Agriculture

5

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Abstract

Recent findings and advancement in technologies in the field of plant–microbe interactions have led to deeper investigations on the role of soil and endophytic microbial communities in the growth and development of their respective host plants. The process of recruitment of the rhizospheric bacteria inside the host plant tissues through various active and passive passages is also an interesting and established phenomenon. The mutualistic relationship thus developed plays important role in shaping the functionalities of the host plant through different plant growth-promoting mechanisms such as auxin production, nitrogen fixation, and phosphorus solubilization. Nevertheless, these bacteria also enhance the stress tolerance capabilities of the host plant leading to their widespread utilization in sustainable agricultural practices. This chapter focuses on the role of the soil bacterial communities, their recruitment inside the host plants, and their further endophytic life and functionalities. Further, some of the recent applications of seed endophytic bacteria have also been discussed.

5.1 Introduction

Excessive use of chemical fertilizers and pesticides, and climate change have forced the scientific community to find alternative approaches in order to increase the crop productivity sustainably. The interaction of the diverse microbial community in the soil with a number of physiological processes inside the plants has yielded significant observations leading to more intensified research for utilization of this microbial community in agricultural practices (Baker et al. 1997; Berg 2009; Kumar and

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Verma 2018). Many rhizospheric microorganisms particularly bacteria have been shown to increase crop productivity. Major functional roles of these bacteria include stimulation of plant growth through enhanced nutrient availability or production of plant growth hormones such as auxins, inhibition of phytopathogens through antibiosis or production of antifungal compounds, tolerance toward abiotic stresses through reactive oxygen species (ROS) production, and improvement of soil health through the bioremediation process (Arshad and Frankenberger Jr 1997; Richardson et al. 2009; Hayat et al. 2010; Pal et al. 2021a; Verma et al. 2021a, b). These functional aspects of beneficial bacteria can be employed in sustainable agricultural practices for improving crop productivity in place of chemical fertilizers. A variety of symbiotic as well as non-symbiotic bacterial species including *Rhizobium*, *Azotobacter*, *Bacillus*, *Azospirillum*, and *Klebsiella* have been widely used to enhance the plant production (Burd et al. 2000; Cocking 2003). Such soil-inhabiting bacterial species that contribute in improving plant growth are commonly known as plant growth-promoting rhizobacteria (PGPRs).

Effective colonization of the rhizospheric region of the plant by such beneficial bacteria is an essential criterion for their optimal functionality (Lugtenberg et al. 2001). Bacterial colonization in symbiotic bacteria takes place inside the plant cells in specialized structures known as nodules as observed in *Rhizobium* sp., which occur in leguminous plants (Burd et al. 2000). *Mesorhizobium*, *Bradyrhizobium*, *Azorhizobium*, and *Allorhizobium* are some of the *Rhizobia* species that have been reported globally in successfully establishing a symbiotic relationship with the leguminous plants and performing the process of nitrogen fixation (Bottomley and Maggard 1990; Bottomley and Dughri 1989). However, many bacterial species colonize the plant tissues firstly inside the roots followed by shoots and other aerial parts of the plant systematically leading to a more intricate relationship between the two, which further results in better development and stress tolerance capabilities of the host (James et al. 2002; Compant et al. 2005b; Pal et al. 2021b). Such bacterial colonists do not cause any disease symptoms inside the host tissues and are called endophytes (Schulz and Boyle 2006). The recruitment of these mutualists from the rhizospheric region to the inside of the plant is a complex phenomenon and can be achieved through a number of intrinsic and extrinsic factors. The bacterial community present in the soil shows chemotactic movement toward the root exudates released by the plant in the rhizosphere allowing them to efficiently colonize the rhizosphere as well as the rhizoplane (Walker et al. 2003; Bais et al. 2006; Lugtenberg and Kamilova 2009). The plant-beneficial bacteria possessing competitive advantages such as production of lytic enzymes, antibiotics, and siderophores colonize the internal tissues of the plant roots (van Loon and Bakker 2005; Raaijmakers et al. 2002; Haas and Défago 2005). Once inside the plant system, a number of active and passive processes are involved leading to widespread colonization (Compant et al. 2010). However, the community structure as well as number of the endophytic bacterial population varies in response to the stress conditions faced by the host plant (Podolich et al. 2015; Walitang et al. 2018). The utilization of such endophytic species in agricultural systems for improving crop productivity is a more viable and environmentally sustainable approach as these mutualists possess important functionalities supporting plant growth and preventing stress conditions (Welbaum et al. 2004; van Loon and Bakker 2005; Lugtenberg and Kamilova 2009; Pal et al. 2019). Plant growth-promoting properties of endophytes include production of growth hormones, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity, enhanced nutrient acquisition, and nitrogen fixation while the synthesis of antifungal compounds, antibiotics, siderophores, lytic enzymes, etc. contributes to the properties of biological control (Hardoim et al. 2008; Chaturvedi et al. 2016; Herrera et al. 2016; Santoyo et al. 2016). These endophytes also provide tolerance to different types of abiotic stress conditions such as salt stress, drought stress, and water stress.

The present chapter focuses on the role of microbial communities present in soil as well as inside the plants in shaping modern agricultural practices. PGPRs, their colonization pattern in the plant, and how they form the endophytic community of the host plant are also discussed. Furthermore, this chapter also covers the functional aspects of these endophytes in plant growth promotion and disease prevention (Table 5.1).

5.2 PGPRs and Their Recruitment Inside Plants (Bacterial-Plant Symbiosis)

Modern researches in the field of rhizosphere biology have thrown ample light on the interesting functional roles and mechanisms of associated microbial communities. Reports suggest that these microbial communities could be an important tool that can aid plant as well as soil health. The various biotic activities performed by such microbes make the soil dynamic for nutrient availability and sustainable for agricultural productivity (Koul et al. 2019). The PGPRs that inhabit the rhizosphere (rhizoplane, superficial intercellular spaces, or dead root cell layer) are known as extracellular plant growth-promoting rhizobacteria (e-PGPRs) while those existing in the internal living tissues (nodules, apoplastic spaces of the root cortex, meristematic region, etc.) are called as intracellular plant growth-promoting rhizobacteria (i-PGPRs; Vessey 2003; Gray and Smith 2005). These i-PGPRs are now commonly called endophytic bacteria. Bacterial genera such as *Azotobacter*, *Azospirillum*, *Agrobacterium*, *Bacillus*, *Serratia*, *Arthrobacter*, *Pseudomonas*, *Micrococcus*, and *Burkholderia* are commonly included under e-PGPRs and *Rhizobium*, *Bradyrhizobium*, *Allorhizobium*, *Frankia* sp., etc. are examples of i-PGPRs (Gray and Smith 2005). The most commonly found genera of bacterial endophytes include *Bacillus*, *Burkholderia*, *Pantoea*, *Pseudomonas*, *Micrococcus*, and *Microbacterium* (Santoyo et al. 2016).

PGPRs are known to influence plant growth, increase crop yield, reduce the incidence of disease, and help the plants to cope up with the abiotic stresses (Welbaum et al. 2004; van Loon and Bakker 2005; Lugtenberg and Kamilova 2009). These plant growth-promoting bacteria (PGPBs) can be utilized in agriculture as biofertilizers, biopesticides, or for phytoremediation purposes (Berg 2009; Lugtenberg and Kamilova 2009; Weyens et al. 2009). The inoculation of PGPRs

Table 5.1 List of crop-plant-associated bacteria with their functional role in plant growth promotion

Plant-associated bacteria	Crop/plant(s)	Functional role(s)	Reference
Kluyvera ascorbata	Canola, tomato	IAA production, siderophores	Burd et al. (2000)
Rhizobium leguminosarum	Rice	IAA production	Dazzo et al. (2000)
Azotobacter sp.	Maize	IAA production	Zahir et al. (2000)
Aeromonas veronii	Rice	IAA production	Mehnaz et al. (2001)
Enterobacter sakazakii, *Pseudomonas* sp., *Klebsiella oxytoca*	Maize	ACC deaminase activity	Babalola et al. (2003)
Pseudomonas fluorescens	Groundnut	IAA production, siderophores	Dey et al. (2004)
Pseudomonas denitrificans, *Pseudomonas rathonis*	Wheat, maize	IAA production	Egamberdiyeva (2005)
Azotobacter sp., *Pseudomonas* sp.	Sesbania, mung bean	IAA production	Ahmad et al. (2005)
Pseudomonas sp.	Wheat	IAA production	Roesti et al. (2006)
Bacillus cereus, *Bacillus licheniformis*	Wheat, spinach	IAA production	Çakmakçi et al. (2007)
Pseudomonas tolaasii	Brassica	IAA production, siderophores	Dell'Amico et al. (2008)
Bacillus sp., *Paenibacillus* sp.	Rice	IAA production	Beneduzi et al. (2008)
Streptomyces acidiscabies	Cowpea	Hydroxamate compounds	Dimkpa et al. (2008)
Achromobacter xylosoxidans	*Brassica juncea*	Bioremediation of heavy metals (copper)	Ma et al. (2009a)
Pseudomonas sp., *Bacillus cereus*	*Brassica juncea*, *B. oxyrrhiza*	Bioremediation of heavy metals (nickel)	Ma et al. (2009b)
Bacillus sp.	*Cicer arietinum*	Bioremediation of heavy metals (chromium)	Wani and Khan (2010)
Pseudomonas sp.	Wheat	IAA production and phosphate solubilization	Sharma et al. (2011)
Bradyrhizobium sp.	*Vigna radiata*	IAA production and phosphate solubilization	Ahemad and Khan (2011, 2012)
Pseudomonas	*Cassia tora*	IAA production	Kumar et al. (2015)
Pseudomonas, *Bacillus*	Turmeric	IAA production	Kumar et al. (2016)

ACC, 1-aminocyclopropane-1-carboxylate; *IAA*, indole acetic acid including *Azospirillum*, *Bacillus*, *Enterobacter*, *Klebsiella*, and *Pseudomonas* with seeds/or substrate has been shown to enhance the seedling vigor, production of growth regulators, better tolerance to biotic and abiotic stresses, and improved efficiency in the use of fertilizers (Kokalis-Burelle et al. 2006; Lamsal et al. 2013; Park et al. 2013). However, it has also been shown that PGPRs fail to generate desired response in several cases under field conditions (Compant et al. 2010). This situation might arise because of insufficient colonization (rhizosphere/plant) by the PGPRs, a significant process needed for yielding beneficial traits (Lugtenberg et al. 2001).

The process of colonization takes place in response to the root exudates (carbohydrates, amino acids, organic acids, etc.) released by the plant acting as a nutrient source for rhizospheric bacteria (Lugtenberg and Dekkers 1999; Walker et al. 2003; Bais et al. 2006). Bacteria perform chemotactic movement toward the released exudates as a result of which these bacteria colonize the rhizosphere as well as rhizoplane region of the plant root (Lugtenberg and Kamilova 2009). A study demonstrated the colonization of rhizosphere by bacterial cells through *gfp* or *gus* labeling of the strains and visualizing them by florescence in situ hybridization (Gamalero et al. 2003). Further extension of the study done with *Pseudomonas fluorescens* and tomato roots suggested that non-uniform distribution and densities of the strain vary in accordance with the root zone (Gamalero et al. 2004). Reduced chemotactic movement, as well as decreased colonization, was observed when a mutant strain of *P. fluorescens* lacking *cheA* gene (responsible for chemotactic movement) was inoculated in the tomato rhizosphere (de Weert et al. 2002). For successful colonization, the PGPBs need to be highly rhizo-competent and production of secondary metabolites including siderophores, antibiotics, and cyclic lipopeptides help the producing strains in having the competitive advantage over other strains (Compant et al. 2005a; van Loon and Bakker 2005; Haas and Défago 2005; Raaijmakers et al. 2008). A number of other determinants such as quorum sensing, bacterial flagella, and production of enzymes are also involved in the process of colonization (Turnbull et al. 2001; Latour et al. 2008; Compant et al. 2010).

Following the rhizospheric colonization, numerous bacteria have been reported to colonize the internal tissues of the plant and exhibit plant growth-promoting traits (Hallmann 2001; Compant et al. 2008; Verma et al. 2018; Kumar et al. 2020). It is now a well-established fact that almost all the plants harbor diverse endophytic bacterial communities (Berg et al. 2005). A number of active as well as passive mechanisms can be employed by the bacterial strains for gaining entry inside the host plant (Hardoim et al. 2008). Cracks at root emergence sites and fissures at the lateral root base can act as sites from where the bacterial strains can gain entry inside the host plant (Reinhold-Hurek and Hurek 1998; Goormachtig et al. 2004). Secretion of cell-wall-degrading enzymes, lipopolysaccharides, presence of flagella and pili, etc. are some of the other factors that affect the colonization process (Duijff et al. 1997; Dörr et al. 1998; Krause et al. 2006; Böhm et al. 2007). Once the bacterium enters the root cortex, further colonization takes place with the secretion of cell-wall-degrading enzymes, which enable them to pass through the endodermis, allowing their entry into the central cylinder (James et al. 2002). Further colonization of the endophytic bacteria takes place either through lumen of xylem vessels or by passing through different xylem vessels via perforated plates from where they can migrate to the above-ground parts of the host plant (Bartz 2005; Compant et al. 2008). Many studies have suggested the presence of endophytic bacteria in flowers, fruits, and seeds as well (Mundt and Hinkle 1976; Misaghi and Donndelinger 1990; Hallmann 2001; Verma et al. 2018.; Kumar et al. 2020).

5.3 Bacterial Endophytes in Plant Growth Promotion and Stress Tolerance

The present working definition of endophytes includes bacterial communities that are present inside the living tissues of host plant asymptomatically for complete or a part of their life cycle (Wilson 1995; Hallmann et al. 1997). Once inside the host plant, the endophytic bacterial community can directly confer the beneficial effects to the plant cell and can easily evade the competition with their rhizospheric counterparts (Santoyo et al. 2016). An established endophytic community inside the tissues of the host plant remains undisturbed by the change in the outside soil environment (Hallmann 2001). Endophytic PGPBs are known to facilitate plant growth and provide better tolerance abilities against different types of stresses. The facilitation of plant growth by endophytic PGPBs can occur by a number of mechanisms including enhanced acquisition of nutrients from the soil (nitrogen fixation, phosphate solubilization, siderophore production), modulation of levels of plant growth hormones (auxin, cytokinin, or ethylene), or by improving the plant fitness through the production of antibiotics, lytic enzymes, volatile compounds, etc. (Ryan et al. 2008; Santoyo et al. 2016; Pal et al. 2021a, b).

Production of phytohormones by bacterial endophytes is one of the most commonly employed mechanisms for plant growth promotion (Bloemberg and Lugtenberg 2001; Verma et al. 2021a, b). Many bacterial endophytes have been reported to produce indole acetic acid (IAA), the most common auxin. Nearly 50% of the bacterial isolates from banana trees were found to be producing IAA in the presence of L-tryptophan (Gomes et al. 2017). A study showed that three IAA-producing bacteria isolated from sugarbeet roots when inoculated under gnotobiotic and glasshouse conditions significantly enhanced plant height, fresh weight, dry weight, and number of leaves per plant (Shi et al. 2009). Similarly, cytokinins, gibberellins, and abscisic acid are also produced by different bacterial endophytes but their roles are less commonly understood in the regulation of plant growth (Bhore et al. 2010; Khan et al. 2014; Shahzad et al. 2017). ACC (1-aminocyclopropane-1-carboxylate) deaminase activity of many endophytic bacteria is also known to regulate plant growth by lowering the levels of plant hormone ethylene (Gaiero et al. 2013). In a study, bacterial endophytes (*Arthrobacter* sp. and *Bacillus* sp.) isolated from pepper (*Capsicum annuum* L.) showing ACC deaminase activity resulted in reduced abiotic stress under tested conditions (Sziderics et al. 2007). Similarly, ACC deaminase containing *Pseudomonas putida* and

Rhodococcus spp. in peas (*Pisum sativum*) has been shown to mitigate heavy metal stress conditions (Belimov et al. 2001).

Endophytes have shown great potential in the mitigation of abiotic stress tolerance in plants (Lata et al. 2018). Gond et al. (2015) reported increased biomass under salinity stress (1 mole L^{-1}) after treatment with *Pantoea agglomerans*, which was isolated from teosinte (*Zea mays mexicana*) roots. They also observed upregulation of aquaporin gene family (mainly *ZmPIP* and *PIP2-1* genes for plasma membrane integral protein) in the treated plants through gene expression analysis. Also, *Pseudomonas* sp. was shown to induce osmotic stress tolerance in tomato plants under glasshouse and field conditions, as the treatment resulted in increased biomass as well as fruit yield (Sarma et al. 2011).

Endophytes have also been known to activate pathogen defense response through a process known as induced systemic resistance (ISR). ISR results in protection of unaffected parts of the plant against a future pathogen or insect attack through a jasmonic acid mediated pathway (Miliute et al. 2015). Endophytic *Pseudomonas fluorescens* was shown to induce such response against cucumber anthracnose in cucumber plants (Wei et al. 1991). Similarly, another pathogen-induced resistance called as systemic acquired resistance (SAR) mediated by salicylic acid is known to confer resistance to host plants against a wide range of pathogens (Pieterse et al. 2014) (Table 5.2).

5.4 Functionality of Seed-Inhabiting Bacterial Endophytes in Modern Agricultural Practices

Bacterial communities residing inside the seed have shown promising results in plant growth promotion as well as in stress tolerance and are therefore considered as an important alternative in shaping modern agriculture (Hardoim et al. 2008; Kumar et al. 2021). These seed-inhabiting endophytic bacteria face much less competition and share a more close relationship with the germinating embryo than their rhizospheric counterparts, thereby possessing the ability to directly confer their beneficial traits to the developing seedling and contribute to its overall growth and development (Verma and White 2018; Kumar et al. 2020; Pal et al. 2019). However, it has been suggested that seed microbial communities are dynamic and are subject to changes in internal and external environmental factors (Liu et al. 2013). Further, seed status, nutrient composition, germination process, host plant tissue, as well as the genotype of the plant influence the seed microbiome (Mundt and Hinkle 1976; Song et al. 2006; Coombs and Franco 2003; Cankar et al. 2005; Adams and Kloepper 2002). The action mechanism of such bacterial endophytes is similar to the PGPRs such as modulation of plant hormones (auxin, cytokinins, ethylene, etc.), enhanced acquisition of nutrients (nitrogen, phosphorus, iron, potassium, etc.), and providing tolerance against various types of biotic and abiotic stresses. Verma and White (2018) demonstrated the role of indigenous seed bacteria in promoting seedling development of browntop millet (*Urochloa ramosa* L.), as suggested by increased root–shoot lengths, biomass, and chlorophyll content with respect to the

Table 5.2 List of PGPRs that have also been reported to control disease in crop plants against various pathogens

PGPR strain(s)	Crop	Biocontrol	Reference
Bacillus amyloliquefaciens, *Bacillus subtilis*, *Bacillus pumilus*	Tomato	Tomato mottle virus	Murphy et al. (2000)
Bacillus amyloliquefaciens, *B. subtilis*, *B. pumilus*	Cucumber	Cucumber mosaic virus	Zehnder et al. (2000)
Bacillus pumilus	Cucumber	Bacterial wilt	Zehnder et al. (2001)
Bacillus pumilus	Tobacco	Blue mold	Zhang et al. (2002)
Pseudomonas sp.	White clover, *Medicago truncatula*	Acyrthosiphon Kondoi	Kempster et al. (2002)
Bacillus subtilis, *B. pumilus*	Pearl millet	Downy mildew	Raj et al. (2003)
Bacillus cereus	Tomato	Foliar diseases	Silva et al. (2004)
Bacillus spp.	Bell pepper	Blight of bell pepper	Jiang et al. (2006)
Paenibacillus polymyxa	Sesame	Fungal disease	Ryu et al. (2006)
Enterobacter sp.	Chickpea	*Fusarium avenaceum*	Hynes et al. (2008)
Bacillus subtilis	Cucumber, pepper	Soil-borne pathogens	Chung et al. (2008)
Burkholderia sp.	Maize	Maize rot	Hernández-Rodríguez et al. (2008)
Azospirillum spp.	Rice	Rice blast disease	Naureen et al. (2009)
Pseudomonas fluorescens	Banana	Banana bunchy top virus	Kavino et al. (2010)

uninoculated controls. Further, it was also shown that these endophytes were also protecting the seedlings from fungal phytopathogens, including *Fusarium oxysporum*, *Curvularia* sp., *Alternaria* sp., and *Sclerotinia homoeocarpa* (Verma and White 2018). In another study, bacterial endophytes from seeds of finger millet (*Eleusine coracana* L.) were shown to modulate seedling growth and development (Kumar et al. 2020). A study done on abscisic acid producing seed endophyte *Bacillus amyloliquefaciens* showed a significant increase in plant growth attributes of rice (*Oryza sativa*) in saline conditions (Shahzad et al. 2017). *Pseudomonas* spp. strains isolated from seeds of tomato (*Solanum lycopersicum*) were shown to mitigate cold stress by coding cryoproteins, and reducing membrane damage and ROS levels (Subramanian et al. 2015).

5.5 Conclusion

Soil microflora holds immense potential in shaping modern agricultural practices. PGPRs under optimal conditions can effectively colonize the plant tissues and form a more close relationship with the host plant performing a number of functions that affect the plant growth and development. Plant-associated bacteria fix atmospheric nitrogen into ammonia supporting plant as well as soil health. Moreover, functions like phosphate solubilization and siderophore production also aid in nutrient cycling within the soil. Production of plant growth hormones such as auxin results in changes in the root architecture contributing to improved plant fitness under stress conditions. Antagonistic activity against various phytopathogens and providing tolerance under different types of abiotic stress also make them a more obvious candidate for agricultural applications. Positive outcomes have been reported when these microbial inoculants were used under controlled conditions whether in the laboratory or in the greenhouse. Field trials have also shown promising but heterogeneous results. However, more strategized field trials with the development of more stable and effective microbial consortium as inoculums are needed to be done for ensuring their sustainability in agriculture.

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Arbuscular Mycorrhizal Fungal Symbiosis for Mutual Benefit: More Than Expectation

6

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Abstract

Arbuscular mycorrhizal fungi (AMF) prevail in most natural terrestrial ecosystems and are widespread geographically having a very extensive host range. AMF form symbiotic relationships with the majority of plant species and play an essential role in ecosystem services, especially plant growth, disease protection and overall soil quality. AMF symbiotic associations are very useful for agriculture and horticulture. It is considered that they have the ability to not only improve crop disease and fertility management but also alter the accumulation of contaminants in plants in various cropping patterns including commercial field and greenhouse crop production. AMF produce glomalin-related soil protein and extra-radical hyphae significantly influence the soil carbon dynamics. The role of AMF is largely overlooked in terrestrial C cycling and climate change. AMF have been recognized as significantly involved in net primary productivity agumentation and further this accumulated additional photosynthets fixed in soil as soil corbon with help of their extended hyphae. AMF colonization also modulates plant defence responses and is found effective in the activation of plant immune responses locally and also systemically against a number of biotic stresses. This chapter highlights the potential of AMF beyond the C sequestration of terrestrial ecosystem concerning the way towards a better understanding of possible AMF mechanisms by which it can be utilized for sustainable agriculture looking the needs of hours the research can be moved forward in a more positive direction.

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6.1 Introduction

Symbiosis and mutualism among rhizospheric microorganisms and plants are common phenomena going on continuously in the soil. Some microorganisms have strong symbiosis while others have loose, but they have defined niches. Mycorrhizae have a strong symbiosis with the roots of nearly 90% of crops (Pressel et al. 2010). Interactions between plants and microbes for mutual benefits are frequent. They form a symbiosis in general for increased nutritional absorption of plants and/or to cope with adverse conditions. Arbuscular mycorrhizal fungi (AMF) received food and shelter from their host plant. AMF symbiosis can alter host plant physiology, and so their mineral nutrition acquisition capacity is enhanced and the resistance capability of plants is increased for both abiotic and biotic stresses. The resistance capability of plants due to AMF association varied with AMF species associated. It has been studied that AMF colonized plants often produce increased biomass and have increased productivity (Fiorilli et al. 2018). The resistance/tolerance in the plant to soil-borne pathogens due to AMF colonization has been extensively reported (Whipps 2004). AMF are soil-borne fungi and can significantly induce plant resistance to several abiotic stresses due to increased nutrient absorption (Sun et al. 2018). AMF are the group of Glomeromycota order having the colonizing ability of plant roots mostly persisting in cortical cells to form endosymbiosis relationships in nearly 80% of land plants (Ahanger et al. 2014). They form arbuscular structures at the heart of endosymbiosis. AMF take photosynthetically produced carbon compounds in exchange for nutrient supply and provide protection for hosts against pathogens and environmental stress (Sikes 2010). However, the ability of the AMF to adapt to a broad host range, the formation of these arbuscular and the molecular mechanisms involved in the process have not been clarified so far. The name "arbuscular" is derived from characteristic structures called arbuscules formed within the cortical cells of many plant roots as a result of AMF colonization (Smith and Read 2008). Among all mycorrhizae, AMF are the most important and common. First genome sequences of the *Rhizophagus irregularis* DAOM197198 isolate has been published (Lin et al. 2014). Mycelial networks of mycorrhizal fungi often connect plant root systems over broad areas. These fungi frequently comprise the largest portion of soil microbial biomass (Mahmoudi et al. 2019, 2020). AMF are zygomycetes belonging to the order Glomales. Fossil evidence (Remy et al. 1994) and DNA sequence analysis (Simon et al. 1993) revealed that both AMF and plants are almost more than 400 million years old. AMF have the most peculiar characteristics that occur during the symbiotic relationship with plant roots, it is significantly increases root surface area result of extensive hypha production. These structures help plants to grow under relatively harsh conditions. Several ecophysiological studies have demonstrated that AMF symbiosis is a key component in helping plants to cope with water stress and in increasing drought resistance through altering host root morphology to form a direct pathway of water uptake by extraradical hyphae (Dar et al. 2018).

Mycorrhizal symbiosis is one of the most fundamental types of mutualistic plant-microbe interaction. Being the most common mycorrhizal symbiosis found with

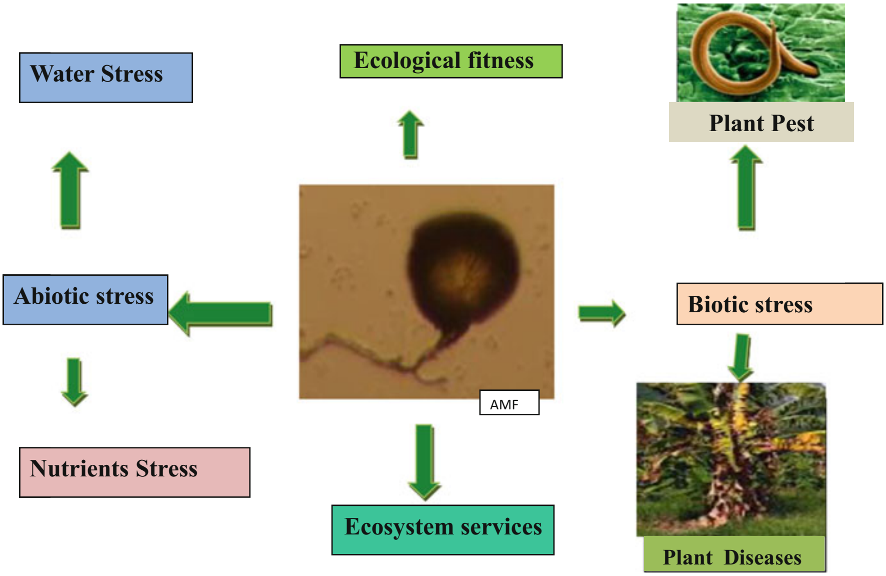

Fig. 6.1 Common benefits of AMF association to plant

AMF but at the genetic level most of AMF species are not fully characterized (Jiang et al. 2017). AMF are beneficial to the ecosystem by improving soil quality and the carbon cycle. AMF facilitate host plants to grow vigorously under stressful conditions (Begum et al. 2019). AMF mediate a series of complex communication events between the plant and the fungus leading to enhanced photosynthetic rate and other gas exchange-related traits (Birhane et al. 2012). Numerous reports describe improved resistance to a variety of stresses including drought, salinity, herbivory, temperature, metals and diseases due to fungal symbiosis (Ahanger et al. 2014; Salam et al. 2017). An increase in plant root surface area by AMF hyphal network significantly enhances the access of roots to a large soil surface area, causing improvement in plant growth (Bowles et al. 2016).

Plants may trade more than 50% of their photosynthetically carbohydrates with AMF and other microbes. AMF play a significant role in soil by mobilizing nutrients available to plants, creating optimized growing conditions, improving soil characteristics and quality and increasing water availability in soil (Fig. 6.1). AMF are thought to have a monophyletic origin in the Ordovician approximately 480 million years ago (Delaux 2017), and they are found in the majority of land plants in most taxa and virtually all ecological niches. Most land plants are facultative symbionts by nature; however, the majority of the plant species have obligate parasitism with the AMF (Graham et al. 2017). On the other end of the scale, some plant taxa, for example, the *Brassicaceae* and *Chenopodiaceae*, became asymbiotic and have no interaction with AMF (Brundrett 2004). AMF symbiosis is largely thought to have an association between more than 100,000 plant species and nearly 100 AMF morphotypes. Sexual reproduction in AMF has never been observed; however, hyphal fusion (anastomoses) occurs to exchange their genetic material for maintaining new genetic diversity in the population (Chagnon 2014). With the advent of large-scale sequencing approaches, AMF taxonomy and systematics rose to a new level (Spatafora et al. 2016). The advent of new molecular techniques and the results obtained with these modern tools indicate that the diversity of AMF has been underestimated (Lee et al. 2013). Characterization of AMF species on the basis of morphological characteristics is not sufficient, only scattered informations is available (Chen et al. 2018; Savary et al. 2018).

AMF offer crucial advantages to the host plant, especially to abiotic stress (nutrient and water) and biotic stress, and protects from diseases and pests (Malhi et al. 2021). Indeed AMF connect whole plant communities of the ecosystem and horizontally transfer plant nutrients. AMF significantly contribute to the uptake of soil nutrients, increase plant biomass and confer on the plant's improved resistance to stress and pathogens under artificial non-symbiotic condition. Looking at the diverse benefits of AMF to plants and the ecosystem, this review is compiled to collect all aspects of these important organisms for their applicability in sustainable agriculture. The present review highlights on AMF plays a significant role as a primary biotic soil component that is important for efficient ecosystem functions. The compiled findings and information may be used to improve plant AMF symbiotic interaction at molecular levels and these improvements open a new avenue to employ AMF as biofertilizers for sustainable agriculture systems.

6.2 Mycorrhiza

The word mycorrhiza is used to define the association of plant roots and biotrophic mycorrhizal fungi. These mycorrhizal fungi form a network of filaments associated with plant roots. The AM fungi release a number of hormones which lead to stimulate plant growth and accelerate root development and thereby enable plant roots to absorb mineral nutrients from the soil. Based on its morphological characteristics, mycorrhiza is classified into five groups as arbuscular, arbutoid, ecto ericoid, monotropoid and orchid (Wang and Qiu 2006). AMF are the most common variety of mycorrhizal fungi; these are microscopic and branched forms found in the cortical cells of roots (Manchanda and Garg 2007). These AMF are involved in diverse roles from accelerating the nutrient uptake to improving plant development and improving soil health and properties of the soil, thereby influencing the ecosystem. It was known that fungal-host interaction can produce microbe-associated molecular patterns (MAMPs), exopolysaccharides, volatile organic compounds (VOCs), Myc factors and Nod factors (Goh et al. 2014). Moreover, VOCs have demonstrated the ability to modulate root system architecture favouring symbiotic associations and also modulating Nod factor signalling pathway enabling AMF colonization (Maillet et al. 2011). Besides the host specificity the host plant recruit their AMF association or AMF choose their host in the ecosystem, a number of recent studies revealed that the initiation of AMF symbiosis with host plant involves varieties of plant genes, hormones and so on and of these strigolactones and fungal derived lipochito-oligosaccharides have been identified as key players (Mohanta and Bae 2015). Additionally, it has been reported that for the development of arbuscules development and AMF infection in legume crops nodulation genes NSP1 and NSP2 and DELLA proteins like SLR1 play key role in AMF infection and nodulation (Jin et al. 2016). To understand the interaction between AMF and the partner plants, identification and characterization of the fungal effectome (means the products of effector condidate genes) are found to be of immense help (Sędzielewska and Brachmann 2016).

6.3 Intracellular Accommodation of AMF

The symbiotic relationship between AMF and the roots of higher plants is widespread. Plants are to be considered to form a complex community with soil microbes and other organisms where plant tissues provide diverse niches for a number of microbes (Kothe and Turnau 2018). AMF are being very long evolutional history (more than 400 million years) to the developed mechanism of the symbiosis (Heckman et al. 2001; Schüssler et al. 2001). This involves plant-derived and fungal signalling molecules that enhance AMF colonization (Gutjahr and Parniske 2013) to form a symbiosis. It includes the extent of adaptation and genetic/metabolic coordination between mycorrhizal partners. Indeed, AMF symbiosis requires a dedicated signalling pathway starting with the root-exudate (strigolactone) acts as signals, which stimulate AMF activity (Kretzschmar et al. 2012). Just before colonization, AMF subsequently secrete a chemical called lipochito-oligosaccharides; host plant sense it and activate a signal transduction pathway. This is shared with root nodule symbiosis and referred to as a common symbiosis signalling pathway (CSSP) (Gutjahr and Parniske 2013). It is well established that generally AMF have very low host specificity. The bidirectional signal exchange involvement during symbiosis is a present-day challenge to our current understanding of communication between both partners. The AMF and their hosts will constantly communicate to establish and maintain the symbiosis. The symbiotic mechanism of AMF and their host comprises many steps: The first step is the searching for the host root. The second step is the penetration of fungi into the host root. The final step is the establishment of mycorrhizal symbiosis. To complete the above-mentioned steps, several molecules (strigolactones) are secreted by the roots and they help AMF to identify their host plants. It also stimulates the growth of AMF and their branching. The AMF interactions are established further with the induction of seven genes (Bonfante and Genre 2010). A multi-level interaction thus will be visible with changed transcriptome, proteome and metabolome patterns. These can be visualized with techniques such as transcriptomics (Nagabhyru et al. 2018), proteomics (Shrivastava et al. 2018) and metabolomics (Rivero et al. 2018; Hill et al. 2018) or combinations thereof (Larsen et al. 2016). Wagner et al. (2015) identified protein effectors as important for the signal exchange between the symbiont and the host. A subgroup of metabolites known as volatile compounds is also addressed for signal exchange (Pistelli et al. 2017). In nature, a partnership between plant and AMF is not alone, and additional interactions with bacteria or other fungi will influence the outcome of these associations. These additional interactions in the vicinity of the root are also considered an important mechanism of cross-talk between the partners (Wagner et al. 2016). However, microscopic evidence shows that at later stages the interaction has a very high degree of coordination at the cellular level by the formation of an infection structure that allows cellular invasion (Genre et al. 2008) leads to the formation of the intracellular arbuscules serve as a nutritional (Food storage) interface between the partners (AMF and Plant) (Harrison 2012). The molecular-genetic basis of structure formation is still elusive. The formations of structures is thought to be a prerequisite for AMF infection of host roots and to require signalling through the common symbiosis signalling pathway (Genre et al. 2005). The establishment of AMF is associated with a fundamental reprogramming of the host cell activation of hundreds of genes (Hogekamp and Küster 2013; Calabrese et al. 2017). These genes are thought to be required for intracellular accommodation of the fungus and for coordination of symbiotic functions with their host.

AMF spores start germination just followed by signalling process and grow in search of the host root surface by branched fungal mycelia. Finally, they reach host root surface and penetrate and colonized it and establish in the cortical region with finely divided hyphae that ultimately develop into arbuscles (Parniske 2008). A membrane protein (SYMPK) is activated, which codes for a receptor-like kinase with the potential to recognize AMF signals directly or indirectly. Gutjahr and Parniske (2013) reported that a second membrane protein transduces these signals from the cytoplasm to the nucleus by phosphorylating an unknown substrate through its kinase domain. This eventually leads to the regulation of other genes and finally root colonization takes place (Parniske 2008).

6.4 Exchange of Benefits in AMF Symbiosis

Arbuscular mycorrhizal fungi (AMF) in the present-day agriculture is believed to be essential (Sosa-Hernández et al. 2019). AMF are considered as evolved from one ancestral group, an extensive group of fungi that form a mutualistic relationship with most land plant species, including agricultural crops (Brundrett and Tedersoo 2018). AMF are known for their ability to increase plant nutrient uptake and productivity (Smith and Smith 2011). AMF biomass abundance, spore numbers and root colonization levels typically decline with increasing soil depth; however, over 50% of AMF biomass was found below 30 cm (Higo et al. 2013) and from natural plantation it was found up to 8 m depth (De Araujo Pereira et al. 2018). AMF communities are highly relevant components of agroecosystems and found below the plough layer (deeper of six inches) generally are overlooked especially in associated with trees.

AMF form finely branched fungal structures called arbuscules which surround the peri-arbuscular membrane of the host and increase the contact surface between the two partners. It has been estimated that it increased the contact area to correspond to a multiple of the entire cell surface (Alexander et al. 1989). Therefore, cells with AMF arbuscules are a suitable site for nutrient exchange. Indeed, the plant host expresses many symbiosis-specific nutrient transporters that are thought to mediate mineral nutrient uptake from the AMF (Rausch et al. 2001) and most one is a symbiotic phosphate transporter (AMF genes responsible for phasphate absorption and translocation) (Yang et al. 2012). Vigneron et al. (2018), based on phylogenomic analysis and its orthologs in other land plants, suggest that the AMF-related phosphate uptake pathway represents an early evolutionary innovation. The available information suggests that the arbuscules are the site of the transfer of phosphate from the fungus to the plant and phosphate delivery is the most important benefit of this symbiosis (MacLean et al. 2017) and many other mineral nutrients (Wang et al. 2017). George (2000) also suggests that nutrient elements such as nitrogen, sulfur, and microminerals (i.e. copper and zinc) may be transferred via the arbuscules. Interestingly, AMF-related pathways can also stimulate plant growth and physiology in nutrient-independent ways. Boldt et al. (2011) found that mycorrhizal plants show enhanced photosynthetic capacity. More prominently, the overexpression of a petunia strigolactone transporter (PDR1), which is involved in AMF signalling, has been reported by Kretzschmar et al. (2012). It is sufficient to improve root and shoot growth in the absence of AMF (Liu et al. 2018a, b). AMF and their signalling can potentially increase plant growth in yet unexplored ways. In return for the symbiotic services of the AMF, photosynthates by partner plant receives fixed carbon from the plant. In correspondence to plant-pathogen interactions, carbon transfer has long been thought to carry on in the form of carbohydrates. Indeed, a large body of evidence has demonstrated that AMF can take up and utilize sugars, but only under symbiotic conditions in the roots (Roth and Paszkowski 2017). The work of Tang et al. (2016) indicates that AMF may generate their abundant lipid reserves in spores and vesicles and genomes of two AMF lack a fatty acid synthase complex (Rich et al. 2017). It is thought that the plant host induces several components of fatty acid biosynthesis and processing in mycorrhizal roots indicating that AMF may also receive fatty acids besides sugars. Indeed, it has been confirmed with the recent evidence (Luginbuehl et al. 2017; Brands et al. 2018). AMF lipids are at least partially derived from the host plant; moreover, many aspects of lipid transfer to AMF stay behind to be clarified.

A number of soil-inhabiting microorganisms interact with AMF (phosphate solubilizers, free-living and symbiotic nitrogen fixers, antibiotics, plant growth hormone, siderophore and chitinase producers, saprophytes, plant pathogens, predators and parasites). Some soil-inhabiting bacteria possess the ability to produce antibiotics or siderophores which are iron chelators that may act as inhibitors against several plant pathogens or may stimulate plant growth. AMF hyphae, in addition to having enhanced nutrient absorption capability of their host plant, provide an area for the interaction of plants with other soil microorganisms that have an effect on root development and performance (Toljander et al. 2006). These interactions may happen positively or negatively and they may be inhibitory or stimulatory and competitive or mutualistic to each other for the plant. Cordier et al. (1999) reported that AMF establishment in the host rhizosphere changes the microbial population both quantitatively and qualitatively. AMF symbiosis and hyphal net formation can directly or indirectly affect microbial communities in the rhizosphere (Barea et al. 1997). Kloepper (1996) observed that rhizobacteria are known to show a specific ability for root colonization and act as plant growth-promoting rhizobacteria

(PGPR). Significant progress has been made in estimating the exchange of resources between plants and AMF and their regulation (Walder and van der Heijden 2015). Kiers et al. (2011) demonstrated that the exchange of carbon for nutrients is reciprocally regulated, such that the most beneficial partner receives the most resources in return. These striking results support the idea that biological market dynamics ensure the evolutionarily stable regulation of resource exchange in AMF symbioses (Noë and Hammerstein 1995).

6.5 Significance of AMF for Plants in Natural and Agroecosystems

AMF have a symbiosis with a number of host species in the different ecosystems and considered are not host-specific (Ingleby 2007). The same species of AMF associated with trees can colonize crop species and therefore enhance both tree and crop growth in agroforestry systems. Therefore, the colonized tree species can act as a reservoir of AMF, from which roots of growing crop seedlings can quickly form mycorrhizal associations. Almost all types of soils harbour AMF spores despite the different structural and chemical differences of the cropping fields (Don-Rodrgue et al. 2013). Jean et al. (2018) reported that AMF associated with agroforestry plantations may colonize maize crop plants and they reported that agroforestry plantations harbour AMF inoculum. AMF are one of the most widespread symbiotic fungi colonizing the majority of agricultural plants (Posta and Duc 2020). The effects of AMF on plant growth and physiological elements contents have been widely studied in many species. The ability of AMF to enhance host plant uptake of relatively immobile nutrients, particularly phosphorus (P) and several other micronutrients, has been the most recognized beneficial effect of AMF symbiosis (Smith and Smith 2011). Growth stimulation in the host plant is the result of AMF extending the absorbing network beyond the nutrient depletion zones of the rhizosphere, which allows access to a larger volume of soil. AMF hyphae are much thinner than roots and are able to penetrate smaller pores and uptake more nutrients (Allen 2011). Numerous studies show that AMF (*Glomus intraradices*) has the ability to solubilize rock phosphate through localized alterations of soil pH and/or by the production of organic acid anions. This alteration through the production of chemicals may act as chelating agents. Additionally, AMF colonization is known to improve plant nitrogen nutrition; however, their role in making N available to plants has not been fully recognized. Uptake of other nutrients, such as Na, K, Mg, Ca, B, Fe, Mn, Cu and Zn, by growing plants is also influenced by AMF colonization (Bucking et al. 2012). The benefit of AMF colonization is not quantified as it depends to a large degree on the environmental conditions. In most natural conditions and under mineral nutrient deficiency coupled with abiotic stress conditions, mycorrhizal plants are thought to have a selective advantage over non-mycorrhizal individuals of the same species. Thus, AMF can potentially promote intraspecific competitiveness and selectively favour mycorrhizal plants. Since numbers of host plant colonized by different species of AMF and similarly AMF species infects numbers of host species, from this point of view it is not clear which partner (AMF or host) gets what types of benefits (nutrients, Protection and shelter). The common mycorrhizal networks (CMNs) add an additional level of complexity to the analysis of benefits in mycorrhizal interactions (Jakobsen and Hammer 2015). These common mycorrhizal networks (CMNs) play a critical role in the long-distance transport of nutrients through soil ecosystems and allow the exchange of signals between interconnected plants. CMNs affect the survival, fitness and competitiveness of the fungal and plant species that interact via these networks, but how the resource transport within these CMNs is controlled is largely unknown. A strongly interconnected plant community can potentially gain stability because weaker individuals could profit from mineral nutrient supply from the CMN at the expense of stronger plants that entertain the CMN. In this way, the stronger plants indirectly benefit less competitive plants, thereby attenuating competition among plant individuals. Such "underground socialism" has been invoked particularly in cases where seedlings grew better when they were connected to a CMN that had been established by older plants (Bücking et al. 2016). In the most extreme version of the theme, achlorophyllous plants obtain all their resources, including carbon, from CMN, thereby parasitizing—indirectly—on other plants that supply the network with their carbon (Bidartondo et al. 2002). This can be liked to the transitional evolutionary phase from autotrophy to mycoheterotrophy (Selosse et al. 2017). Aggarwal et al. (2011) also reported that symbiosis of AMF grants benefits directly the host plant's growth and development. Besides enhancing plant growth and development, they provide a range of benefits from stress alleviation to bioremediation of heavy metals in polluted soils. They may also enhance resistance in a plant against pathogens and increase the plant diversity. Other services provided by AMF in natural and agricultural ecosystems are given in Fig. 6.2.

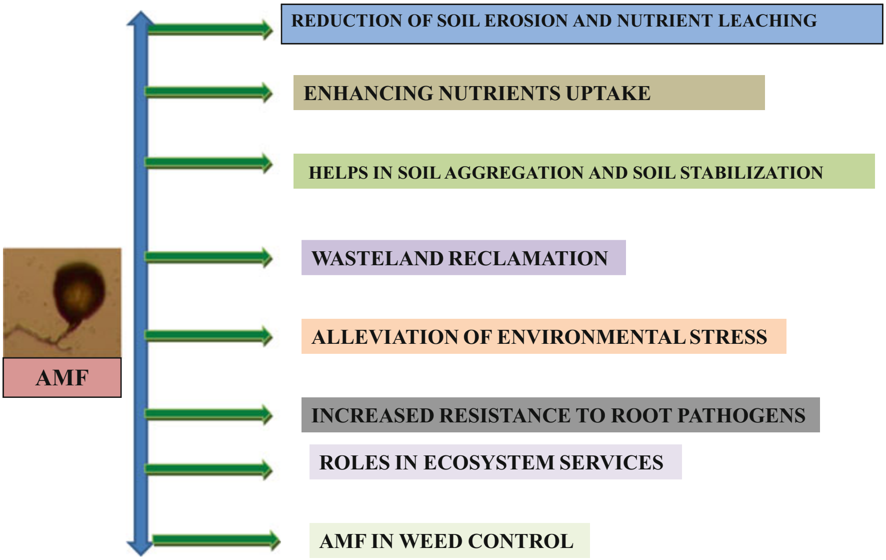

Fig. 6.2 Significance of AMF in natural and agricultural ecosystem

6.6 Functional Specificity in AMF Interactions with Host Plant

AMF-associated soil microorganism is fundamental for the maintenance of plant health, as it can protect plants from soil-borne diseases and various abiotic stresses. Schweiger et al. (2014) reported that the metabolome is next to diverse biological functions and thus critical for ecological interactions between plants, their enemies, and beneficial microorganisms. AMF alter the plant metabolome by a different way (improving water uptake and nutrition of the host plant) primarily due to increased phosphate supply and acting as an additional sink for plant-derived photosynthates. They are also influencing photosynthesis. AMF are well-known symbiont having agricultural importance, as enhance nutritional values and yields of crops, modulate crop pest and stress resistance and influence global C and P cycling, ecosystem services and primary productivity (Parniske 2008). On the other hand, AMF colonization and formation of extra-radicle hyphae and spores are highly dependent on plant host identity (Thirkell et al. 2019). Indeed, a combinatorial study on mycorrhizal benefits employing a large panel of plant and fungal species from different geographical locations showed that the mycorrhizal growth response ranged from −50% to +50% growth promotion (Kaur and Suseela 2020). The mutualistic potential did not correlate with phylogenetic patterns in either partner. Interestingly, combinations of partners isolated from the same location performed better, indicative of co-adaptation (Wyatt et al. 2014). In agreement with functional specialization, soils with a diverse AMF flora can support more diverse plant communities than if only one or few AMF are present (van der Heijden et al. 1998). Thus, despite the very low host specificity of AMF under laboratory conditions, functional specialization within the AMF community shapes the level of the biodiversity and productivity of plant communities at the ecosystem level. Functional diversity of AMF is given in Fig. 6.3.

6.7 Benefit of AMF to Natural and Agroecosystems

Anthropogenic activities in developing countries like India pose a number of ill-effects on the environment. These activities increase accumulation of heavy metals and other organic contaminants into the different eco-system, causing ill effect to environment, contaminates water bodies and accumulate in soil and finally accumulates in crops (Rai et al. 2019). Continuous release of exceeding amounts of contaminants such as geochemicals, agrochemicals and industrial chemicals containing heavy metals pose risks to food safety and human health have much considered nowadays and urgent needs felt for formulating strategies to reduce their effects (Rai et al. 2019). Besides playing an important role in crop and soil health improvement, AMF also reduce the accumulation of contaminants in plants. AMF symbiosis and its role in the reduction of contents of organic contaminants and the underlying mechanisms have been studied in detail. Results of experimentation indicate that AMF widely occur in contaminated sites with organic chemicals. AMF improve plant tolerance to organic contaminants and enhance crop growth,

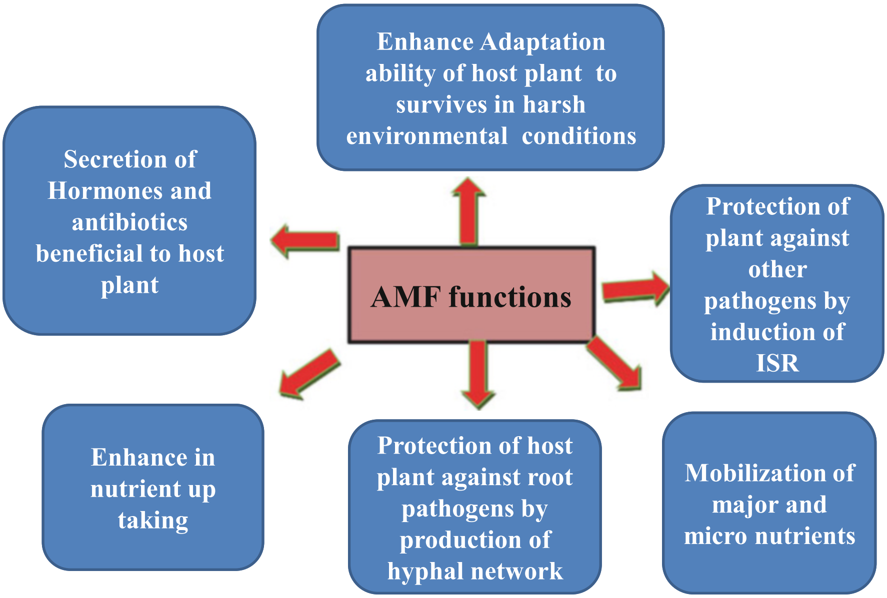

Fig. 6.3 Functional diversity of AMF symbiosis in natural and agricultural ecosystem

leading to increased biomass of the crops. Besides improved nutritional supply to the host plant, AMF interactions also provide other benefits to plants, such as improved drought and salinity tolerance (Augé et al. 2015) and disease resistance (Pozo and Azcón-Aguilar 2007). In the last few years, the mechanisms responsible for the increased plant tolerance to stress have yet to be fully elucidated (Szczałba et al. 2019). Several benefits received by AMF symbiosis and the mechanisms in ameliorating organic contaminant residues in crops can be easily understood with following points: (1) AMF improved mineral nutrition and water availability, (2) they alleviated oxidative stress results of contaminants, (3) they enhanced activities of enzymes related to contaminant degradation, (4) AMF structures like mycelia and arbuscles accumulate and capture of contaminants, (5) glomalin-related soil protein (GRSP)-triggered changes in bioavailability of contaminants, (6) AMF also stimulate the activity of contaminant degrading soil microorganisms, (7) improved soil structure; and (8) reduced pesticide application as act as biopesticides and biofertilizer. (9) Reduced weed population because of inhance plant growth. AMF may provide several benefits to the ecosystem (Chen et al. 2018). A number of studies and reviews have highlighted the AMF contributions to phytoremediation of soils polluted with heavy metals (Wang et al. 2017) and organic contaminants (Rajtor and Piotrowska-Seget 2016). AMF can enhance phytoextraction efficiency by increasing the accumulation of contaminants in plants, particularly in aerial parts (Cabral et al. 2015). In general, the plant faces two major stresses: abiotic related to water, nutrient and environmental stress and the other is biotic, which includes stresses rendered by various pathogenic microorganisms causing diseases and other pests. AMF association mitigates this stress by employing various mechanisms induced by themselves or induced by host plant in response to AMF. Thus AMF contributed in so many ways to host plants and play a significant role in sustaining the ecosystem. Besides the above-mentioned attributes, AMF can also provide other ecological functions by influencing the soil microbial community and chemical environment of the mycorrhizosphere. AMF symbiosis may play a significant role in stabilizing soil aggregates by the production of glomalin and conferring plant tolerance to several abiotic stress (Li et al. 2013; Chitarra et al. 2016) and biotic stress (van der Heijden et al. 2015).

6.7.1 AMF Association for Mitigation of Biotic Stress in Cropland Ecosystem

In addition to the benefits of nutrient cycling and acquisition to plants, AMF-associated plants showed increased tolerance to abiotic and biotic stresses (Rivero et al. 2018; Campo et al. 2020). AMF priming is suggested as the mechanism underlying mycorrhiza-induced resistance (Balmer et al. 2015; Sanmartín et al. 2020). AMF symbiotic plants have more resistance to several pathogens. AMF symbiotic plants scale up defences in a faster and more efficient manner, representing a phenomenon known as defence priming (Martinez-Medina et al. 2016; Mauch-Mani et al. 2017). Out of several defence responses, the most fascinating cellular defence responses against pathogens are the deposition of β-glucan polysaccharide callose. This sugar polymer strengthens plant cell walls against attackers, blocks their entrance and provides the plant with additional time to activate subsequent defence mechanisms from their cascade if needed. Mustafa et al. (2017) demonstrated that AMF symbiotic plants infected with *Blumeria graminis* show increased papillae formation at penetration sites. AMF can trigger callose accumulation in wheat following chitosan infiltration (Pérez-de-Luque et al. 2017). Sanmartín et al. (2020) reported that *B. cinerea* infection in tomato plants can be challenged with priming of *Rhizoglomus irregularis*. Roth and Paszkowski (2017) found that several sugar transporter genes belonging to the SUT family (SUT1, 2, and 4) and SWEET family, some invertase genes (LIN6) and sucrose synthases have higher levels during AMF symbiosis. Plants colonized with AMF act by various mechanisms to combat biotic stresses (Fig. 6.4).

6.7.2 Effects of AMF on Plant Defence and Disease Resistance

Mycorrhizal roots often exhibit intense colonization both intercellularly and intracellularly and sometimes it can be reached more than 90% of the total root length. AMF colonization mostly depends on the right fungal partner and is very much influenced by the environmental conditions; however, fungi have to produce chitin as a molecular signal to activate defence mechanisms of plants (Boller and Felix 2009). Indeed all pathogens usually produce inhibitors of defence known as

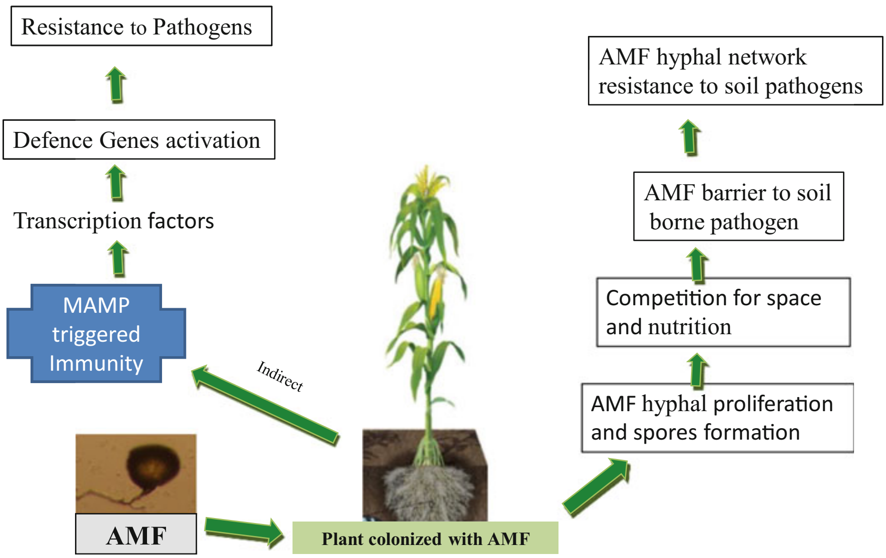

Fig. 6.4 AMF strategies to mitigate biotic stress in plant under crop land ecosystem

effectors. It has been predicted that AMF also have numerous effectors in the genomes (Sedzielewska Toro and Brachmann 2016; Kamel et al. 2017). Only a few of them have been functionally analyzed (Kloppholz et al. 2011). In contrast, AMF symbiotic plants often exhibit increased disease resistance (Cameron et al. 2013). AMF colonization in nature improved plant health due to better nutrition acquisition, or a systemic induction of the defence status, well recognized as systemic acquired resistance (SAR). In addition, AMF, or other microbes associated with their mycelium, can directly interfere with rhizospheric pathogens.

6.7.3 Mechanism of Plant Disease Reduction by AMF

The AMF symbiosis involves several mechanisms in the management of plant biotic stress. A numbers of studies and reports suggested that AMF followed various strategies to combat biotic stress (Dehne 1982; Krishna and Bagyaraj 1984; Reid 1990) and the mechanisms involved are as follows:

(a) Production of lignifications, plant roots become thicker and production of other polysaccharides which in turn restrict the entry of root pathogens.
(b) It creates a mechanical barrier in the host root which hinders the pathogen penetration and subsequent spread.
(c) By producing and accumulating a sufficient quantity of metabolites, which provide resistance to the host tissue against plant pathogen invasion.

(d) Stimulating flavonoid wall infusions which prevented lesion formation by the pathogen *Fusarium oxysporum*.
(e) Increase amount of phenol reduce pathogen growth.
(f) Producing antifungal and antibacterial antibiotics and toxins.
(g) Colonizing the host plant root and rhizosphere and showed competition with the soil-borne pathogens for the uptake of essential nutrients.
(h) Stimulating the rhizospheric microbial activity and creating competition in the root zone that resulted in preventing the pathogen to get access to the roots.
(i) Roots colonized by AMF enhanced the activity of actinomycetes antagonistic to root pathogens.
(j) AMF helps plant in absorption due to extended hyphal growth.
(k) Changing the amount and type of plant root exudates favouring the pathogen growth.

Other workers reported that resistance to fungal diseases was found with inoculation of AMF. Inoculation of *G. mosseae* significantly reduces pink root disease caused by *Pyrenochaeta terrestris*. Abdalla and Abdel (2000) reported that *G. mosseae* protects peanut plants from infection by *F. solani* and *Rhizoctonia solani*. Plant resistance/tolerance to biotic stresses can be enhanced drastically through AMF inoculation (Saghir et al. 2010).

6.7.4 AMF Association for Mitigation of Abiotic Stress in Cropland Ecosystem

Drought stress is one of the most devastating abiotic factors among the different forms of abiotic stress, threatening crop growth and productivity worldwide (Guo et al. 2020). Both salt and drought stresses share some common properties and generally result in impaired key physiological functions in living organisms such as fungi (Daffonchio et al. 2015). Salinity, drought and high temperature have become serious problems in many regions, not only because of a higher risk to public health and the environment but also because of negative effects on the yield. Salinity characterizes as hyperosmotic stress is one important stress, posing a water deficit that is comparable to a drought-induced water deficit (Daffonchio et al. 2015). The application of AMF for the mitigation of such salinity can be achieved (Plouznikoff et al. 2016). With the multiple benefits that AMF confer to their hosts, they hold great promise for application in crop production under various conditions. Most agricultural crops are hosts for AMF and can therefore potentially benefit from inoculation with AMF. Some AMF species were isolated from Arabian arid regions and deserts: *F. mosseae*, *Claroideoglomus etunicatum*, *R. fasciculatus*, *G. aggregatum, Diversispora aurantia*, *D. omaniana*, *S. africanum* and undescribed *Paraglomus* species (Dhar et al. 2015; Symanczik et al. 2015). Indeed, many studies have shown that the application of commercial AMF inoculum benefits crops under agricultural conditions (Weber 2014). Numerous studies have shown that AMF can increase plant health and yield (Rouphael et al. 2015; Hijri 2016). One important

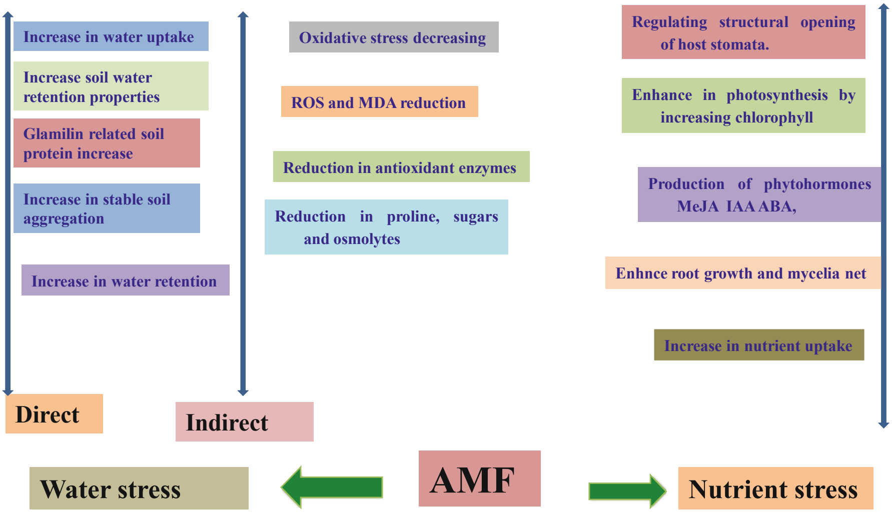

Fig. 6.5 AMF strategies to mitigate abiotic stress of plant

aspect of this is the promotion of root system development (Gutjahr and Paszkowski 2013). Zou et al. (2020) suggested that AMF can regulate physiological and molecular responses to tolerate drought stress, and they have a strong ability for coping with drought-induced oxidative damage through the production of the oxidative burst in the arbuscule-containing root cortical cells. Similar to plants, AMF modulate a fungal network in enzymatic (e.g. Superoxide dismutage SOD encoding genes (*GmarCuZn SOD*) from *Gigaspora margarita* and copper-zinc superoxide dismutase Cu/Zn-SOD gene (*GintSOD1*) from *Glomus intraredices*) and non-enzymatic (e.g. GintMT1, GinPDX1 and GintGRX1) antioxidant defence systems to scavenge ROS. Plants also respond to mycorrhization to enhance stress tolerance via metabolites and the induction of genes. Li et al. (2020) also reported that AMF inoculation alleviated the toxic symptoms under moderate salt levels (100 and 150 mM). Greenhouse study conducted on potatoes clearly indicated that AMF inoculation helps in the mitigation of phosphorus and nitrogen uptake and water use efficiency (Liu et al. 2018a, b). Chakraborty and Saha (2019) in their reviewed paper indicated that AMF are the most important component in the mitigation of several abiotic stress. AMF also help plants to cope with heavy metals, diseases and pathogens. AMF symbiosis mitigates these stresses through various mechanisms, which are increased hydromineral nutrition, ion selectivity, gene regulation, production of osmolytes and the synthesis of phytohormones and antioxidants (Diagne et al. 2020). Different mechanisms and strategies adopted by AMF for the mitigation of abiotic stress are given in Fig. 6.5.

6.8 Significance of AMF in the Managed Ecosystems of Different Climatic Zones

AMF are associated with a majority of plants in natural habitats and are playing a significant role in important ecological services from nutrient mobilization and acquisition to the improvement of soil structures. AMF are an important component of all ecosystems and their diversity and distribution mostly depend on the cop communities, soil types and climatic factors of the ecosystem. These symbiotic associations are found with most crop plants including cereals, vegetables and fruit trees and well recognized components of sustainable agriculture (Chen et al. 2018). Gao and Guo (2010) reported that AMF are present in almost all the ecosystems. Vieira et al. (2019) concluded that the AMF community assemblages in tropical mountains are related to the heterogeneity of habitats of these ecosystems. Different land use patterns affect the diversity and distribution of AMF species in ecosystems. Jansa et al. (2006) observed that AMF functional traits differ considerably among and within species, meaning that the functional properties of a mycorrhizal community depend on its composition. Melo et al. (2020) observed that AMF species varied with the type of crop species in the ecosystem. Deforestation is a major problem in an arid ecosystem. Replantation by adaptation of agroforestry in the degraded land through Government along with local bodies is now executing to restore natural habitat. These are the measures to stabilize degraded and eroding surfaces. Newly established trees are very vulnerable to abiotic stresses. This critical phase can be overcome with mycorrhizal inoculation of the trees before planting. Mycorrhizal inoculation significantly increases the growth and health of young trees, thereby increasing their fitness and survival after planting (Sellal et al. 2017). Another interesting example is stabilizing sand dunes by planting the drought-tolerant mesquite tree (*Prosopis juliflora*), which increases mycorrhizal communities in sand dunes (Moradi et al. 2017)

6.9 Commercialization of AMF

AMF have multifaceted characteristics that raised opportunities for their commercial application. It has been observed that the AMF-related markets considerably grew well during the past decades, with increasing numbers of actors, products and market volume (Vosatka et al. 2008). Commercial AMF products are readily available and targeted at the general public and agricultural industry. In the market, mycorrhizal formulations are sold in granular, powder, liquid and tablet forms. Dry products comprise dusts, granules and wettable powders. Dusts have a particle size ranging from 5 to 20 mm and contain about 10% inoculums of an organism by weight. These products include inert carriers such as charcoal, lignite, clay minerals (perlite, vermiculite and bentonite), starch polymers, dry fertilizers and ground plant residues (Pal et al. 2016). Since the 1990s, the number of companies selling mycorrhizal products in the markets. If we consider globally, the main players are coming from North America, Europe, Asia and Latin America. In the domain of the Americas, the main markets include the United States, Canada, Mexico, Brazil, Argentina, Colombia and Chile. The Asia region is mainly dominated by India, followed by China. The Indian market itself has seen an outstanding growth during the last decade mainly due to the involvement of organizations such as The Energy and Resources Institute (TERI). In general, the AMF are being produced by small- and medium-stakeholders and cover local and regional markets. The European market represents one of the leading markets for mycorrhizal biostimulants. In Europe itself, the number of firms producing and selling AMF products has increased from less than 10 firms in the late 1990s to more than 75 firms in 2017. The largest domains of application include gardening and landscaping, horticulture, agriculture, forestry, golf courses, recultivation of degraded land, roof plantings, soil remediation and research. The cost of mycorrhizal inoculation for professional uses at an agricultural scale is considerably low, with an estimated investment of 135$ per hectare in the case of potatoes in the United States (Hijri 2016). Apart from pure AMF inocula, many products include mixed fungal inocula, sometimes in combination with ectomycorrhizal fungi or with plant growth-promoting rhizobacteria.

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Rhizodeposits: An Essential Component for Microbial Interactions in Rhizosphere

7

Madhurankhi Goswami and Suresh Deka

Abstract

Rhizodeposits are essential rhizosphere-associated constituents synthesized by plants that support various biological and physiochemical activities in soil. They significantly influence microbial root colonization capacity, multiplication of rhizosphere microorganisms, soil microbial activity, soil health and secretion of organic bioactive compounds. Root exudates are a group of vitally important compounds with multifarious functions and are released from the living plant roots. They are a complex group of substances secreted by plant roots consisting of low molecular and high molecular weight constituents. The root exudate composition reflects the opposing-associating trait of plants towards rhizosphere microorganisms. These rhizosphere microorganisms produce a wide range of antibiotics that provide a defence to the host plants against a number of phytopathogens. The root exudates, produced as a part of the rhizodeposition process, exert a direct impact on the biogeochemical cycling of carbon and nitrogen. They help in modulating organic matter decomposition in soil by altering the microbial communities involved in the decomposition of soil organic matter and also affect the soil nitrification process. Root exudates are well known for their activity as chemoattractant and signalling molecules for successful

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interactions between plant and rhizosphere microorganisms. Thus, the current chapter will elaborate on the various roles of root exudates in plant-microbe interaction and rhizosphere functioning, the mechanism of root exudates and the molecular insights of the root exudation process.

7.1 Introduction

7.1.1 Rhizosphere: A Dynamic Ecological Niche Space for Complex Microbial Interactions

The ‘rhizosphere’ which refers to the nutrient-rich region of the soil surrounding the plant roots is a metabolically active and diversely rich hot spot for microorganisms, considered to be the most complex ecosystem on Earth. The various biological and chemical processes occurring in the rhizosphere soil is solely influenced by the roots. The rhizosphere represents the most complex and metabolically active region of the soil which controls plant-microbe interactions. The complexity of the rhizosphere region varies with the variation of plant genotypes and also with the age and architecture of plant roots. Apart from the domain, the width of the rhizosphere region also depends on the plant species. The rhizosphere microzone differs from the bulk soil (commonly known as edaphosphere) in terms of increased microbial population and metabolic activity and also due to higher accessibility of plant root exudates. Over the years, efforts have been made to restructure and regenerate the definition of rhizosphere so as to depict three regions, which include the endorhizosphere, the rhizoplane and the ectorhizosphere. The endorhizosphere, as the name suggests, refers to the apoplastic space between the cells and includes the root cortex and the endodermal cells. The rhizoplane refers to the intermediate region or the root surface adjacent to the roots. The ectorhizosphere is the topmost zone of soil that surrounds the plant roots (De-la-Pena and Loyola Vargas 2014).

The rhizosphere microbiome is a complex, highly dynamic microbial assemblage under the control of a number of environmental factors. Soil and plant genotypes are equally crucial in shaping the rhizosphere microbiome via recruitment of soil microorganisms from the bulk soil. Even during a pathogen attack, the host plant actively takes part in selecting a specific group of microbial cells from the rhizosphere microbial community to control and inhibit the infection. This is how disease suppressiveness is triggered in soil despite the presence of virulent soil pathogens. Disease suppressiveness is solely due to the involvement of soil microbial communities and the intense microbial activity in the rhizosphere zone which is activated with an onset of a disease (Mazzola 2002). The recruitment of specific antagonistic microorganisms from the rhizosphere during pathogen invasion was studied and elaborated by Mavrodi et al. (2012). For example, under circumstances of pathogen attack in wheat plants, that is, *Gaeumannomyces graminis* var. *tritici* (under irrigated conditions) and *Rhizoctonia solani* (under dry conditions), the major soil-borne pathogens attacking wheat plants, the wheat rhizosphere recruits

2,4-diacetylphloroglucinol (DAPG) producing pseudomonads and phenazine producing pseudomonads to suppress the growth of take-all pathogens *G. graminis* var. *tritici* and *R. solani*. Thus, under conditions that favour pathogen attack, the host plant recruits antagonists from the rhizosphere microzone to suppress the ill effects of pathogens on host plants.

The rhizosphere soil is an indication of a high microbial population, increase microbial diversity and metabolic activity. The diversity and functionality in the rhizosphere soil are mainly owing to the production of root exudates due to the secretion of organic carbon by the roots (Bakker et al. 2013). Plants act as the main source of organic carbon in soil due to physiological and biochemical plant processes such as litterfall, plant senescence and the C loss from the plant roots. These plant-derived C inputs constitute approximately 0.5–10% of the total C fixed in the soil. The soil microbes get chemotactically attracted to the C containing compounds secreted by the plant roots leading to their survival and proliferation in this carbon-rich environment. These root-derived C containing compounds are collectively referred to as rhizodeposits (Farrar et al. 2003).

7.2 Rhizodeposits: A Vital Component for Plant-Soil Linkage

Rhizodeposits are grouped into different classes based on their composition, mode of release or function. They include substances like root exudates, actively released secretions like proteins, mucilage, lysates, secondary metabolites and inorganic molecules, shedded border cells, root cap cells and the ageing root tissue (Bowsher et al. 2018). The rhizodeposits released into the soil are differentially used by diverse components of the soil community that includes the rhizosphere microbial communities and other residing soil fauna. They behave specifically towards a different group of soil microorganisms resulting in their attraction or repulsion (Fig. 7.1). The composition, superiority and quantity of root exudates being released by the host plant vary between the type of plant cultivar, plant developmental stage and also on environmental factors such as soil type, soil pH, temperature and the residing microbial communities. This chapter will spotlight the importance of root exudates, based on the large body of literature, with the aim of unveiling the mechanistic insights of root exudation patterns and the potent roles of root exudates in different dimensions.

7.2.1 Root Exudates: A Multifunctional Compound of the Rhizosphere Microzone

Root exudates are considered to be the most essential among the rhizodeposits for plant-microbe interactions and rhizosphere microbial community structure. The living roots of plants release plant photosynthates that include sugars, carbon compounds, inorganic ions, metabolites and amino acids as root exudates (Badri

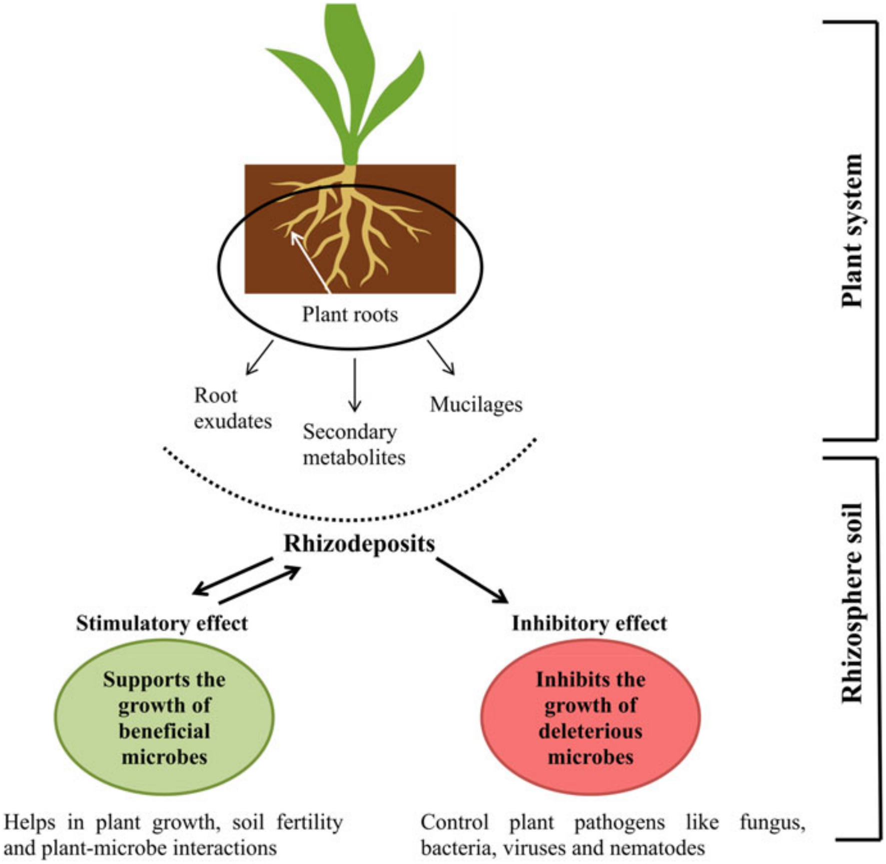

Fig. 7.1 Interaction of rhizodeposits with beneficial or pathogenic rhizosphere soil microorganisms resulting in the stimulatory or inhibitory effects

et al. 2013; Chaparro et al. 2013). The sugars that are present in the root exudates include monosaccharides, disaccharides and five-carbon sugars; amino acids include arginine, asparagine, glutamine, aspartate and cysteine. Root exudates also consists of organic acids such as benzoic acid, acetic acid, ferulic acid, ascorbic acid and malic acid. Apart from the aforementioned constituents, root exudates also contain phenolic compounds and some high molecular weight compounds such as auxin, gibberellin, flavonoids, fatty acids, enzymes, nucleotides, alkaloids, polyacetylenes, tannins, steroids, terpenoids and vitamins (Hayat et al. 2017) (Table 7.1). Root exudates are classified into two groups: the high molecular weight (HMW) compounds such as proteins, terpenoids, vitamins and polysaccharides and low molecular weight (LMW) compounds such as amino acids, sugars, phenols, organic acids and other plant metabolites. The HMW compounds are not easily utilizable by soil microorganisms but make up the majority of C present in the root exudates,

Table 7.1 Different potential roles of root exudates and the compounds detected in root exudates (adapted from Dakora and Phillips 2002; Haichar et al. 2014)

Component in root exudates	Functions	Detected components in root exudates
Amino acids and phytosiderophores	Nutrient source, acts as a chemoattractant and helps in chelating poorly soluble mineral nutrients such as Fe, Al and Ca phosphate	a- and b-alanine, proline, Asparagine, valine, threonine, aspartate, tryptophan cysteine, ornithine, cysteine, histidine, glutamate, arginine, glycine, homoserine, isoleucine, phenylalanine, leucine, -Aminobutyric acid, lysine a-Aminoadipic acid, methionine, serine and homoserine
Organic acids	Nutrient source, acts as a chemoattractant, helps in chelating poorly soluble mineral nutrients and induces the expression of *nod* gene, soil acidifiers and Al detoxifiers	Citric, glutaric, oxalic, malonic, malic, aldonic, fumaric, erythronic, succinic, ferulic, acetic, butanoic, butyric, syringic, valeric, rosmarinic, lactic, glycolic, trans-cinnamic, piscidic, formic, aconitic, pyruvic, vanillic and tetronic acids
Sugars and vitamins	Replaces phosphates from rocks making them available for microorganisms, promotes microbial and plant growth	Glucose, deoxyribose, oligosaccharides galactose, biotin, maltose, thiamine, ribose, niacin, xylose, raffinose pantothenate, rhamnose, riboflavin, arabinose and fructose
Phenolics, inorganic ions and gases	Nutrient source, chemoattractant molecules, microbial growth promoters, *nod* gene inducers as well as *nod* gene inhibitors, Al detoxifiers, chelators of poorly soluble mineral nutrients and resistance	Liquiritigenin, luteolin, daidzein, 4′,7-dihydroxyflavanone, genistein, 4′,7-dihydroxyflavone, coumestrol, 4,4′-dihydroxy-2′-methoxychalcone, eriodictyol, 4′-7-dihydroxyflavone, 3,5,7,3′-tetrahydroxy4’methoxyflavone, Naringenin, isoliquiritigenin, 7,30-dihydroxy-4′-methoxyflavone, umbelliferone, (+)- and (−)- catechin
Proteins and enzymes	Catalysts for P release from organic molecules, biocatalysts for organic matter transformations and plant defence	Acid/alkaline, phosphatase, amylase, invertase, protease, PR proteins, lipases, beta-1,3-glucanases

while the LWC compounds are readily utilizable, more diverse and are accompanied by a wide array of significant functions.

The root morphology serves as an important criterion in influencing the overall make-up and composition of root exudates. The ageing portions of the plant roots were known to exude more of organic acids, while the root tips of primary and lateral roots secrete more of amino acids (McDougall 1968; Rovira 1969). Each part of the plant roots is specialized for the secretion of different compounds as part of root exudates (Frenzel 1957, 1960). For instance, the meristem or the root apex secretes glutamic acid, valine, leucine and phenylalanine as root exudates while the outer layers of the root cap cells and root hair cells are specialized in the secretion of mucilage. Mucilage secretion can also occur due to degradation of the root epidermal cells. Aspartic acid is secreted overall by the entire root system. The zone following the root tip region is considered to be the primary site for root exudation in plants (Badri and Vivanco 2009).

Root exudation by the plants is due to the tremendous root pressure at the growing root tips in order to push their way through the soil. The root exudates mediate multipartite interactions in the rhizosphere region. They behave as a food source attracting neutral, beneficial and pathogenic soil microorganisms. As a response, the host plant introduces compositional modulations that cause the recruitment of beneficial soil microbes, thereby inhibiting/suppressing the pathogenic ones to avoid the flourishing of the non-beneficial microbial community in the rhizosphere microzone as well as defending the plants from pathogens (Zhang et al. 2009).

Root exudations occur by both active and passive transport systems wherein LMW compounds are transported by the process of direct passive diffusion. The movement of these compounds depends on cell membrane permeability, intracellular fluidic pH and polarity of the molecules that are being exuded by the plant roots while the HMW compounds are transported via membrane-bound transporter proteins including the ATP binding cassette (ABC) transporters (Badri et al. 2009; Yuan et al. 2018). Additionally, substrates such as indoles, pyrrolidines, quinolines and isoquinolines belonging to different classes of alkaloids produced by plants; flavonoids and phenolic acids that refer to plant-derived phenolic compounds and antimicrobials are transported from the living roots to the rhizosphere via multidrug and toxic compound extrusion (MATE) active transporters by using ion electrochemical gradient (Weston et al. 2012).

The movement of exuded root secretions in soil depends on diverse factors such as the quantity of root exudation, the nature of root secretions, the receptiveness of the compounds by the soil microorganisms for microbial assimilation and degradation, the nature and quantity of clay in the soil and the total water load of the soil. The zone that extends up to 1–2 mm from the root is considered the distance that is travelled by the root exudates. This rhizosphere microzone observes the maximum microbial load due to the high influence of root exudates that comprises sugars, organic acids and amino acids (Rovira 1969).

7.2.2 Factors Influencing Root Exudation Pattern

There are a number of factors that influence the exudation of compounds from the living plant roots. Soil moisture, plant biotype, microorganisms residing in the vicinity of the roots, damage to the living plant roots and the prevailing environmental conditions influence and modulate the root exudation pattern in plants.

The quality and quantity of root exudation vary among plant species. For instance, both the root exudates of wheat and barley plants contain sugars but the difference in both the exudates occurs in the case of certain sugars such as galactose, glucose and rhamnose, which is due to species variation. Variations in root exudation among species may be due to fluctuations in the distribution potential of the photosynthates which serve as a basic substrate for root exudates. Apart from plant biotype, plant root architecture also influences the exudation pattern as well as the distribution capacity of photosynthates. The morphological characteristics of roots including the surface area of roots, the root branching type, root tip density or the size of the root system determine the rate and extent of the distribution of photosynthates in soil. The absorbing roots that include the primary roots exhibit higher physiological activity than the xylem and phloem vessels present in the plant roots. These roots help in the exchange of materials across the plant roots. These differences in the architecture of the absorptive roots among species or within species, that is, the overall root architecture, play a decisive role in determining the root exudation rates (Yang et al. 2020). Additionally, the leaf habits also exhibit a strong influence over the root exudation pattern of plants. For instance, the studies undertaken by Sun et al. (2017) and Wang et al. (2019) have observed that there occurs higher exudation in deciduous trees than the evergreen trees, which was due to the differences in the leaf habit of both the plants.

Furthermore, environmental conditions and soil characteristics exert a strong relationship on the root exudation pattern of plants through direct or indirect effects. In consideration of environmental conditions, soil warming or an increase in soil temperature plays a crucial role in root exudation patterns. Several studies have investigated its influence on root exudation. Husain and McKeen (1963) have reported that the exudates obtained from strawberry plants grown at 20–30 °C contained higher amounts of amino acids than those grown at 5–10 °C. But the influence of soil warming on root exudation varies from plant to plant. For example, Schroth et al. (1966) observed that root exudation from cotton and bean plants was higher at a temperature of 37 °C, whereas it was lower in case of pea plants. In the case of pea plants, the exudation rate was observed to be higher at 27 °C. A similar pattern was observed for *Vicia faba* plants where the exudation of tannins and phenolics showed a profound increase at 30 °C, which was comparatively low when grown at 4 °C (Bekkara et al. 1998). An increase in temperature influences the root exudation pattern not only quantitatively but also qualitatively. Reports suggest that differences in the release of exudates due to temperature variations were due to alterations in the permeability of the cellular membranes or changes in cellular metabolism. Lower metabolic energy at lower temperatures allows substances to leak out of cells (Hale et al. 1971). Light intensity is one of the other factors responsible for exudation variations in plants. It modifies the exudation rate of secondary metabolites because of alterations in the biological phenomenon of photosynthesis. For instance, a few studies have reported that root exudation strictly depends on diurnal rhythms, which means an increase in root exudation during the light periods while declining during dark periods (Watt and Evans 1999). For example, Hughes et al. (1999) have reported that under light conditions the root exudates of *Alnus glutinosa* (L.) contain more of flavonoid content than the root exudates that were produced during dark conditions. As for tomato and subterranean clover plants, a similar root exudation pattern was observed. Clover plants grown under diurnal light exuded more serine, glutamic acid and a-alanine as part of root exudates than those growing under shady (60%) conditions. In the case of tomato plants, levels of aspartic acid, glutamic acids, phenylalanine and leucine in exudate were high during light periods, which were reported to be reduced by shading (Rovira 1969). Soil moisture significantly influences the exudation patterns in plants. As in the case of high soil moisture, there is less availability of oxygen in the soil, which results in hypoxia. Under hypoxic conditions, there occurs a shift in the respiration process from aerobic to anaerobic. This shift in respiration results in the accumulation of ethanol, lactic acid and alanine at phytotoxic levels in the plant root system (Rivoal and Hanson 1994). The plants defend themselves from the harmful effects of accumulated ethanol and lactic acid by secretion of a wide range of metabolites as part of root exudates (Xia and Roberts 1994).

Any damage whether physical or chemical to the living plant roots can cause significant changes in root exudation patterns. The extent of influence on root exudation is more pronounced for physical damage to roots than chemical ones. Physical damage to the plant roots can occur during digging, trenching or roto-tilling within the root area of the existing plants, whereas chemical damage occurs due to the excessive application of chemicals to the plants. There are studies suggesting that physical damage can cause a sharp increase in the release of amino acids by 73–120% in comparison to the intact, undamaged root system (Ayers and Thornton 1968). In addition to amino acids, physical damage to plant roots can also significantly influence carboxylate exudation in plants (Tiziani et al. 2020). In reference to chemical damage, Martin (1957) has reported that there was increased exudation of root exudates (scopoletin) from oat and wheat roots (C^{14}-1abeled organic compounds) (McDougall and Rovira 1965) when immersed in distilled water in comparison to the nutrient solution. Distilled water is hypotonic while a plant cell is hypertonic. So, when a plant cell is placed in distilled water, water moves from the outside of the cell to the inside, resulting in cell swelling which leads to increased membrane permeability. Thus it can be observed that both physical and chemical damage influences root exudation patterns in plants although the influence shown due to physical damage was more pronounced than that of chemical.

7.2.3 Mechanism of Root Exudation and Genes Involved

Root exudation is a biological process involving multiple mechanisms facilitating transport of soil C to the living plant roots and its release to the surrounding soil. The C produced in the source organs in the phloem is translocated by a specialized mechanism known as Munch's pressure-driven mechanism that drives the phloem mass flow by use of a pressure gradient in the phloem (Liesche and Schulz 1930). It works principally based on the turgor pressure difference that exists between sink and source organs. During phloem unloading, the low molecular weight compounds are diverted to the phloem pericycle where they are unloaded and eventually move out of the phloem pole pericycle. The high molecular weight compounds like protein compounds remain restricted to the phloem pole pericycle. In no time, the low molecular weight molecules move out of the plant roots to the surrounding soil environment. In order to do so, these molecules need to move across the plasma membrane to reach the apoplast. The plasma membrane is permeable to small, gaseous molecules, while it is impermeable to larger, charged as well as uncharged polar molecules (e.g. glucose) (Canarini et al. 2019).

The small polar molecules and the uncharged molecules are transported through simple passive diffusion using the permeability nature of the lipid membrane as a criterion. The movement of smaller molecules through the lipid membrane is solely dependent on the electrochemical gradient between the source and the sink. The electrochemical gradient helps in the translocation of the molecules from the cytoplasm of root cells to the vicinity soil. The larger molecules pass through the membrane by interacting with specific transmembrane proteins, which is known as facilitated diffusion. These proteins help in transiting the molecules by forming small pores through the lipid bilayer or phospholipid bilayer membrane (Sasse et al. 2018). The efflux of the larger compounds such as sugars, amino acids and organic acids can also take place through specific efflux pumps and channels. A few of the transporters have been characterized for amino acids such as Usually multiple acids move in an out Transporter (UMAMIT), Cationic Amino acid Transporter (CAT), Lys His Transporter (LHT), Glutamine Dumper transporters (GDU) (Pratelli et al. 2010; Besnard et al. 2016). Similarly, for the transport of sugars, transporters like Sugars will be eventually exported as Transporter (SWEET), Sucrose Transporter (SUTs) and Monosaccharide Transporters (MUTs) (Hennion et al. 2019) and organic acid transporters such as Multi-drug And Toxic compound Extrusion/citrate Transporters (MATE) and Aluminum-activated Malate Transporters (ALMT) (Wu et al. 2018) have been characterized. Excretion of high molecular weight metabolites by roots can also take place via vesicular transport (Badri and Vivanco 2009). The newly synthesized secondary metabolites are transported by the vesicles to other storage compartments or the plasma membrane for efflux. This process of exudation of metabolites involving vesicles is known as exocytosis. In some cases, the allelopathic compounds secreted by the plant roots are cytotoxic to plant cells. These allelopathic compounds are separated from the cytosol by membrane-bound vesicles by a process known as vesicular trafficking. Vesicular trafficking and exocytosis are known to be involved in combating attacks by plant pathogens (Grotewold 2001).

Ion channels are responsible for the secretion of carbohydrates and carboxylate ions such as malate and oxalate. These exuded compounds are transported across the lipid membrane through a transport mechanism mediated by proteins. The most widely studied transporter is the aluminum-activated-malate transporter (ALMT). They consist of a group of proteins, responsible for several physiological plant processes like exudation of organic acid in the presence of toxic Al^{3+} ions in the soil, conferring aluminium tolerance in plants under aluminium stress conditions (Sharma et al. 2016). For instance, the organic acid (OA) anions were known to render Al^{3+} resistance to plants under Al toxicity, but their exudation from the plant roots involves a cascade of events. The cascade starts with Al^{3+}/H^+ activating unknown receptors. Activation of the receptors results in an increase of cytosolic Ca^{2+} ions which activate the calcium sensor proteins or calmodulin. The activated calmodulin protein binds to the glutamate decarboxylase enzyme converting it from an inactive form to an active form. The activated form of glutamate decarboxylase enzyme converts glutamate to ɣ-aminobutyric acid, which is involved in the regulation of expression of ALMT1 activity in *Arabidopsis thaliana* plants. In *Arabidopsis,* Al-tolerance genes such as *AtALMT1*, *AtMATE*, *ALS3* and different H^+ tolerance genes are regulated by zinc finger protein sensitive to proton rhizotoxicity1 (STOP1). STOP1 is considered a core component for controlling Al and H^+ tolerance in *Arabidopsis,* while STOP2 regulated by STOP1 protein confers only H^+ tolerance in *Arabidopsis* due to low expression of *AtMATE* and *ALS3* genes. In addition to these, there are a few other factors like calmodulin-binding transcription activator2 (CAMTA2) that is involved in the regulation of expression of the *AtALMT1* gene (Kobayashi et al. 2014). Just like in *Arabidopsis*, in the case of *Oryza sativa*, the *ART1* gene encodes for a transcription factor that is involved in the regulation of 31 downstream genes implicated in Al tolerance. Briefly, Al tolerance in rice is basically due to the involvement of a number of genes that are associated with the process of detoxification of Al at different cellular levels (Tsutsui et al. 2011). The downstream genes that are regulated by *ART1* were characterized to be STAR1 and STAR2 (SENSITIVE TO AL RHIZOTOXICITY1) encoding ATP binding and transmembrane domain of a bacterial-type ATP-binding cassette transporter, Nrat1 (Nramp Al transporter 1), located in the plasma membrane of the root cells and functions as a transporter for trivalent Al into the plants which is essential for the prior step of final Al detoxification, OsALS1, CDT3 and OsFRDLA1 encoding an Al-induced MATE transporter. The ALMT transporters are not the only ones that confer stress tolerance in plants but there are MATE active transporters that also confer tolerance in plants against different stresses (Liu et al. 2009). MATE transporters play a vital role in the transport of a wide variety of molecular substrates, hormones and secondary metabolites (Takanashi et al. 2014). These are the independently activated transporters that confer stress tolerance to plants and are actively involved in citrate exudation.

Secretion of root exudates can also take place via proteins located in the root plasmatic membrane through an active transport mechanism. The active transport mechanism consists of two classes of membrane transporters, namely, ABC and MATE transporters. The root exudation from the living roots involving proteins occurs in three different situations depending on their specificity: transporters that are involved in the secretion of various metabolites, membrane transporters that are involved in translocation of different metabolites to the rhizosphere soil and the unique and highly specific transporters that are involved in the exudation of compounds from plant roots (Jones and George 2002; Orelle et al. 2018). The ABC group of transporters are considered the primary transporters while the MATE transporters are considered the secondary active transporters, and this is due to the fact that the former utilizes the energy from ATP (adenosine triphosphate) hydrolysis while the latter uses electrochemical gradient from the transportation of compounds across the membrane (Weston et al. 2012).

7.3 Root Exudates Mediating Belowground Interactions

Root exudates play a vital role in the root rhizosphere region. Through the exudation of a wide range of compounds as root exudates, the plant roots can structure the soil microbial community and regulate their existence in the immediate vicinity of the roots. Moreover, root exudates can help the soil microbiota in coping up with herbivores, stimulating the process of plant-microbe symbioses, modulating the soil physical and chemical properties and playing a pivotal role in the growth suppression of different pathogenic microorganisms and competing for plant species (Nardi et al. 2000; Walker et al. 2003).

7.3.1 Root Exudates Mediating Plant-Microbe Interactions

From the previous literature, it is well known that plant roots are the most potent source for the recruitment of soil microbes. These soil microbes help in promoting the growth of the plants, protecting the host plant from diverse plant pathogens and increasing plant tolerance to abiotic stress conditions. The predominant factor that helps the host plant to recruit rhizosphere soil microbes are the root exudates which commute with the rhizosphere-residing beneficial microbes while inhibiting the non-beneficial soil microbes.

7.3.1.1 Root Exudates: An Essential Chemoattractant for Microbial Host Root Colonization

Plants exude high levels of C as root exudates that behave as chemoattractant for bacteria. Most of the motile bacteria direct their movement in response to these chemical gradients, a bacterial response known as chemotaxis, for initiating a communication between the plant roots and the soil bacteria and colonizing the root region of the host plants (root colonization). The chemotactic response of bacteria increases their root colonization efficiency in the rhizosphere. The various molecules exuded by the plants help in initiating a positive bacterial chemotaxis towards different host plants leading to root colonization (Brencic and Winans 2005). Bacterial chemotaxis is initiated by the binding of a signalling molecule to a chemoreceptor. This transmembrane chemoreceptor which is responsible for bacterial chemotaxis is also known as methyl-accepting chemotaxis protein and consists of a number of domains. The transmembrane consists of a periplasmic or cytosolic ligand-binding domain, cytoplasmic region consisting of HAMP (histidine kinase, adenyl cyclase, methyl-accepting chemotaxis protein [MCP] and phosphatase) linker and a signalling domain. The ligand-binding domain is responsible for binding extracellular compounds. Once the ligand binds itself to the binding LBD, autophosphorylation of the histidine kinase CheA gets altered, which in turn transfers the phosphoryl groups to the response regulator CheY. Subsequently, the generated CheY-P permits its interaction with the flagellar motor to control cell swimming or tumbling to ultimately mediate chemotaxis (Feng et al. 2018). Existing literature reported bacterial chemotaxis towards various molecules exuded by host plant roots as root exudates mediated by specific chemoreceptors in establishing plant-microbe interaction following host root colonization (Webb et al. 2014; Allard-Massicotte et al. 2016).

Root colonization is one of the most important factors that aids in plant-microbe interaction. Root colonization is considered a key process that controls plant growth and induces systemic tolerance against different biotic and abiotic stresses (Sachdev and Singh 2018). The metabolites and the other organic secretions as a part of root exudates favour microbial colonization of root surfaces. Root colonization occurs when several groups of soil bacteria form microcolonies or biofilms on root surfaces. Biofilms are an assemblage of microbial communities embedded in a matrix of extracellular polymeric compounds (Bogino et al. 2013). The extent of biofilm formation varies between different root regions. In accordance with the previous literature, the depth and thickness of microbial biofilms are higher in the apical region of the roots than in ageing or mature root regions. This is due to the fluctuations in the composition of the root exudates and nutrient availability at the root plane or specific secretion of antimicrobials from the root tip (Rudrappa et al. 2008).

7.3.1.2 Root Exudates as Signalling Molecules

The organisms in the rhizosphere region interact with each other as well as plants via chemical communication established in the rhizosphere microzone. The plants secrete a wide array of metabolites as a response to altered gene expression, which is a result of signalling molecules secreted by the rhizosphere microorganisms. Overall, plants produce a compositionally diverse array of more than 100,000 different low molecular mass natural products known as secondary metabolites. In this chapter, we will explain a few of the molecules that are involved in legume-rhizobia, plant-AMF and actinorhizal plant-Frankia interactions.

Rhizobia

Bacteria belonging to the Rhizobiaceae family produce flavonoids and non-flavonoid signalling molecules that play a significant role in symbiotic interactions. The symbiotic interaction between any strain of bacteria belonging to the Rhizobiaceae family and legume is the result of a molecular interaction based on the signal molecules produced and secreted by both the associated partners involving a succession of recognition events. Initially, the interaction starts with the exudation of signal molecules by the host plant that expresses the genes involved in the process of nodulation.

Flavonoids are a group of plant secondary metabolites that are well known for their activity in plant-microbe interaction. They are synthesized via the central phenylpropanoid pathway and the acetate-malonate pathway. Flavonoids consist of two benzene rings connected by a three-carbon linking chain. They consist of two aromatic rings which are synthesized by different biosynthetic pathways. The flavonoids are categorized into different subgroups based on their molecular structure including flavonols, flavones, flavanones, isoflavonoids, chalcones, catechins, anthocyanidins and dihydroflavonols. There are several studies that have reported its role in in vitro nodule formation process using reporter genes (Shaw et al. 2006). The legume roots are known to secrete flavonoids into the surrounding soil. The quantity and concentration of the flavonoids increase with the presence of *Rhizobium* species in the soil. The flavonoid molecule behaves as a signalling molecule expressing the *nod* genes in rhizobia. The expressions of nod genes are responsible for the synthesis of Nod factors that initiate the nodulation process (Haichar et al. 2012). The Nod factors are lipochitooligosaccharides that act as signalling molecules triggering a sequence of events in host plants such as root hair curling or shepherd's crook, infection thread formation to allow the entry of rhizobia to the host root cells and nodule development (Cullimore et al. 2001). The process of nodule formation is regulated by both rhizobia and the plant and involves mechanisms that are controlled by two factors, that is, nodule numbers and nodule position (Sasse et al. 2018). The existing literature suggests that Nod factors induce certain flavonoids to initiate nodule formation through their action as auxin transport inhibitors. By inhibiting auxin transport inhibitors, there involves a local accumulation of auxin at the nodule initiation site initiating the development of nodule primordial. Similarly, the non-flavonoid molecules are also involved in inducing the expression of *nod* genes. There are several studies that documented the activity of various non-flavonoid molecules in inducing the expression of *nod* genes. For example, trigonelline and stachydrine from alfalfa seeds (Phillips et al. 1992), aldonic, erythronic and tetronic acid (Gagnon and Ibrahim 1998), xanthones (Yuen et al. 1995), vanillin and isovanillin from wheat seeds (Le Strange et al. 1990) can induce expression of *nod* genes in *S. meliloti* by activating the regulatory NodD protein, nod genes in *Mesorhizobium loti*, *Rhizobium lupini*, and *Sinorhizobium meliloti, B. japonicum, Rhizobium* sp. respectively

Frankia

Actinorhizal symbiosis is a type of symbiotic interaction occurring between actinobacterium Frankia and dicotyledonous plants. The symbiotic interaction between the two partners initiates with the infection process. As in *Rhizobium*, 'shepherds crook' is also observed in *Frankia*, but unlike *Rhizobium* Nod factors, the extracellular deforming factors in the case of *Frankia* are quite different both structurally and functionally (Bagnarol et al. 2007). As in the case of *Rhizobium*/ legume interaction, the main factor is the host-derived flavonoids that help the rhizobia to interact specifically with their hosts, and similar is the case with *Frankia*. The root hair curling in *Frankia* is initiated on interaction with the host root filtrate. There are few studies that have correlated the strain specificity in *Myricaceae-Frankia* symbiosis with root phenolics. The main root exudates that were affected by *Frankia* inoculation are phenols, flavonoids and hydroxycinnamic acids. The existing literature suggests that flavonoids determine the microsymbiont specificity as the host plant adapts to their secondary metabolism in accordance with the compatibility status of bacterial strains (Popovici et al. 2011). There are studies that reported that *Frankia* inoculation to host plant *A. glutinosa* activates the genes coding for phenyl ammonia lyase (pal) and chalcone synthase (chs), involved in flavonoid biosynthesis. Similarly, the study by Auguy et al. (2011) revealed that

Frankia interacts with the tropical tree *Casuana glauca* Sieb. ex Spreng. and results in the activation of eight *Casuana glauca* genes coding for enzymes involved in flavonoid biosynthesis.

Arbuscular Mycorrhizal Fungi (AMF)

Arbuscular mycorrhizae are found to colonize the majority of land plants. Mycorrhizal colonization of plant roots is mediated by controlled exudation by the plant roots and sensing of specific secondary metabolites released by the roots. During plant root colonization, the roots communicate with the mycorrhizal association in a complex way. The mycorrhizal fungi obtain a substantial fraction of soil C as a primary source of energy for the metabolic activities in the soil while at the same time benefitting the plants by delivering soil nutrients in return. One of the crucial steps in AMF development in plant roots is the formation of extraradical hyphae induced by signal molecules exuded by plant roots that initialize AMF-induced symbiosis. The signalling molecules secreted by the plant roots as a result of AMF interaction are known as strigolactones.

Strigolactones are known as branching factors exuded by plant roots. They are well known for their activity in stimulating fungal hyphae branching in symbiotic AMF. Strigolactones also help in signalling directional growth of AMF towards roots. Most important, strigolactones are considered an important signal molecule for the establishment of the AM symbiosis (Akiyama and Hayashi 2006). Strigolactones are synthesized from the carotenoid pathway and any alterations in the carotenoid mechanism affect strigolactone synthesis which ultimately affects AMF-host interaction. Recently it was reported that strigolactones play a crucial role even at a later stage of the AMF-plant interaction, when the fungus is already established in the root (Steinkellner et al. 2007).

7.3.1.3 Root Exudates as Carbon Cycling Activator and Nitrification Inhibitor

The exuded materials from the plant roots contain high amounts of C compounds that directly impact the rhizosphere microbial population. Root exudates can provide the rhizosphere microorganisms with precursors that are essential for phytohormone synthesis. Studies reported that plant exudates also contain higher quantities of aminocyclopropane-1-carboxylic acid (ACC) which serves as the main source of carbon and nitrogen for the rhizosphere microbes. This was shown by *acd*S expression by root exudates. Living plant roots also exude certain sugars such as glucose and sucrose that are involved in exopolysaccharides production, which is an essential step in microbial biofilm formation (Haichar et al. 2012). For instance, on growing *Paenibacillus polymyxa* in a culture medium containing a high concentration of sucrose, a high level of levan production was observed. Levan is a naturally occurring fructan present in many plants and microorganism species. High levels of sugar induce the expression of gene *sacB* involved in levan synthesis. Thus, it was clearly understood that the exuded sucrose from the root apex of wheat roots in the rhizosphere ecosystem can easily induce the expression of the *P. polymyxa sacB* gene (Bezzate et al. 2000). These Exopolysaccharides play a pivotal role in legume-rhizobia interactions via taking part in microbial biofilm formation around the plant roots. Since EPS can be produced at high levels of sugar concentration (that is either glucose or sucrose), it points out to facts that (a) root exudates contain a high concentration of these molecules and (b) in the presence of gene expression inducer bacteria may produce EPSs with low glucose or sucrose concentrations. Plant roots exude a wide array of organic substances as a part of root exudates. The organic compounds exuded by the roots were reported to accelerate the process of decomposition of soil organic matter (SOM) with the help of soil-dwelling, rhizosphere-associated microorganisms. The increased solubilization of soil nutrients depends solely on the activation of rhizosphere microbial activity by labile C released by roots. The existing literature suggests that some of the primers like root exudates, even at meagre amounts, can stimulate SOM turnover, which increases nutrient availability, especially nitrogen or phosphorus to plants (Jones et al. 2004; Cheng et al. 2014).

The nitrification process is a key step in the global nitrogen cycle that links the oxidation of ammonia to the loss of fixed nitrogen in the form of dinitrogen gas brought about by specific nitrifying microbial activity. The process involves converting a non-utilizable form of N in the soil to a simple, readily utilizable form that contributes significantly towards plant productivity and environmental quality. The existing literature demonstrated the importance of using recombinant luminescent bacterial strains (*Nitrosomonas europaea*) to monitor nitrification inhibitors released from plant roots (Subbarao et al. 2007). Among the cereals, legume crops, groundnut, pearl millet and sorghum showed detectable biological nitrification inhibition (BNI) in root exudates while among pasture grasses, *Brachiaria humidicola* (Rendle) Schweick and *B. decumbens* showed the highest BNI capacity. In another study, Zakir et al. (2008) have reported for the first time the root exuded compound methyl 3-(3-hydroxyphenyl)propionate (MHPP) responsible for BNI by sorghum. Similarly, Subbarao et al. (2013) have reported a root exuded compound, sorgoleone, 1-p-benzoquinone (exuded from sorghum roots) to contribute significantly to the BNI capacity in sorghum.

7.3.1.4 Role of Root Exudates in Nutrient Deficiency

The living plant roots produce various organic and inorganic compounds to enhance the adaptation rate of plants to different environmental conditions. The exudation of organic compounds by the plant roots increases with a decrease in soil nutrients. Plants increase their exudation of organic compounds into the rhizosphere under nutrient deficiency. Under Fe deficit conditions, plants exude phytosiderophores into the rhizosphere to adapt to the prevailing condition. Herbaceous or woody plants acquire Fe from the soil by secretion of phytosiderophores that facilitates in acquiring of ferrated-phytosiderophore complexes aided by a highly precise and efficient nutrient uptake system (Römheld 1991; von Wiren et al. 1994, 1995). The rate of root exudation increases is positively related to the tolerance of a plant species to Fe deficiency. Under Fe deficiency, the Fe-deficiency tolerant plant genotypes show more root exudation rates than the plant genotypes sensitive to Fe deficiency (Rengel 2002). The process of phytosiderophore release and its uptake by the plants is solely under genetic control (Römheld and Marschner 1990). Several genes are involved in the biosynthesis of phytosiderophores and their precursor nicotianamine that codes for the enzyme nicotianamine synthase (Higuchi et al. 1999). According to the literature, Fe deficiency demands more phytosiderophore production than that under Zn deficiency (Rengel et al. 1998; Walter et al. 1994). This implies to effortless, undeviating and less complicated series of events that are involved in the phytosiderophore triggering process in Fe deficiency than the highly complex triggering process in case of Zn deficiency. Plant genotypes that are tolerant to Zn and Fe deficiency are reported to exude a lesser amount of phytosiderophores than the Zn and Fe sensitive ones in order to increase mobilization of Zn and Fe from sparingly soluble sources (Rengel 2002).

7.3.2 Other Novel Functions of Root Exudates

Root exudates are known to involve in different positive and negative types of interactions that include interactions between plants, plant and soil microorganisms and also between plants, soil microbes and nematodes (tritrophic interactions). Plant -microbe interactions is one of the most common interactions that exist in the rhizosphere microzone which illustrates the tritrophic interactions and the role of root exudates in these interactions. There are a few studies that explained the tritrophic interactions and their occurrence, which is when both the partners the soil microbes and nematodes act synergistically to stimulate growth in plants. A study by Horiuchi et al. (2005) demonstrated that soil-dwelling nematode *Caenorhabditis elegans* helps in establishing a positive connection between roots and soil rhizobia resulting in healthy and high nodulation in legumes. Briefly, the study elucidated the role of *C. elegans* in initiating plant-microbe symbiosis. On the release of plant root volatiles, the nematode, *C. elegans* carry the bacterium to the roots of the legumes resulting in the initiation of plant-microbe symbiosis.

Root exudates contain compounds that are known to exert direct influence on shaping the rhizosphere microbial community. The different components in root exudates show positive chemotactic responses in bacteria and the capacity for different microbial species to utilize and compete for substrates (Somers et al. 2004). For example, in one of the reports by Naim (1965), it was documented that the microbial density in the root apex of Libyan desert grass (*Aristida coerulescens*) was significantly higher than the microbial population found at the base of the plant. A similar pattern was observed for wheat roots (Van Vuurde and Schippers 1980). Looking at the colonizing pattern shown by the microbial communities, it was suggested that this was due to the exudation of certain rhizodeposits predominantly at the root tip. These include root exudates and lysates that is highly predominant at the bases of plant roots.

Furthermore, plant roots secrete a wide range of inhibitory substances as secondary metabolites that help the host plants to cope with different bacterial and fungal pathogens in response to inducers that initiate a defence response (Walker et al. 2003). Under natural conditions, plant defence responses remain constantly stimulated but exudation of secondary metabolites by plant roots such as saponins, glucosinolates and hydroxamic acids accounts for the resistance or the susceptibility of particular plant species/cultivars to root pathogens. There are several studies that have highlighted the antimicrobial activity exhibited by LMW compounds and the plant defence proteins which are released as a part of plant root exudates. For instance, Lanoue et al. (2010) reported that the barley root system secreted phenolic compounds such as vanillic acid, p-coumaric acid and with antimicrobial activity under *Fusarium* attack. Similarly, during *Pseudomonas aeruginosa* infection of basil roots, the basil root system secreted rosmarinic acid that exhibited antibacterial activity against *P. aeruginosa* under in vitro conditions. Flavonoids were recently reported to show a significant inhibitory effect against different bacterial and fungal plant pathogens. Flavonoids were considered to be an important molecule for conferring resistance to plants against different phytopathogens. The chemiosmotic potential between cytoplasmic matrix and vacuole acts as the primary driving force for the transport of flavonoid molecules to the plant infection site. At the site of infection, the flavonoids induce hypersensitivity reaction and programmed cell death. The antimicrobial activity of flavonoids depends on their ability to inhibit microbial adhesion and the inactivation of cell envelope transport proteins. They can disrupt the microbial membrane, alter membrane permeability and inhibit cell envelope and nucleic acid synthesis by forming hydrogen bonds with the stacking of nucleic acid bases, electron transport chain and ATP synthesis (Mierziak et al. 2014). Additionally, plant roots secrete various proteins to protect the plant against different soil-borne pathogens. For instance, Park et al. (2002) have reported that the roots of the plant *Phytolacca americana* (pokeweed) are well known for their ability to secrete various plant defence proteins including PAP-H. PAP-H is a ribosome-inactivating protein (RIP) that helps in the inhibition of protein synthesis by acting on the ribosome in a highly specific order. They also exhibit in vitro N-glycosidase activity against fungal ribosomes, whereby they can recognize and depurinate fungal ribosomes.

Root exudates have also been shown to alter the genetic make-up of the microbial communities (Baudoin et al. 2003) and enhance the number of pollutant degraders in rhizosphere soil (Joner et al. 2002). Marschner et al. (2002) in one of their studies examined the changes in microbial community profile associated with roots varying in architecture and maturity as a result of variations in root exudation pattern using DGGE analysis. DGGE analysis revealed a prominent difference in microbial communities on the basis of the root type which ultimately affects the compositional content of root exudates. The significant differences in fungal and bacterial community structure were recognized to be the difference in organic acid production by both the communities. For instance, the fungal community is known to produce high concentrations of citric acid, while the bacterial community produces *cis*-aconitic and malic acid in addition to citric acid. In relation to root exudates stimulating hydrocarbon degrader populations in rhizosphere soil, Cebron et al. (2011) have observed that the major microbial strains in a phenanthrene contaminated soil were *Pseudoxanthomonas* sp. and *Microbacterium* sp. before the addition of root exudates, but on the addition of root exudates of ryegrass, the growth of

Pseudomonas sp. and *Arthrobacter* sp. were also favoured. This suggests that root exudates have a direct impact on enhancing the microbial diversity of any contaminated piece of land while increasing the abundance of PAH-RHD$_{\alpha}$ gene containing microorganisms for faster remediation of PAH contaminated sites. Secondary plant metabolites, a key component of rhizodeposits, also play a crucial role in plant survival in environments contaminated with different hydrocarbons such as polyvinyl chloride (PVC) and polyaromatic hydrocarbons (PAHs). There are studies reporting that the presence of high concentrations of secondary plant metabolites in the rhizosphere ecosystem enhances the number of pollutant degraders in rhizosphere soil (Donnelly et al. 1994).

7.4 Conclusion and Future Perspectives

The current chapter highlighted a few of the research findings to understand the mechanistic insights of exudation-related plant-microbe interactions as well as rhizosphere functioning. Overall, the chapter provided an illustrated explanation based on the research findings to establish the root exudates as key mediators for successful interaction between plants and rhizosphere microbes in the rhizosphere microzone. But due to a bottleneck in a comprehensive understanding of root exudates chemistry, there is a lack of information and knowledge about belowground plant-microbe interactions. Although several components of root exudates have been characterized, studied and explained in detail, the metabolic profiling of the root exudates has not been explored in detail except for a few components such as organic acids, flavonoids and fatty acids. This can be majorly due to the inadequacy and limitation of technology to isolate and characterize the minute portions of the natural products that are highly alterable in nature.

Moreover, experimental works corresponding to spatiotemporal pattern study of root exudates are mostly under axenic or monoxenic laboratory conditions and thus require more attention for conducting *in-planta* studies to understand the mechanistic insights of root exudates in belowground plant-microbe interactions as well as their dynamic role in plant growth and development. Although much is known in regard to the role of root exudates in an interactive activity, a still more is required to have a good understanding of the exudation pattern of different plant roots belonging to different species, the variations in exudation with respect to plant age, soil type, environmental parameters and rhizosphere microbiome. Furthermore, the fate of root exudates in rhizosphere soil and the function of these exuded materials in microbial physiology and functions have not been explored in detail. Thus there is a huge scope for researchers to undertake metabolomic studies to improve the understanding and knowledge of the same. Moreover, it opens up several opportunities for the researchers to take up studies to understand the interlink between bacterial gene expression and the nature of exuded material from the living plant roots.

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Rhizospheric Microbial Diversity: Organic Versus Inorganic Farming Systems

8

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Abstract

The agricultural system is very much dependent on the interaction and association of plants with the rhizospheric soil microbes. The microbial diversity found in the rhizosphere is unique and distinct from the other parts of the soil. The rhizospheric population is very important in terms of plant nutrients. Field studies reveal that different farming practices, namely, organic and inorganic farming systems, decide the soil microbial population and diversity, but information on the effect of these systems on the rhizospheric group is meagre. This chapter is an attempt to review the effects of organic and inorganic systems on rhizospheric plant-soil-microbe interactions.

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8.1 Introduction

8.1.1 Plant-Microbe Relationship and Rhizosphere

Overexploitation of the soil for more crop production to feed the ever-increasing population has led to the deterioration of soil quality and its health. The capacity or potential of soil in terms of crop productivity and soil fertility defines soil quality or soil health (Williamson et al. 2011). Agricultural ecosystems will be resilient, resistant and redundant only when the health of the soil is good, and thus soil health or quality is redefined as the capacity of soil in terms of living organisms (Karlen et al. 2003), becoming also increasingly relevant as soil ecosystem services (Garbisu and Epelde 2011). The most important and critical point is to find out the indicators for sustainable agriculture which are associated with effective nutrient cycling (Rooney et al. 2009), particularly mediated by soil microbiological activities, its diversity and functionality (Babalola et al. 2007; Allison and Martiny 2008). Thus, the soil microbial biomass/communities are conceded as very important indicators as well as regulators of agroecosystems. It is a fact that these regulators and their spatial and temporal functions depend on the type of agricultural practices or systems (Hartman et al. 2018).

Effects of different soil management systems, namely, organic system (where no synthetic inputs are provided) versus inorganic systems, showed diversified soil properties and microbial communities (Azevedo Jr. et al. 2017). Also, long-term field experiment studies revealed contrasting effects of different agroecosystems on physicochemical properties of soil (Crittenden and de Goede 2016), microbial biomass (Amaral et al. 2011) and habitat-specific bacterial and fungal taxa (Chen et al. 2018). Contextual variables in an agroecosystem such as soil type, climate and cropping system mostly influence the soil properties at a spatial scale. Dependency on chemical fertilizers is more than the application of organic manure in a conventional inorganic system although excessive use of chemical fertilizers affects soil quality, reduces nutrient uptake by the crop and increases environmental hazards such as greenhouse emissions and eutrophication (Zhu et al. 2016).

With the need to achieve sustainability in agriculture and to address the environmental concerns adhered with the use of chemical fertilizers, researchers are finding out a way to either substitute chemical inputs with inorganic or promote the balanced/integrated nutrient system by combining both the inputs (Bhattacharyya et al. 2008). It was proved in the recent past that irrespective of climatic conditions and experimental sites, the microbial diversity and the functionality were more in the organic system because of the nutrient enrichment of the soil (Hamm et al. 2016). Owing to the improved soil carbon and nitrogen, an enhanced population of certain bacterial strains was observed in the compost (organic manure) applied field (Chaudhry et al. 2012). Nevertheless, plants also regulate rhizospheric microbial communities either through rhizodeposition or by moisture and temperature control (Denef et al. 2009). Thus, it can be concluded that microbes play a vital role in the biogeochemical cycling processes in the soil and also the bulk soil communities

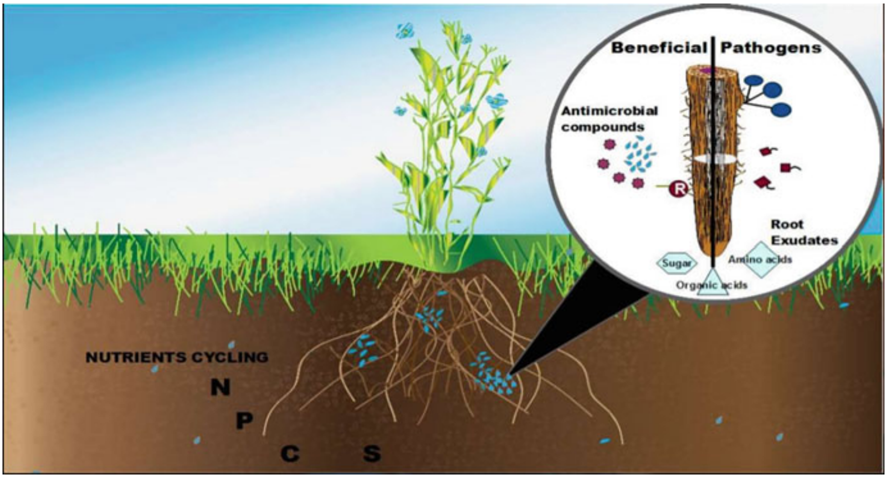

Fig. 8.1 Rhizospheric soil with plant-microbe interaction (https://www.slideshare.net/ashwincheke/plant-microbe-interaction-by-dr-ashwin-cheke)

affect the unique environment of rhizospheric microbial communities (Zhang et al. 2018; York et al. 2016).

The soil rhizosphere is known as a hotspot where dynamic relationship and interaction occurs between plant roots and microbial communities present in the soil (Fig. 8.1). The plant roots release exudates in the form of organic acids, amino acids and sugars to attract microbes, which in turn release antimicrobial compounds to protect plants against pathogens. These allow the microbes such as fungi and bacteria to cause the breakdown of the organic matter and thus help in nutrient release and cycling (Kuzyakov 2002), promoting plant growth directly or indirectly (Lugtenberg and Kamilova 2009) and suppressing plant pathogens (Hayat et al. 2010). If we see the agricultural system management from ecosystem services or nutrient flux point, it becomes necessary to analyze soil processes and soil properties to understand the complex relationship between management systems, particularly focused on the rhizosphere (Ryan et al. 2009; Luo et al. 2018). But the unique mechanisms of rhizospheric microbes (Guo et al. 2018) are likely linked to the performance of the plants (Rout and Southworth 2013). Since the rhizospheric soil is controlled by the plant and soil microbial processes, it is very difficult to predict the functional implications of rhizospheric communities.

8.1.2 Rhizosphere Exudates and Nutrient Availability

The rhizosphere is called the interface of root and soil, where the soil is influenced by plant roots. The rhizosphere is characterized by the prevalence of greater microbial activity than the non-rhizosphere or soil away from the roots of the plants. The term ‘rhizospheric effect’ indicates the overall influence of plant roots on soil microorganisms which include species of bacteria, fungi, actinomycetes and other organisms such as nematodes, protozoa and algae. The rhizosphere effect is due to the rhizodeposits from the roots, which attract some microorganisms.

In the rhizosphere, about 50% of the photosynthates are transferred into the roots, only about 1% are actively released as root exudates and 10% are lost as root debris (Uren 2007). Rhizodeposits/exudates include a variety of substances that originate from sloughed-off root cells and tissues, mucilages, volatiles, soluble lysates and exudates that are released from damaged and intact cells (Dakora and Phillips 2002). Root exudates comprise the largest fraction of non-volatile rhizodeposits (Meharg and Killham 1988) and are complex mixtures of low molecular weight organic compounds including carbohydrates, amino acids, organic acids, phenolics, fatty acids, sterols, vitamins, enzymes, purines/nucleosides as well as inorganic ions (HCO_3^-, H^+) and gaseous molecules (i.e. CO_2, H_2) (Dakora and Phillips 2002) (Table 8.1). Among these components, organic acids, amino acids and carbohydrates are generally released in the largest quantities (Farrar et al. 2003).

8.2 Microbial Diversity in Different Land Systems

Microorganisms play an important role in many ecosystem services and also provide support to all living forms. The land system has complex and huge microbial biodiversity, which plays important role in the management of natural habitats (Table 8.2).

8.3 Inorganic Versus Organic Microbial Shifting

Microbes are the key drivers in maintaining the functionality of the agroecosystem. It is also important to study the impact of the different farming practices on the microbial population or communities in the soil. Shifting the conventional inorganic farming practices to organic practices has also shifted the microbial communities of the system towards a positive organization for a sustainable ecosystem. A number of studies suggest that microbial abundance and diversity were more in soil under organic management system as compared to the soil under conventional (inorganic) practice. This upsurge in microbial diversity could be associated with reduced tillage, cover cropping and usage of organic fertilizers. As a result of this, soil organic carbon increases and serves as an energy source for heterotrophic microbiota (Liao et al. 2018). If organic farming practices have been applied for long term, it can be assumed that the soil microbial composition has shifted and improved the turnover of the soil organic matter. The relative abundance of microbes varies with the system as shown in Fig. 8.2 and Table 8.3.

The shifting of the microbial community is owed to the change in the nutrient content of the soil, especially for the increased salinity and accumulated nutrients. An example of different farm practices can be seen in the shifting of iron redox bacterial communities that influence the iron cycle in the soil. The soil that is

Table 8.1 Different root exudates and their function in nutrient availability in soil

Root exudates	Substances identified	Functions in nutrient availability	References
Sugar	Arabinose, fructose, fucose, galactose, glucose, maltose, oligosaccharide, raffinose, rhamnose, ribose, sucrose, xylose	Sugar serves as C and N source for microorganisms Greater effect on P mobilization in soil	Gunina and Kuzyakov (2015), Ratnayake et al. (2013), Gransee (2001)
Amino acid and amide	Asparagine, aspartic acid, α-alanine, β-alanine, arginine, cystine/cysteine, glutamine, glycine, histidine, lysine, methionine, phenylalanine, proline, serine/homoserine	The C and N present in amino acid and amide easily be taken up by microorganisms Increases N availability in soil	Moe (2013), Gent and Forde (2017), Phillips et al. (2004)
Carboxylic acids or organic acid	Acetic, butyric, citric, glutaric, lactic, maleic, malic, malonic, oxalic, propionic, pyruvic, succinic, tartaric, valeric	C and N source for microorganisms P mobilization by maleic, citric, tartaric acid P acquisition Mobilization of P, Fe, Mn and Zn in the soil (chelation and reduction) Increases P availability by carboxylates Detoxification of Al^{3+} (chelate and complexation) K mobilization by organic acids	Jones (1998), Wang and Lambers (2020), Nadira et al. (2016), Shane and Lambers (2005), Ryan et al. (2014), Dinkelaker et al. (1993), Neumann and Romheld (2001)
Phytosiderophore	Nonproteinogenic amino acids, for example, mugineic acid and its derivatives	Mobilization of trace elements, for example, Fe (barley > wheat > oat > rye > corn > sorghum > rice) Mobilization of Zn, Cu and Mn is reported due to rhizosphere acidification	Neumann and Romheld (2001)
Enzyme	Amylase, invertase, phosphatase or phosphohydrolase, protease, polygalacturonase	Amylase helps in N mineralization Invertase enhances C mineralization Protease plays a role in N mineralization Phosphatase- P solubilization (acid phosphatases and phytases) Polygalactoturase- N mineralization	Das and Varma (2010), Song et al. (2020), Das and Varma (2010), Tarafdar and Marschner (1994), Neumann and Romheld (2001), Arshad Jr and Frankenberger (1998)

(continued)

Table 8.1 (continued)

Root exudates	Substances identified	Functions in nutrient availability	References
Growth factor	*p*-Amino benzoic acid, auxins, biotin, choline, inositol, *n*-methyl nicotinic acid, niacin, pantothenate, pyridoxine, thiamine	Increase nutrient availability (N, P), micronutrient solubilization	
Phenolic acid and coumarin	Coumarin, cinnamic acid, caffeic acid, ferulic acid, salicylic acid, syringic acid, vanillic acid	Mobilization of Fe and Mn and Fe mobility Detoxification of Al^{3+}	Clemens and Weber (2016), Römheld (1987), Dakora and Phillips (2002)
Others	Flavanone, fatty acids, proteins, sterols, lipids, aliphatics, aromatics, vitamins, carbohydrates	Riboflavin enhances Fe acquisition Aliphatic and aromatic organic acids, vitamins and carbohydrates moderate trace metal toxicity Soil carbohydrates for rhizobium improve N fixation	Chen et al. (2017), Vranova et al. (2013), Abd-Alla et al. (2014)

Table 8.2 Different microbial diversity in different land systems

Land system/ landscape	Types of microorganisms	Reference
Forest soils	Alpha- and Delta proteobacteria, Actinobacteria and Acidobacteria	Min Song et al. (2018), Lladó et al. (2017), Uroz et al. (2013)
Pine forest soils	Proteobacteria (Burkholderiales, Caulobacteriales, Rhizobiales and Xanthomonadales), Bacteroidetes (Sphingobacteriales) and Acidobacteria	Lladó et al. (2017)
Deciduous forests	Proteobacteria, Actinobacteria, Bacteroidetes and Acidobacteria	López-Mondéjar et al. (2015)
Acidic soils of coniferous forests	Alpha Proteobacteria, Acidobacteria and Actinobacteria	Lladó et al. (2017)
Agriculture soils	Proteobacteria, Acidobacteria, Actinobacteria, Gemmatimonadetes, Firmicutes and Verrucomicrobia, Bacteroidetes, *Microvirga*, *Nocardioides*, Oligotrophic taxa, Chlorofexi, mineral weathering bacteria and bacteria associated with ECM fungi	Lauber et al. (2009)
Landfills	Epsilon proteobacteria, Gamma proteobacteria, Clostridia and candidate division OP3	Blake et al. (2016)
Rhizosphere	Actinomycetes Bacillus, Paenibacillus, Pseudomonas, methanotrophic bacteria, ammonia-oxidizing bacteria, and N_2-fixing bacteria, *Clostridium* spp., *Arthrobacter* spp., *Brevibacterium* spp., *Corynebacterium* spp., Serratia, Enterobacter and *Rhizobium* spp. Acinetobacter, Agrobacterium (α-Proteobacteria), Alcaligenes (β-Proteobacteria) and Xanthomonas (γ-Proteobacteria). Acidobacterium, Bacteriodetes, copiotrophic bacterial strains and ECM Fungi	Garbeva et al. (2004), Latif et al. (2020)
Litter and deadwood	Proteobacteria and Bacteriodetes, Cellulolytic taxa and fungal mycelia degraders, Nitrogen-fixing Bacteria, litter and dead wood decomposing bacteria (Burkholderia, Phenylobacterium and Methylovirgula) and fungi *Chitinophaga, Ewingella, Pseudomonas, Pedobacter, Variovorax, Stenotrophomonas* genera that are known to produce chitinolytic enzymes	Brabcová et al. (2016), Kielak et al. (2016)
Grasslands	*Imperata cylindrica*, *Microstegium fasciculatum* and *Murdannia triquetra*. *Lotus wrangelianus*, *Hemizoniacongesta*, *Holocarpha virgata*, *Plantago erecta* and *Lasthenia californica*	Batten et al. (2006)
Shrubland	Pyracantha fortuneana, Vitex negundo and Alchorneatrewioides	Batten et al. (2006)

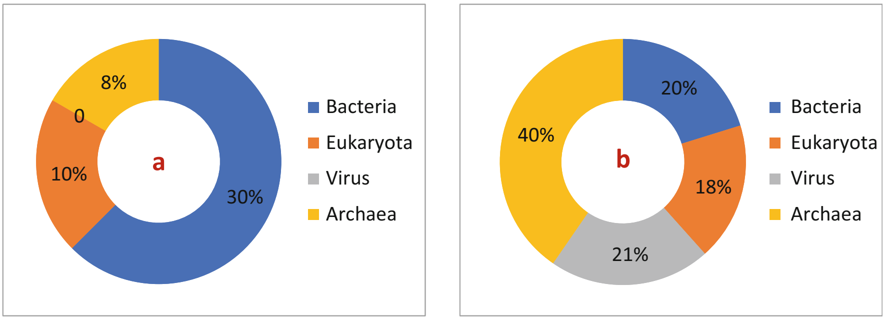

Fig. 8.2 Relative abundance of microbes in percentage. (**a**) Organic system. (**b**) Inorganic system

maintained under inorganic fertilization shows a higher abundance of ferric ($Fe^{3+)}$ reducing bacteria (e.g. *Geobacter*), while the soil under organic fertilization regimes shows a higher amount of *Pseudomonas* and *Anaerolinea*, which are Fe^{2+} oxidizers (Wen et al. 2018). In addition to this, Nakhro and Dkhar's findings (2010) revealed that organic carbon inputs showed a significant positive correlation with fungal and bacterial populations as organic farming practices elevated the carbon content, thereby increasing the microbial counts in the soil. The soil under conventional practice had a low organic carbon content that affected the microbial population. It was observed that members of the phyla *Bacteroidetes* and *Planctomycetes* were more abundant under organic management, whereas members of the *Actinobacteria* and *Verrucomicrobia* tended to be more abundant in the soil under conventional practices (Fernandez et al. 2020).

Luo et al. (2017) had performed a research on the agri-soil and shown the increased ratios of fungi to bacteria (F/B), Gram-positive to Gram-negative (G+/G−) and arbuscular mycorrhizal fungi (AMF) to saprotrophic fungi (AMF/SF) upon increased manure input for 8 years. This suggested that certain microbial groups, such as G+ bacteria, fungi and AMF, are more adapting to organic farming than the other ones. It has also been observed that the application of the organic manures accelerates the aggregation of the soil, that is, the formation of macroaggregates increases the organic carbon concentration in the aggregates than the conventional inorganic NPK fertilizer. These aggregates provide more suitable microhabitats for anaerobic microbes (Zhang et al. 2014).

Applying organic manures or organic farm practices not only increase the biomass of individual microbial group but also alter their community structure. When the native microbes utilize the exogenous supply of the organic carbon source, it leads to a change in community composition; that is, as soon the organic compounds are added to the soil, the fast-growing Gram negative bacteria start proliferating. After a while, their population decreases allowing the growth of other groups such as Gram positive bacteria and fungi (Lazcano et al. 2013). Also, as we discussed above, organic inputs may accelerate the soil aggregation, generating pores larger in size. This facilitates the fungal growth and explains better the higher number of fungus and actinomycetes (G+ bacteria) in organically managed soils (Strickland & Rousk

Table 8.3 Relative abundance of microbes in the organic and inorganic systems

Relatively abundant microbes in an organic system	Relatively abundant microbes in the inorganic system	References
Bacteria: *Bacillus (Firmicutes), Butyrivibrio (Firmicutes), Glycomyces (Actinobacteria), Leptolyngbya (Cyanobacteria), Microbulbifer (Proteobacteria), Methylocaldum (Proteobacteria), Nostoc (Cyanobacteria), Planctomyces, Pseudoxanthomonas (Proteobacteria), Roseiflexus*	**Bacteria:** The genera *Rhodoplanes (Proteobacteria)* and *Skermanella (Proteobacteria), Archeal phylum Crenarchaeota* and the genus *Candidatus Nitrososphaera*	Liao et al. (2018)
Bacteria: *Alpha-Proteobacteria, Gammaproteobacteria, Firmicutes, Rhodobium* **Fungi:** *Mortierella, Verticillium*	**Bacteria:** *Actinobacteria, Chloroflexi, Acidobacteria, Nitrospirae, Nocardioides, Marmicola* **Fungi:** *Ascomycota, Alternaria, Davidiella Dothideomycetes, Leotiomycetes*	Francioli et al. (2016)
Bacteria*:* *Actinobacteria* and *Nitrospirae*	**Bacteria***:* *Proteobacteria* and *Fibrobacteres*	Ai et al. (2015)
Viral families: *Circoviridae, Inoviridae, Microviridae* **Bacteria:** *Korarchaeta, Eukaryarchaeota, Saccharomonospora, Sorangium*	**Viral families:** *Myoviridae, Podoviridae, Siphoviridae* **Bacteria:** *Nanoarchaeota, Crenarchaeota, Firmicutes, Thaumarchaeota, Nocardiodes*	Enebe and Babalola (2020)
Bacteria: *Pseudomonas, Anaerolinea, Aquincola, Clostridiales, Dechloromonas*	**Bacteria***:* *Geobacter, Clostridium, Desulfosporosinus, Desulfitobacter, Peptocaccaceae, Desulfurispora, Bateroidales*	Wen et al. (2018)
Bacteria: *Desulfuromonadales, Clostridiales, Erysipelotrichales, Rhodocyclales and Rhodobacterales* **Fungi:** *Glomeromycetes (unclassified class), Cantharellales, Saccharomycetales, Trichosporonales, Agaricales and Onygenales* **Protist:** *ThraustochytridaThecamoebida, Labyrinthula and Heterolobosea*	**Bacteria:** *Bacillales, Deinococcales, Micrococcales, Acidobacteriales, Kineosporiales and Streptomycetales* **Fungi:** *Paraglomerales, Eurotiales, Neocallimastigales and Chaetothyriales* **Protist:** *Prasiolales, Tribonematales, Cryptofilida, Phytiales, Dermamoebida and Bicoecales*	Harkes et al. (2019)

2010). Such microbial groups accumulate more SOC, proving better for the soil fertility and biological quality (Luan et al. 2020). From soils under organic farming at different locations, the average microbial population of fungi, bacteria and actinomycetes was recorded as 56.9%, 55.2% and 49.5%, respectively, which was comparatively higher than those in the conventionally managed soils (Sheoran et al. 2018). Moreover, a study showed that in some cases the same genera can be prevalent in both conventionally and organically managed soils with limited differences. Some microbial groups showed highly reliable differences on the account of statistics among *Bacillus* and *Gemmatimonas*, which were more prevalent under conventional practice; also, among the genera with the higher count in organically managed soil, namely, *Holophaga*, *Acidobacteriaceae*, *Hyphomicrobium*, *Flavobacterium* and *Nocardioides* ($p < 0.05$). They also studied the relative abundance of the phylum *Actinobacteria* in the organic wheat soil, finding the lower taxa contributing utmost to the change. With a relative abundance of more than 1%, the orders of Actibacteria-Rubrobacter, *Acidimicrobium* and *Solirubrobacterales* constituted 5.83% in soil under organic farming, that is, twice of those in the conventional soil system (Armalytė et al. 2019).

Thus, it can be concluded that the organic system exerts positive and beneficial effects on soil biological properties, particularly microbial community, diversities, heterogeneity and richness. Nevertheless, the response of farming systems to rhizospheric microbial communities is very complex and magnificent.

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Rhizomicrobes: The Underground Life for Sustainable Agriculture

9

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Abstract

Sustainable agriculture can be considered the first step towards a sustainable world. Besides other human activities, agriculture is one of the root causes of the introduction of harmful chemicals into the environment. Therefore, one cannot imagine the existence of a sustainable world without realizing the existence of sustainable agriculture. Sustainable agriculture is the integration of efforts towards achieving socio-economic equality, economic growth and environmental preservation. Harnessing the plant beneficial microbes in agriculture for minimizing the use of non-biodegradable chemicals is a progressive endeavour towards sustainable agriculture. Plant microbiomes are emerging as promising niches to be explored for plant beneficial microbes. Plant microbiomes harbour endophytes, epiphytes and rhizomicrobes as well. In this chapter, we will discuss the possibilities of harnessing rhizomicrobes in the field. Microbes associated with the rhizosphere of the host plant are known as *Rhizomicrobes*. Rhizomicrobes are known to bestow their host plant with numerous plant growth promotion properties such as Biocontrol, Biofertilization, Phytostimulation, Rhizoremediation, Stress resistance and many more. Rhizomicrobes contribute not only to plant health but also to the improvement of soil fertility. A deep understanding of the role and reality of rhizomicrobiomes of different plants is the need of the hour as such microbes can be successfully harnessed in agriculture as biofertilizers in place of chemical fertilizers. Therefore, rhizomicrobes can play an important role in transforming the present agriculture system into the sustainable one.

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9.1 Introduction

Continuous use of chemical fertilizers in the agriculture system is not only polluting the environment but also posing threats to human health (Gupta et al. 2015). Application of green manures and biofertilizers is the need of the hour to save the environment as well as life on the earth. Plant microbiome studies can prove to be helpful in the isolation and selection of plant beneficial microbes to be used as green manures/biofertilizers. Plants are known to be associated with complex microbial communities. Such complex microbial communities constitute the holomicrobiome of the host plant. Broadly, the holomicrobiome of a plant can be differentiated into three compartments, namely, phyllosphere, endosphere and rhizosphere. The phyllosphere of a plant is harboured by epiphytes, the endosphere by endophytes and the rhizosphere by rhizomicrobes. These microbes may be associated with the host plant through symbiotic, mutualistic or pathogenic interactions.

The rhizosphere and its associated microbes together constitute Rhizomicrobiome. Based on the interactions with the host plants, rhizomicrobes can be differentiated as symbiotic rhizomicrobes and free-living rhizomicrobes. Root exudates of the host plant influence the composition of microbial communities associated with the rhizosphere (Hartmann et al. 2008). Morgan et al. (2001) have defined the phenomenon of attraction of active soil microbial communities towards roots exudates as the Rhizospheric effect. The rhizosphere is richer in nutrients as compared to the bulk soil. Therefore, in the rhizospheric zone of a plant, there are 10–100 times more microbes as compared to that present in the bulk soil. The rhizosphere is found to be inhabited by fungi, bacteria, archaea, protozoa, algae, viruses, oomycetes, microarthropods and nematodes. Bacteria and fungi are the prominent microbial groups among rhizospheric microbiota but rhizospheric fungi are less explored microbiota colonizing the rhizosphere as compared to the rhizospheric bacteria (Pattnaik and Busi 2019). Rhizomicrobes act as plant growth promoters directly or indirectly by synthesizing growth regulators, antibiotics, lytic enzymes, siderophores and nitrogen fixation etc. In the direct mechanism, they promote plant growth *via* biofertilization, rhizoremediation, root growth stimulation and stress tolerance. In the indirect mechanism, they act *via* antibiosis, posing competition for nutrients and niches, inducing systemic resistance. Having the vision of sustainable agriculture, advanced crops are expected to be resistant/tolerant of different diseases and stress conditions. Plant growth promoting rhizomicrobes can thus be successfully used as biofertilizers to achieve agricultural sustainability (Fig. 9.1).

9.2 Modern Techniques to Explore Rhizomicrobes

Rhizomicrobes are known to bestow their host plant with numerous plant growth promotion properties such as Biocontrol, Biofertilization, Phytostimulation, Rhizoremediation and Stress resistance. Deep and detailed studies of rhizomicrobes can prove to be helpful to plan the strategies for crop improvement using

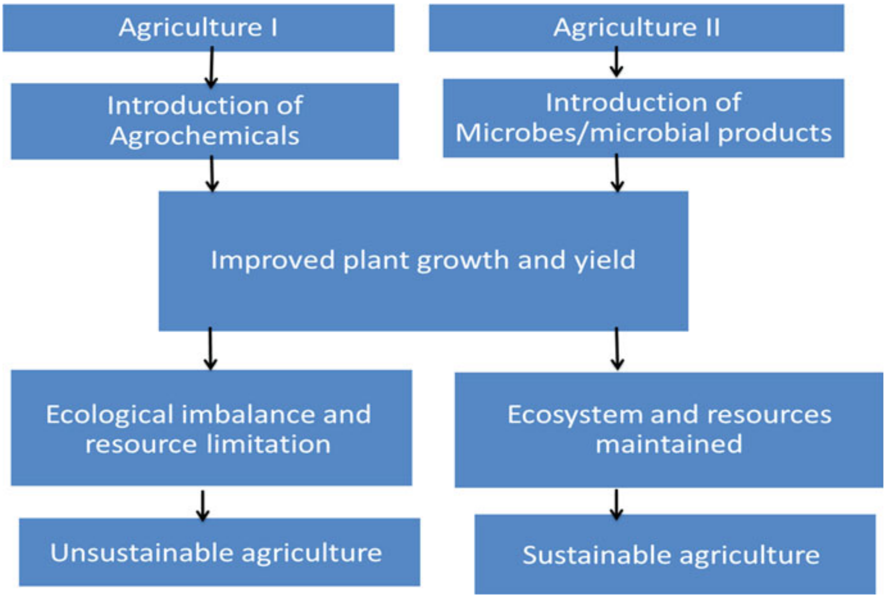

Fig. 9.1 Unsustainable versus sustainable agriculture

rhizomicrobes for sustainable agriculture. The application of modern tools and techniques in the field can give us a closer view of the interactions among the different rhizomicrobes as well as with the host plant. Modern Omics and Meta-omics techniques can be successfully used to study the rhizospheric microbial communities in terms of their composition and colonization, diversity and distribution and their associations and interactions (Fig. 9.2) (Pascual et al. 2016; Baeshen 2017). Metagenomic studies of the plant rhizospheres can prove to be helpful in unravelling the metabolic potential and other plant beneficial attributes of the rhizomicrobes without even culturing them. The comparative metagenomics technique can be used to study the functional diversity among the rhizomicrobiomes of different plants or the same plant under different environmental conditions. Advanced Next-generation sequencing technology has made metagenomic studies comparatively easier. Transcriptomic and metatranscriptomic studies can reveal the differential expression of genes among symbiotic and free-living rhizomicrobes. Comparative transcriptomic studies can prove to be helpful in finding the differential gene expressions in the same microbial species residing in the rhizosphere and bulk soil or some other habitat. Such information is needed for realizing the use of PGPR (plant growth promoting rhizobacteria) in agriculture as biofertilizers. Further, proteomic and metaproteomic studies of the rhizospheres are helpful in determining the actual functional roles of the rhizomicrobes in the plant health and productivity. The mass spectroscopy technique has made proteomic studies comparatively easier. Metaproteogenomic studies link the genomic and proteomic studies and the data so obtained can prove to be helpful in determining the actual potential of the microbes

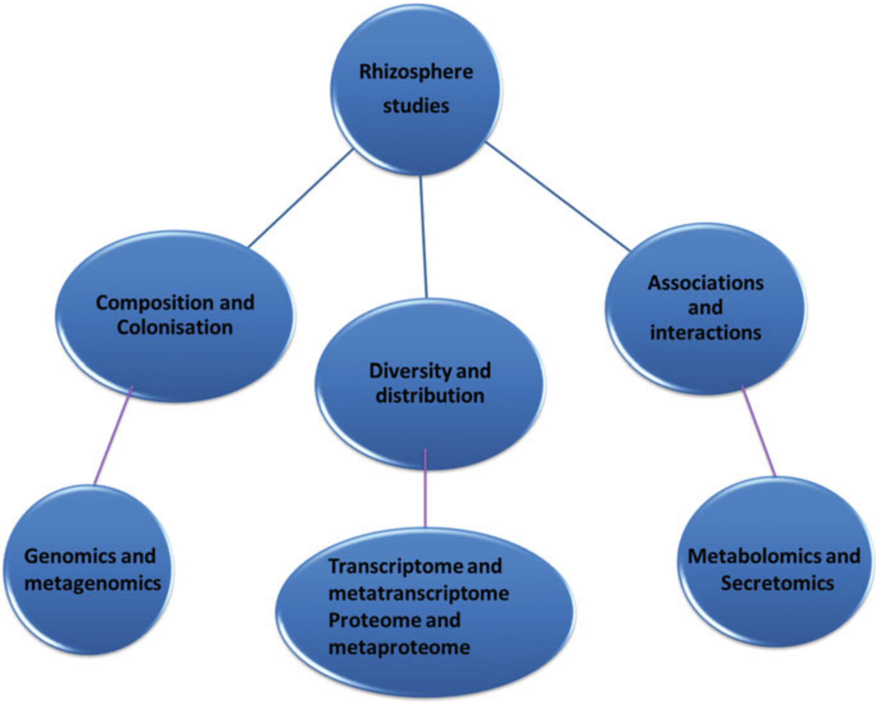

Fig. 9.2 Techniques to study different aspects of rhizomicrobiome

for sustainable agriculture. Besides using classical tools such as Microscopy counts, Phospholipid fatty acid analysis (PLFA), Fatty acid methylated esters analysis (FAME) and DNA fingerprinting, modern tools such as Microarray, next-generation sequencing (NGS), pyrosequencing, stable isotope probing (SIP), fluorescence in situ hybridization (FISH) and metabarcoding can also be successfully used for quick and detailed analysis of the rhizomicrobiomes (Abdelfattah et al. 2018). Therefore, multidisciplinary studies are needed to be done in the field of rhizomicrobiome for a better understanding of the niche as a whole so that the actual potential of the rhizomicrobes can be harnessed in a more efficient and sustainable way.

9.3 Techniques to Introduce PGP Microbes in Agriculture

Owing to their plant beneficial attributes, the introduction of rhizomicrobes in agriculture will lead to increased agronomic efficiency in a sustainable way. Successful microbial inoculation in the target host plant is the first step towards the colonization and plant-microbe interactions. The introduction of microbes to the target host cell is termed *bioinoculation* and the microbes used are termed *bioinoculants*. Plant growth promoting rhizomicrobes can be used as bioinoculants in plants, seeds, roots and soil (Bashan et al. 2014). Successful bioinoculation of selected potential rhizomicrobes in crops would result in phytopathogen-resistant plants with improved plant growth and productivity (Parasuraman et al. 2020). Liquid inoculation and nano-encapsulation techniques can prove to be helpful in realizing the use of plant growth promoting microbes as biofertilizers. de Oliveira et al. (2018) have successfully used the liquid inoculation method to inoculate *Azospirillum lipoferum* in *Myracrodruon urundeuva* seeds. The inoculated plants have been found to be more tolerant to drought stress. Conversely, Montiel et al. (2017) have found the microencapsulation method to be more efficient as compared to the liquid inoculation method. It has been observed that alginate microencapsulation-based immobilization of *P. putida*, associated with the tomato rhizosphere, results in improved growth and productivity in tomato plants (Montiel et al. 2017). Microencapsulation method based inoculation is preferred for improved microbial adhesion, microbial colonization of root cells and permanency. The success of the inoculation method is assessed in terms of the plant response. The response of plants to the microbe inoculation is affected by different factors, such as inoculation method, environmental conditions, inoculant species, inoculant density and soil type. Also, multistrain bioinoculants are found to be more potent in terms of plant growth promotion as compared to monostrain-bioinoculants. Bioinoculants supplemented with nanocompounds are also found to be more effective. Chaudhary et al. (2021) have studied the combined effect of nanocompounds and PGPR bioinoculants on the growth and yield of *Zea mays* plants. The study revealed that the combined treatment of nano-zeolites and PGP *Bacillus*-based bioinoculants to the maize plant leads to improved plant growth in terms of weight and length of roots and shoots as well as carotenoids, chlorophyll, proteins, carbohydrates and phenol content in the inoculated plant as compared to the control. Pereira et al. (2010) have studied the effects of the size of bioinocula on the inoculated plants. The study has revealed that bio-inoculum with a high concentration of multiple PGPR is more effective towards improved plant growth under drought stress. Therefore, keeping eye on the impacts of all the studied factors, new efficient methods of bioinoculation need to be explored for the successful harnessing of rhizomicrobes towards sustainable agriculture.

9.4 Importance of Rhizomicrobes

Rhizomicrobes are well-known plant growth promoters bestowing the host plant with improved health and yield. Besides their importance in the agriculture sector, rhizomicrobes also find applications in the field of Biomedicine, textile and food processing industries and many more for environmental sustainability (Pattnaik and Busi 2019), but in the present chapter, we will discuss the importance of rhizomicrobes towards the achievement of agriculture sustainability. Rhizomicrobes lead to plant growth promotion in three different modes, namely, Biofertilization, Biostimulation and Biocontrol (Fig. 9.3). Biofertilization involves facilitating the assimilation of soil nutrients, Biostimulation involves the synthesis of

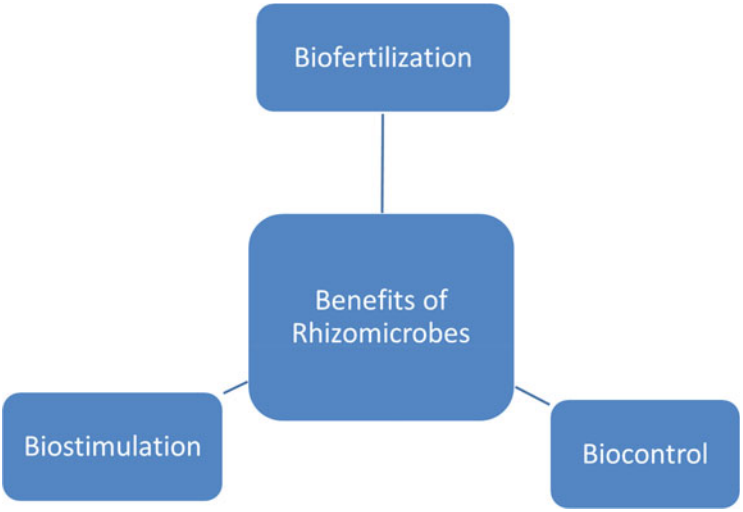

Fig. 9.3 Benefits of rhizomicrobes

phytostimulating hormones and Biocontrol involves the suppression of plant diseases.

9.4.1 Biofertilization

Indiscriminate use of chemical fertilizers is leading to a continuous drop in the Nutrient use efficiency (NUE) in crops, which is posing a serious threat to future food security. Owing to the non-availability of the complex and insoluble soil nutrients to the plants for direct use, the chemical fertilizers appear as the easy alternatives to meet the nutrient demands of the crop plants. As a result, there is a continuous accumulation of harmful chemicals in the environment. Therefore, there is a need to find out some eco-friendly alternatives to meet the nutrient supply of crops. Rhizomicrobes are known to solubilize complex soil nutrients into simple and soluble forms for the direct use of host plants. Biofertilization seems to be one of the characteristics of the rhizomicrobes which can be harnessed to fulfil nutrient demands of the crop plants in a more sustainable way. Biofertilization includes phosphate solubilization, siderophore production and nitrogen fixation potentials of the rhizomicrobes.

9.4.1.1 Phosphate Solubilization

Rhizomicrobes are not only beneficial to the host plant but also contribute to enhancing the fertility of the soil. The soil improvement and plant growth promotion characteristics of the rhizomicrobes are the result of their ability to solubilize, mineralize and decompose available nutrients (Satyaprakash et al. 2017). Phosphorus is one of the essential nutrients needed for the proper growth and development of plants. Phosphorus is present in the soil but in a complex insoluble form, which is difficult for the plants to absorb. As a result, artificial Phosphorus fertilizers are used to meet the crop demands, which leads to heavy metal accumulation in the soil. Therefore, eco-friendly alternatives are needed to be searched to avoid the adverse effects of chemical fertilizers on the environment. Interestingly, rhizomicrobes are reported as potential phosphate solubilizers (Gupta et al. 2015). They play an important role in P cycling. Rhizomicrobes are found to follow two types of mechanisms to solubilize phosphorus (Wang et al. 2017). One mechanism includes the secretion of phosphate solubilizing enzymes like phosphatases and the second mechanism includes the production and release of organic acids such as citric acid, acetic acid, lactic acid, succinic acid and malic acid by the rhizomicrobes to solubilize phosphorus. The efficiency or amount of phosphate solubilization is found to be affected by the microbial strain, type of organic acid produced, type of C source used and the type of phosphate to be solubilized (Marra and de Oliveira-Longatti 2019). Various studies are available regarding the P solubilizing ability of rhizosphere-associated bacteria as well as fungi. More specifically, Rhizobacteria are found to be more efficient P solubilizers as compared to their fungal counterparts (Satyaprakash et al. 2017). Different rhizobacterial genera, namely, *Pseudomonas*, *Rhizobium*, *Bacillus*, *Arthrobacter*, *Serratia*, *Enterobacter* and many more, are reported as potential phosphate solubilizers (Gupta et al. 2015; Chauhan et al. 2017), whereas *Aspergillus*, *Penicillium* and *Fusarium* are reported as potential phosphate solubilizing fungal genera (Elias et al. 2016). Singh et al. (2020) have isolated 18 bacterial strains from the rhizosphere of sugarcane plants with phosphate solubilization potential. The fungi associated with the rhizosphere of *Arabis alpina* have been found to improve plant growth and P uptake in P-poor soil (Almario et al. 2017). Therefore, the use of phosphate solubilizing rhizomicrobes in the soil as biofertilizers can prove to be an eco-friendly and sustainable alternative to the chemical (P) fertilizers.

9.4.1.2 Nitrogen Fixation

Nitrogen is the most essential plant macronutrient. Atmospheric nitrogen cannot be directly used by plants. Therefore, chemical nitrogen fertilizers generally in the form of urea are used to meet the nitrogen demands of the crops. This, in turn, results in acidification of soil as well as water and also causes air pollution. Hence, there is a need to find sustainable nitrogen source alternatives for the crops. The plant growth promoting rhizomicrobes with nitrogen-fixing potential can be considered a reliable nitrogen source for sustainable crop production while maintaining the fertility of the soil. Nitrogen-fixing microbes use a nitrogenase enzyme system to fix atmospheric nitrogen which is otherwise unavailable to the plants for direct use (Gaby and Buckley 2012). The efficiency of nitrogen fixation is mainly influenced by the type of soil and soil conditions, nitrogen-fixing microbial strain, plant species and so on (Singh et al. 2020). The rhizobacterial genera such as *Azotobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Cyanobacteria*, *Enterobacter*, *Pseudomonas* and *Diazotrophicus* are reported as potential nitrogen fixers for the host plants (Bhattacharyya and Jha 2012; Singh et al. 2020). Singh et al. (2020) have isolated

22 bacterial strains from the sugarcane rhizosphere with nitrogen-fixing potential. They have studied the nitrogen fixation in sugarcane plants inoculated with *B. megaterium* and *B. mycoides* strains. Therefore, nitrogen-fixing *Bacillus* species associated with rhizospheres can be successfully used as biofertilizers. The significance of studying the nitrogen-fixing rhizomicrobes lies in the fact that the biological nitrogen-fixing ability of such microbes can be extended to non-leguminous plants also. There is a need to isolate and identify PGPR associated with different crops that can perform potentially under different environmental conditions.

9.4.1.3 Siderophore Production

Iron is also one of the essential nutrients for plant growth and development, but it is not present freely in the environment for plant use. Plant-associated microbes play an important role in iron assimilation by the host plants. Such microbes produce low molecular weight, iron chelating agents known as siderophores (Winkelmann 2007). Siderophores produced by plant growth promoting rhizomicrobes mostly belong to the carboxylate, hydroxamate and catecholate families (Beneduzi et al. 2012). Siderophore production potential of rhizomicrobes results in both direct and indirect growth promotion of host plants. In the direct plant growth promotion mechanism, rhizomicrobes synthesize siderophores for improved iron assimilation for the host plant. Enhancement in the overall uptake of iron by host plants results in improved plant growth and yield. In the case of indirect plant growth promotion mechanism, rhizomicrobes produce iron sequestering siderophores to create an iron-deficient environment for the pathogenic microbes sharing the same niche. In this way, rhizomicrobes promote the growth and yield of host plants in an indirect way by restricting the proliferation of pathogenic microbes. *Bacillus*, *Azotobacter*, *Burkholderia*, *Pseudomonas*, *Serratia*, *Rhizobium* and *Streptomyces* are the well-known rhizobacterial genera with siderophore production potential (Gupta et al. 2015). Various rhizospheric *Pseudomonas* species, namely, *P. fluorescens*, *P. putida* and *P. syringae*, are reported to possess biocontrol potential against phytopathogens *via* siderophore production (Shanmugaiah et al. 2015). Patil et al. (2014) have reported siderophore producing rhizospheric *B. subtilis* species with biocontrol potential against phytopathogenic fungi. Siderophore producing rhizomicrobes are also found to increase the chlorophyll II level in the host plants (Sujatha and Ammani 2013). Singh et al. (2020) have isolated 10 bacterial strains from the rhizosphere of sugarcane with siderophore production potential, whereas Hussein et al. (2019) have studied the siderophore production potential of rhizosphere fungi associated with *Panax ginseng*. The fungal species *P. commune*, associated with *P. ginseng* rhizosphere, has been reported as a potent siderophore producer (Hussein and Joo 2019). Khamna et al. (2009) have isolated 75 actinomycetes isolates, from the rhizosphere of different medicinal plants, with siderophore production potential. Among actinomycetes strains, *Streptomyces* spp. are reported as potent siderophore producers (Ref, if any?).

9.4.2 Biostimulation

Biostimulation is also known as Phytostimulation. It includes the synthesis of phytostimulating hormones by the plant-associated microbes for plant growth promotion. Rhizomicrobes are known to synthesize different phytohormones, namely, auxins, cytokinins, ethylene and gibberellins. Phytohormones synthesized by rhizomicrobes result in plant growth promotion *via* stress resistance, overproliferation of lateral roots, increased nutrient and water uptake and so on. Rhizomicrobes are reported as potential Indole acetic acid producers (Spaepen and Vanderleyden 2011; Syamala and Sivaji 2017). Singh et al. (2020) have isolated 22 bacterial strains, with indole acetic acid production potential, from the rhizosphere of sugarcane plants. Production of IAA by rhizomicrobes leads to overproduction of lateral roots and root hairs, which further enhances the soil nutrient uptake ability of the plant. *Agrobacterium*, *Rhizobium*, *Bradyrhizobium*, *Klebsiella*, *Pseudomonas* and *Enterobacter* are reported as potential IAA producing rhizobacterial genera (Mohite 2013; Bal et al. 2017). Khamna et al. (2009) have isolated 36 actinomycetes strains, from the rhizosphere of different medicinal plants, with IAA production potential. Among different actinomycetes strains, *Streptomyces* spp. are reported as potent IAA producers. Besides IAA, some rhizobacterial species such as *B. subtilis*, *P. fluorescens*, *Pantoea agglomerans* and *Rhodospirillum rubrum* have also been reported to synthesize cytokinins and gibberellins for the plants (Gupta et al. 2015). Ethylene is a plant stress hormone. Under stressful conditions, ethylene hormone halts the important plant cellular processes and leads to premature senescence. 1-Aminocyclopropane-1-carboxylate (ACC) is the precursor of ethylene synthesized by plants in response to environmental stress (Vejan et al. 2016). Rhizobacteria bestow their host plants with stress resistance by synthesizing the ACC deaminase enzyme (Noumavo et al. 2016). The enzyme converts the ACC precursor into α-ketoglutarate and ammonia, thus surpassing the negative effects induced by ethylene hormone under abiotic stress. Rhizomicrobes associated with the rhizosphere of *Papaver somniferum* and *Jatropha* are reported as potential ACC deaminase producers (Barnawal et al. 2017). Two rhizospheric *P. aeroginosa* strains have been found to enhance the synthesis of phytohormones and salicylic acid in the tomato plant (Parasuraman et al. 2020). Bioinoculation of the tomato seeds with the rhizobacterial strains leads to improved seed germination and growth of the tomato plant (Parasuraman et al. 2020). Rhizobacterial strains are also found to be effective in bestowing the improved nutrient uptake and water stress tolerance among plants with high water demands under drought conditions (Pereira et al. 2010). PGPR are known to minimize the adverse effects of drought stress by inducing the production of antioxidant enzymes, hormones or metabolites. *Myracrodruon urundeuva* plants inoculated with the PGPR *A. lipoferum* are more tolerant to drought stress (de Oliveira et al. 2018). Significantly lower superoxide dismutase enzyme activity was found in the inoculated plants as compared to the uninoculated ones under stress conditions. Therefore, the bioinoculation of PGPR in crops can be considered an effective biotechnological tool to alleviate different stress effects among crop plants.

9.4.3 Biocontrol

Biological stress caused by phytopathogens greatly affects the health and yield of plants. To avoid such losses, chemical pesticides and fungicides are being used, which leads to environmental havoc. The phenomenon by which organisms prevent or reduce the severity of the plant diseases caused by phytopathogens is termed biocontrol and the antagonistic microbes are known as biocontrol agents. Biological control seems to be a sustainable approach for plant disease management as improper use of chemical pesticides leads to the development of resistance among phytopathogens as well as ecological contamination (Tariq et al. 2020). Rhizomicrobes are known as potential biocontrol agents (Beneduzi et al. 2012). Successful bioinoculation of potential rhizomicrobes in crop plants would ensure defence against phytopathogens as well as improvement in plant growth and productivity (Parasuraman et al. 2020). Therefore, the application of rhizomicrobes as biocontrol agents seems to be a potential and eco-friendly approach for sustainable plant growth and development.

Rhizosphere inhabiting *Bacillus* and *Pseudomonas* genera are potential biocontrol agents (Dorjey et al. 2017). Rhizomicrobes suppress the deleterious effects of phytopathogens by following different mechanisms, namely, production of antibiotics, competition for niche and nutrients, production of lytic enzymes and induced systemic resistance. The most common mechanism for biocontrol among rhizomicrobes is, however, the production of antibiotics. A diverse range of antibiotics have been reported from both rhizobacteria and fungi including polyketides, lipopeptides, alcohols, aldehydes, ketones, heterocyclic nitrogeneous compounds, phenazine derivatives, pyrrole derivatives and many more (Gupta et al. 2015; Singh et al. 2017). Different antibiotics suppress phytopathogens through different mechanisms. Most of these antibiotics attack cellular constituents such as cell walls, cell membranes or ribosomes (Singh et al. 2017). Some of the antibiotics also act as stimulants and induce systemic resistance among the plants. Rhizomicrobes are also able to induce systemic resistance among host plants against phytopathogenic bacteria, fungi, viruses, insects and nematodes. Under the influence of induced systemic resistance (ISR), the plant or its parts become more resistant to the damage-causing pathogens. Jasmonate, ethylene, acetoin, homoserine lactones, siderophores, lipopolysaccharides and so on are the main elicitor compounds used by rhizomicrobes to induce systemic resistance among plants and save them from the biological stress (Gupta et al. 2015). Rhizomicrobes are also potential multienzyme producers. They produce different extracellular lytic enzymes such as chitinases, lipases, phosphatases, proteases, dehydrogenase and glucanase which are used to antagonize the growth of phytopathogens directly or indirectly. Singh et al. (2020) have reported enhanced expression of various enzymatic genes, such as chitinases, glucanases, catalase, superoxide dismutase and phenylalanine ammonia lyase, in sugarcane plants inoculated with selected rhizobacterial *Bacillus* strains. Chitinase and β-glucanase enzymes produced by rhizomicrobes are particularly effective against pathogenic fungi (Vejan et al. 2016). *P. fluorescens* associated with the rhizosphere of tomatoes has been found to produce chitinase and β-glucanase enzymes to suppress the growth of *F. udum* causing *Fusarium* wilt (Kumar et al. 2010). *Trichoderma* sp. associated with the rhizosphere of strawberry has been found to possess antagonistic potential against the causal agents of root rot of strawberry (Ahmed and El-Fiki 2017). Two rhizospheric *P. aeruginosa* strains have been found to possess significant antagonistic potential against the phytopathogens of tomato, namely, *F. oxysporum* and *Alternaria solani* (Parasuraman et al. 2020). *Bacillus* strains associated with the rhizosphere of the sugarcane plant have been reported to possess antagonistic potential against the sugarcane rot pathogens, namely, *Sporisorium scitamineum* and *Ceratocystis paradoxa* (Singh et al. 2020). Khamna et al. (2009) have isolated 23 actinomycetes strains, from the rhizosphere of different medicinal plants, with antifungal potential. Among actinomycetes strains, *Streptomyces* spp. are reported as potent antifungal agents. Thus, rhizomicrobes can be considered as potential biocontrol agents, thereby reducing the disease incidence and inducing the systemic resistance among plants.

9.5 Conclusions

Rhizomicrobiome represents a complex niche which involves continuous interactions among the host plant and the associated microbes. Such interactions result in improved plant health, productivity and soil fertility. Rhizomicrobes are progressively gaining agricultural and biotechnological relevance owing to their promising plant growth promotion potential. More research is focused on plant growth promoting rhizobacteria (PGPR), whereas rhizosphere-associated fungi are also of huge significance in terms of plant growth promotion characteristics. There is a need to design efficient strategies for successful isolation and utilization of rhizomicrobes as biofertilizers. There exists a gap between the mechanism of plant growth promotion of host plants by PGPR and their application as biofertilizers in agriculture. To overcome this gap, systemic research is needed to be conducted in the area of rhizomicrobiome for a better understanding of their colonization and their complex interactions. Successful harnessing of this underground treasure, termed as plant growth promoting rhizomicrobes, as biofertilizers, biostimulants and biocontrol agents shall prove to be a big contribution towards sustainable development in general and sustainable agriculture in particular.

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Synthetic Biology Tools in Cyanobacterial Biotechnology: Recent Developments and Opportunities

10

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Abstract

Cyanobacteria are ubiquitous microorganisms that play a significant role in the maintenance of the earth's ecology. Owing to the smaller and completely sequenced genome, some strains have emerged as appropriate candidates for manipulating their genetic sequences to enhance growth and photosynthesis under distinct environmental fluctuations. Synthetic biology tools have arisen as an indispensable means for scaling up the natural circadian rhythm of prokaryotes and eukaryotes, thus improving the physiological and metabolic processes to promote their growth under adverse environmental conditions. Although the availability of synthetic biology tools for engineering multiple pathways in cyanobacteria is still limited, in the past few years significant progress has been made in developing genetic tools including promoters, sRNA, RBS, riboswitches and CRISPR (clustered regulatory interspaced short palindromic repeats)/Cas-9 systems for engineering cyanobacteria with improved biomass production and product development. Systematic rewiring of physiological, biochemical and molecular pathways may significantly improve the growth and production of engineered cyanobacteria under stressful environments. In this chapter, recent advancement in synthetic biology tools and their application in cyanobacteria for sustainable biotechnologies is reviewed. Furthermore, it also provides valuable insights into their future developments.

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10.1 Introduction

Cyanobacteria are an evolutionarily ancient and ecologically important diverse clade of photosynthetic prokaryotes contributing significantly towards primary productivity (Banerjee et al. 2013; Yadav et al. 2018). The arrangement of chloroplasts of higher plants has been postulated to be the product of endosymbiotic uptake of cyanobacteria (Singh et al. 2013). Consequently, they are recognized as ubiquitous model organisms for contemplating physiological processes such as photosynthesis, electron transport chain and biochemical process that are considerably similar to most of the eukaryotic algae and higher plants (Tan et al. 2015). Recently, the CO_2 concentrating mechanism of cyanobacteria has been shown to be involved in the improvement of growth and productivity of various agricultural crop plants (McGrath and Long 2014); however, it is still a daunting task for producing ample food in changing climate to bridge demand and supply gap of the ever-growing population (Singh et al. 2013).

Substantial research investigations have been made to characterize cyanobacterial genomes using next-generation sequencing techniques and sequence assembly tools (Tan et al. 2015). These investigations have revealed novel information about cyanobacterial genes and their functions which can be exploited to modulate the metabolism of cyanobacteria for the biosynthesis of desired products using advanced synthetic tools (Carter and Warner 2018). Genome editing through various site-specific nucleases such as zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and newly identified clustered regulatory interspaced short palindromic repeats (CRISPR)-CRISPR associated protein (Cas) has provided a singular platform for gene editing. Despite the technological advancement and increased application, few attempts have been made in the implementation of the CRISPR-Cas9 system for cyanobacterial improvement (Sengupta et al. 2018). The major constraint for its application in cyanobacteria is to develop a rapid and robust protocol of genetic transformation which can be leveraged for improving the photosynthetic process in crop plants which may in turn improve their growth and productivity under climate extremes.

Synthetic biology is an emerging discipline that intercepts biotechnology and advanced molecular biology from an analytical schematic approach to manipulate host cell metabolism and regulatory systems with high efficiency (Hagemann and Hess 2018). Initially, research involving synthetic biology was mainly focused on model species like *E. coli* and yeast. However, the exploitation of synthetic biology has been lagging in developing an efficient cyanobacterial transformation system (Johnson et al. 2016). While cyanobacteria are an ideal candidate for metabolic engineering, a relatively small number of cyanobacterial strains have been used as models for example, *Synechococcus elongatus* PCC 7942, *Synechocystis* sp. PCC 6803 and *Nostoc* sp. PCC 7120. Owing to fast growth rates, capacity to withstand high light intensities and elevated temperatures, *Synechococcus* PCC 7002, *S. elongatus* UTEX 2973 and *S. elongatus* PCC 11801 are further included in this list (Johnson et al. 2016). This chapter is an attempt to dissect recent advancements and bottlenecks that hinder efficient cyanobacterial transformation via the implementation of ultra-throughput genome editing CRISPR-based tools.

10.2 Development of BioBricks for Tailoring Cyanobacterial Genome

BioBricks are the arrangement and/or reconfiguration of new/existing biological parts that can be efficiently mobilized into living organisms (Lindblad 2018). These BioBricks are the intersecting elements involved in the precise modulation of genetic engineering principles for manipulating host cells. These BioBricks are also referred to as 'Genetic toolboxes' which are the main players catalysing manipulation of the genome with high efficiency (Sengupta et al. 2018). BioBricks include a wide variety of genetic tools being developed to carry out both primitive and advanced molecular research in a biological system such as inducible or constitutive promoters, plasmid or shuttle vectors, ribosome binding site (RBS), riboswitches, CRISPR-Cas systems, small RNA mediated tools which have been exploited efficaciously for gene editing to stimulate stress tolerance and metabolic manipulations in *E. coli*, yeast and in model cyanobacterial species (Santos-Merino et al. 2019). Cyanobacterial synthetic biology research has been accelerated in the last 8–10 years as delineated by an increasing number of publications. Efforts are on way to strengthen genetic toolboxes in cyanobacteria, which will be discussed in the following sections. An overview of genome editing and the application of various synthetic biology tools in metabolic engineering of cyanobacteria is summarized in Fig. 10.1.

10.2.1 Inducible and Constitutive Promoters

Inducible promoters can regulate the expression of specific genes under the influence of certain inducers, whereas constitutive promoters are programmed to facilitate persistent transcription of endogenous genes in an unregulated manner (Liu and Pakrasi 2018). A promoter is considered an ideal promoter if it is capable of generating a predictable response at a specific concentration of inducer or repressor and should be stable/lie dormant under normal conditions of growth and development (Behle et al. 2019). A plethora of research has indicated that most of the inducible promoters have metal-binding activity that modulates host cell metabolisms under distinct environmental stresses which otherwise may cause protein denaturation (Zess et al. 2016). For example, some promoters named *coaA* and P_{nrsB} from *Synechocystis* sp. PCC 6803 and P_{smt} and P_{isiAB} from *Synechococcus* sp. PCC 7942 were significantly induced under various heavy metal stresses. An isopropyl-β-D-thiogalactoside (IPTG) induced promoter with a repressed *LacI* promoter has been shown to differentially regulate the genes involved in photosynthesis and nitrogen fixation in *S. elongatus* PCC 7942. Interestingly, *E. coli* promoter L03 induced by anhydrotetracycline (aTc) was able to regulate the expression of genes

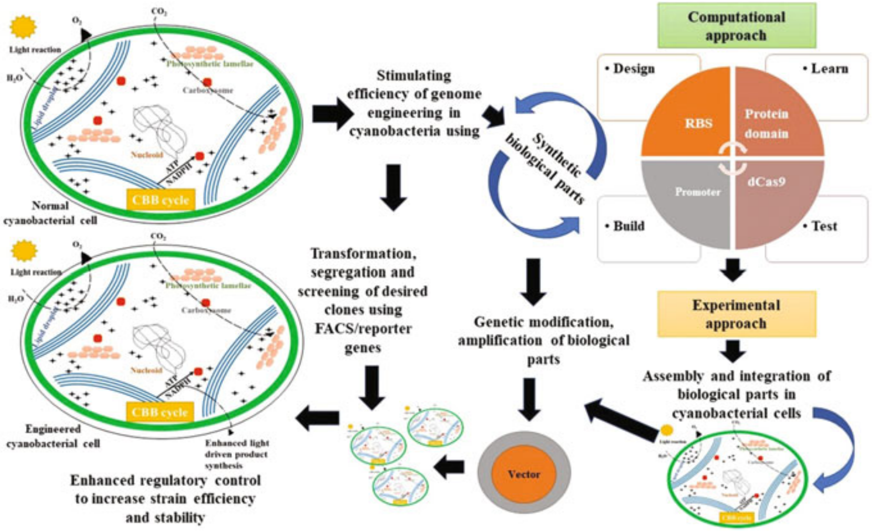

Fig. 10.1 Strategy for engineering cyanobacterial genome incorporating both computational and experimental aspects by exploiting novel synthetic biology tools. First, the synthetic biological parts are identified, tailored and tested for enhancing light-driven product synthesis in cyanobacteria. The models are then further refined based on the data sets generated to create efficient/improved synthetic biology toolkits. These advanced toolkits are then used to modulate the expression of genes/proteins of metabolic pathways to increase light-driven product synthesis in cyanobacteria and at the same time minimize the instability in the engineered strains. *CBB*, Calvin-Benson-Bassham cycle; *RBS*, Ribosome binding sites; *dCas9*, CRISPR-associated protein 9; *FACS,* Fluorescence-activated cell sorter

involved in the photosynthetic process, circadian rhythm and stress defence pathway (Jin et al. 2019). Simultaneously, overexpression of T03 promoter with tetR repressor causes significant induction of T03 promoter up to 200-fold in *Synechococcus* sp. PCC 7002, 7942 and *Synechocystis* sp. PCC 6803, thus confirming its wide adaptability (Zess et al. 2016). Researchers have also attempted to improve cyanobacterial growth and biomass production by stimulating the uptake of xylose or glucose by overexpressing arabinose-inducible promoter P_{BAD} (Dong et al. 2018). Additionally, heterologous expression of *E. coli* P*rha*BAD promoter which is induced in the presence of rhamnose in *Synechocystis* sp. PCC 6803 causes significant induction of the RhaS transcription factor which in turn stimulates the glucose-xylose-rhamnose metabolic pathway leading to improved growth and biomass production (Santos-Merino et al. 2019). Nonetheless, the feasibility and efficiency of these promoters need to be further validated and evaluated in other cyanobacterial species. In oxygenic photosynthetic cyanobacteria, the oxygen-sensitive enzymes/metabolites such as dehydrogenase significantly alter the production of 1-butanol more readily compared to anoxic conditions (Immethun et al. 2016). This concern was overcome by constructing two strong cyanobacteria-specific promoters by employing the fumarate and nitrate reduction (FNR) system of *E. coli*. In an attempt, the fnr gene system was introduced in *Synechocystis* sp. PCC 6803 to develop an FNR-activated promoter that was able to induce the expression of concerned genes under oxygen-free conditions (Immethun et al. 2016). Another attempt involved the introduction of the dark sensing protein Cph1 of *Synechocystis* in *E. coli*, which is capable of phosphorylating histidine kinase resulting in the modulation of transcriptional regulation of the OmpR gene in *E. coli* leading to its activation under dark conditions (Sun et al. 2018). Furthermore, several researchers have shown that the most effective way to design promoters is by modulating the interaction between response regulators (RRs) and transcription factors (TFs) (Vasudevan et al. 2019). Huang et al. (2016) engineered slr1037 and sll0039 (RR encoding genes) and TF encoding gene Sll1626 that tremendously enhanced the tolerance against 1-butanol and fatty acid biosynthesis in some cyanobacterial strains.

Constitutive promoters such as PpsbA2, PrnpB and PcpcB have been systematically elucidated in cyanobacteria for producing fuels and chemicals by regulating the expression of genes of various metabolic pathways (Yao et al. 2015; Wang et al. 2017). In addition, several attempts have also been made to develop new promoters in various cyanobacteria either by modifying existing promoters or by using various computational approaches and online databases. For instance, Sun et al. (2018) significantly modified the tandem promoter rRNA from *Synechococcus* sp. PCC 7942 using Ps promoter from *E. coli* which increased the production of soluble proteins compared to their native form. Similarly, several endogenous promoters such as Pcpc560 (from P*cpcB*) and PpsbA2S (from P*psbA2*) have been modified/ optimized to efficiently regulate the production of functional proteins; these were also able to regulate the expression of several reporter genes (Sun et al. 2018). Furthermore, several researchers have also confirmed the superiority of truncated promoters over their native form (Behle et al. 2019). Some strong promoters such as P*cpc*560 and P*rbc* designed by using bioinformatic tools in *Synechococcus* sp. PCC 7002 and *Synechocystis* sp. PCC 6803 were able to regulate gene expression up to eightfold compared to control cells. Additionally, the efficiency of promoters involved in regulating genes of the photosynthetic pathway was examined using yellow fluorescent protein as a probe. This study led to the discovery of promoter Psll1626 which exhibited strong activity (up to 1000-fold) compared to the native PrbcL promoter (Miao et al. 2017).

10.2.2 Optimizing RBS Modifications

The ribosome binding site (RBS) is the sequence located upstream of the start codon of mRNA and specifically involved in the recruitment of ribosomes during translation. RBS has the ability to affect gene expression in terms of translational efficiency up to 10,000-fold (Sengupta et al. 2018; Lindblad 2018). It is essential for modulating the coordinated translation of several downstream genes by facilitating the interaction between ribosome and Shine-Dalgarno (SD) sequences (Wendt et al. 2016; Sun et al. 2018). Bacterial transcription and translation systems primarily consist of promoters, terminators, coding sequences (CDS) and RBS sequences

(Singh et al. 2016). Among all, RBS sequences are the ones that strategically perform critical binding between mRNA and ribosomes. RBS engineering is mainly achieved by changing the nucleotide sequences present upstream of the START codon through transgenic approaches. Upstream of the START codon through transgenic approaches.

Some efforts have been made during the last couple of years to engineer RBS sequences in both algae and cyanobacteria for enhancing the productivity of several metabolic pathways (Sakamoto et al. 2018). Furthermore, attempts have been made to engineer RBSs to increase the production of 2,3-butanediol in *Synechococcus* sp. PCC 7942 by improving the transcription of three genes, namely, *alsS*, *alsD* and *adh*, of 2,3-butanediol biosynthesis pathways (McEwen et al. 2016). Further, Zhou et al. (2016) were also able to stimulate the biosynthesis of 2,3-butanediol in *Synechococcus* sp. PCC 7942 by employing different RBS promoters with contrasting translation competencies and reported approximately 1.8–2.0-fold increase in the final production. Attempts are on to modify the existing toolboxes especially promoter and RBS sequences to optimize the production. Veetil et al. (2017) selected the content-specific RBS features in one of the strongest plant promoters, namely, strongest plant promoters, namely, PpsbA from pea, and transferred it into *Synechosystis* sp. PCC 6803 which resulted in increased expression of ethylene-forming enzyme, thereby enhancing ethylene production up to threefold. Likewise, Wang et al. (2017) developed a P*psbA* promoter targeting RBS to enhance the expression of the limonene synthase (LS) gene in *Synechococcus* sp. PCC 7942, thus accelerating the production of limonene by 20–30-fold.

Tailoring RBS sequences can have an erratic effect on specific gene functions which can instigate severe repercussions on the expression of genes/proteins in different microorganisms (Carroll et al. 2018). Therefore, in order to achieve the desired RBS modifications, one needs to validate the competency of edited RBSs by performing several in silico-based prediction programs such as RBS calculator, RBS designer and UTR designer that efficiently use statistics based thermodynamic algorithm to predict the efficiency of specific ribosome-mRNA pairs (Vijay et al. 2019). Advancement in these tools has facilitated the development of RBS libraries *per se* in *Synechococcus* sp. PCC 7002 and *Synechocystis* sp. PCC 6803 (Thiel et al. 2018). The efficiency and experimental validation for all the three tools have been evaluated and predicted based on the determination coefficient (R^2) in cyanobacteria.

10.2.3 Riboswitches Mediated Regulation of Gene Expression

Riboswitches are cis-acting elements located at 5′-untranslated regions (5′-UTR) with the ability to regulate the expression of mRNA upon binding to small molecules called ligands. However, in the absence of these ligands, the conformation of riboswitches is altered which may lead to inhibition of transcription/translation due to self-cleavage of target mRNA (Sengupta et al. 2018; Klahn et al. 2018; Taton et al. 2017). Riboswitches have presented themselves as an exceptional tool that efficiently regulates gene expression with a high degree of modularity. Furthermore, they are also able to induce the expression of several genes within a short time even in the absence of an inducer/enhancer, thereby abating their leaky expression (Ohbayashi et al. 2016).

Singh et al. (2018) successfully manipulated theophylline-dependent riboswitch for controlling gene expression in distinct microbial systems such as *Prochlorococcus marinus*, *Synechococcus* sp. PCC 7335 and *Synechocystis* sp. However, their use in cyanobacteria is well known where Nakahira et al. (2013) introduced a modified theophylline-dependent riboswitch that regulated the expression of proteins in *Synechococcus* sp. PCC 7942 even in the absence of ligand. Additionally, this riboswitch has also been exploited in other cyanobacterial species such as *Synechocystis* PCC 6803, *Leptolyngbya* BL0902, *Nostoc* sp. PCC 7120 and *Anabaena* sp. PCC 7120 (Taton et al. 2017) where the modified theophylline riboswitch under the control of transcriptional repressors resulted in reduced expression of genes. A novel and native cobalamin-responsive riboswitch capable of functioning well has also been discovered in *Synechocystis* sp. PCC 7002 (Perez et al. 2016). However, its implementation remains elusive in those cyanobacterial species which are not cobalamin autotrophs. In addition, recently theophylline-dependent riboswitch was also engineered in *Synechococcus* sp. PCC7942 to control the expression of ADP-glucose pyrophosphorylase (*glgC*) expression which resulted in a 40–300% increase in the intracellular concentration of *glgC* and glycogen synthesis (Chi et al. 2019). Subsequently, the modified theophylline-responsive riboswitch (theophylline riboswitch) resulted in decreased expression of NADP-dependent sorbitol-6-phosphate dehydrogenase (S6PDH) compared to simple *trc* promoter that resulted in four- to fivefold decrease in the rate of sorbitol production in *Synechocystis* sp. PCC 6803 (Chin et al. 2019). However, efforts are constantly being made to discover new riboswitches detecting metabolites such as TPP, FMN, queuosine, roseoflavin and cyclic dinucleotides (Cano et al. 2018). Nonetheless, their suitability, efficiency and genetic stability in cyanobacteria need to be experimentally verified.

10.2.4 Small RNA (sRNA) as Functional Tools

Bacterial sRNA belongs to a class of non-coding RNA of approximately 50–300 nucleotides that are able to regulate mRNA via proper or improper base pairing (Jagadevan et al. 2018). Small RNA (sRNA) aided synthetic biology tools have shown to be effective in regulating important cellular and biological processes such as replication, virulence and plasmid regulation in bacteria (Chappell et al. 2015). In addition, sRNAs are also able to regulate various signal transduction processes at distinct developmental stages that effectively improve their growth and adaptability under different abiotic stresses (Brenes-Álvarez et al. 2016). Furthermore, strategic engineering of sRNA can help in designing effective regulatory tools by removing non-essential genes to target distinct metabolic pathways having a toxic effect on host cells (Brenes-Álvarez et al. 2016). Nowadays, sRNA is being explicably used and functionally characterized in cyanobacteria, but to date fewer studies have reported their successful application as a genetic tool (Pei et al. 2017). For instance, Abe et al. (2014) successfully exploited sRNA-based genetic tool for improving the translation of cis-repressive mRNA using a trans-activating RNA. Additionally, they also devised and efficiently optimized the MicF and chaperone Hfq system in *E. coli* utilizing a trans-activating sRNA scaffold molecule that significantly improved intermolecular hybridization with cis-repressive sRNA (Dersch et al. 2017).

A complete list of researchers who have used sRNA as genetic tools for engineering metabolic pathways in cyanobacteria is compiled in Table 10.1. Hu et al. (2017) identified an antisense RNA (asRNA) named RblR which is 113 nucleotides in length in *Synechocystis* sp. PCC 6803 that exhibited appreciable complementarity to its target gene rbcL, thus efficiently modulating its expression under various abiotic stresses. Simultaneously, Ueno et al. (2017) used this tool to activate the transcription of the cyAbrB2 gene for enhancing glycogen biosynthesis in *Synechocystis* sp. PCC 6803. Overexpression of CoaR resulted in the downregulation of slr0847 (coaD) and slr0848 operon, thereby enhancing tolerance of *Synechocystis* sp. PCC 6803 to 1-butanol and acting as a negative regulator for the synthesis of CoA (Sun et al. 2017). Georg et al. (2017) modified the photosynthetic apparatus of cyanobacteria by using trans-acting IsaR1 sRNA which induced oxygenic photosynthesis and improved survival under iron-deficient conditions. Furthermore, Srivastava et al. (2017) have also reported the inability of a trans-encodings RNA in regulating transcription of sigJ, a sigma factor encoding gene in *Anabaena* PCC 7120. Knocked down of this trans-encoding RNA enhanced the expression of sigJ gene which in turn improves the tolerance of *Anabaena* PCC 7120 against photooxidative stress. Analysis of transcriptome through comparative approach identified 119 upregulated genes of which *slr0007* significantly enhanced the tolerance of *Synechocystis* sp. PCC 6803 by stimulating the synthesis of lipopolysaccharide (Bi et al. 2018). Simultaneously, Sun et al. (2018) devised two new sRNA mediated genetic tools in *Synechocystis* sp. PCC 6803 using paired termini RNAs (PTRNAs) and Hfq-MicC scaffold RNA analogous to *E. coli* which were able to knock down the expression of target genes up to 90% leading to an increased synthesis of malonyl-CoA up to 50%. Most recently, Olmedo-Verd et al. (2019) functionally characterized a novel sRNA in *Nostoc* PCC 7120 named *as_glpX* that encodes two important enzymes of the Calvin cycle, namely, sedoheptulose 1,7 bisphosphatase and fructose 1,6 bisphosphatase (SBPase). Transcription of *as_glpX* is induced in the early differentiation process of heterocyst development and its overexpression leads to decreased expression of *glpX* mRNA. So, they targeted *as_glpX* mRNA as a tool to shut down the process of CO_2 fixation to regulate metabolic transformation in *Nostoc* heterocyst. Besides their role in post-transcriptional regulation, sRNA has also been known to efficiently regulate gene expression under extreme climatic conditions, thus strengthening their growth and biomass by enhancing their abiotic stress tolerance (Pei et al. 2017). These technological advancements together with in silico approaches have remarkably facilitated the identification of several sRNAs that are able to differentially regulate gene expression under various biotic and abiotic stresses (Giner-Lamia et al. 2018; Sun et al. 2018).

Table 10.1 List of recently developed genome-scale models (GSMs) in cyanobacteria

Cyanobacterial species	Genome-based model	Target genes/metabolites	Functional role	Algorithm used	References
Synechocystis sp. PCC 6803	*i*mSyn617	Calvin cycle and photorespiratory system	Improved growth and enhanced synthesis of biochemicals	^{13}C-metabolic flux analysis	Hendry et al. (2019)
Synechocystis sp. PCC 6803	CycleSyn	Metabolic pathways	Improved pyruvate metabolism and cellular growth	Flux balance analysis	Sarkar et al. (2019)
Arthrospira platensis	*i*AK888	Glycogen metabolism	Increased glycogen biosynthesis	Flux balance analysis	Klanchui et al. (2018)
Synechocystis sp. PCC 6803	*i*mSyn716	TCA cycle, Calvin-Benson cycle	Enhanced glyoxylate metabolism and corrinoid biosynthesis	^{13}C-metabolic flux analysis	Gopalakrishnan and Maranas (2015)
Synechocystis sp. PCC 6803	*i*SynCJ816	Photosynthesis and oxidative phosphorylation processes	Increased CO_2 fixation up to 35%	Flux balance analysis	Joshi et al. (2017)
Anabaena PCC 7120	Manually created a stoichiometric model	Carbon and nitrogen metabolism	Improved growth	Flux balance analysis	Malatinszky et al. (2017)
Synechococcus elongatus PCC 7942	*i*JB785	Production of high-value chemicals	Enhanced photosynthesis leading to increased metabolism	^{13}C-metabolic flux analysis	Broddrick et al. (2016)
Synechococcus sp. PCC 7002	*i*Syp821	Gluconeogenesis and carbon fixation	Enhanced glycogen and lipid synthesis under nitrogen depletion	Flux balance analysis	Qian et al. (2017a)
A. platensis NIES-39	GSMApNIES-39	TCA and pentose phosphate pathways	Enhanced glycogen production under nitrogen deprivation condition	Flux balance analysis	Yoshikawa et al. (2015)

10.2.5 Utilization and Function of Reporter Genes

An efficient way to detect and validate synthetic biology tools in terms of their reproducibility and efficacy is by applying a suitable reporter gene. The reporter genes facilitate sophisticated detection of important biological circuits without causing any lethality to target organisms (Taton et al. 2017). The efficacy and reproducibility of any reporter gene depend upon its localization ability and fluorescence intensity. The reporter genes such as luciferase and fluorescent proteins are most frequently and robustly used in most bacterial systems. On the contrary, in the cyanobacterial system, *lux* operon is well studied and used for predicting the real-time location of genes (Thiel et al. 2018). Furthermore, several reporter genes have been devised and functionally characterized as physical and metabolic sensors based on input signals sensed by them (Immethun et al. 2016). Physico-chemical sensors comprise environmental signals such as light, temperature and CO_2/O_2 levels; for example, a two-component system associated histidine kinase (CcaS) reporter is an endogenous reporter present in *Synechocystis* sp. PCC 6803 which gets activated in response to green light and deactivated upon exposure to red light (Brand and Owttrim 2017). Likewise, an oxygen responsive genetic circuit has also been developed in *Synechocystis* sp. PCC 6803 using fumarate and nitrate reduction (FNR) system which was capable of working under both aerobic and anaerobic conditions and efficiently transcribed *nifHDK* under oxygen-deprived conditions, thus stimulating the transcription of *fnr* gene (Immethun et al. 2016).

Reporter genes directing arabinose signalling have also been created using arabinose-responsive promoter (P_{BAD}) in *Synechocystis* sp. PCC 6803. Similarly, reporter genes regulating gene expression of various metabolic pathways have also been documented in *Synechocystis* sp. PCC 6803. Expression of NtcA-responsive reporters under the influence of NtcA-activated promoters along with RNA polymerase II promoters and sigma factor efficiently regulated the expression of flavin-binding fluorescent protein (*fbfp*), thus stimulating the conversion of iso-citrate to 2-oxoglutarate under varying nitrate concentrations (Immethun et al. 2017). More recently, Cotterill et al. (2019) developed a Cyclops-7 phycocyanin reporter gene for measuring intracellular phycocyanin concentration in cyanobacteria. The developed Cyclops-7 phycocyanin reporter gene reliably predicted phycocyanin levels at different cyanobacterial biovolume. Likewise, Kumar et al. (2019) exploited the *gfp*-reporter construct fused with *nifD* and *nifH* genes to activate the transcription of hglD and hglE genes involved in the differentiation of the heterocyst and glycolipid layer production.

10.3 Advancing Cyanobacterial Transformation Through the CRISPR-Cas System

Genome editing tools based on CRISPR-Cas systems have recently gained much attention for manipulating gene expression in various organisms. This technique was primarily identified in *Streptococcus pyogenes* which protects them from various pathogenic microorganisms by stimulating their adaptive immune response (Ungerer and Pakrasi 2016). In recent years, the CRISPR-Cas systems have reformed the genetic engineering technology which uses spacer sequences that guide CRISPR RNA (crRNA) and trans-activating crRNA (tracrRNA) to induce double-stranded break in target DNA (Wendt et al. 2016). In eukaryotes, the CRISPR-Cas system is being widely used to engineer crop plants against various biotic and abiotic stresses, whereas its implication in prokaryotes such as cyanobacteria is still lagging (Mougiakos et al. 2018). The implementation of the CRISPR-Cas system in cyanobacteria was incepted by targeting *nonbleaching protein A (nblA)* mutation which impedes the depigmentation process by stimulating phycobilisome degradation under nitrogen deficit conditions (Wendt et al. 2016). The depigmentation process in cyanobacteria is an easily recognizable trait developed when all *nblA* copies are mutated, thus presenting themselves as an excellent observable phenotype during the segregation process. Therefore, the cyanobacterial scientific community began exploiting the CRISPR-Cas system for advancing the genome editing process. In their study, a group of researchers observed that approximately 70% of CRISPR transformed cells were able to segregate without using selection markers (Behler et al. 2018). Nonetheless, the CRISPR-Cas system has substantially impacted the field of cyanobacterial biotechnology by precisely engineering its genome and improving its growth and biomass under stress conditions (Masepohl et al. 1996; Barrangou and Horvath 2017).

In addition, the efficiency of the CRISPR-Cas system was also validated by another research group where they have reported that the efficiency of homologous recombination was significantly improved in conjunction with the CRISPR-Cas system genome editing process in *S. elongatus* sp. PCC7942 (Huang et al. 2016). Following successful validation in cyanobacteria, Li et al. (2016) employed the CRISPR-Cas system for stimulating the production of succinate compounds with multiple industrial applications. For this, they used the CRISPR-Cas system to mutate the *glgC* gene encoding glucose-1-phosphate adenylyl transferase enzyme that stimulates glycogenesis by diverting carbon away from succinate which resulted in increased synthesis of succinate. Furthermore, the same group also generated a knock-in mutant comprising *ppc* and *gltA* genes using the CRISPR-Cas system in the *glgC* locus which resulted in a twofold increase in succinate production by improving the tricarboxylic acid (TCA) cycle (Behler et al. 2018). The above findings undeniably confirmed the practical applicability of the CRISPR-Cas system for cyanobacterial engineering. Apart from their role in improving gene editing efficiency, CRISPR-Cas systems are also impeccably used in the improvement of homologous recombination in cyanobacteria by generating short homology arms of about 400 bp compared to previously used 700–1000 bp arms. The advantage of using a short arm is that it opens up the possibility of integrating genes having shorter genomic target sites, thus efficiently confronting random recombinational events that otherwise have severe repercussions on the nature and behaviour of host performance. Additionally, the CRISPR-Cas system uses a small template DNA for genome editing as well as for homologous recombination from 2000 ng to 200–300 ng (Fokum et al. 2019).

The CRISPR-Cas system has some limitations in cyanobacteria and needs further refinement before it suitably replaces traditional engineering techniques. One of the major constraints in the application of the CRISPR-Cas system in cyanobacteria is the high rate of false-positive colonies for desired DNA modification. Several findings on cyanobacterial transformation have reported around 40–60% mutation in the CRISPR-transformed colonies (Wendt et al. 2016; Xiao et al. 2018). In addition, the CRISPR-Cas system has also been reported to be toxic in some cyanobacterial species like *S. elongatus,* which could be due to the lethality of Cas9 protein or the unavailability of homologous DNA for the repair process (Xiao et al. 2018). In the meantime, efforts are being diverted towards developing a target-based inducible system in cyanobacteria to reduce the lethal effect of the Cas9 system by exploiting a new CRISPR-based Cpf1 system (CRISPR from Prevotella and Francisella 1) which is able to improve the transformation efficiency with minimum leakage (Behler et al. 2018; Sun et al. 2018). Given the importance of the CRISPR-Cas system for genome editing, researchers are now exploiting a new variant of Cas9 for cyanobacteria named Cpf1 (previously known as Cas12a protein), a non-toxic RNA-directed dsDNA nuclease that generates a cohesive cut using crRNA (Higo et al. 2017). This CRISPR-Cpf1 system has been effectively applied in *Synechococcus* sp. PCC 7942, *Synechococcus* sp. PCC 2973, *Synechocystis* sp. PCC 6803 and more recently in *Anabaena* PCC 7120 for generating mutants and knockouts without having any lethal effect on the host (Niu et al. 2018; Santos-Merino et al. 2019). CRISPR-Cpf1 has also been successfully used to upregulate the expression of various genes by stimulating transcription factors and enhancer sequences (Higo et al. 2017). An overview of the CRISPR-Cas9 system and its implementation in improving cyanobacterial growth and metabolism is depicted in Fig. 10.2.

10.4 Genome-Scale Metabolic Models

Genome-scale models (GSMs) are computation-based tools that are able to simulate gene-protein interaction to predict metabolic fluxes for various 'OMICS'-based studies. These models can be easily generated using genomic sequences and their feasibility can be readily validated by various computational approaches (O'Brien et al. 2017). Some recent findings on the automation of genome-based models in cyanobacteria have been documented; nonetheless, they are way behind compared to heterotrophic microorganisms (Gudmundsson et al. 2017). Heterologous pathways in cyanobacteria have been targeted using a stoichiometrically designed model for enhancing the production of alcohol, ethylene and isoprene (Knoop and Steuer 2015). The genome-based models in *Synechocystis* sp. PCC 6803 have been used for increasing the production of alcohols, terpenes and fatty acids by improving fermentation and beta-oxidation processes on the one hand and maintaining an adequate balance of intracellular ATP and NADPH through exterminating NADH sinks on the other hand (Shabestary and Hudson 2016). The cyanobacterial metabolic network has been integrated and reconstructed for enhancing the production of

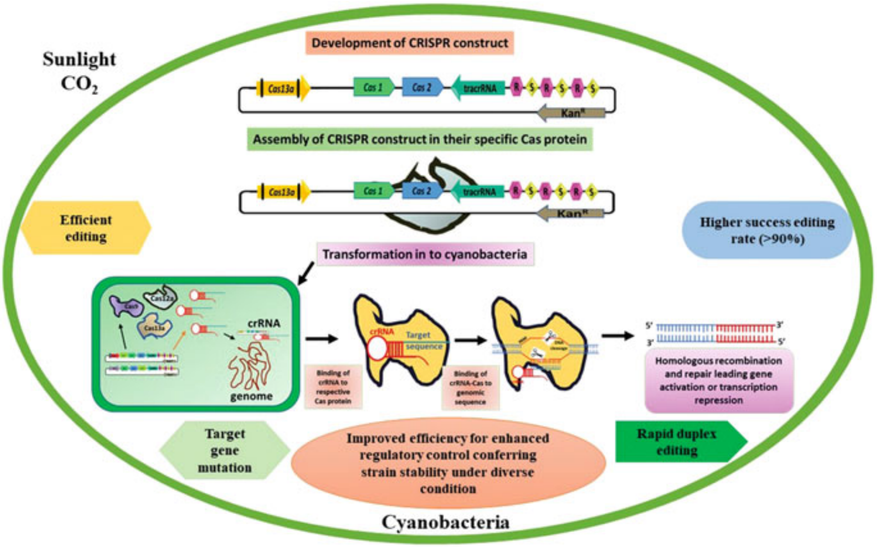

Fig. 10.2 Overview of CRISPR-Cas system for engineering cyanobacteria. The figure represents the systematic layout of CRISPR systems including locus and domains. This system uses crRNA and trans-activating crRNA guided by spacer sequences to induce double-stranded break using Cas proteins. The construct containing essential elements is then used to induce double-stranded break in cyanobacteria for cleaving the desired DNA sequence. Following transformation, the double-stranded break is then repaired by either homologous recombination or non-homologous end joining method as mediated by template plasmid. The selection of respective transconjugants is done on Kanamycin supplemented agar plates and the positive colonies are then streaked on BG11 medium supplemented with Kanamycin for the selection of mutants with desired traits

limonene and isobutyraldehyde (Mohammadi et al. 2018). Similarly, Wang et al. (2017) devised a computational tool to monitor limonene flux and photosynthesis rate and concluded that limonene synthase is the key enzyme involved in the limonene synthesis pathway. Metabolic flux analysis based on ^{13}C between normal and recombinant *Synechococcus* sp. PCC 7942 has outlined important genes involved in glycolysis, as well as associated pathways and overexpression of these genes, resulting in the increased production of pyruvate kinase (Jazmin et al. 2017).

In many cyanobacterial species such as *Anabaena* PCC 7120 and *Synechocystis* sp. PCC 6803, approximately 50% of proteins are uncharacterized ‘hypothetical proteins’ (Pandey et al. 2013; Agrawal et al. 2015; Rai et al. 2019). However, proteomics studies have been able to identify several hypothetical proteins that are up-accumulated in response to stress conditions which can be further classified based on cellular and biochemical functions they perform using interaction studies. Additionally, these interaction studies have led to the identification of genes linked to the metabolic and other signalling pathways (Agrawal et al. 2014). Inclusion of these studies along with genome-based models will facilitate the detailed characterization of these hypothetical proteins, discover new signalling pathways, establish a mechanistic link between metabolic pathways and thus contribute significantly towards the engineering of cyanobacteria for biotechnological application (Agrawal et al. 2017, Chaurasia et al. 2017; Singh et al. 2017; Shrivastava et al. 2016). In this context, some attempt has been made to provide functional insight into various metabolic pathways by targeting essential genes via genome-scale models in *Synechococcus* sp. PCC 7942, thus constructing sophisticated models to provide a better understanding of the nucleic acid metabolism (Qian et al. 2017; Abernathy et al. 2019). Similarly, Klanchui et al. (2018) have constructed a genome-scale model known as iAK888 for predicting cellular/biochemical behaviour as a tool to enhance glycogen production that could eventually increase bioethanol production in *Arthrospira platensis*. The simulated model iAK888 can further be modified and used in other cyanobacterial species for enhancing growth, biomass and biofuel production; however, it requires experimental validation before its application. Most recently, Hendry et al. (2019) have reported solar-based production of biofuels/biochemicals by generating a genome-based mapping model, namely, imSyu593, in *Synechocystis* sp. PCC 6803. The constructed model was an improvement over previously designed imSyn617 that significantly improved not only carbon metabolism (up to 96%) but also increased the rate of carbon uptake, thus increasing the biomass of *Synechococcus* sp. PCC 2973 (Hendry et al. 2019). Altogether, the above-mentioned research findings demonstrated the potential role of genome-based models and their application in synthetic biology (Table 10.1).

10.5 Development of Modular Cloning Suite for Cyanobacterial Transformation

Synthetic biology tools have revolutionized the engineering of the microbial genome by remodelling the traditional method of recombinant DNA technologies and accelerating the analysis, assembly and synthesis of metabolic pathways/compounds (Vasudevan et al. 2019). Compared to the traditional method of genetic engineering such as chemical, mechanical and electrical, synthetic biology involves contemplation, dissolution and systemization of basic biological elements such as promoters, terminators, RBS, riboswitches and sRNA to develop an efficient biological system with high accuracy (Santos-Merino et al. 2019). Owing to its small genome size and the availability of the complete genome sequence, the precise and efficient synthetic engineering of cyanobacteria can result in the sustainable production of essential metabolites and biofuels for agricultural and industrial applications (Singh et al. 2016). The upper hand of synthetic engineering involves high accuracy, increased pyramiding of transgene and enhanced gene expressions, thus eventually reducing the risk of epigenetic modifications and off-target contaminations (Zess et al. 2016). One of the fundamental aims of synthetic biology mediated tools is to engineer the existing metabolic pathways for enhancing the bioproduction of high-value metabolites/compounds which is otherwise impossible to be achieved by traditional cloning methods (Du et al. 2018).

To expedite high-throughput cloning in cyanobacteria, a robust and powerful approach called the golden gate cloning system has been described which is capable of assembling several genes in one construct (Nagel 2019). This golden gate system involves the use of IIS restriction enzymes capable of digesting nucleic acid away from their restriction sites with the probability of generating 4^4 nucleotide overhangs with a high degree of ligation to facilitate the assembly of multiple genes in one construct in a systematic orientation to perform high-throughput cloning in cyanobacteria (Sebesta et al. 2019). In addition, the technique also facilitates the easy construction of multiple combinations by using the same four-nucleotide overhangs to both 3′ and 5′ ends. This new modular cloning (MoClo) method has been widely adopted in bacteria, plant and animal systems and has significantly improved the efficiency of assembling different genetic parts with complex vectors in a highly economical and accessible way without the application of any proprietary tools and reagents (Engler et al. 2014). However, in cyanobacteria development of a modular cloning suite has been adopted by Vasudevan et al. (2019) by modifying the plant golden gate cloning toolkit. They designed vectors for assembling different individual genetic elements including 21 terminators and 12 constitutive and 33 inducible promoters in *Synechocystis* sp. PCC 6803 and *Synechococcus* sp. UTEX 2973 and efficiently merged them with CRISPR/Cas system to generate knock-out mutants. They constructed a robust CyanoGate system linking cyanobacteria with algal and plant systems by integrating golden gate MoClo syntax and genomic libraries of plants from different platforms for the characterization of different genetic elements such as promoters, terminators and RBS. Further, they also constructed level T acceptor vectors for scaling up the integrative or replicative transformation process and generated both knock-in and knock-out mutants more efficiently. Above workers (Vasudevan et al. 2019) assembled T vectors with eYFP expression cassette ($P_{cpc560\text{-}e}$YFP-T_{rrnB}) to generate pPMQAK1-T-eYFP and pSEVA421-T-eYFP trans-conjugates in *Synechocystis*.

The expression cassettes were then assembled on the CRISPRi system to study gene repression by designing small regulatory RNA to recruit Hfq protein (highly conserved among bacteria). The trans-conjugates expressing only sgRNA showed no reduction in eYFP expression. On the contrary, the trans-conjugates carrying Cas9 construct showed a 40–90% reduction in the expression as observed previously for *Synechococcus* PCC 7002 and *Synechocystis*, thus confirming the utility of CyanoGate kit with CRISPRi system (Vasudevan et al. 2019). Overall, the designed CyanoGate-based MoClo kit efficiently improved the availability of genomic elements in cyanobacteria which can be extended in other non-cyanobacterial species. In addition, an online DNA design portal has also been established to increase the accessibility and usability of CyanoGate which is currently maintained by Edinburgh Genome Foundry.

10.6 Engineering Cyanobacteria for Enhancing Abiotic Stress Tolerance

Abiotic stresses affect cyanobacterial growth either by imposing high light intensities or temperatures that disturb their natural circadian rhythm and photosynthetic apparatus, or heavy salinization of groundwater along with heavy metals may severely affect their growth and metabolism (Marques et al. 2016; Saraf et al. 2017). Being capable of adapting to diverse ecological niches and the presence of a smaller genome, cyanobacteria offer a suitable model to modify their metabolic and signalling pathways for arming them against different environmental stresses (Luan and Lu 2018). Recent findings have indicated that physiological regulatory networks significantly upregulate the transcription of various downstream stress-responsive genes which in turn activate the protective mechanisms by efficiently maintaining ion/osmotic homeostasis of cyanobacterial cells under environmental fluctuations (Rai et al. 2019). So, efforts are needed to tailor innate immune response systems using synthetic biology tools to enhance their survival under climate extremes. In this context, the engineering of *GroESL* system-dependent heat shock proteins (HSPs) offers a unique possibility to prevent protein aggregation and simultaneously improve the proper folding of stress-responsive proteins. A body of literature has suggested that the engineering of native HSPs of g*roESL* has improved the growth of *Anabaena* PCC 7120 at 42 °C and also under 50 mM salinity stress (Nakamoto et al. 2000; Luan and Lu 2018). Similarly, overexpression of HSP-dependent clpB1 gene enhanced re-solubilization and disaggregation of target protein, thus enabling the survival of *Synechococcus* sp. PCC 7942 at 48–50 °C (Eriksson and Clarke 1996; Chaurasia and Apte 2009). Su et al. (2017) engineered and overexpressed HSP encoding gene hspA which improved the growth of *Synechococcus* PCC 7942 not only under high-temperature conditions but also under salinity stress. Manipulation of heterologous endogenous regulators and their subsequent integration can revitalize the regulatory and signalling networks leading to improved growth and adaptation of cyanobacterial cells to specific stresses (Aikawa et al. 2015). Modifying *Synechocystis* sp. PCC 6803 by integrating engineered group II sigma factor (SigB) resulted in improved growth of *Synechocystis* under high-temperature stress and butanol production (Kaczmarzyk et al. 2014). Similarly, *Anabaena* PCC 7120 overexpressing DNA-binding protein (*all3940*), *all1122*, *alr0750* and phytochelatin synthase (*alr0975*) have shown multiple stress tolerance including UV, salinity, heavy metals and desiccation due to differential expression of their proteome pattern (Narayan et al. 2010; Chaurasia et al. 2017; Sen et al. 2019). Researchers have also exploited Na^+/H^+ antiporter encoding *nhaP* gene from *Aphanothece halophytica* to enhance the growth of engineered cyanobacterial strain *Synechococcus* sp. PCC 7942 under 0.5 M NaCl (Waditee-Sirisattha et al. 2012). Metabolic engineering for substantial and improved production of stress-responsive metabolites such as proline or glycine betaine could be a promising approach to overcome abiotic stress-induced oxidative damages and Waditee-Sirisattha et al. (2012) have successfully engineered freshwater cyanobacteria *Anabaena* PCC 7120 and *Anabaena doliolum* by modulating glycine betaine synthesis pathway to improve their growth under salinity stress. In addition, they also modified serine hydroxymethyl transferase (SHMT), an enzyme which catalyzes the conversion of serine to glycine for nucleic acid biosynthesis and other macromolecules, and integrated successfully in model cyanobacteria *Synechococcus* sp. PCC 7942 to improve their growth under salinity stress up to twofold (Waditee-Sirisattha et al. 2017).

10.7 Frontline Approaches for Modifying Cyanobacterial Genome

Significant progress has been made in recent years and various genome-based approaches can also be exploited in developing more synthetic biology tools for cyanobacteria. The development of suitable phenotyping methods is of utmost requirement to accelerate cyanobacterial engineering. Screening methods such as single-cell phenotyping, high-throughput droplet phenotyping method and their subsequent modifications in the future can enhance the screening of RBS-based promoters in the recombinant libraries. Flux balance analysis (FBA), a mathematical algorithm used for characterizing the metabolic and signalling networks in different microorganisms (Orth et al. 2010), helps to analyze genome-scale reconstructions of several organisms (Qian et al. 2017a). Modifying FBA and related methods can be efficiently used to create gene knock-outs, overexpression constructs to increase the physiological ability of an organism to improve growth and sustainable bioproduction under climate extremes. Efforts have been directed to refine the existing genome-based models or construct the new ones with high fidelity, wider application and increased accuracy using computer-based algorithms. Genome-based models such as Optgene, OptKnock and OptORF and minimization of metabolic adjustment (MOMA) have been refined using in silico-based algorithms to predict target insertions and deletions controlling the synthesis of essential metabolites (Shabestary and Hudson 2016; Lin et al. 2017). Therefore, developing more in silico-based algorithms with wider applicability in different cyanobacterial strains will boost the metabolic engineering strategies for their possible biotechnological applications.

10.8 Conclusion and Future Direction

Cyanobacteria are arguably the most promising microbial platform for sustainable biotechnologies, still unravelling their full potential demands application of cutting-edge synthetic biology techniques. Significant efforts have been made to characterize genetic tools such as promoters, RBS, riboswitches, sRNA and CRISPR/Cas systems for developing robust expression libraries in heterologous organisms such as *E. coli* and yeast, while cyanobacteria still lag behind in the optimization of these synthetic biology tools for improving the efficiency of genetic engineering and understanding its complex regulatory networks. In view of that, this chapter provides significant insights into recent developments made in the synthetic biology tools and their application for engineering cyanobacteria to withstand environmental perturbation during their outdoor cultivation as well as to increase the bioproduction of essential metabolites for the biotechnological and agricultural endeavour. Successful utilization of cyanobacteria for industrial/agricultural applications requires the development of genetically modified strains that are more compatible with their outdoor cultivation. The application of the CRISPR/Cas tool has opened the possibility of using synthetic biology tools for engineering cyanobacterial species endowed with enhanced tolerance to abiotic stresses.

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The Potential of Rhizobacteria for Plant Growth and Stress Adaptation

11

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Abstract

Plants host a very rich microbiome comprising fungi, bacteria, and protozoa. Bacteria proliferate inside tissues or around roots, where carbon-rich compounds exert positive chemotaxis. Many physiological functions in crops are related to symbiotic events with Gram-positive or Gram-negative bacteria, which influence growth, development, nutrition, and immunity and fine-tune root tropisms. This potentiates the exploration of the substrate and may confer stress tolerance. The production of secondary metabolites and volatile compounds has been thoroughly investigated in Gram-positive bacteria in recent years, and a few of these info-chemicals increase the endogenous auxin levels and/or response, whereas Gram-negative species releases quorum-sensing signals of the *N*-acyl-homoserine type that are recognized by roots to strengthen root branching and immunity. This chapter reviews the contributions of rhizobacteria to enhance crop fitness in the field to make agriculture safer and more sustainable and highlights a few examples of how cross-kingdom signaling influences root behavior.

11.1 Introduction

Agriculture is the primary economic activity enabling food supply worldwide. Starting from the so-called "green revolution" driven by Dr. Norman Borlaug and his coworkers, through the development of crops more efficient to take up nutrients and water and more resistant to environmental challenges along with industrial

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production of fertilizers, now we may have access to fruits and fiber to satisfy world's demand in the few following years. Nevertheless, continuous population growth has led to the undesirable situation of the opening of natural ecosystems to agriculture, despite these lands may not be adequate to support plantations in the long term (Gomiero 2016). Another challenging trend is that the excessive use of agrochemicals has led to soil salinity, water contamination, and environmental risks (Atafar et al. 2008). Recent approaches to make agriculture more sustainable take advantage of the mutualistic relationships that plants establish with microorganisms, mainly bacteria and fungi. The root microbiome contains a great diversity of rhizobacteria that positively impact plant growth, help in phytoremediation, nutrient solubilization, or prevent diseases (Alori et al. 2017; Köhl et al. 2019; Jiang and Fan 2008). Besides, through chemical communication with root cells, rhizobacteria amplify host responses associated with the tolerance to biotic and abiotic challenges (Bano and Muqarab 2017; Chiappero et al. 2019).

The establishment of rhizobacterial populations is promoted by root exudates, which are mainly constituted of carbohydrates, amino acids, organic acids, and secondary metabolites. Changes in the chemical composition of the rhizosphere influence the microbiome and, consequently, modify the overall plant adaptive responses (Sasse et al. 2018). The competition of soil microorganisms for space along roots drives the association between different bacterial taxa (Philippot et al. 2013). Consortia establishment allows the integration of diverse traits related to the growth of the host and health maintenance (Kumar et al. 2016). Recent studies have identified compatible rhizobacteria for consortia formulation and farming practices. Here, we present an overview of the rhizobacterial mechanisms that benefit crops and how these can be modulated when bacteria coexist within plant roots.

11.2 Rhizobacterial Functions in Plant Growth and Development

Plant developmental processes such as embryogenesis, germination, growth, flowering, and reproduction are regulated by a wide range of compounds named phytohormones, many of which can be produced by rhizobacteria. Indole-3-acetic acid (IAA) is the most widely distributed auxin in plants and microorganisms that stimulates root growth and organogenesis (Perrot-Rechenmann 2010). Through changes in the configuration of the root system, bacteria improve soil exploration and, therefore, nutrient uptake (Fig. 11.1). *Pseudomonas*, *Bradyrhizobium*, *Streptomyces*, *Azospirillum*, *Sphingomonas*, and *Acinetobacter* species that actively produce IAA constitute important options to enhance crop performance (Chouyia et al. 2020; Hashmi et al. 2019; Ijaz et al. 2019; Kumawat et al. 2019; Molina-Romero et al. 2017).

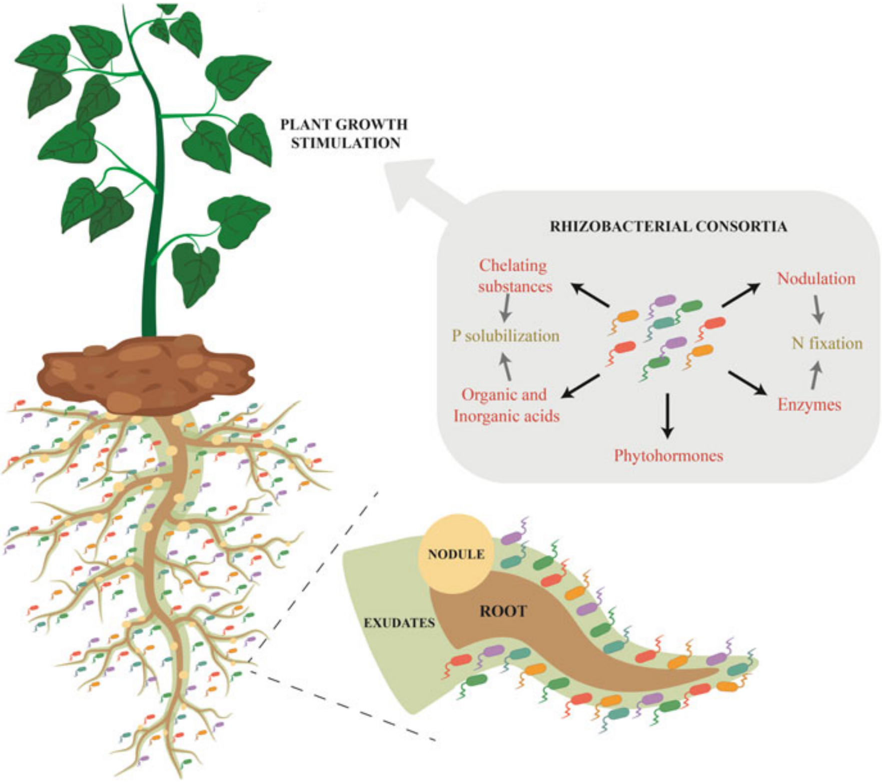

Fig. 11.1 Plant growth enhancement by rhizobacterial species. Soil bacteria can improve plant growth and development contributing to P solubilization by the production of chelating substances and organic acids. The enzymatic machinery of some rhizobacteria allows to convert the atmospheric nitrogen into assimilable N forms for plants. Phytohormones produced by rhizobacteria reconfigure the root system architecture in a way that improves its ability to explore the soil and therefore boosts the uptake of nutrients

11.3 Enhanced Nutrient Uptake

Plants rely on an adequate supply of minerals for growth and development. When a given soil lacks any macro- or micronutrients, various physiological processes are altered, decreasing their productivity. Phosphorus (P) and nitrogen (N) are the most limiting elements for the success of crops due to their structural, metabolic, and signaling functions. Most nutrients should be supplied in their ionic forms as part of chemical fertilizers or as part of organic amendments that farmers use to warrant high productivity (Maathuis 2009). Phosphorus commonly remains in the soil as insoluble phosphate complexes, not easily available for uptake by the root system. The production of organic acids, chelating substances, and enzymes from rhizobacterial species promotes solubilization of organic and inorganic P forms, which can be taken up by root hairs and lateral roots (Alori et al. 2017). Such ability to solubilize P is an interesting trait of bacteria that increases the growth and yield parameters in tomatoes and maize (Bradáčová et al. 2019, 2020) and supports growth in P-deficient soils (Bradáčová et al. 2019). Noteworthy, a mixture of *Pseudomonas* sp., *Burkholderia* sp., *Enterobacter* sp., and *Serratia* sp. improved both the available P content in soil and its uptake in aloe plants (Gupta et al. 2012).

Rhizobacteria may support N reduction to form nitrate (NO_3^-) and ammonium (NH_4^+) for plant nutrition. Particularly, the *Rhizobium* genus stimulates the growth of legumes through N fixation in root nodules (Vargas et al. 2017). The mineralization of organic matter contributes to enriching the pool of available N in the soil by the activity of lytic enzymes from saprophytic bacteria and fungi (Geisseler et al. 2010). Soybean and common bean seeds inoculated with *Bradyrhizobium* sp. and co-inoculated with *Azospirillum* sp. generated the same yield under N deficiency conditions as in fertilized soils. This consortium is an example of alternatives less expensive for farmers to support productivity (Hungria et al. 2013). Integration of rhizobia species including *Bradyrhizobium* sp., *R. leguminosarum*, and *Sinorhizobium meliloti* synergistically increased the nodule number, biomass, and the N uptake in soybean and pigeon pea (Kumar et al. 2016; Kumawat et al. 2019; Pandey and Maheshwari 2007).

Plant nutrition also demands several trace elements that play critical roles in redox reactions and function as cofactors of several enzymes such as RNA polymerase, cytochromes, and superoxide dismutase (Nagajyoti et al. 2010). Bacteria can help plants, either by solubilizing these micronutrients through acidifying the soil or by releasing siderophores that act as metal chelators (Abbaszadeh-Dahaji et al. 2016; Scavino and Pedraza 2013). Wheat plants whose seeds were treated with *Bacillus* sp., *Providencia* sp., and *Brevundimonas* sp. mixtures showed better content of Fe, Zn, Cu, and Mn (Rana et al. 2012). Another triple consortium (*B. megaterium*, *Arthrobacter chlorophenolicus*, and *Enterobacter* sp.) applied to wheat seeds increased the content of microelements in the harvested grains (Kumar et al. 2014).

11.4 Tolerance to Abiotic Stress

Soil pollution caused by natural or anthropogenic factors and extreme weather conditions are detrimental to agriculture. Heavy metals, salinity, drought, and drastic temperature fluctuations are the principal abiotic stresses that negatively impact crop growth and productivity (Farooq et al. 2012; Nagajyoti et al. 2010; Parihar et al. 2015; Mathur and Jajoo 2014). Physiological consequences of abiotic stress reside in overproduction of reactive oxygen species (ROS) and the emission of the gaseous phytohormone ethylene (Kumar and Verma 2018). ROS include free radicals that act as messengers in cell signaling through the so-called oxidative stress response. Disturbed ROS homeostasis compromises cell integrity and may cause cell death (Sharma et al. 2012). ROS affect membrane stability via lipoperoxidation and damage proteins and nucleic acids, and thus plants employ scavenging enzymes such as peroxidase, superoxide dismutase, catalase, polyphenol oxidase, and

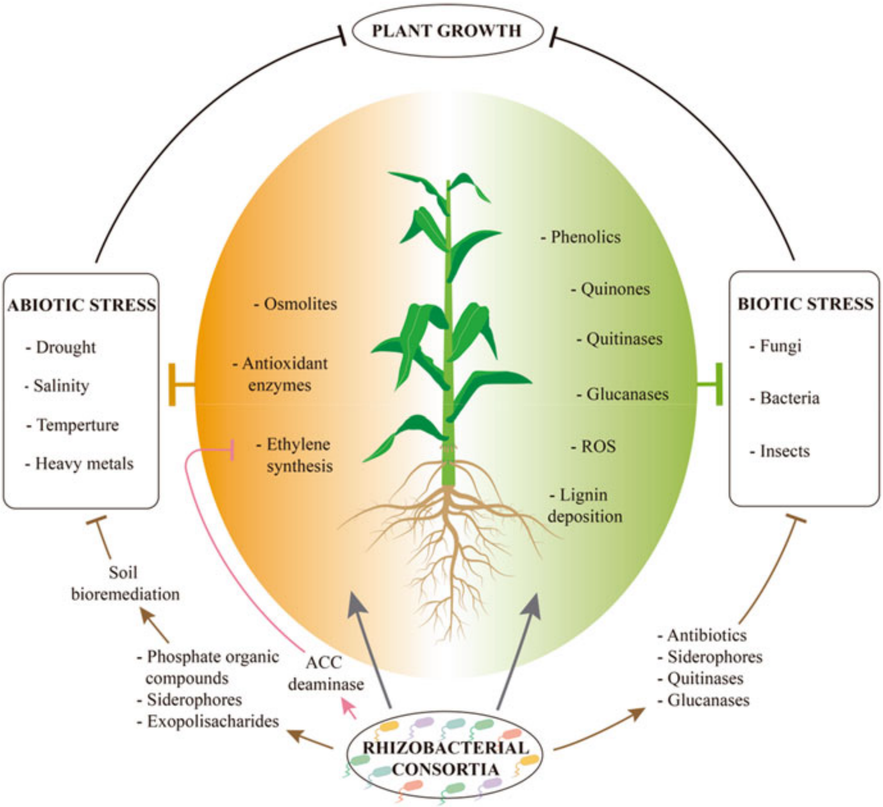

Fig. 11.2 Rhizobacteria mitigate abiotic and biotic stress in plants. Arrows represent positive or negative regulation. Soil bacteria produce numerous enzymes and compounds that directly reduce the soil pollution caused by heavy metals and eliminate the soilborne phytopathogens (brown arrows). The induced systemic tolerance and the induced systemic resistance in plants, which involve components that attenuate the impact of abiotic and biotic stresses, can be activated by rhizobacteria (grey arrows). ACC deaminase from bacteria can decrease ethylene overproduction triggered in plants in response to several stresses (pink arrows). Overall, growth/defense trade-offs are finely modulated in symbiotic interactions

antioxidant compounds including proline, glutathione, ascorbic acid, carotenoids, vitamins, and flavonoids to diminish the oxidative stress (Mehla et al. 2017). Also, as part of adaptive strategies, plants maintain a precise balance in the amount of ethylene synthesized from aminocyclopropane-1-carboxylic acid (AAC) through ACC deaminase enzyme activity that degrades ACC to α-ketobutyrate and ammonia (Shi et al. 2012; Singh et al. 2015).

A practical and eco-friendly strategy to mitigate these nonbiological stresses is the use of probiotic microbes (Fig. 11.2). Rhizobacteria can restore soil fertility by eliminating contaminants and inducing morphological, physiological, and gene expression changes in plants to improve their resistance through a mechanism termed induced systemic tolerance (IST) (Etesami and Maheshwari 2018). Indeed, rhizobacteria provide a combination of different and potential properties that reinforce the plant tolerance to abiotic stresses (Rajput et al. 2018; Saikia et al. 2018; Silambarasan et al. 2019).

11.4.1 Heavy Metals

Fe, Zn, Mn, Mo, Cu, and Ni are heavy metals considered micronutrients for plants in low micromolar or nanomolar concentrations. However, higher concentrations of these minerals (i.e., millimolar range) or other nonessential metals led to adverse effects on germination and shoot and root development not only by oxidative damage, but also by causing enzymatic dysfunction, chlorosis, and senescence (Nagajyoti et al. 2010).

Heavy metal pollution in soils represents a problem for agriculture and human health, and its origin can be natural (i.e., volcanic eruptions and rocks) or industrial (mining and smelting) (Alloway 2013; Ma et al. 2019; Wei et al. 2018). Long-term application of fertilizers and pesticides may also pollute the soils with heavy metals (Atafar et al. 2008). The use of microorganisms is one of the cheapest and most ecological alternatives for the restoration of contaminated soils. Certain bacteria can precipitate, methylate, reduce, oxidize, and chelate heavy metals to attenuate their toxicity (Jiang and Fan 2008; Ojuederie and Babalola 2017). Phosphate organic compounds, as well as siderophores, can interact and bind heavy metals. Shilev et al. (2020) isolated a bacterial consortium (*Pseudomonas* spp. and *Bacillus* sp.) tolerant to heavy metals and inoculated it to spinach seeds in soils contaminated with Pb, Zn, and Cd. The consortium showed the capacity to precipitate or immobilize heavy metals forming insoluble complexes and at the same time increased phosphate solubilization and siderophore production. Plants treated with the bacterial mixture had less accumulation of heavy metals in leaves, stems, and roots and sustained remarkable growth. Another bacterial consortium supported the Pb and Cd immobilization, increasing the phosphate solubilization (Yuan et al. 2017). Through interaction with their functional groups, exopolysaccharides from bacteria also retain various heavy metals (Morillo Pérez et al. 2008). Phosphate solubilizing activity, siderophore, and exopolysaccharide production are traits of *B. megaterium* and *Pantoea agglomerans* that enhance the growth of mung bean plants in soils polluted with Al reducing its concentration in tissues (Silambarasan et al. 2019).

Plants possess mechanisms to counteract the toxicity of heavy metals. For instance, compounds from root exudates such as organic acids, carbohydrates, and siderophores retain toxic elements by chelation, avoiding their absorption by crops (Nagajyoti et al. 2010). These mechanisms can be over-induced by rhizobacteria, allowing the plants to continue growing, even at high concentrations of heavy metals (Silambarasan et al. 2019).

11.4.2 Salinity

Salinization is a severe problem for plant development that has spread to 20% of irrigated lands. Unlike halophytes, most crops exhibit physiological problems when grown in soils with moderate or high salt concentrations (Abogadallah 2010; Farooq et al. 2015). Saline stress increases the Na^+ and Cl^- levels in the cytoplasm and chloroplasts, provoking an osmotic imbalance that stimulates ROS and ethylene overproduction and affects photosynthesis and plant growth (Amjad et al. 2014; Dar et al. 2017; Farooq et al. 2015). Cellular responses to salinity imply the production of osmoregulatory solutes, increased antioxidant activity, and improved selectivity of ions (Ahanger et al. 2017; Amjad et al. 2014). Highly efficient mechanisms are employed by rhizobacteria isolated from halophytes to improve the host adaptation to saline soils (Abbas et al. 2019; Jiménez-Vázquez et al. 2020). These microbes regulate the rhizosphere osmolality and promote adaptation through counteracting saline shock (Bharti et al. 2016; Sarma et al. 2012). Halotolerant rhizobacteria such as *Achromobacter* sp., *Serratia* sp., and *Enterobacter* sp., which presented ACC deaminase activity and IAA production, were found to increase the length of the shoot and root system in avocado plants exposed to saline stress conditions. The consortium intensified the superoxide dismutase activity and diminished lipoperoxidation (Barra et al. 2017). Halotolerant *Aeromonas* spp. mixture protected wheat plants from saline stress improving the root and leaf biomass and increasing the grain yield under half chemical fertilization (Rajput et al. 2018).

11.4.3 Drought

Comparable to salt toxicity, drought increases osmotic stress. The main physiological processes altered by drought are photosynthesis and the uptake and transport of nutrients (Farooq et al. 2012). Therefore, the germination, growth, and development of plants are drastically disrupted under this adverse condition (Asrar and Elhindi 2011; Liu et al. 2011; Samarah and Alqudah 2011). Under drought, plants raise the synthesis of abscisic acid, a phytohormone that induces stomatal closure and prevents water loss through transpiration (Tombesi et al. 2015). Rhizobacteria may support plant growth in water-deficient conditions via decreasing stomatal aperture and reducing ethylene levels (Chiappero et al. 2019; Cho et al. 2008; Mayak et al. 2004). Rhizobacterial consortia alleviated the growth repression of common bean and mung bean triggered by drought stress (Figueiredo et al. 2008; Silambarasan et al. 2019). Crops exposed to drought and grown under pretreatments with bacteria showed more superoxide dismutase, peroxidase, and catalase activity (Barra et al. 2017; Kohler et al. 2008). The mixture of *Ochrobactrum* sp., *Pseudomonas* sp., and *Bacillus* sp. had a synergic effect on the growth of different plant species exposed to drought. Also, they reduce the ACC levels in plants (Saikia et al. 2018).

11.4.4 Temperature

Extreme temperatures either heat or cold affect crop productivity. Production of ROS, heat shock proteins, and abscisic acid and the activation of mitogen-activated protein kinases (MAPKs) are part of the signaling mechanisms triggered by heat stress (Andrási et al. 2019; Evrard et al. 2013; Guo et al. 2016; Link et al. 2002; Yu et al. 2019). Elevated temperatures impair water content, photosynthesis, and membrane stability (Liu and Huang 2000; Mathur et al. 2011; Wassie et al. 2019). Thermotolerant rhizobacteria can enhance plant mechanisms associated with tolerance to heat stress, such as the production of osmolytes, activation of antioxidant enzymes, and reduction of ethylene levels (Ali et al. 2011; Khan et al. 2020; Mukhtar et al. 2020; Sarkar et al. 2018).

The foliage is very sensitive to cold stress and is one of the organs more impacted by chilling, exhibiting wilting, necrosis, chlorosis, and deformation, which affect early leaf growth and flowering (Kumar et al. 2010; Rymen et al. 2007; Yadav 2010). Acclimation to low temperatures is regulated positively by abscisic acid since mutants altered in the signaling of this phytohormone are hypersensitive to cold stress (Shi and Yang 2014). Adverse effects of cold stress can be attenuated by an overproduction of solutes such as sugars and amino acids, whose accumulation is stimulated through interaction with rhizobacteria (Ait Barka et al. 2006; Fernandez et al. 2012; Mishra et al. 2011). Tomato plants grown in soil inoculated with *B. cereus*, *B. subtilis*, and *Serratia* sp. were exposed to cold stress and showed a high level of hydrogen peroxide (H_2O_2) than non-inoculated plants. After days, the consortium moderated the oxidative stress caused by low temperatures mainly through the activation of superoxide dismutase and peroxidase activities. Besides, rhizobacteria increased the production of osmoprotectants such as proline and sugars. These effects helped to reach almost 100% plant survival (Wang et al. 2016). Kakar et al. (2016) applied a combination of *Bacillus* sp. and *Brevibacillus* sp. in rice seedlings and then applied cold stress treatment. The bacteria improved cold tolerance, upregulated the activity of antioxidant enzymes, and increased the proline content of plants, stimulating their growth and survival.

11.5 Biotic Challenges

Considerable crop losses occur due to nematode, viral, bacterial, oomycete, and fungal pathogens, which cause damage at each developmental stage (Savary et al. 2019). The control of plant diseases has been possible in the last decades by applying biocides that pollute air, water, and soil, damage pollinators, and ultimately enter the food chain (Popp et al. 2013; He et al. 2016). Thus, an important goal to achieving sustainable productivity is to develop new alternatives based on understanding plant-microbe interactions in an ecological context (Fig. 11.2).

Plants manifest constitutive and inducible defenses to protect themselves from pathogens. The properties of their immune system can be further modulated by abiotic and biotic stresses (Jain et al. 2016; Miura and Tada 2014). Detailed information on how plant defense is mounted has been gathered in leaves, and until recently little was known about how roots react under attack. It has become evident that damaged roots unlock a protective mechanism in coordination with probiotic bacteria surrounding them that boosts the defense reaction (Berendsen et al. 2012; Hematy et al. 2009).

11.5.1 Disease Suppressive Soils

Some soils have the property to suppress plant diseases because their microbiomes inhibit the spread of phytopathogens. On the other hand, the so-called conducive soils favor the incidence of plant diseases (Schlatter et al. 2017). Abiotic factors and certain farming practices can influence the growth of microorganisms related to the balance required for plants to survive and thrive (Jansson and Hofmockel 2020; Mehta et al. 2014). Continuous cropping is a practice that diminishes rhizobacterial diversity, generating a higher vulnerability to the root rot disease (Tan et al. 2017). In contrast, monocropping systems triggered the reduction of take-all disease, a phenomenon linked to the rising of *P. fluorescens* (Sanguin et al. 2009). The release of root exudates attracts specific groups of bacteria, causing changes in the soil microbial composition that reinforces defensive barriers (Berendsen et al. 2012). Proteobacteria, Firmicutes, Acidobacteria, and Actinobacteria strains predominate in the bacterial community of suppressive soils associated with tolerance to distinct crop diseases (Cha et al. 2016; Kyselkova et al. 2009; Sanguin et al. 2009). Mendes et al. (2011) dissected the rhizobacterial microbiome of sugar beet cultivated in suppressive soils of damping-off disease, concluding that the bacterial taxa contribute to disease mitigation. Seven rhizobacterial strains delayed the *Fusarium verticillioides* mycelial growth on maize seeds and decreased the blight disease incidence in seedlings. Each bacterial strain had a biocontrol effect on spreading the fungus and disease incidence, but not to the same degree as the consortium did (Niu et al. 2017). Other rhizobacteria showed a synergistic biocontrol on the blast disease in rice and collar rot disease in betelvine (Lucas et al. 2009; Singh et al. 2003). This protective capacity could persist for more than one season (Zhang et al. 2019). Therefore, multiple mechanisms depending on the composition of the microbial consortia account for the natural root antagonism to soilborne pathogens.

11.5.2 Fighting Plant Diseases

The production of antibiotics such as 2,4-diacetylphloroglucinol (2,4-DAPG), phenazine-1-carboxylic acid, pyrrolnitrin, oomycin, aerugine, and kanosamine is an important mechanism used by rhizobacteria to suppress the growth of phytopathogens (Kenawy et al. 2019). These compounds damage cell membranes, interfere with the electron transport chain, and affect enzymatic activity (Janiak and Milewski 2001; Raaijmakers et al. 2009; Troppens et al. 2013). Santhanam et al. (2019) evaluated biofilm development during the colonization of tobacco roots by

Bacillus sp., *Pseudomonas* sp., and *Arthrobacter* sp. This consortium increased plant survival against the sudden wilt disease, and one of the factors associated with the resistance was the production of surfactin, an antifungal compound. A single antibiotic can control diverse phytopathogens; for example, 2,4-DAPG, produced by several *Pseudomonas* strains, suppressed take-all, root rot, and damping-off fungal diseases and crown gall caused by *Agrobacterium tumefaciens* (Weller et al. 2007).

Cell wall degrading enzymes can be secreted by rhizobacteria that affect fungal phytopathogens. Chitinase and glucanase production is one of the desirable determinants to select rhizobacteria for biocontrol (Pliego et al. 2011). In this manner, the attenuation of *F. oxysporum* in maize was made possible, and at the same time plant growth and yield increases were obtained upon applying a quadruple bacterial inoculum composed of *B. megaterium*, *P. aeruginosa*, *Serratia* sp., and *P. fluorescens* (Akhtar et al. 2018).

Micronutrient availability is an ecological determinant for plant-microbe interactions. Under deficiency of Fe, an essential micronutrient, specific bacteria produce and secrete siderophores to chelate the available iron, rendering it unavailable for phytopathogens (Kramer et al. 2020). Treatments of *Pseudomonas* sp. or its siderophores protected plants against *Gaeumannomyces graminis* and *F. oxysporum* (Kloepper et al. 1980). Besides, some consortia had suppressive effects related to the activity of siderophores (Akhtar et al. 2018; Estevez de Jensen et al. 2002; Santhanam et al. 2019).

11.5.3 Plant Defensive Reactions

The plant immune system is reinforced by gene expression related to lytic enzymes and the production of antimicrobial compounds (Jain et al. 2016). Two main pathways control the defense reactions, the first involves systemic acquired resistance (SAR), stimulated after an attack of pathogens and dependent on the phytohormone salicylic acid. SAR induces pathogenesis-related (PR) genes, and it is initially manifested in the damaged site but later spreads systemically to distant tissues (Klessig et al. 2018). In the second pathway, the so-called induced systemic resistance (ISR) involves plant interactions with nonpathogenic rhizobacteria, which promote defense-related gene expression through signaling of the phytohormones ethylene and jasmonic acid. ISR has been reported to occur in the aerial and root system and also in leaves, keeping the plant defense machinery ready and alert to respond quickly to single and multiple biotic stimuli (Pieterse et al. 2014).

Berendsen et al. (2018) isolated three bacterial strains from the *Arabidopsis thaliana* rhizosphere, which prevailed during foliage infection caused by the oomycete *Hyaloperonospora arabidopsidis*. The plants grown in soil previously inoculated with these rhizobacteria showed less incidence of the oomycete in leaves. Besides, a combination of rhizobacteria and plant growth-promoting fungi (PGPF) protected cucumber plants against *F. oxysporum* infection in stems, enhancing the expression of genes encoding chitinases, glucanases, and enzymes involved in the synthesis of antifungal compounds (Alizadeh et al. 2013). Peroxidase is another defense component of plants that limits the pathogen spread as it mediates the establishment of physical barriers such as lignin and suberin in cells (Passardi et al. 2005). Adjusting the levels of reactive oxygen species, both peroxidase and superoxide dismutase, can create a toxic environment for pathogens in plant cells (Torres et al. 2006). *Bacillus* spp. diminished the bacterial and fungal infection in tomato and pepper apparently by induction of the peroxidase and superoxide dismutase activity (Jetiyanon 2007).

The plant phenylpropanoid pathway directs the production of phenolic compounds with antimicrobial properties. Phenylalanine ammonia-lyase converts L-phenylalanine into *trans*-cinnamic acid, a precursor for the synthesis of phenolics (Cheynier et al. 2013). Indeed, polyphenol oxidase oxidizes phenols to generate quinones, which also inhibit phytopathogen growth (Jukanti 2017). *Pseudomonas* sp., *Rhizobium* sp., and *Trichoderma* sp. reduced the mortality in chickpea plants infected by *Sclerotium rolfsii*. The triple microbial consortium enhanced the gene expression of peroxidase and superoxide dismutase and increased phenylalanine ammonia-lyase, polyphenol oxidase activity, and lignin deposition (Singh et al. 2013).

11.5.4 Quorum-Sensing-Mediated Antagonistic Activity

Colonization of roots, either by beneficial microorganisms or by plant pathogens, initiates a complex interrelationship where root exudates and mineral nutrients play an essential role. The formation of biofilms mainly composed of bacteria is a biological barrier that extends beyond the root epidermis. This process is under the control of a cell-to-cell chemical communication program termed quorum sensing (QS). In Gram-negative bacteria, QS controls the transcription of genes by producing small molecules, mainly the *N*-acyl-L-homoserine lactones (AHL) (Dwivedi et al. 2017). These compounds have strong activity not only in bacteria but also in roots, which suggests that they modulate the inter-kingdom plant-bacteria language (Ortíz-Castro et al. 2008).

Mutants of *P. chlororaphis* deficient in QS regulation lost their ability to inhibit the growth of plant pathogens. These variants exhibited low expression of *phzA*/*prnA* genes related to the production of two broad-spectrum antibiotics phenazine-1-carboxylic acid and pyrrolnitrin. AHL supplements induced the *prnA* expression, indicating that the QS compounds directly trigger pyrrolnitrin synthesis (Selin et al. 2012; Shah et al. 2020). Kim et al. (2017) concluded that the QS in *Chromobacterium* sp. supports its capacity to suppress fungal diseases in plants since the mutation of the gene encoding homoserine lactone synthetase reduced its chitinolytic activity. Other rhizobacterial enzymes attenuated the virulence of phytopathogenic bacteria by degrading QS molecules. For instance, Actinobacteria releases lactonases, which degrade AHLs in *Pectobacterium carotovorum* and affect its virulence (Vesuna and Nerurkar 2020). The interaction between bacterial communities also upregulated or downregulated the production of siderophores via QS signals (Shah et al. 2020; Stintzi et al. 1998; Whiteley et al. 1999).

11.6 Perspectives

Plants and microorganisms have developed inter-kingdom relations, which have been gaining special attention in agriculture since they improve crop yields and may reduce fertilization and pest control costs. The combination of selected traits from two or more bacteria further intensifies the probiotic effects on crops. Competition for energy and nutrient sources from exudates has led rhizobacteria to influence the host defense responses against bacteria and fungi that could be a threat. Therefore, it is crucial to identify and understand the mechanisms that define rhizobacteria compatibility and antagonism to develop successful inoculants.

Exploration of natural ecosystems has proven to be a valuable strategy to identify novel traits in both bacteria and plants useful to resist stress. The search for halophyte bacteria in a largely untouched sink hole termed the “Poza Salada,” located in the Chihuahua Desert, enabled the identification of a group of plant beneficial bacteria living in association with roots of well-adapted plant species, which grow healthy despite the very high salt concentrations. Three bacterial isolates typified molecularly as *Bacillus* sp., *P. lini*, and *Achromobacter* sp. promoted the growth of *A. thaliana*, *Cucumis sativus*, and *Citrullus lanatus* in vitro and in soil under standard and saline growth conditions (Jiménez-Vázquez et al. 2020; Palacio-Rodríguez et al. 2017). Noteworthy, *Achromobacter* sp. interfered with the root gravity response and caused the formation of waves and circles called coils, which primed roots for enhanced branching through the proliferation of lateral roots (Jiménez-Vázquez et al. 2020). The mechanisms underlying the beneficial properties of halophytes to plants are far from being understood; however, their protection from salinity in different plant species opens new avenues for agriculture. Regardless of the plant growth-promoting traits that rhizobacteria have in their natural niche, their survival should be tested under different environmental conditions to warrant their prevalence on crops. Generating more knowledge about the ways rhizobacteria interact with plants will facilitate access to biological tools for more productive and sustainable agriculture.

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Mycoremediation: An Emerging Technology for Mitigating Environmental Contaminants

12

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Abstract

Mycoremediation is a technique that transmutes toxic, recalcitrant pollutants into environmentally safe products by organic treatments. It is a green method for cleaning up polluted sites. Because of a breakthrough in technology, exceedingly harmful contaminants are persistently released into the environment via industries. Polycyclic aromatic hydrocarbons (PAHs), heavy metals, polychlorinated, and pharmaceutical compounds (PhC) are mutagenic. They are freed by petroleum refineries, textile mills, and vehicle exhaust. Human exposure has risen because of their rampant use in certain industrial, agricultural, and domestic fields. Recently, there has been a growing ecological and global public health concern accompanying environmental contamination. The traditional methods applied to remove them pose risk to the ecosystem. Remediation of polluted sites has become a center of attention within society because of accelerating public awareness. The theory of mycoremediation has come up from the chief role of fungi within the ecosystem, which is to decompose. Nonetheless, the dominating biomass in soil are fungi, which still have not been exploited aptly for mycoremediation. Microfungi and macrofungi both contribute to the feasibility of mycoremediation. Their wealthy enzyme compositions assist the process. The objective of this chapter is to review the role of contaminants on the environment as well as to focus on the part of fungi in eliminating them. We have discussed in detail the various works and the contemporary advancements; futuristic omics approaches that are in the midst of progress.

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12.1 Introduction

The industrial revolution is one of the noteworthy incidents in society. It commenced in Britain around the eighteenth century and is still ongoing. It altered our civilization from an agricultural unit to a manufacturing hub. It changed the grassroots organization and has its *pros* and *cons*. Collectively with all this came pollution! Previously, there were minor factories, but lately this business flourished into full-scale industries, and environmental pollution gained momentum. Moreover, many factories still work with age-old technologies creating huge waste. Soil contamination occurs due to the extraction of mineral ores from the earth's crust which are essential for use as industrial raw materials. Leading complications affecting solid waste management are irrational plans and inappropriate waste collection, leading to threats such as environmental degradation and pollution of water, soil, and atmosphere. Open junkyards are a breeding place for harmful microbes and generate health hazards. Both surface and groundwater are negatively affected by this. The scarcity of genuine policies and improper enforcement protocols allowed industries to neglect the laws. Costly conventional remedial techniques and the obligations related to the use of such practices have stimulated industries to search for novel techniques. To achieve proper remedies, the scientific fraternity and technology firms are testing and discovering emerging technologies. One of these is bioremediation, which is significant nowadays. Far-reaching toxic pollutants constituted a threat to living creatures. The elimination of such contaminants is a prerequisite of present times. The physical-chemical or a union of such methods has been harnessed already. Yet factories use poisonous chemicals and generate dumping issues. The bioremediation strategy directs the use of biological microbes or fungi to do the cleanout. Methods used for remediation of toxic contaminants are rhizoremediation, phytoremediation, microbial remediation, and biosurfactants, which is a leading-edge tool. Mycoremediation in contaminated sites points at pulling out harmful pollutants. Frequently, contaminated sites can become toxin-free. The bioremediation process can be performed by the following:

1. **In situ**: The process is done just at the contamination site. Polluted soil or water is cured at its root source. It is a desirable method since it demands a lesser workforce. Also, it controls the spreading of contaminants to farther locations.
2. **Ex situ**: It requires locating contaminated material to a distant treatment spot. It is troublesome in the case of contaminated water bodies. These methods are risky as chances of contamination spreading exist. The remediation of contaminated sites has become a preference for society because of the rise in standards of living and the recognition of environmental issues. Rhizoremediation integrates plants and microbes associated with them.

12.1.1 Benefits of Bioremediation

- Bioremediation is a spontaneous process.
- It is carried out mostly in situ.

- It has consent of environmental panelists.
- Minimal tool necessity.
- Organic and eco-friendly method.
- Slight power expenditure.
- Budget-friendly compared to other technologies.
- Less alarming to the environment.

12.1.2 Drawbacks of Bioremediation

- The bioremediation process is limited to the compounds that are biodegradable since all the compounds cannot be entirely degraded.
- It is a tremendously specific task.
- It requires an extended period than conventional methods.
- If mushrooms are incorporated into the process, they can turn fatal when consumed as such since these are hyperaccumulators of toxic compounds.

We can protect the deteriorating quality of our surroundings by adopting innovative technology for waste disposal and by being aware of raw materials that can alleviate pollution.

12.2 Fungi as Bioremediators

Fungi are powerful decomposers. They are responsible for breaking down most of the earth's plants and wooden scrap into life-giving soil by breaking complex plant cell components such as cellulose and lignin into final forms (Dutta and Hyder 2017). Organic pollutants contain PAH, heavy metals, and synthetic dyes that are carcinogenic and steadily present in the surroundings. They bring about disadvantageous effects on human health. Many studies revolve around microfungi, but the focus has been shifted to macrofungi that grow abruptly on the soil. They can assemble heavy metals in their fruit bodies and can be visible in the form of mushrooms. Incorporating endemic fungi in the remediation process needs vast mycelium. Mycelia are a dense network of vegetal and thread-like hyphae. Enzymes are secreted from mycelia. An expanded hyphal network aids in the employment of toxic compounds. Their growth substrate frame filamentous fungi as superior in bioremediation to other microorganisms. Mycoremediation is a biotechnique that integrates fungi in the decontamination of altered surroundings (Kapahi and Sachdeva 2017). Enzymes secreted are extracellular and break larger molecules into smaller ones, which enter the cells for further reactions (Levasseur et al. 2014). There are diverse parts that apply mushrooms in the remediation process. Mushroom spawn is spread on contaminated sites to trap toxic compounds. Mycoremediation potential depends on the ease of accessibility of nutrient distribution in soil. Nutrients are supplemented to the soil to amplify the process (Adams et al. 2015). Introducing mushroom beds can decrease soil erosion and aid in curing the disrupted place. Mushrooms need ample dampness, air, shadow, and fair temperature to grow. Different mushroom species can effectively decontaminate polluted sites. Biodegradation of recalcitrant pollutants as PAHs is noticed by white-rot fungi. Combining certain strains of mushrooms can be more successful as they can together modify contaminants and hold on to the water in the soil. Befitting fungi used in soil decontamination are basidiomycetes. Two ecological groups are used in the bioremediation process (Treu and Falandysz 2017). They are as follows.

Saprotrophic basidiomycetes—These are white-rot fungi and brown-rot fungi. These wood-decaying fungi use dead organic matter as a carbon source. Saprophytic fungi use their digestive enzymes to reduce harmful hydrocarbons and defoliants. The examples include *Agaricus bisporus*, *Trametes versicolor*, *Pleurotus ostreatus*, *Irpex lacteus*, *Lentinula edodes*, *P. tuber-regium*, *P. pulmonaris*, and *Phanerochaete chrysosporium.*

Biotrophic basidiomycetes—They include ectomycorrhizal associations. They gain optimum nutrition by secreting enzymes that degrade the molecules present in soil organic matter. The examples are *Lactarius* spp., *Amanita* spp., *Morchella* spp., and *Boletus* spp. Fungi have great power of endurance in contrast to bacteria in fore bearing higher concentrations of pollutants. Mycelia of white-rot fungi pierce the cell cavity to release ligninolytic enzymes (Tišma et al. 2010).

12.2.1 Biosorption Mechanism

Mushrooms incorporate various paths to concentrate heavy metal contaminants. Adsorption is the binding of molecules or ions upon the surface of other molecules. The surface is adsorbent and the compound accumulated is adsorbate. Bioaccumulation relates to employing living cells except for biosorption that involves lifeless biomass. Biosorption by mushrooms is productive and nontoxic as compared to the bioaccumulation process. Being a passive process, it does not require media for growth due to no damage to the biomass with contamination or cell death (Dhankhar and Hooda 2011). The solid phase is known as biosorbent. Biosorption can be listed into three types—cell surface sorption, extracellular accumulation, and intracellular accumulation. Cell surface sorption happens by interactivity between metals and functional groups present on the fungal surfaces. It focuses on adsorption and chelation properties. Intracellular accumulation can occur only in a living cell by transport across the cells (Vegliò and Beolchini 1997).

12.3 Enzymes Used by Fungi in the Remediation Process

Fungal enzymes comprise proteases, amylases, catalases, laccases, peroxidases, and so on. Polymeric compounds such as starch, cellulose, lipids, proteins, or other complex biomolecules are hydrolyzed by these enzymes. Ligninolytic enzymes secreted by white-rot fungi for lignin oxidation make up two categories—

peroxidases, that is, lignin and manganese peroxidases (LiP, MnP), and laccases. Biodegradable extracellular enzymes are laccases and class II peroxidases. Cytochrome P450 monooxygenases and glutathione transferase are intracellular enzymes. The intracellular metabolic breakdown pathway present in mycoremediation shows affinity with secondary metabolism in fungi, especially in mycotoxin production (Chanda et al. 2016). Oxidative enzymes from fungi are prioritized because they are less substrate-specific enzymes.

12.3.1 Ligninolytic Fungal Enzymes

Laccases are multicopper oxidases. Laccases can oxidize a range of phenolic and non-phenolic compounds. They function as a catalyst in industries and have bioremediation potential (Vishwanath et al. 2014). Reactive oxygen species (ROS) demolish cellular membrane that is harmful to the cell entity. *Aspergillus foetidus* had resilience toward Pb^{2+} and an inflated level of antioxidative enzymes intracellularly (Chakraborty et al. 2013). The peroxidase enzyme needs hydrogen peroxide for reactions. Fungi have innated oxidative enzyme machinery that withdraws harmful compounds. The tribromophenol level is decreased by the fungal laccase of *T. versicolor* (Donoso et al. 2008). *Coriolus versicolor* can degrade PAHs with manganese phosphate and lignin phosphate enzymes (Jang et al. 2009). Laccase of *Trichoderma* species decays phenanthrene (Han et al. 2004). Laccases hold a low shelf-life. Nanobiotechnology-driven studies involving laccases could be a boost.

12.3.2 Non-ligninolytic Fungal Enzymes

Fungi have intracellular components that are composed of cytochrome P450 monooxygenase and glutathione transferases (Morel et al. 2013). Cytochrome P450 monooxygenase is the predecessor to degradation plans involving aromatic pollutants. Lignin-degrading enzymes do not work accordingly due to insufficient lignocellulosic substrate on contaminated soil. This limitation can be prevailed by enzymes such as cytochrome P450 monooxygenase as reported in ascomycetes (Marco-Urrea et al. 2015).

12.4 Mycoremediation of Polycyclic Aromatic Hydrocarbons

PAHs (polycyclic aromatic hydrocarbons) are emitted as a consequence of pyrolytic processes due to insufficient combustion of organic materials. They are released in industrial and other activities such as mining, combustion of gases, and smoking (Fig. 12.1). PAHs are a crowd of organic pollutants with two or more fused aromatic rings. A few examples of PAHs are naphthalene, anthracene (AC), phenanthrene, fluorene, pyrene, and chrysene. The best-known PAH is benzo[*a*]pyrene (BaP). Carcinogenic PAHs are prevalent in all surface soils. Exposure to benzo[*a*] anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, and BaP is frightening. They are soluble in lipids but slightly less soluble in water. They get absorbed from the lungs, gut, and skin of mammals. Food is regarded as the leading source of human exposure to such compounds due to the generation of PAH through cooking oils or from the atmospheric accumulation of PAHs on grains, fruits, and vegetables (de Vos et al. 1990). Biodegradation methods are engaged to transform into nonhazardous forms in an ecologically sound way. Bioremediation is a crucial tool to rehabilitate the PAH-contaminated sites. The plant-microbe interaction for the disintegration of soil pollutants consequences in rhizoremediation. Hussein and Mansour (2016) reported general usage of some PAHs:

1. Anthracene: Dial event for food preservatives and red dye manufacturing
2. Fluoranthene: Manufacturing of agrochemicals dyes and insulating oils
3. Phenanthrene: Employed in resins and pesticides, plastics, and explosives
4. Pyrenes: Manufacturing of pigment dyes and their precursors

Trichoderma lixii strain was isolated from PAH-contaminated soil and might grow on phenanthrene using it as a personal carbon source. *Trichoderma* species can reside in a broad spectrum of substrates by using them (Venice et al. 2020). Biodegradation of PAHs occurs under either aerobic or anaerobic conditions and could be increased by physicochemical-free treatment of contaminated soil (Cerniglia 1993). Oxygenases, peroxidases, dehydrogenases, and ligninolytic enzymes are involved in PAH degradation (Chang et al. 2002). *P. ostreatus*, a

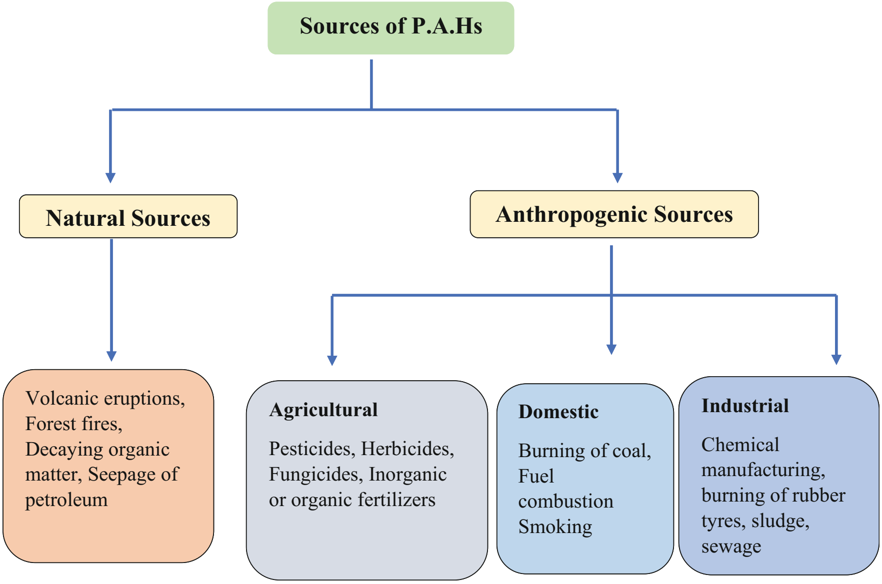

Fig. 12.1 Various sources of polycyclic aromatic hydrocarbons

white-rot fungus, and its substrate are used to recycle Nigerian oil drill cuttings containing PAH under laboratory conditions (Okparanma et al. 2011). Healthy soil shelters plants and microorganisms. Treatment of soil is making it unsuitable for plant nourishment. Both ligninolytic and non-ligninolytic fungi can break down PAHs by extracellular lignin-degrading enzymes. Species belonging to *Aspergillus*, *Penicillium*, *Rhizobium*, and *Trichoderma* can degrade PAHs (Aydin et al. 2017). Toxic soil was collected from a diesel eroded site; naphthalene (a PAH) was chosen for hydrocarbon degradation studies. DNA barcode identified the involvement of *Fusarium oxysporum* in it (Romauld et al. 2019).

Bhatt et al. (2002) selected two white-rot fungi *I. lacteus* and *P. ostreatus* for inspecting the degradation of polycyclic aromatic hydrocarbons in contaminated industrial soils. Ecotoxicity assay had also been executed and it was observed that they degraded fluorene, anthracene, chrysene, and pyrene in contaminated places. *P. chrysosporium* was able to degrade fluorene in the soil (George and Neufield 1989). Vyas et al. (1994) said that anthracene was degraded up to 60% by white-rot fungi. They oxidized anthracene (AC) to anthraquinone (AQ). AQ was also found to degrade further in some selected cultures of white-rot fungi. Fungi implement monooxygenase enzyme-mediated polycyclic aromatic hydrocarbon degradation (Gupta and Pathak 2020). Mahajan et al. (2021) showed that PAH is xenobiotic. Studies were conducted in the Gulf of Kutch and the surrounding coastal areas of Gujarat. Fungal growth was observed in the collected samples. The isolates of *P. ilerdanum* and *Aspergillus* showed around 75% ability to degrade several PAHs. *Pycnoporus sanguineus* degraded roughly 70% of anthracene. Extracellular laccase and cytochrome P450 enzyme played role in this (Zhang et al. 2015). *P. eryngii* was studied for its efficiency in getting rid of manganese and phenanthrene. They removed them by more than 90% in a half-a-month span (Wu et al. 2016). Synthetic surfactant, tween 80, enhanced the mycoremediation process as compared to plant-based surfactant saponin. Marine fungi and *Marasmiellus* species displayed an increased level of pyrene and BaP degradation. It degraded pyrene completely without generating toxic compounds (Vieira et al. 2018). PAHs are present in the marine environment, but their degradation plans are not quite understood. Fungus improvised cytochrome P450 and epoxide hydrolases for toxin degradation. PAHs have low solubility in water and it can be enhanced by using surfactants to reduce the surface tension (Lamichhane et al. 2017).

Surfactants have hydrophobic and hydrophilic moieties. They fill the gap in the little information about surfactant enhanced remediation (SER) of PAHs. Tween 80 has already been preemptively widespread as a surfactant. In *T. versicolor*, PAH degradation was raised by tween 80 and it degraded fluorene, anthracene, phenanthrene, and pyrene (Rodríguez-Escales et al. 2013). SER is a noteworthy technique to treat the PAH in the soil and aquatic environment with the support of surfactants that are either synthetic or natural (biosurfactant). Agrawal et al. (2018) used *Ganoderma lucidum* for the biodegradation of phenanthrene and pyrene. They were able to degrade at least 90% of these with ligninolytic enzymes. The mechanism of PAH metabolism by non-ligninolytic fungi has been already studied in detail (Gupte et al. 2016). It involves the oxidation of aromatic rings by cytochrome P450 monooxygenase. Atmosphere receives a higher quantity of PAH by environmental lead (Pb) since lead is now ubiquitous. PAHs expand by the combustion of organic matter. They are cohesive to soil particles and get settled down by landfills and dumping activities of man. Exposure to them leads to asthma, respiratory diseases, deterioration of air quality, and chronic sickness when consumed via food.

12.5 Mycoremediation of Heavy Metals

Heavy metals are naturally occurring elements found throughout the earth's crust. Their high toxicity levels are due to several factors—their dose and path of exposure. Examples include lead (Pb), zinc (Zn), cadmium (Cd), chromium (Cr), mercury (Hg), and arsenic (As) (Tchounwou et al. 2012). *Pleurotus* species are universally adopted and high-yielding varieties employed in the mycoremediation process. They have biosorption capacity because of their vast hyphal biomass. Such fungi gather high levels of heavy metals. Biosorption is a process by which heavy metals get absorbed on the surface of the biosorbent (Velásquez and Dussan 2009). Procedures as chelation (extracellular) and binding to proteins (intracellular) lead to heavy metal remediation and endurance in fungi (Fawzy et al. 2017). These methods are for decontaminating polluted environments. Mushroom laccase and manganese peroxidase (MnP) enzymes degrade the lignocellulosic remnants and allow them to grow on agricultural wastes. Accumulation of heavy metal within the fruit body inclines to increase with a rise of the metal in the substrate (Ogbo and Okhuoya 2011). The residue after mushroom harvest is employed for mycoremediation in required contaminated sites. Akhta and Mannan (2020) reported that soil erosion and weathering of the earth's crust are some natural methods of heavy metal pollution. Anthropogenic activities result in industrial effluents, fertilizers, and pesticides that release tons of heavy metals. They enter the human body via the consumption of contaminated food and water. The accumulation of heavy metals in plants hinders their activities. The effects of some heavy metals on plants are as follow:

1. Obstruction in the electron transport system (ETS)
2. Decrease in water potential
3. Inhibition of antioxidative defense systems
4. Loss of essential metal ions and inhibition of plant growth

Ion exchange and electrochemical analysis methods are frequently done to dispose heavy metals, but they have their restrictions. Various fungi associated with the genus *Aspergillus* and *Fusarium* were isolated from land contaminated with arsenic. They could tolerate high concentrations of arsenic (As). Moreover, *T. reesei* and *Fomitopsis meliae* can handle copper, cadmium, arsenic, lead, and iron (Oladipo et al. 2018). They can also boost the soil quality. It is crucial to develop methods by which the remediating fungi are pulled out as they accumulate metal ions inside them. Heavy metal pollution renders negative consequences on soil and crop quality. *Galerina vittiformis* was functional in eradicating Cu, Pb, Cd, and Zn within a month in a district of Karnataka, India (Damodaran et al. 2013). *Marasmius oreades* can remove bismuth and titanium (Elekes and Busuioc 2010). Microorganisms have unfolded several mechanisms to change or reduce the toxicity of metallic contaminants through pH change, biosorption, or bioaccumulation. In bioaccumulation, enzymes are actively transported within and outside the cell and create vacuoles where metal ions are gathered and immobilized. Production of metal-binding compounds takes place. Khan et al. (2019) isolated specific fungal strains of *Aspergillus* from lead and mercury-contaminated industrial sites. *Aspergillus* (M and M7) strains could be used for in situ or ex situ remediation of lead and mercury-contaminated soil. de Wet et al. (2020) reported that *A. piperis* is an applicable candidate for bioremediation of lead, copper, manganese, and magnesium. He proved it through the agar well diffusion method. Pihurov et al. (2019) stated some challenges faced in incorporating fungi as bioremediators:

1. Difficulty in producing fungal inoculum in bulk amounts.
2. There should be accuracy in the application of inoculum for obtaining ample growth in soil.
3. Choice of a carrier material.
4. Piling up heavy metals in soil declines the produce.

Numerous fungi are microscopic in the soil and play a relevant role in recycling complex organic compounds and might tolerate heavy metal metalloids (Chan et al. 2016). A peculiar cell wall structure furnishes fungi with metal binding property (Gupta et al. 2000). Cell surface sorption of metals and metalloids is a repercussion of physicochemical interaction between the metal ions and the functional groups on cell surface proteins (Dhankhar and Hooda 2011). Heavy metals are exclusively collected within fungal cell walls in the form of precipitates such as oxalates, phosphates, and sulfates (Wei et al. 2013). Channeling of heavy metals to fungal cell walls can be assisted by siderophores. They enhance the mobility of heavy metals such as Cd, Cu, Zn, and Pb (Schalk et al. 2011). Antioxidant enzymes also play an extensive role in responses to metal vulnerability (Raab et al. 2004). These enzymes have been claimed to displace reactive oxygen species from fungi. They restore the impairment caused by reactive oxygen species (Bai et al. 2003). The synergy of biosynthesis of nanomaterials and the possibility of fungi to oust toxic metals from contaminated land have been suggested by many researchers. *Fusarium* species isolated from a Zn-contaminated mine in South Korea were able to absorb up to 320 mg/L of zinc and have the ability to produce zinc oxide nanoparticles (Velmurugan et al. 2010). *P. eryngii* can remove aluminum oxide nanoparticles up to around 90% (Jakubiak et al. 2014). Kumari et al. (2019) stated that *Cladosporium* species have very high absorption power and can easily absorb heavy metals such as lead, nickel, chromium, and arsenic. *Komagataella phaffii* mitigates contamination of cadmium, chromium, and lead (Liaquat et al. 2020). It had raised tolerance against increasing levels of these heavy metals. Biosorption is the binding and concentration of heavy metal from an aqueous solution by microbial biomass. Adsorption is subject to pH and dose (Prasad et al. 2013). Approximately 90% of lead (Pb^{2+})

was withdrawn by *T. viride* at a pH of 6 and 100% at pH 7 by *P. florida*. It is pertinent to get rid of lead from wastewater. Ligninolytic enzymes actively participating in their degradation were expressed in the presence of the substrate. A variety of investigations were performed using *P. chrysosporium*, which degrades a high number of organic pollutants (Pointing 2001). The existence of heavy metals in soil is catastrophic for agronomic businesses and consumers. The use of pharmaceutical compounds (PhC) has increased over time. They are resistive to degradation by light, water, and chemical compounds that produce them. They are hard to handle when liberated within the aquatic ecosystem. Solid-state fermentation, a biological procedure based on living microbes, has stimulated curiosity due to its minimal environmental damage. It presents several gains because it takes place under requirements for the expansion of filamentous fungi like white-rot fungi (Thomas et al. 2013).

12.6 Mycoremediation of Plastics

Polythene is the chiefly used plastic across the globe. Among the complete plastic waste produced, polythene contributes the maximum share of 65%. Synthetic plastic is nonbiodegradable. Polythene or polyethylene is a polymer of ethylene gas. Terrestrial animals unknowingly consume discarded plastic bags together with commercial waste, which is fatal for them. Polythene shows detrimental consequences on all the major kinds of biomes. A different plan of action is needed to cope with the ever-increasing rate of plastic waste. Use of bioremediation is an eco-friendly and long-lasting method for their degradation. Most effective polythene deteriorating fungal isolates are known to be of *Aspergillus* strains. Sangale et al. (2019) collected some samples to isolate polythene degrading fungi from coastal regions of India. It has been reported that the breakdown of polythenes was a crucial part of the degradation path followed by ligninolytic fungi. Primary degradation terminates in the genesis of methane, carbon dioxide gas, and water (Grover et al. 2015). Commercial fields have relied on plastic production, but major difficulties lie in modern times because of their imperishable nature, which is tricky to manage. Marine biology is adversely affected by plastic dumps. It is often problematic for all folks as microplastics are ingested by marine organisms, getting somewhere incorporated into the food chain phenomenon. Munir et al. (2017) made frequent attempts to downplay the aggregation of plastic waste within the environment over some decades. Microbial degradation of plastics has been holding much notice lately because of the pressure on developing countries to curtail plastic waste. Filamentous fungi like *Trichoderma* and *Aspergillus* have promising leads to the longer term of plastic degradation strategies. Mushrooms like *P. tuber-regium* grow swiftly on media having polythene powder by utilizing it as a carbon and energy source. FTIR analysis shows absorbance at regions near carbonyl groups suggesting that polythene was degraded oxidatively. Monocultures of *P. tuber-regium* and *P. pulmonaris* were used within the testing procedure. It may be due to their capability of higher production of extracellular enzymes that helped in utilizing the polyethylene powder (Nwogu et al. 2012).

Plastic disposal in a landfill may be a method applied to eliminate solid plastic waste. Steady piling up of such wastes in landfills is a concern as plastics take around thousands of years to decompose and therefore the land becomes inferior for residential projects. It also impedes water flow. White-rot fungi, *P. ostreatus*, could be a strong degrader of lignin and cellulose. Mycelial formulations are noted in the substrate with biodegradation. This was done by combining abiotic and biotic factors and exposing the plastic bags for 4 months in the sun and the other 4 months in fungal incubation (Luz et al. 2020). Raaman et al. (2012) have also reported the usefulness of *Aspergillus* species and the potential of their various strains in polythene degradation. Plastics have greater tensile force. Indiscriminate use of plastic bags by people has presented an alarming situation. It is the foremost report on the degradation of rarity polythene (LDPE) under laboratory conditions by *A. japonicus*.

Polyurethanes aid in environmental issues because of belonging to a very repellent polymer family. Their degradation may be a matter of question for sustainable waste management strategies. They need a negative impact on terrestrial and water bodies. Fungi are more effective in their degradation as compared to bacteria. Magnin et al. (2018) isolated 30 strains of fungi from polyurethane wastes. Some species of *Alternaria* and *Penicillium* utilized polyurethanes as a carbon source and these species were found to be helpful. Polyurethane treatment may be a challenge within the current scenario. *Penicillium* species proved to be more important in handling plastic pollution. An enormous number of health care institutes and hospitals are producing enormous biomedical waste. Biomedical waste generation by hospitals may be a huge problem as its management requires power expenditure and warm temperature treatments. Most health care centers install incinerators for quick treatment and management of biomedical waste generated in their respective hospitals. Incineration methods unleash smoke and ash into the environment resulting in system and cancer issues. These procedures are extravagant, liberating toxic compounds such as dioxins, furans, and ash. Coprophilic fungi, *Periconiella* species (found in cow dung), were employed for the degradation of medical wastes. It is a cheap and less demanding method for biomedical and plastic waste degradation. The biomedical scrap was smeared on the pure culture of the fungal species. The degradation started on the fourth day. It can degrade plastic from the ninth day without polluting the environment (Deshkar et al. 2019).

12.7 Mycoremediation of Dyes and Agricultural Contaminants

P. chrysosporium was the primarily described fungus to be ready to degrade synthetic dyes (Ruiz et al. 2018). Turquoise blue dye was learned as a pollutant and the efficiency of *P. ostreatus* was evaluated for its degradation. Detoxification of malachite green and fuchsin was observed by *A. niger* and *P. chrysosporium* (Rani et al. 2014). Different chemical methods are used for the remediation of colored effluents, especially in textile industries. The key advantage of those fungi in dye degradation relies on the oxygenation of the fungus and gradual contact of released enzymes with the molecules. Biodegradation occurs due to disruption of the chromophore in dye molecules because of extracellular enzyme formation by fungi. Rodríguez et al. (1999) purified laccase enzyme from *T. hispida* and watched its decolorization act. Lignin and manganese peroxide enzymes were found to play the part. Distinct industrial dyes were decolorized biocatalytically by extracellular enzymes from different strains of white-rot fungi (Table 12.1).

Textile dye effluents discarded into the aquatic habitats increase the biological oxygen demand. The requirement of an enormous amount of water in cloth manufacturing units is deleterious as the wastewater gets enriched chemically. Poor handling of dyes ends up in contaminated habitats as plenty of harmful metals are present in them (Singh 2017). Kuhar et al. (2015) illustrated malachite green combined and solo degradation by *G. lucidum* and *T. versicolor*. Jimenez et al. (2018) estimated the performance of fungal segments in dye degradation. *P. pulmonaris*, *P. ostreatus*, *T. versicolor*, and plenty of other white-rot fungi were tested. Usage of fungal consortia has not caused a synergy between the species to amend the bioremediation of dyes. It can be discovered through the race for space or nutrients between selected species in solid-state fermentation (SSF) that microorganisms need a definite area to grow that starts to be restricted when one species uses the identical substrate for growth. *T. reesei* isolates metabolized benzo alpha pyrene with glucose as a co-metabolite substrate in a PAH-contaminated soil (Yao et al. 2015). Nonidentical strains of *Aspergillus* and *Fusarium* were isolated

Table 12.1 Decolorization of a few dyes using White-Rot Fungi

WRF	Dye	References
Cladosporium cladosporoides	Synthetic dyes	Nilsson et al. (2006)
	Azo dyes and triphenylmethane dyes	Vijaykumar et al. (2006)
Phanerochaete chrysosporium	Azo dyes [Red-80 and Mordant Blue-9]	Singh and Pakshirajan (2010)
Coriolus versicolor	Textile effluents	Asgher et al. (2009)
Pleurotus ostreatus	Remazol Brilliant Blue Royal	Erkurt et al. (2007)
Pleurotus ostreatus	Acid Orange 7, Acid Orange 8, Mordant violet 5	Lu et al. (2008)
Pleurotus ostreatus	Direct Red 80	Singh et al. (2009)
Pleurotus ostreatus and *Stereum ostrea*	Triphenylmethane Dye	Usha et al. (2020)
Cordyceps militaris	Reactive Green 19, Reactive Yellow 18, Reactive Red 31, Reactive Red 74	Kaur et al. (2015)
Pleurotus pulmonaris	Malachite Green, Brilliant Blue, Phenol Red Coomassie, Victoria Blue B	Sanga et al. (2018)

from soil contaminated with arsenic and reduced arsenic under in situ conditions (Singh et al. 2015).

The chemical and physical properties of soil such as pH, temperature, and water availability add adequately to the microbial domain in the soil besides the success of the bioremediation process. Filamentous fungi can cause the degradation of pharmaceutical compounds at a relatively faster rate than bacteria (Agunbiade and Moodle 2014). Accessibility to the contaminants hastens the process. Many fungi generate biosurfactants that assist in the alteration of contaminants (Prakash 2017). Additionally, litter-degrading fungi too help out in breaking down soil organic matter. Lindane is an imperishable chlorinated insecticide. It has been employed in the agriculture field for pest control prior to being banned. It lasts long within the environment and gathers in fat tissues due to the greater dissolving power in lipids (Zucchini-Pascal et al. 2009). The efficiency of white-rot fungi to degrade the insecticide lindane (a chlorinated insecticide) was observed in *T. versicolor* and *Pleurotus* species (Ulčnik et al. 2012).

12.8 Advances in Mycoremediation Technology

Evolutionary biology techniques like transcriptomics, proteomics, and metagenomics have ushered in better environmental management plans (Plewniak et al. 2018). Multi-omics studies conducted as a single omics program cannot reveal all the functional and physiological activities of any community (Meena et al. 2018). Metagenomics analysis begins with the nucleic acid separation of the collected samples. Transcriptomics enlightens us about the regulation of gene mRNA expression. Microarray and sequencing techniques are engaged in this tool. Microarray throws light on gene expression. DNA microarray is a widely used technique in transcriptomics. It gives an insight into every gene that constitutes a living being and its mRNA expression (Maroli et al. 2018). Proteomics is the set of proteins found inside an organism that is known as the proteome. It includes posttranslational modifications and metabolism paths inside the cell. It has made possible the discovery of proteins present in microorganisms at contaminated sites. Community-level proteome examination is known as metaproteomics. Community proteome extraction involves the following:

- Cell fractionation
- Protein extraction via ultrasonication
- Generation of protein pool
- Protein identification

Protein obtained is identified by gel electrophoresis methods or by enzymatic digestion followed by mass spectra analysis using modern technical analysis such as LC-MS/MS. The ultimate steps are protein identification and data interpretation (Chandran et al. 2020). Metabolomics is the study of metabolites account of a cell

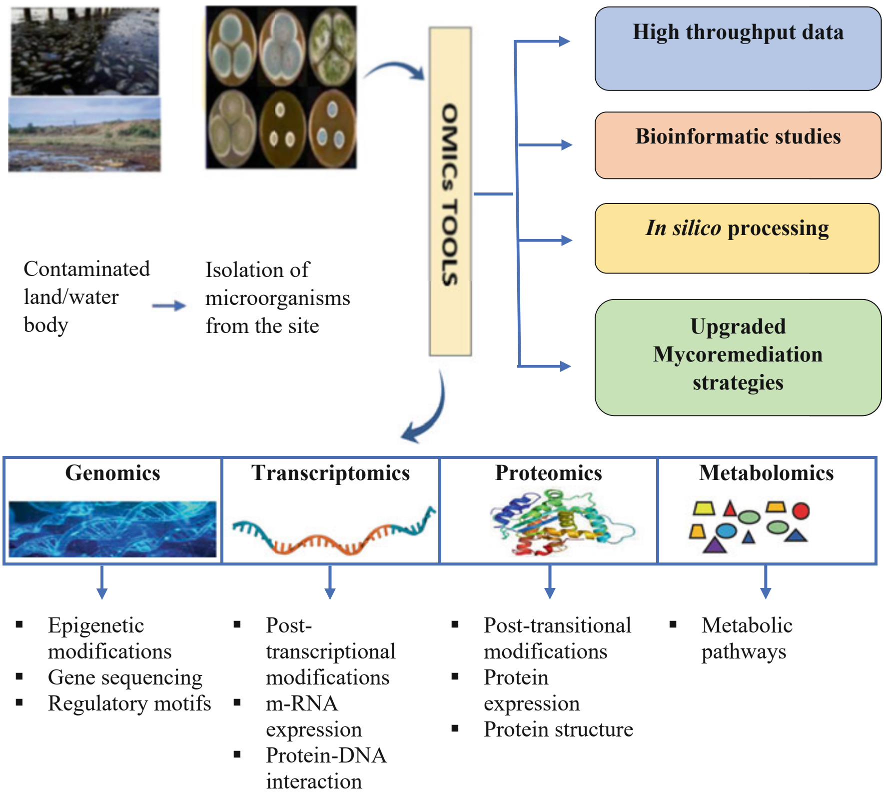

Fig. 12.2 Illustrating modern omics approaches

(Fig. 12.2). Under stress conditions, cells of living beings secrete various metabolites to cope up.

The metabolomics approach analyses the effect of environmental conditions on metabolites (Malla et al. 2018). It may help understand the primary and secondary metabolites emitted by microorganisms when subjected to contaminated areas and check their stress response. The discovery of a conserved and varying gene sequence, 16S rRNA, has reformed molecular biology. Phylogenic and analogical experiments are conducted to differentiate closely related microorganisms. Further, it is being incorporated to study the microbial diversity isolated from contaminated sites (Lovely 2003). Two mainstream advances in remediation technology are biostimulation and bioaugmentation methods. Bioaugmentation is the inclusion of favorable microbes. The fungal cell wall is rich in chitin, a polymer of *N*-acetyl D-glucosamine that intensifies metal intake. Supplemented microorganisms can metabolize a particular pollutant when added to the soil. In fungal augmentation, superior inocula are developed. In biostimulation, nutrients, electron acceptors, or donors are injected into the contaminated areas to arouse the degrading property of microbes.

The shortcoming of biostimulation is that if the toxicity levels within the soil are high, the indigenous microbes might not be sufficient. In bioaugmentation, there is a possibility that the induced microbial population might not be able to survive in the new environment.

12.9 Conclusion and Prospects

Integrated practice is a combination of two different methods to achieve a collaborative and constructive plan to treat noxious compounds. The positive link among the environmental components serves as the basis for all living organisms but leaps in science and technology have contributed to the pollution of resources. Lesser knowledge on proper disposal of effluents and negligence to implement strict regulatory standards by policymakers have added to the worsening of resources. The physiochemical treatment methods exercised to rectify contaminated sites are flawed when it comes to larger-scale implementation. It will be solved by integrated processes that are of a fixed duration, nonpolluting, and achievable. The endemic fungi growing in polluted habitats should be investigated for futuristic studies as they are accustomed to the higher concentration of pollutants, which will help in more organic technology buildup. It is not very easy to completely get rid of contamination but synergistic approaches have such potential. The application of ligninolytic fungi and their enzymes could be beneficial. Recombinant DNA technology can be applied to upgrade the genetic material of fungi for their use in mycoremediation. Establishment of Genetic microbial tools to decay pollutants needs to be analyzed. Genetically modified cultures have a rich effect on the ecosystem. Some pollutants are mild but catastrophic. The biosorption mechanism also requires future research. Additional studies should be done to address the gap in the methods employed by fungal communities in the bioremediation process. Interdisciplinary methods are needed to focus on either the regeneration of biomass or the retrieved metal being converted to a fruitful form.

Omics approach carries the prospective to assume the metabolism of fungi in polluted areas. They bring a new vision to record mechanisms included in polycyclic aromatic hydrocarbons, heavy metal, and chlorinated compounds remediation at the species or community level. Bioinformatic tools together with metabolomics have empowered a thorough perception of mechanisms operating inside microbial communities and the genes accountable. Metabolite detection is done by combining bioinformatics software and analytical methods. Progress in nanotechnology might help in studying the role of membrane-associated oxidoreductases in alleviation by white-rot fungi. The fungal biomass eliminated from fermentation processes is used in the mycoremediation process. Regardless of being new, there are a lot of possibilities for research in this field. We are capable enough to discover new dimensions. We only need a proper endorsement, management skills, implementation strategies, and sharp resources!

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Exploration of Plant Growth-Promoting Rhizobacteria (PGPR) for Improving Productivity and Soil Fertility Under Sustainable Agricultural Practices

13

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Abstract

Under green revolution practices, the imbalanced use of chemical fertilizers and pesticides causes a negative impact on soil health due to the loss of soil microbial flora and fauna. To overcome this negative impact of the green revolution and to increase sustainable agricultural production without damaging further agricultural lands, the only alternative and effective means is to reduce the use of chemicals in agriculture specifically for plant nutrition and plant protection. Under sustainable agricultural practices, plant growth-promoting rhizobacteria (PGPR) can be effective tools to increase productivity while ensuring sustainability in agriculture. PGPR colonize the rhizosphere zone and help in promoting plant growth and development by regulating nutrient acquisition, modulation of plant hormones, and ameliorating various negative effects of various pathogens. PGPR also help sustain the plant growth productivity and significantly increase soil fertility and health under different biotic and abiotic stresses. As per the literature, many studies prove to increase agriculture productivity due to the use of PGPR as eco-friendly microbial inoculants for promoting plant growth attaributes through various direct and indirect mechanisms. The mechanisms of PGPR include biological nitrogen fixation, phytohormones production, Phosphate, potassium, and zinc solubilization, siderophores production, and secretion of other secondary metabolites (phenolic compounds (phenylpropanoids and flavonoids)) that enhance crop productivity and control phytopathogens. Therefore, this chapter focuses on a detailed description of PGPR keeping in view their functional mechanisms as eco-friendly approaches to increase productivity and enhance

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soil fertility. PGPR can be used as an eco-friendly, socially acceptable, and cost-effective technology for challenges in the future.

13.1 Introduction

Microorganisms inhabiting almost every part of the biosphere support the life system on the planet Earth. At present, there is limited knowledge about the microbial world of the Earth's crust and its members that are present in the environments such as soil, water, and air. These microorganisms can live either in an individual plant or in specific plant organs (e.g., roots, shoots, leaves, seeds, nodules, sprouts of legumes, flowers, and fruits). Other than that, the microorganisms reside in a narrow zone of soil, influenced by plant roots, and are associated with roots and the rhizosphere (Rout and Southworth 2013; Chouhan et al. 2021c; Mukherjee et al. 2019, 2020a, b). The plant-associated microbes migrate from bulk soil to the rhizosphere region of plants (Kloepper 1978). These rhizosphere bacteria act as symbionts for plants and considered plant growth-promoting rhizobacteria (PGPR) (Kapulnik 1991). Plants secrete flavonoids such as amino acids and sugars that provide a rich source of energy and nutrients for the microorganisms, which results in higher bacterial population in the rhizosphere region as compared to the non-rhizosphere region. A variety of bacterial genera are present in this region, most commonly species of *Pseudomonas, Arthrobacter*, *Bacillus*, *Erwinia*, *Flavobacterium*, *Burkholderia*, *Caulobacter*, *Serratia*, *Hyphomicrobium*, *Micrococcus*, *Agrobacterium*, and free-living nitrogen-fixing bacteria (*Azotobacter* and *Azospirillum*) (Foster et al. 1983; Prithiviraj et al. 2003; Gray and Smith 2005; Mukherjee et al. 2020a, b). Rhizobacteria represent an important group of microorganisms and show more intimate associations with plant roots referred to as endophytes (Hardoim et al. 2015). Most members of bacterial taxa have been isolated from the endorhizospheric region, comprising endophytic species (Hallmann and Berg 2006). The bacterial taxa present within this region include species of *Pseudomonas*, *Enterobacter*, *Bacillus*, *Burkholderia*, and *Azospirillum*. There are a number of factors involved in the establishment of the rhizosphere and endophytic microbiome (Lakshmanan et al. 2014). It depends upon soil and host type (Bulgarelli et al. 2012; Lundberg et al. 2012). There are, however, other factors such as biotic and abiotic circumstances, climatic conditions, and anthropogenic effects that also play important roles in microbial population dynamics in specific plant species and soil types (Lakshmanan et al. 2014).

13.2 Rhizosphere

The term rhizosphere was first coined by Lorenz Hiltner (Hiltner 1904). The rhizosphere is the narrow area around the root surface that supports highly diversified microbial activity (Fageria and Stone 2006; Lakshmanan et al. 2014).

Based on the microbial colonization, the rhizosphere area can be categorized into three zones, namely, rhizosphere, rhizoplane, and endorhizosphere. The rhizosphere microbiomes are influenced by the quantity and quality of root exudates. The rhizoplane is the root surface strongly associated with soil particles. The endorhizosphere is the interior part of the root that is inhabited by some specific type of microbes called endophytes (Barea et al. 2005). Highly diversified microorganisms that compete with each other to occupy space and food (Raaijmakers et al. 2002) colonize the rhizosphere zone. Therefore, these microorganisms could be beneficial or pathogenic to the associated plant. Mutualistic symbionts of the rhizosphere microbiome colonize the plant tissue and obtain nutrition and in return they provide factors for the enhancement of growth and development of the host plant (Haas and Keel 2003). Different kinds of interactions take place in the rhizosphere that can occur between plant–plant, microbe–microbe, plant–microbe, and interaction with other eukaryotic microbes (De-la-Peña et al. 2012). To reveal the complex interactions between these microorganisms, there is a need for the development of an understanding of the chemical communication between plants and their rhizosphere microbes as to how they coordinate their behavior and interact with each other. A number of studies have been carried out on molecules and the mechanisms of microorganisms that addressed their coordination in the rhizosphere with the plant growth and productivity (Pieterse et al. 2009; Miller and Oldroyd 2012; Morel and Castro-Sowinski 2013; Rosier et al. 2018). Microorganisms associated with rhizosphere function as plant growth-promoting rhizobacteria (El-Tarabily et al. 2008; Merzaeva and Shirokikh 2010), as anti-phytopathogenic through their activities such as protecting the plant from disease (Kloepper et al. 2004) and making nutrients available to the plant (Pradhan and Sukla 2005; Martínez-Hidalgo et al. 2014). Despite many reports on rhizosphere microbes, communication mechanisms and interactions between different taxa and different communities are largely unknown.

13.3 Plant Growth-Promoting Rhizobacteria (PGPR)

Plant growth-promoting rhizobacteria are the microbes that aid plants in their growth through direct and indirect mechanisms such as nitrogen fixation, production of phytohormones, and various other secondary metabolites as attributes of biocontrol agents (Mukherjee et al. 2020a, b, 2021a, b, c). Rhizobacteria affect plant growth and metabolism in multiple ways, such as regulating the nutrient acquisition, modulation of phytohormones, and ameliorating negative effects of various pathogens and also different abiotic stresses (Fahad et al. 2015; Chouhan et al. 2021a). PGPR have also been shown to help in the remediation of the environment through detoxification of various heavy metals, such as arsenic and cadmium, and also pesticides, such as monocrotophos and chlorpyrifos. The process of enhancement of plant growth and development by the PGPR is achieved through the modulation of plant and soil chemistry, which is aided by the regulation of various hormones and nutrients (Nadeem et al. 2014). PGPRs are also known to ameliorate the negative effects of various abiotic stresses such as salinity stress and heavy metal stress through the production of different secondary metabolites.

13.3.1 Functional Attributes of PGPR for Sustainable Agriculture

The functional properties of PGPR are of two types, that is, direct and indirect mechanisms. The direct mechanism includes biological nitrogen fixation, solubilization of phosphate, zinc, potassium, and other minerals, and production of phytohormones, ammonia, and siderophore (Fig. 13.1) (Mukherjee et al. 2020a, b). The indirect mechanisms are the production of hydrogen cyanide (HCN), chitinase, antibiotics, and various other secondary metabolites produced for biocontrol of disease-causing agents (Mukherjee et al. 2020a, b). These functional properties are very useful for enhancing plant growth attributes and soil fertility, and health contributing to sustainable agricultural production. The production of various secondary metabolites helps in the amelioration of the different abiotic stresses such as salinity stress and drought stress (Sunita et al. 2020; Chouhan et al. 2021b). The nitrogen-fixing rhizobacteria present around the roots of the leguminous plants cause the formation of root nodules to help in fixing atmospheric nitrogen and making nitrates available to the plants. The production of ammonia is an important aspect of PGPR to support nitrogen nutrition and thereby increase crop productivity. The production of HCN by microbes protects plants from soilborne phytopathogens (Hayat et al. 2010). The PGPRs also enhance nutrient acquisition

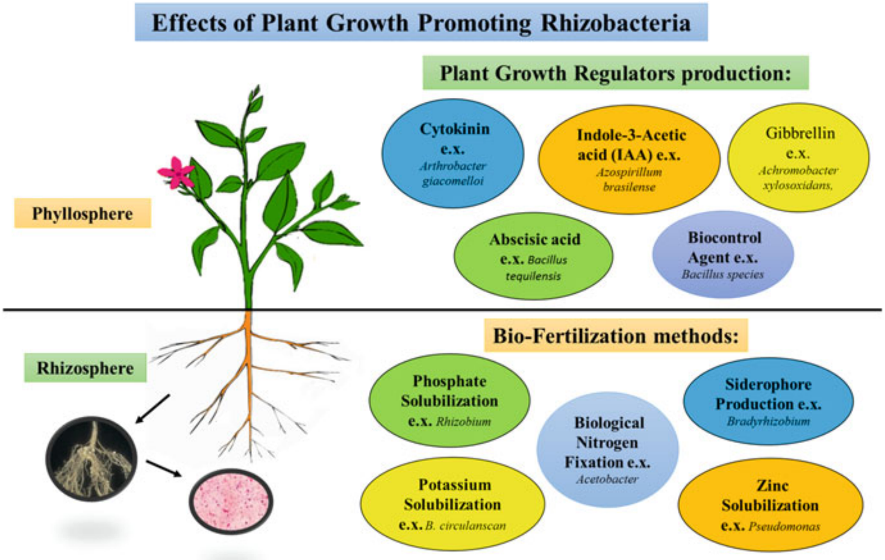

Fig. 13.1 Mechanisms of plant growth-promoting rhizobacteria for sustainable agriculture

and can be used as nutrient solubilizers. Microbial species such as *Azotobacter*, *Pseudomonas*, *Bacillus*, *Burkholderia*, *Enterobacter*, and *Azospirillum* are well-reported PGPR strains known to promote plant growth effectively through increased uptake of nutrients (Mukherjee et al. 2020a, b, 2021a, b, c; Rasool et al. 2021). Other functional attributes of the PGPRs can be the minimization of the deleterious effects posed by pathogens and stress conditions and enhancing plant growth simultaneously (Safdarian et al. 2020).

13.3.1.1 Biofertilizer

Indiscriminate use of chemical fertilizers and pesticides in agricultural production has disturbed various ecosystems. Synthetic chemicals contaminate the environment, increase soil salinity, and also decrease the nutritional quality of food products. The increased chemical contents in soil indirectly lead to an increase in greenhouse gas emissions, thereby increasing climate change concerns. They make it more vulnerable to plant pathogens by weakening the root system. The symbiotic association of the plants and microbes plays a significant role in the biofertilizer industry. It can help boost agricultural production with its management in a sustainable way. Nitrogen from the atmosphere is converted to ammonium and nitrate and made available to the plants. Microbes such as *Rhizobium*, *Azorhizobium*, and *Sinorhizobium* act as biofertilizers (Prasad et al. 2019). Recently, a large number of biofertilizers have been used to harness the symbiotic relationship between microbes and their host plants in boosting crop production under sustainable agriculture (Kumar et al. 2009). However, detailed studies are required under adverse biotic and abiotic conditions such as drought, salinity, high soil temperatures, adverse soil pH, and presence of organic acids to understand the mechanism and also enhance fertility and crop production through biofertilization process. Biofertilizer is a microbial culture developed in solid and liquid bioformulation for boosting crop productivity. This can be used for multiple plant growth-promoting agents that directly and indirectly enhance plant growth attributes and productivity. Liquid biofertilizer is more effective than solid biofertilizers due to more cell viability and effectiveness. Biofertilizers are used as seed, soil, and root dip treatments for enhancing agricultural productivity in a truly sustainable way.

13.4 Mechanisms of PGPR

13.4.1 Biological Nitrogen Fixation (BNF)

Nitrogen is a crucial element present in many compounds of the plant playing a vital role in plant growth, which can be fixed by both *Rhizobium* and some free-living bacteria in soils and plants. Nitrogen fixation is a process through which microbes can fix atmospheric N_2 and provide it to plants for their use. The nitrogen fixation process is catalyzed by specific symbiotic, nonsymbiotic, or endophytic microbes. More than 80% of nitrogen is present in the atmosphere but is unavailable to plants. Only some microbes are able to convert gaseous nitrogen to ammonia and nitrates and make it available to the host plant (Franche et al. 2009). A diazotrophic enzyme, nitrogenase causes the conversion of atmospheric nitrogen to ammonia. These microbes show complex interactions with leguminous and nonleguminous plants to form a nodule. Among diazotrophs, rhizobia-legume symbiosis is the most studied in agriculture systems (Fenchel et al. 2012). In addition to symbiotic bacteria, many bacteria present in the rhizosphere have the ability to fix atmospheric (e.g., *Bacillus*, *Acetobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Corynebacterium*, *Beijerinckia*, *Clostridium*, *Derxia*, *Enterobacter*, *Klebsiella*, *Rhodospirillum*, *Xanthobacter*, *Pseudomonas*, and *Rhodopseudomonas*) (Latt et al. 2018) (Table 13.1).

13.4.2 Phosphate Solubilization

Phosphorus is the second most important element for plant growth, which is required by the plant in higher quantities. Phosphorus is necessary for various processes including DNA and RNA synthesis. Most of the phosphorus is found in an insoluble state in the soil, being not readily available to plants. Plants obtain phosphorus in the soluble form such as HPO_4^{-2} and $H_2PO_4^{-1}$, which depends on the pH of the soil (Rodríguez and Fraga 1999; Richardson 2001). To fulfill the deficiency of phosphorus, chemical fertilizers are used in soil but a great amount of phosphorus is again converted into less available forms by the process of precipitation with other elements such as Al, Fe, and Ca. The precipitation of phosphorus depends on the pH of the soil (Stevenson and Cole 1999; Richardson 2001). Approximate 40% of phosphorus can be solubilized by bacterial culture (Richardson 2001). Many microbes can solubilize the phosphorus in a simpler form and make it available to the plant and hence are called phosphate solubilizing microbes (Rodríguez and Fraga 1999). A diverse number of microbes have been reported that solubilize phosphorus. They include species of *Pseudomonas*, *Bacillus*, *Rhizobium*, *Burkholderia*, *Achromobacter*, *Agrobacterium*, *Micrococcus*, *Aereobacter*, *Flavobacterium*, and *Erwinia* (Bardiya et al. 1974; Chen et al. 2006; De Freitas, et al. 1997; Dey 1988; Richardson 2001; Rodríguez and Fraga 1999; Verma et al. 2010, 2017; Mukherjee et al. 2020a, b). Phosphorus is a limiting factor for plant growth mainly for legume plants (Raman and Selvaraj 2006). However, the mechanism of phosphorus uptake by PGPM and induced plant growth is not known (Mukerji et al. 2006). PSB may decrease the pH of the soil by producing many organic acids such as citrate, lactate, and succinate. Some species of rhizobacteria have been investigated for their ability to convert glucose to gluconic acid by their membrane-bound enzymes and ultimately into 2-keto-gluconic acid (Mukerji et al. 2006; Verma et al. 2017). However, the organic acid secreted by plants presumably has the capacity to solubilize P than the acid secreted by rhizobacteria (Jones 1998). A variety of organic substrates are reported that can be a major source of P for plant growth. It can be possible when the organic substrate is converted to inorganic P with the help of acid and alkaline phosphatase enzymes. Since soil pH ranges from acidic to neutral value, in this environment acid phosphatases may work efficiently (Rodríguez and Fraga 1999).

Table 13.1 Plant growth-promoting rhizobacteria, its mode of action, and possible outcome

Plant growth-promoting bacteria	Plant tested	Activity	Mode of action	Reference
Bacillus strains	*Solanum nigrum* L.	PGPR-assisted phytoremediation	Inoculation with QX8 and QX13 also enhanced the dry weight of shoots (1.36- and 1.7-fold, respectively) and roots (1.42- and 1.96-fold, respectively) of plants growing in cd- and Pb-contaminated soil and significantly increased total Cd (1.28- to 1.81-fold) and Pb (1.08- to 1.55-fold) content in aerial organs compared to non-inoculated controls	He et al. (2020)
Brevibacterium frigoritolerans (AIS-3), *Alcaligenes faecalis* subsp. *phenolicus* (AIS-8) and *B. aryabhattai* (AIS-10)	*Crocus sativus* L.	Biocontrol activity—*Sclerotium rolfsii* and *Fusarium oxysporum*	The PGPR enhanced plant growth through the production of plant hormones and also checked the growth of phytopathogens	Rasool et al. (2021)
Arthrobacter aurescens, *B. atrophaeus*, *Enterobacter asburiae*, and *Pseudomonas fluorescens*	*Triticum aestivum* L.	Promote growth, regulate Na+/K+ balance, and decrease ethylene emissions in plants	Upregulation of H^+-PPase, HKT1, NHX7, CAT, and APX expression in roots	Safdarian et al. (2020)
Bacterial isolates	*Mucuna pruriens* L.	Mitigation of drought stress	Lower root ACC content (−45%) in water-stressed inoculated plants, maintain higher levels of both isoprene emission, and carbon assimilation	Brunetti et al. (2021)
B. cereus, *Serratia marcescens*, and *P. aeruginosa*	*T. aestivum*	Mitigation of salinity stress	Indole-3-acetic acid (IAA) and hydrogen cyanide (HCN) productions, N2-fixation, and P solubilization enhanced.	Desoky et al. (2020)
Alcaligenesfaecalis	*T. aestivum*	Mitigation of salinity stress	Maintained ionic imbalance by regulating Na^+ and K^+ ions and significantly increased growth parameters and plant biomass by	Babar et al. (2021)

(continued)

Table 13.1 (continued)

Plant growth-promoting bacteria	Plant tested	Activity	Mode of action	Reference
			decreasing reactive oxygen species induced lipid peroxidation while increased accumulation of osmolyte, photosynthetic pigments and improved photosystem II efficiency as compared to uninoculated plants	
Enterobacter sp. C1D, *Pseudomonas* sp. G22, *Rhizobium* sp. IC3109	*Cajanus cajan*	Plant growth promotion in an intercropping system	Cocultivation resulted in a decrease of the exudation of secondary metabolites	Vora et al. (2021)
P. Alcaliphila and *P. hunanensis*	*Phaseolus vulgaris* L.	Plant growth promotion	Nitrogen fixation, solubilization of inorganic phosphate, and production of phytohormones, ammonia, and acetoin	AlAli et al. (2021)
P. Plecoglossicida, *Acinetobacter calcoaceticus*, *B. flexus,* and *B. safensis*	*Bacopa monnieri* (L.)	Mitigation of salinity stress and plant growth promotion	Increase in shoot Na^+:K^+ ratio, shoot and root biomass (fresh and dry weights), soil enzymes, and soil nutrient parameters showed significant positive correlations with the shoot Na^+:K^+ ratio	Pankaj et al. (2020)
Flavobacterium sp.	*Oryza sativa*	Mitigation of salinity stress and plant growth promotion	Increased plant hormone production and maintenance of Na^+:K^+ ratio	Menon et al. (2020)
P. putida NBRIRA	*Chickpea* (*Cicer arietinum* L.)	Improvement in drought stress tolerance and enhancement in chickpea	Modulation of membrane integrity, osmolyte accumulation (proline, glycine betaine), and scavenging of ROS. Differential expression of genes involved in ethylene biosynthesis (ACO and ACS), salicylic acid (PR1), jasmonate (MYC2) transcription activation, SOD, CAT, APX, and GST (code for antioxidant enzymes), DREB1A (dehydration responsive	Tiwari et al. (2016)

			element binding), NAC1 (transcription factors expressed under abiotic stress), LEA, and DHN (dehydrins), which was the result of positive modulation of stress by bacteria	
Rhizobium sp.	Pea *(Pisum sativum)*	Plant growth promotion	IAA, siderophores, HCN, ammonia, EPS	Ahemad and Khan (2012a)
Bacillus spp.	Corn (*Zea mays* L.)	Plant growth and nutrient enhancement	Facilitates nutrient acquisition such as nitrogen, phosphorus, and iron essential for plant development	Calvo et al. (2017)
B. methylotrophicus M4–96	*Arabidopsis thaliana*	Biostimulation of plant growth and development	IAA production, production of bacterial volatile compounds such as four classes of compounds, including 10 ketones, 8 alcohols, 1 aldehyde, and 2 hydrocarbons	Pérez et al. (2017)
P. fluorescens strains	*Medicago truncatula*	Biocontrol and plant growth promoting activities	Production of antifungal volatile compounds such as methanethiol, dimethyl sulfide, DMDS, and dimethyl trisulfide. Plant growth promoting and biocontrol traits such as siderophore production, protease activity, production of indole-3-acetic acid (IAA). Production of phenazines (gene-*phzCD*), DAPG (gene-*phlD*), hydrogen cyanide (gene-*hcnAB*), and ACC deaminase (gene-*acdS*)	Hernández et al. (2015)
P. putida Rs-198	Cotton	Plant protection and promotion against salt stress	Improvement in production of endogenous indole acetic acid (IAA) content while reduction in abscisic acid (ABA) content under salt stress in cotton	Yao et al. (2010)

(continued)

Table 13.1 (continued)

Plant growth-promoting bacteria	Plant tested	Activity	Mode of action	Reference
			seedling increase in absorption of the Mg^{2+}, K^+, and Ca^{2+} and decrease uptake of Na^+ from the soil	
B. amyloliquefaciens BChi1 and *Paraburkholderia fungorum* BRRh-4	Strawberry	Improvement in growth, yield, and content of antioxidants	Total anthocyanins, phenolics, flavonoids, carotenoids, and antioxidant activities in fresh strawberry fruits were increased	Rehman et al. (2018)
B. pumilus str. DH-11 and *B. firmus str. 40*	*S. tuberosum*	Plant growth promotion and abiotic stress tolerance	It showed enhanced 1-aminocyclopropane-1-carboxylic acid deaminase activity, phosphate solubilization, and siderophore production. Abiotic conditions tiggered enhanced mRNA expression levels of the various ROS-scavenging enzymes and higher proline content in tubers induced by PGPR-treated plants, thus increasing plant abiotic stress tolerance. There was a positive influence of bacterial strain on the PSII phytochemistry of plant, which was confirmed by photosynthetic performance indices	Gururani et al. (2013)
Bradyrhizobium sp. *MRM6* strain	Greengram	Abiotic stress tolerance and plant protection	Synthesizing of plant growth-promoting substances such as IAA, siderophores, EPS, HCN, and ammonia	Ahemad and Khan (2012b)

P. fluorescens	(*Cuminum cyminum* L.) (Cumin)	Biocontrol and plant protection	Production of pathogenesis-related enzymes: Chitinase, β1, 3 glucanase, and protease	Rathore et al. (2020)
Bradyrhizobium sp., *Pseudomonas* sp. and *Ochrobactrumcytisi*	*Lupinus luteus*	Heavy metal tolerance and plant growth promotion	Nitrogen fixation, improvement in plant biomass, and decrease in accumulation of heavy metals in roots and shoots as a bioprotective effect	Dary et al. (2010)

All these reports provide important information about the role of bacterial microbes in making phosphate available to the plant. Therefore, the use of these microbes as a bioinoculant to enhance agricultural production is of great interest to agricultural scientists.

13.4.3 Potassium Solubilization

Potassium (K) is available in four forms in the soil and the process of solubilization is mainly done by a wide range of saprophytic bacteria, fungi, and actinomycetes strains (Bakhshandeh et al. 2017). Many studies show that soil bacteria can transform the insoluble potassium into a soluble form that is easily available to the plant (Mukherjee et al. 2019; Meena et al. 2016). There is a considerable amount of potassium solubilizing (KS) aerobic and anaerobic bacteria in soil and mostly KSB are, however, aerobic. The comparatively higher concentration of KSB is present in the rhizospheric region as compared to the non-rhizospheric region (Padma and Sukumar 2015). A number of KSB such as *B. mucilaginosus*, *B. circulanscan*, *B. edaphicus*, *Burkholderia*, *A. ferrooxidans*, *Arthrobacter* sp., *E. hormaechei*, *Paenibacillus mucilaginosus*, *P. frequentans*, *Cladosporium*, *Aminobacter*, *Sphingomonas*, *Burkholderia*, and *P. glucanolyticus* have been reported that can solubilize the silicate rocks (Meena et al. 2016). Among KSB in the soil, some potential bacteria such as *B. mucilaginosus*, *B. edaphicus*, and *B. circulanscan* have been reported to possess very effective potassium solubilizing abilities (Meena et al. 2015, 2016). These potassium solubilizing microbes can be isolated from different regions of rhizosphere and non-rhizosphere soil, paddy soil (Bakhshandeh et al. 2017), and saline soil (Bhattacharya et al. 2016).

13.4.4 Siderophore Production (Iron Chelation)

Iron (Fe) is the core element for life on earth including plants for their growth and development. Iron acts as a cofactor of many enzymes with redox activity that catalyzes a number of biochemical reactions, which is essential for the growth of nearly all organisms (Sharma et al. 2003; Crowley 2006). For N_2 fixation in the leguminous root by the *Rhizobium*, Fe protein is required for the nitrogenase enzyme (Graham 2005). Siderophore is a low-molecular-weight Fe-chelating compound produced by some microbes in low availability of iron for the plant in soil (Crowley 2006). Many bacteria (Ali and Vidhale 2013) and fungi (Kümmerli et al. 2014; Renshaw et al. 2002) under iron-restricted conditions produce siderophores by producing iron-chelating molecules, and graminaceous plants (Hider and Kong 2010). Diverse microbes are able to synthesize siderophore in the rhizosphere and approximately 500 siderophore structures have been identified (Crowley 2006). Some bacterial genera *Bradyrhizobium*, *Rhizobium*, *Serratia*, and *Streptomyces* are known to synthesize siderophore in rhizospheric soil. Some plant-associated microbes can also have the capability to form siderophores (Sessitsch et al. 2004).

The siderophore-producing microbes can be used as a biofertilizer because these make immobilized Fe available to plants and act as a biocontrol agent (Glick 1995). Plants and microbes have evolved a specific mechanism by which they solubilize the insoluble form of Fe with the help of their outer membrane receptor proteins, periplasmic binding proteins, and inner membrane transport proteins in an iron-restricted environment (Matzanke 1991; Sharma and Johri 2003). Previous studies reported that many plant species are capable of using Fe^{3+} siderophore complexes formed by bacterial microbes (Bar-Ness et al. 1991). Nevertheless, using bacterial siderophores in plant nutrition remains controversial (Vessey 2003). Glick (1995) has shown that the overall requirement of bacterial siderophores by plants is low. The plant cannot use some bacterial siderophores (e.g., pseudobactin and ferrioxamine B) and some siderophore-producing bacteria compete with plants for iron use by producing different types of siderophores (Bar-Ness et al. 1992). Moreover, the siderophore formed by microbes plays a role in biocontrol activities and not just in plant nutrition (Vessey 2003; Mukherjee et al. 2020a, b).

13.4.5 Zinc Solubilization

Zinc (Zn) is an important micronutrient required relatively in lower concentrations and is known for its role in catalyzing many metabolic reactions in plants for their healthy growth and development. Zn deficiency in a plant can cause various metabolic problems including a reduction in the formation of carbohydrates, auxins, nucleotides, cytochromes, and chlorophyll, which leads to reduced membrane integrity and ultimately the plant is susceptible to heat stress (Singh et al. 2005; Prasad et al. 2019). Studies reveal that Zn deficiency in crops is not due to the low availability of Zn in soil, but rather it is due to the low solubility of Zn (Gontia-Mishra et al. 2017). The bioavailability of Zn in the soil is hampered by different factors, such as an increase in the pH, soil organic matter, bicarbonate concentration, high magnesium to calcium ratio, and high availability of phosphorus and iron (Li et al. 2016). Commonly 96–99% of applied inorganic zinc is not used by the plant and is converted into different insoluble forms (Saravanan et al. 2004). These details indicate that the applied Zn fertilizers are readily insoluble and remain in the soil and are not assimilated by the plant leading to Zn deficiency. Zn solubilizing microbes are the potential candidates that can convert the complex forms of Zn to its soluble forms and can thus cater to plant zinc requirements. There are a number of potential microbes that have been reported to increase growth and zinc content when inoculated in crops such as *Bacillus* sp. (Hussain et al. 2015), *Pseudomonas*, *Rhizobium* (Deepak et al. 2013; Naz et al. 2016), and *B. aryabhattai* strains (Ramesh et al. 2014). Zn solubilizing PGPR help solubilize the complex forms of Zn into soluble forms and make them easily available to the plant leading to the fortification of grains with Zn (Barbagelata and Mallarino 2013). Therefore, Zn is a limiting factor in sustainable agricultural production and Zn solubilizing PGPR are an important candidate for zinc nutrition in plants (Gontia-Mishra et al. 2017).

13.4.6 Plant Growth Regulators (PGRs)

The production of phytohormones is one of the direct plant growth promotion mechanisms by which a diverse number of bacterial and fungal microbes secrete plant growth regulators and phytohormones including auxins, cytokinins, gibberellins, ethylene, and abscisic acids (Glick 1995; Mukherjee et al. 2018). When these hormones are applied to plants, they increase the root surface area (Vessey 2003). Among all these phytohormones, most of the research has been occurring on the auxin (indole-3-acetic acid) (IAA) (Glick 1995; Verma et al. 2013, 2014) (Fig. 13.1, Table 13.1).

13.4.6.1 Indole-3-Acetic Acid (IAA)

Indole-3-acetic acid represents one of the most important plant hormones produced in plant shoot and transported to root (Rashotte et al. 2000) and plays an essential role in plant growth and development in many aspects such as plant cell cycle, cell division, cell enlargement, root initiation, and apical dominance (Vessey 2003). Modulation of these actions by auxin is believed to occur by a change of gene expression of auxin (Guilfoyle et al. 1998). Owing to such desirable modification in plant roots, they can absorb more water and nutrients from the soil, which in turn induces the growth of the plant (Gravel et al. 2007). The role of IAA produced by microbes has received the attention of a number of researchers (Spaepen and Vanderleyden 2011).

A diverse number of microbial flora including bacterial and fungal species can produce IAA. On the other hand, some endophytic microbes can also produce IAA (Sessitsch et al. 2004). Two types of pathways determine plant-microbe interactions. If beneficial plant-associated bacteria synthesize IAA, then it follows the indole-3-pyruvate pathway, whereas pathogenic bacterial microbes follow the indole-3-acetamide pathway (Patten and Glick 1996; Hardoim et al. 2008). Usually, root elongation is based on the amount of IAA, which could have to regulate plant-microbe interaction. The phytohormone IAA is also taking part in the promotion of symbiosis between legumes and *Rhizobium* Spaepen et al. (2007) and Molla et al. (2001) found that co-inoculation of *Glycine max* with *A. brasilense* and *B. japonicum* remarkably increases the root surface area, length, number, and dry weight of the root as well as the number and size of root nodules. Some other effects of IAA between plants and phytopathogenic bacteria have also been reported including inhibition of plant growth by disturbing auxin balance in plants resulting in tumors and galls (Jameson et al. 2000; Mole et al. 2007). Based on a recent report, IAA has been found to act as a signaling molecule in several microorganisms, which can affect the gene expression of microorganisms (Prusty et al. 2004; Yuan et al. 2008). There may occur a crucial impact on interactions between IAA-producing microbes. Several research articles have been published that indicate that auxin can act as an effector molecule between auxin-producing microbe-microbe interaction and plant-microbe interaction (Lambrecht et al. 2000; Spaepen et al. 2007; Spaepen and Vanderleyden 2011). Nevertheless, the biological mechanism and role of auxin in fungal ecology have not been widely investigated (Reineke et al. 2008; Rao et al. 2010).

13.4.6.2 Cytokinin

Cytokinins are mostly adenine derivative produced by a number of known microbes such as *Azotobacter* sp., *A. giacomelloi*, *Agrobacterium* sp., *A. brasilense*, *Achromobacter* sp., *Enterobacter* sp., *Klebsiella* sp., *B. japonicum*, *B. licheniformis*, *P. fluorescens*, and *P. polymyxa* (Akiyoshi et al. 1987; Cacciari et al. 1989; Taller and Wong 1989; Timmusk et al. 1999; Donderski and Głuchowska, 2000; de García Salamone et al. 2001; Kämpfer et al. 2005; Perrig et al. 2007; Hussain and Hasnain 2009). Cytokinin has the ability to regulate the morphological and physiological processes of plants with the help of differential influence. Cytokinins stimulate cell division, cell cycle, apical dominance, and root hair multiplication, but it also shows lateral root inhibition and inhibits primary root lengthening (Silverman et al. 1998; Riefler et al. 2006). When cytokinin-producing microbes were used as bioinoculant with plants, shoot growth was increased and root to shoot ratio was reduced (Arkhipova et al. 2007). Based on in silico analysis in cytokinin production, it was found that bacterial genes are involved but their role is still not known through functional analyses (Frébort et al. 2011). Therefore, the role of cytokinin produced by PGPR, which influences the root system, remains hypothetical.

13.4.6.3 Gibberellin

Kurosawa first isolated gibberellin in 1962 from *Fusarium moniliforme*. Gibberellins modify plant morphology by elongation and extension of plant roots (Yaxley et al. 2001). Plants, as well as some fungi and bacterial species (Morrone et al. 2009), synthesize gibberellins.Various PGPR produce gibberellins such as *A. xylosoxidans*, *B. cereus*, *Acinetobacter calcoaceticus*, *A. lipoferum*, *A. brasilense*, *Azotobacter* spp., *B. pumilus*, *B. macroides*, *Herbaspirillum seropedicae*, *Gluconobacter diazotrophicus*, *Promicromonospora* sp., *B. cepacia*, and *A. diazotrophicus* (Bottini et al. 1989; Kang et al. 2009; Bastián et al. 1998; Gutiérrez-Mañero et al. 2001; Bottini et al. 2004; Janzen et al. 1992; Joo et al. 2005; Dodd et al. 2010). Gibberellins produced by plants or microbes facilitate plant modification in various aspects such as stem lengthening, germination, flowering, budding, fruiting, and various other modifications that may be significant for plant growth and its development.

13.4.6.4 Abscisic Acid

Abscisic acid is a phytohormone produced by microbes, plants, and animals (Gomez-Cadenas et al. 2015; Karadeniz et al. 2006). Abscisic acid can modulate several aspects of the plant including plant development by stomatal closer (Herrera-Medina et al. 2007) and inhibition of the growth of primary roots (Pilet and Chanson 1981). Based on the reports, abscisic acid high in concentration leads to inhibition of the growth of *Brassica*, beans, and maize (He and Cramer 1996; Cramer and Quarrie 2002).

13.4.7 Biocontrol Agents

In the agriculture system, soilborne pathogenic microbes are the major problem, which hinders agricultural development around the world. Among four groups of pathogenic soil microbes, fungi and nematodes are the major candidates that cause diseases in plants, whereas some bacterial genera are reported to cause diseases in plants (Raaijmakers et al. 2009). Several techniques have been implemented in the agricultural system to enhance crop productivity through better plant nutrition and to protect crop plants from a range of pathogenic microbes. These approaches for agricultural development include the use of organic manures, chemical pesticides, cultivation techniques, compost, and other techniques (Whipps and Gerhardson 2007). At present, chemical fertilizers and manipulated resistant varieties are the most popular measures to protect plants from pathogens (Vassilev et al. 2006). Many biocontrol agents have been isolated that reduce the risk or severity of plant diseases. For example, *Pseudomonas*, *Bacillus* species, and *Trichoderma* species act as biocontrol agents showing excellent antipathogenic activity (Gerhardson 2002). Some bacterial endophytes are also reported to show biocontrol activity against a wide range of fungal pathogens (Berg and Hallmann 2006; Chouhan et al. 2021c). Many PGPR have been identified that show antagonistic activity through several mechanisms such as the production of antibiotics, enzymes, siderophore, and HCN (Podile and Kishore 2006). Diverse PGPR can produce some specific peptide hormone that inhibits the synthesis of cell wall and cell membrane structure and hinders the formation of protein in microbes (Maksimov et al. 2011). Many bacterial species have been identified that produce antibiotics, for example, *Pseudomonas* spp. and the other bacterial species such as *Stenotrophomonas Bacillus, Streptomyces secrets zwittermicin A, xanthobaccin, oligomycin A*, and *kanosamine*, which secrete phenazine, hydrogen cyanide, amphisin, 2,4-diacetyl phloroglucinol, and cyclic lipopeptides (Compant et al. 2005).

13.5 PGPR for Stress Management

Plant growth-promoting rhizobacteria have also been shown to possess properties for the management of different abiotic stresses in plants. They have been proved to be beneficial in stresses such as salinity stress, drought stress, acidity stress, nutrient deficiency, suboptimal root zone temperature, heavy metal stress, and also different biotic stresses. For instance, salinity stress in plants is ameliorated by PGPR by ACC (aminocyclopropane-1-carboxylate) deaminase and ROS (reactive oxygen species) scavenging enzyme activities (Bharti and Barnwal 2019; Vaishnav et al. 2016; Mishra et al. 2018). Under salinity stress conditions, PGPR produce ACC deaminase, osmolytes, antioxidants, and other secondary metabolites that help the plant in coping with stress conditions (Babar et al. 2021). The current strategy for the management of abiotic stress conditions is the isolation of salinity-tolerant PGPR from the rhizosphere of a plant followed by its exogenous application in the rhizosphere of an identified abiotically stressed soil. Drought stress in plants is mitigated by PGPR through the modulation of IAA, ABA, ethylene, and various other plant hormones inside the plant tissues under drought stress conditions (Desoky et al. 2020). The regulation of plant hormone levels helps in the enhancement of root and shoot growth, thereby ameliorating the negative effects of drought stress. The production of osmoprotectants also aids in combating drought stress.

13.6 Future Perspective and Challenges

Plant growth-promoting rhizobacteria have been greatly used in the enhancement of plant growth and development. They have also shown great significance in the management of abiotic and biotic stresses. Further, the importance of PGPR in recent times has been realized for the degradation of xenobiotic compounds for potential bioremediation. Different aspects of PGPR with respect to beneficial effects on plants need to be explored. The deleterious effects of weed can be minimized along with the enhancement of plant growth promotion and development by the exploitation of PGPR along with rhizobia. The complex interactions between PGPR microbes need to be studied more deeply to understand the mechanisms behind such interactions. This could help explore more dimensions in the study of PGPR interactions with the plants for growth and development. A deeper look into the genetics of root colonization along with the molecular interactions of plant microbial signaling may help better understand the mechanisms behind plant growth promotion and development.

13.7 Conclusions

PGPR aid plants in their growth and development even in adverse conditions by modulation of soil chemistry and regulating various parameters involved in it. These can be both symbiotic and nonsymbiotic depending upon their relationship with their interacting host plant but one thing is for sure they help release various plant hormones and secondary metabolites that aid the growth and development of various plant parameters. PGPR also help in the mitigation of various stresses by secretion of different secondary metabolites. Apart from their use in plant growth and development, PGPR have also been shown to play an important role in the bioremediation of various heavy metals and side by side aiding in plant growth and development. Their biocontrol properties in the management of various weeds and pests also add to the list of beneficial properties possessed by PGPR. PGPR can be used as effective biofertilizers for enhancing sustainable agricultural productivity. These microbial inoculants will be cost-effective, environmentally friendly, and economically viable. In the future, this seems to be the best alternative to chemicals in farming.

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Rhizosphere Engineering for Systemic Resistance/Tolerance to Biotic and Abiotic Stress

14

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Abstract

In the wake of climate change and global warming, plants are continuously confronting environmental stress. Environmental stress inexhaustibly impacts plant productivity and sustainability worldwide. The engineering of the rhizosphere could be an option to alleviate both biotic and abiotic environmental stresses. The rhizosphere is the soil–root interface that encompasses a dynamic physical, chemical, and biological ambiance, favoring varied micro-organisms activity. The microbiome of the rhizosphere influences the plant nutrition, root architecture, and soil quality. The rhizosphere microbiomes have intrinsic metabolic and genetic capabilities in regulating plant stress responses too. The knowledge of the rhizosphere and its components is necessary to create strategies for contouring the rhizosphere to enhance plant fitness and productivity. The advancement of meta-omics technologies and bioinformatics tools have unveiled

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the organization, function and dynamics of the rhizoshphere microbiome of diverse environments. The scientists and researchers are more focused on reshaping the rhizosphere using breeding, biotechnological tools, or supplementing with microbial inoculants, etc. This chapter deals with the various strategies of rhizosphere engineering for the alleviation of abiotic and biotic stress to plants.

14.1 Introduction

In the twentieth century, the green revolution bestowed the world with remarkable gain in food production owing to the use of chemical inputs, high-yielding varieties, etc. However, the enhanced use of chemicals caused a detrimental impact on environmental health. Moreover, climate change and climate-related scourges have aggravated their negative impact on plant productivity and sustainability worldwide (Pachauri et al. 2014). The scenario of climate change created an immediacy to look for the new revolution in agriculture to sustain the food, feed, and fiber requirements of the ever-growing population. The new revolution is possibly including a bio-revolution based on the application of biological inputs (e.g., bio-inoculants, the product thereof) and improved varieties (targeting the microbiome community structure) (Timmusk et al. 2017; Backer et al. 2018). The bio-revolution concept is centered on the manipulation of the rhizosphere by various means. Hence, an in-depth understanding of the rhizosphere and its components is essential to generate strategies for reshaping the rhizosphere to enhance plant fitness and productivity. The omics technology and molecular tools are continuously unveiling the mystery of the rhizosphere. The organization, function, and dynamics of rhizosphere microbiome have been explored by researchers in a diverse environment.

14.1.1 An Insight into the Rhizosphere and Its Components

The rhizosphere is the confined zone of contact between plant roots and soil particles, inhabited by a diverse group of micro-organisms (Dessaux et al. 2016; Ahkami et al. 2017). McNear Jr (2013) has illustrated the rhizosphere as having three regions: the endorhizosphere, rhizoplane, and ectorhizosphere (Fig. 14.1). Endorhizosphere is the section of the root cortex and endodermis in which the apoplastic spaces harbor microbes and mineral ions. The rhizoplane is the central region beside the root epidermal cells and mucilage. The ectorhizosphere is the outermost zone which extends from the rhizoplane out into the bulk soil. Plant metabolism greatly influences the rhizosphere by releasing metabolites (root exudates), plant debris (dead cells, mucilage), and carbon dioxide (Dessaux et al. 2016; Ahkami et al. 2017) that assist different and distinctive patterns of microbial colonization. This plant root–soil interface creates a dynamic physical, chemical, and biological ambiance for micro-organisms to perform inter- and intra-species

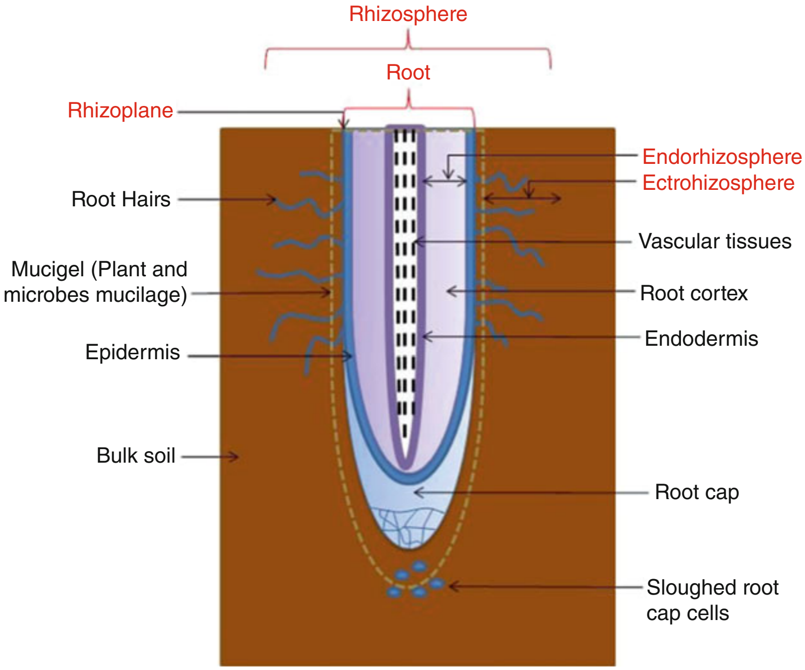

Fig. 14.1 A simplified diagram of rhizosphere region

communication. The microbiome of the rhizosphere plays pivotal roles in soil formation, acquisition of nutrients, suppressing pathogen pressure, secretion and modulation of extracellular compounds like secondary metabolites, hormones, signaling molecules, antibiotics, etc. favoring plant growth promotion and protection. Bacteria, fungi, archaea, and arbuscular mycorrhizal fungi (AMF) are prevalent micro-organisms that help in the nutrition recycling process (Van Der Heijden et al. 2008). The microbiome of rhizosphere is also known for plant protection using various mechanisms for disease suppression, and also salinity and drought stress alleviation, etc. However, the rhizosphere zone is more vulnerable to environmental stress. It is found that the salinity stress in the rhizosphere reduces the survivability of plants (Damodaran and Mishra 2016).

14.2 Rhizosphere Engineering and Different Approaches

The biochemistry of soil and root interface is the effect of various interacting and competing processes based on plant physiology, soil type and water content and microbiome composition (Pinton et al. 2007). For the betterment of plant fitness and productivity, the entire rhizosphere component can be engineered. Till now, research has presented that rhizosphere can be engineered by proper selection of crop genotype, by application of microorganisms or soil amendments, and by genetic manipulation of plant and microbiome activities. Application of microbes or soil amendments improves soil quality via changing its physicochemical properties (Dessaux et al. 2016). Plants can be engineered to select or introduce beneficial and novel traits such as the rhizosphere pH or to release compounds that improve nutrient availability, protect against biotic and abiotic stresses, or encourage the proliferation of beneficial microorganisms (Bowen and Rovira 1999; Damodaran and Mishra 2016). Currently, for microbiome engineering, the whole microbial population (present in plant and rhizosphere) instead of single strain engineering are selected to stimulate plant growth promotion and protection. Similarly, the emergence of “omics” approaches has unraveled the molecular basis of plant–microbe interaction mechanisms responsible for the physiological changes. Hence, engineering of plant–microbe interaction (holobiome approaches/ecological engineering) like an exciting strategy came into the picture. In the coming section, engineering strategies and relevant instances are explained in detail.

14.2.1 Soil Amendments

Rhizosphere is the soil compartment around the roots that influence the root activity and plant growth. The physical and chemical properties of the rhizosphere depend on the soil type, water content, soil microbes as well as the biological activities of the plants (Ryan et al. 2009). So, engineering any of these factors will help us tailor the rhizosphere thereby influencing the biogeochemical activities in the root zone. The microbiome present in the root zone is diverse with both beneficial and pathogenic microbes which regulate the nutrient dynamics as well as the well-being of the crop plants. So rather than increasing the nutrient concentration through fertilization, it is wiser to go for efficient root zone management approaches utilizing the hidden potential of the root zone.

The amendments can be done by modifying the physical and chemical properties of the soil in the root zone. These properties of soil are a function of the percentage of clay, nutrient status, organic matter, and the water-holding capacity (Bowen and Rovira 1999). The soil is a substrate that determines the survival rate as well as the growth of several microbes. Several studies suggest that the clay particle could promote microbial growth as it plays an important role in determining the water-holding capacity and the pore size of the soil bulk. It promotes the useful microflora by adsorbing inhibitory substances as well as conferring desiccation tolerance to the cells. The soil moisture will affect the survival of different microorganisms, especially fungi. The bacteria are more desiccation-tolerant due to their ability to produce spores. The pores in the clay particles can also provide physical protection of the microflora against their predators.

The soil temperature is another factor which affects the rhizosphere and an increase in temperature reduces the antagonistic microbes but it also affects the growth of beneficial ones (Moyano et al. 2007). It is found that physical processes like tillage generally reduce the microbial count by destroying the vegetative propagules of microbes, especially some useful fungi. But the tillage practices like conservative tillage will help improve the growth and colonization of microbes as well as the physical properties of soil (Mirás-Avalos et al. 2011; Wang et al. 2017, 2020).

The nutrient availability in agroecosystems has resulted from the interaction between fertility management strategies and the microbial processes including biological nutrient fixation and mineral mobilization (Schmidt et al. 2019). So, another approach is to influence the plant root architecture by altering the soil nutrient status. The intensity of soil nutrients can affect the root proliferation which decides the adsorption area of the roots. An optimum supply of soil nutrients in the initial phase of crop growth will help to develop an extensive root system. Also, it will affect the growth of beneficial rhizosphere microbes like nitrogen-fixing bacteria and phosphate-solubilizing bacteria. The site-specific application of the nutrients like N and P can encourage root proliferation which can result in enhanced growth, biomass and nutrient absorption rates (Kumar et al. 2017). The strategic nutrient supply can be adopted to stimulate the root growth, to exploit the root-mobilizing potential, and to delay the root senescence (Shen et al. 2013).

The application of synthetic fertilizers also alters the pH of the soil thereby affecting the rhizosphere activities. The use of nitrogenous fertilizers usually lowers the soil pH. This can increase the growth of acidobacteria and decrease the abundance of beneficial ammonia-oxidizing archaea. The deficiency of the nutrients like Zinc and Manganese will make the plants susceptible to some soil pathogens (Bowen and Rovira 1999; Schmidt et al. 2019). Even though the agrochemicals like herbicides can destroy the plant pathogenic rhizosphere microflora, they detrimentally affect the colonization of beneficial ones (Bowen and Rovira 1999).

The major energy source of the rhizosphere microbiome of a specific crop is the plant organic matter and the microbial colonies in the root residues act as the inoculum for the successive crops. So, the addition of plant organic matter is one of the methods to alter the microbial diversity in the rhizosphere (Bowen and Rovira 1999). The addition of organic fertilizers such as composts and crop residues can alter the diversity, abundance, and activity of various soil microbes, especially the nitrogen-fixing bacteria (Schmidt et al. 2019). Biofertilizers containing different types of plant growth regulating rhizobacteria and fungi can drastically change the rhizosphere microbiome status as well as the nutrient availability in the rhizosphere. The combination of organic fertilizers, effective microorganisms, and biofertilizers is proved to be the most efficient way to engineer the rhizosphere through soil amendments without compromising environmental safety (Jilani et al. 2007).

14.2.2 Plant as a Management Tool

The plant roots are the complex resource-rich hotspots that can selectively control the microflora in the rhizosphere which is different from the bulk soil. So, for a long-term improvement of the rhizosphere, the improvement of plant itself through conventional or using biotechnological methods is inevitable. This can be done by influencing the root itself or by influencing the rhizosphere microbiome by selective

Table 14.1 Genes responsible for the root system architecture traits for important crops

Crop	Gene	Phenotype/traits associated	Reference
Rice	*OsPIN2*	Large root angle	Wang et al. (2018)
	DRO1	Deeper rooting	Uga et al. (2013)
	OsEXPA8	Longer primary roots, more lateral roots and root hairs	Ma et al. (2013)
	OsEXPB2	Root hair formation	Zou et al. (2015)
	OsWRKY28	Lateral root formation, root length, phosphate and arsenate accumulation	Zhao (2018)
	RDWN6^{XB}	Root system architecture in low nitrogen availability	Anis et al. (2019)
Wheat	VRN1	Root length and root angle	Voss-Fels et al. (2018)
	TaMOR	Number of roots	Li et al. (2016)
	qSRA-6A	Seminal root angle	Alahmad et al. (2019)
Maize	ZmRAP2.7	Brace root development	Li et al. (2019a)
	ZmTIP1	Root elongation	Zhang et al. (2020)
	ZmPTF1	Root development and drought stress signaling	Li et al. (2019b)
	LRL5	Root hair growth	Wang et al. (2019)
	LOS5/ABA3	Increased root biomass under water stress	Mohammed et al. (2019)
	AtLOS5	Root ion fluxes under salt stresses	Zhang et al. (2012)
	ZmbZIP4	Lateral and primary root development during stress	Ma et al. (2018)
Barley	VRN1	Root length and root angle	Voss-Fels et al. (2018)
	Beta-Expansin	Root hair formation	Kwasniewski and Szarejko (2006)
Soybean	GmEXP1	Root elongation	Lee et al. (2003)

modification of rhizodeposition (organic and inorganic compounds released by roots) (Ryan et al. 2009).

The root system architecture (RSA) of the plant is an important factor that determines the rhizosphere environment. So many genes are associated with the processes that determine this complex trait in different plants (Table 14.1). Several component traits contribute to RSA and thus the rhizosphere which includes the root depth, root hairs, primary and lateral root number, root angle, etc. The degree of root penetrations differs according to the genotype which determines not only the soil physical properties in the rhizosphere but also the degree of root–soil contact and hence the nutrient availability (Gregory et al. 2013). Due to the difficulties in the phenotyping techniques of these traits, the studies are limited and in the developing stage.

The plant roots harbor several genes controlling nutrient fixation processes. These genes could increase the nutrient use efficiency and reduce the demand for chemical fertilizers. These genes will interact with useful microbes like nitrogen-fixing bacteria, phosphate-solubilizing bacteria, vascular arbuscular mycorrhizae, archaea, etc. and stimulate their colonization and growth. The genes like the N transformation-associated genes will interact with the soil microbiome and provide a specific rhizosphere ecosystem. The maize genes like *nifH* (nitrogen fixation), *gdh, ureC* (ammonification), *amoA, hao* (nitrification), and *narG, nirS/nirK, norB, nosZ* (denitrification) are examples for this (Schmidt et al. 2019). The researchers are also aiming to increase the P-acquiring ability of the plants from common forms of soil organic phosphates. As a result, transgenic plants with microbial phytase genes have been developed and they can hydrolyze P from inositol phosphates with 20-folds increase in root phytase activity (Gregory et al. 2013).

The rhizosphere compositions of plants are controlled by the organic materials around the roots which include exudates, secretions, lysates, and plant mucilages. The exudates and secretions are the compounds released by the roots and promote the growth of beneficial microbes. The lysates are the compounds released from senescent or ageing cells, which help the plant to control the pathogenic microbes (Rovira et al. 1978). The mucilage is a gelatinous polysaccharide produced by the root cap which helps roots in penetration. The composition of the root mucilage varies according to the species and genotype of the plant. For example, the rice and the maize mucilages are rich in fructose while the grasses in the *Lolium* spp. contain glucose and xylose. The composition of mucilage will affect the rhizosphere by influencing the biological, viscoelastic, and surface tension properties of soil (Gregory et al. 2013). This rhizodeposition will, directly and indirectly, affect the rhizosphere composition as well as the rhizosphere microbiome. So, engineering the plant genes responsible for these compounds is another way of rhizosphere engineering. The unexplored area of root biology can be explored with novel phenotypic techniques. Then these traits can be engineered by using conventional plant breeding approaches coupled with novel biotechnological tools.

14.2.3 Microbe Engineering

Engineering of microbe community responsible for plant growth, biotic and abiotic stress resistance, and nutrient mobilization for rhizosphere presents a unique opportunity for enhancing crop performance. Microbe cortege surrounding plant root system has been studied for its direct and indirect interactions. For rhizosphere engineering, PGPM are of particular interest. These growth-promoting bacteria directly benefit plants by (1) mobilizing and fixing nutrients, (2) Modulating plant growth by interacting plant growth hormones, and indirectly by (1) Efficient root colonization, (2) Biopesticidal and biocontrol activities, and induction of defense mechanism by SAR (Tailor and Joshi 2014). Most of the studies focused on selecting an effective combination of plant growth-promoting rhizobacteria (PGPR) for inoculating rhizosphere and only a few reports explain the engineering of PGPR for its effective use (Raaijmakers et al. 1995). Introduced heterologous siderophore in *Pseudomonas* spp. confer a competitive advantage in interactions in the rhizosphere. Many studies have been done where microbes are engineered to improve nodulation efficiency of plant, for example, *Sinorhizobium meliloti* strain constitutively expressing *putA* gene resulted in increased activity of proline dehydrogenase, an enzyme crucial for colonization. Better colonization of engineered strain resulted in better nodulation in alfalfa roots (Van Dillewijn et al. 2001). Introduction of ACC deaminase structural gene (*acdS*) and its upstream regulatory gene *lrpL*, from *R. leguminosarum* bv. *viciae* 128C53K in *Sinorhizobium meliloti* also increased nodulation efficiency of alfalfa (Ma et al. 2004). Similarly, overexpression of trehalose-6-phosphate synthase in *Rhizobium etli* improved nodulation, drought tolerance, and yield in *Phaseolus vulgaris* (Suárez et al. 2008). Several cryptogamic diseases got suppressed when the soil was inoculated with genetically modified *Burkholderia vietnamiensis* PGPR strain P418. This engineered strain having chitinase from *Bacillus subtilis* leads to suppression of wheat sheath blight, cotton Fusarium wilt, and tomato grey mold (Zhang et al. 2012). The transgenic strain of *Ensifermedicae* MA11 carrying *copAB* genes from a Cu-resistant *Pseudomonas fluorescens* strain is able to alleviate Cu tolerance in *Medicago truncatula* in Cu-contaminated soil and successfully forming nodules (Delgadillo et al. 2015).

Population engineering rather than single microbe engineering is a novel aspect but ecological interaction among microbes also needs to be considered (Großkopf and Soyer 2014). Types of ecological interactions are commensalism, competition, predation, no interaction, cooperation, and amensalism. Our goal of maximizing beneficial interaction and minimizing negative interaction is particularly challenging as negative interaction tends to dominate the event in two strain co-culture (Foster and Bell 2012). Vast knowledge about naturally occurring PGP microbes that colonize rhizosphere is available in the public domain. Some of these genera like *Pseudomonas, Bacillus, Paenibacillus, Streptomyces, Rhizobium* have genome sequences available and genetic systems amenable for engineering (Rothmel et al. 1991; Dong and Zhang 2014; Kim and Timmusk 2013; Medema et al. 2011; Patel and Sinha 2011). For making a synthetic community of microbes most suitable species will be *Bacillus* as its sequencing information is available in higher depth, transformation is relatively easy (Dong and Zhang 2014), many isolates having plant growth property (Köberl et al. 2013, 2015), and also used as a biocontrol agent. *Bacillus* species are broad-spectrum antagonists to the soil-borne pathogen (Köberl et al. 2013) hence different antibiotic-producing pathways from different strains may be clubbed together to form a synthetic strain. *Bacillus* spp. could be engineered for producing a high concentration of hormones or for nitrogen-fixing machinery (Arkhipova et al. 2005; Kim and Timmusk 2013). These engineered bacillus strains could be combined with some naturally occurring nitrogen fixer from *Rhizobium* and/or *Bradyrhizobium* genera to generate consortium (Ahkami et al. 2017). For increasing cooperation among microbes of synthetic community, strains should be engineered in such a manner that each species would depend on cofactor produced by other species for survival. In this way symbiotic relationship between different synthetic species will develop and competition will be eliminated. Some factors which must be considered for selecting microbe for engineering rhizosphere are:

(1) colonization efficiency of microbe in root surface, rhizosphere, and target host plant; (2) its survival and competition with other microbes in consortia; (3) attachment to the root surface; (4) its plant growth-promoting activity and relation with other PGPR microbial species; (5) abiotic stress tolerance; (6) density of growth; (7) tolerance to herbicides, pesticides, and fertilizers.

14.2.4 Plant–Microbes Interaction Engineering

The profile of rhizospheric microbes in rhizosphere depends on the rhizospheric deposition. Substrate from root exudates determines establishment of host-specific microbe community in rhizosphere. So, genotype of plant and rhizospheric microorganism profile is very fine-tuned. 5–21% of photosynthetically fixed carbon are secreted in root exudates in the form of different metabolites (Bais et al. 2006; Zhang et al. 2015). For plant–microbe interaction, phytohormone plays an important role. Many PGPR microbes are known to be producing phytohormone, including auxin, gibberellin, cytokinin. Other than these, inoculation with nonpathogenic microbes also induces cross-talk between jasmonic acid, salicylic acid, and ethylene to induce systemic acquired resistance (SAR) and induce systemic resistance (ISR), which further protect plants from pathogenic microbes. Plant–microbe interaction engineering is an exciting field of study which also involves plant engineering strategies concerning the plant immune system and mainly focuses on phytohormone pathways and crosstalks. Several studies related to endophyte and phytoremediation are already present, for example, endophytic bacterium *Burkholderia cepacia* engineered for toluene degradation pathway by adding pTOM toluene-degradation plasmid improves toluene degradation capacity of lupine. Lupine seeds when inoculated with this engineered bacterium resulted in reduced phytotoxicity and 50–70% reduction in leaf evapotranspiration. This kind of engineered plant–microbe association is promising in improving the efficiency of phytoremediation volatile organic contaminants (Barac et al. 2004). In another experiment, poplar hybrids (*P. trichocarpa* X *P. deltoides*) were inoculated with *Burkholderia cepacia* VM1468 carrying Toulene degrading plasmid *pTOM-Bu61*. Control plants were treated with soil bacterium *B. cepacia* Bu61 (pTOM-Bu61). The treatment plant showed positive growth in presence of toluene and reduced evapotranspiration of toluene. An interesting result of this experiment was that *Burkholderia cepacia* did not form endophytic relationship with poplar. Indeed, toluene tolerance resulted from horizontal gene transfer to endogenous bacteria of endophytic community (Taghavi et al. 2005). These kinds of *in planta* horizontal gene transfer among plant-associated endophytic bacteria could be used to change natural endophytic microbial communities for phytoremediation. Endophytic bacterial isolates of eggplant, cucumber, and groundnut mainly (more than 50%) consisting of *Pseudomonas fluorescens* reduced the incidence of wilt and damping-off caused by *Ralstonia solanacearum*. Most of the selected antagonists produced an antibiotic, DAPG, which inhibited *R. solanacearum* in vitro (Ramesh et al. 2009). Several studies on plant endophyte interaction in relation to phytoremediation and PGPR have been done. Major difficulties in these cases are maintaining the desired level of microbial population in the rhizosphere. The rhizosphere is influenced by many environmental and edaphic factors. Also, microbial population depends upon genotype of the plant. So, for maintaining the desired level of microbes in the root zone of the plant engineering for root exudates is the proposed idea. This idea is based on opine production in *Agrobacterium* infected plant (Tempe and Petit 1982). Opines are low molecular weight compounds used as a carbon source by *Agrobacterium. Agrobacterium* transfers a TDNA containing opine synthesis gene present in their plasmid to the plant genome which results in opine production in plant roots. These opines are secreted by plant roots gall and facilitate *Agrobacterium* growth. Based on this concept, some studies have been conducted where plants are transformed to produce opines that facilitate growth of indigenous or introduced opine-degrading enzymes. These methods showed their independence from the plant species and soil but nature and concentration of opine lead to the selection of different profiles of indigenous microbes (Tempe et al. 1982; Murphy et al. 1987; Oger et al. 2004; Mondy et al. 2014). To maintain a sufficient level of microbes, engineering trophic link is a promising approach, a natural example of which is nodulating N fixing bacteria. The bacteroids formed in nitrogen-fixing nodules synthesize opine-like compounds that specifically favor the growth of free-living bacteria. In *Arabidopsis* root was modified after foliar application of flagellar peptide flg22 or coronatine, a bacterial toxin. Application of these compounds increased the expression of malic acid transporter AML1 leading to increased concentration of malic acid in the root zone. Increased malic acid favored PGPR *Bacillus subtilis* strain FB1A7 and induced systemic resistance response in plants against *P. syringae* pv. *tomato* (Lakshmanan et al. 2012).

Plant–microbe interaction research can be accelerated by combining metagenomics metabolomics and culture-dependent synthetic communities (SynComs) approach (Liu et al. 2020). Metagenomics will be helpful in determining the structure and function of microbial profile in the rhizosphere followed by culture of microbes to form SynComs. These Syncom axenic plants are manipulated. Plant growth properties of Syncom can be studied using metabolomic approach. Change in plant interacting microbial profile due to environmental stress will be better understood by utilizing all the above approaches.

14.3 3Rhizosphere Engineering to Confer Abiotic Stress Tolerance to Plants

14.3.1 Temperature Extreme

High temperature/heat stress is one of the most challenging environmental risks for plant growth and development. Heat stress causes severe cellular disorganization, denaturation, and aggregation of cellular proteins ultimately leading to cell death. To survive this stress, plants develop multiple strategies which are achieved by the regulation of heat stress-induced genes. Adopting plant breeding and genome editing strategies are widely followed approaches for developing heat-tolerant crop cultivars but each strategy has its own drawbacks. Plant breeding programs are very time-consuming and depend on the availability of diverse germplasm. Strategies like transgenics and gene/genome editing technology requires the identification of the candidate genes which is quite complicated and costly. Additionally, the adoption rate of this technology is quite low due to biosafety issues. An alternative and cheap approach for attaining heat stress tolerance in plants can be the exploitation of soil microbes that positively interact with the plant system. Certain soil-inhabiting bacteria like plant growth-promoting rhizobacteria (PGPR) isolates have been identified that accelerate plant growth under heat stress. For example, treatment of wheat seeds with *Bacillus amyloliquefaciens* UCMB5113 or *Azospirillum brasilense* NO40 resulted in improved heat stress tolerance in wheat (Abd El-Daim et al. 2014). In another study, *Bacillus cereus* was assessed for plant growth-promoting activities under heat stress conditions in tomato varieties. The control plants were drastically affected by heat stress while bacterial inoculation promoted different morphological traits like positive shoot and root growth, increase in fresh and dry weight (Mukhtar et al. 2020). These instances suggest that PGPR are good candidates for improving crop productivity and imparting heat stress tolerance in crops. Fungal endophytes have also been reported to play a significant role in plant survival under various abiotic stress conditions such as drought, salinity, extreme temperature (cold/ heat), heavy metal pollution, etc. (Singh et al. 2011).

Chilling/freezing temperature causes plant cells to expand as the water inside them turns to ice which can rupture and lead to cell death. The effect of cold temperature on plant system is alleviated by activating various metabolic pathways via activation of large arrays of transcription factors. As in heat stress, there are also reports of various microbes which are known to impart cold tolerance in crops. For example, grapevine plants inoculated with *Bacillus phytofirmans* strain PsJN accumulated higher levels of carbohydrates, proline, phenols, and showed elevated rates of photosynthesis and starch deposition compared to control plants during cold stress (Barka et al. 2006). Moreover, inoculation of grapevine with the same PGPR strain lowered the rate of biomass reduction and electrolyte leakage (an indicator of cell membrane injury) during cold treatment at 4 °C, and also promoted post-chilling recovery. Table 14.2 enlists a few experimental evidences for the role of microbial symbionts in plant response to chilling/freezing stresses (Acuña-Rodríguez et al. 2020).

Studies suggest that priming of these beneficial microbes with plants enhances/ stimulates induced systemic resistance which facilitates abiotic stress (heat) tolerance in various crops. Therefore, rhizosphere engineering is a feasible option for developing heat stress tolerant cultivars which can be accomplished either by redesigning the plant metabolism or introduction of synthetic microbial communities into the rhizosphere.

14.3.1.1 Redesigning the Plant Metabolism

Plant root exudates act as chemoattractants for the rhizospheric microbial communities and it has been recently reported that variation in the composition of

Table 14.2 Experimental evidences for the role of microbial symbionts in plant response to chilling/freezing stresses (Acuña-Rodríguez et al. 2020)

Host species	Symbiont	Plant tissue	Salient findings
Solanum lycopersicum (crop—herbaceous)	*Pseudomonas vancouverensis* and *P. fredericksbergensis* (bacteria)	Roots	B+ tomato plants showed high chilling tolerance under chilling stress (10–12 °C), showed lesser cell membrane damage and ROS concentrations, which might be due to the expression of cold acclimation genes (LeCBF1 and LeCBF3)
O. sativa (crop—graminoid)	*B. amyloliquefaciens, Brevibacillus laterosporus* (bacteria)	Roots	B+ plants showed higher proline and chlorophyll concentrations than B- plants under cold stress (0–5 °C)
Vaccinium ashei and *V. corymbosum* (crop—shrub)	*G. mosseae* (AMF)	Roots	AMF inoculation increased leaf antioxidant activity under cold stress (10 °C)
C. sativus (crop - gourd)	*R. irregularis* (AMF)	Roots	Large array of genes were upregulated in AMF inoculated plants and down-regulated in non-AMF plants under cold stress (13 °C)
S. lycopersicum (crop—herbaceous)	*Trichoderma harzianum* (fungus)	Seeds	E+ plants under cold stress (8 °C) exhibited improved photochemical PS II efficiency, growth, electrolyte retention and proline concentration as compared to E- plants
H. vulgare (crop—graminoid)	*Glomus versiforme, R. irregularis* (AMF)	Roots	Improved plant growth, photosynthesis, phosphorus uptake and osmotic regulation at 5 and 25 °C, and enhanced post freezing survival at −5 °C
C. sativus (crop—gourd)	*R. irregularis* (AMF)	Roots	The AMF enhanced plant photosynthetic efficiency at lower temperature. This enhancement might be due to the higher carbon-sink strength observed on AMF-inoculated plants

B-, not bacterized; B+, bacterized; E-, endophyte-free; E+, endophyte-inoculated

root exudates can change the preference for different microbial community assemblages and functions (Sasse et al. 2018). For example, foliar application of jasmonic acid changes the chemical composition of the root exudate and thereby the rhizosphere microbiome community composition in *Arabidopsis* (Doornbos et al. 2011; Carvalhais et al. 2013). Abiotic stress environment can modify root exudation patterns through various mechanisms and genes involved in these mechanisms mostly belong to ABC family and MATE family (Zhou et al. 2019). Augmented exudation rate has been evident under abiotic stress environment with the release of primary and secondary metabolites to the rhizosphere when plants are subjected to drought, high salinity heat stress Al-toxicity, or Pb-toxicity and flooding. All these metabolites can attract the microbiota which will help in establishing mutualistic relationships between plants and plant growth promoting rhizobacteria or mycorrhizal fungi (Vives-Peris et al. 2020). This positive interaction can induce a variety of benefits for the plants through different mechanisms, including production of phytohormones, siderophore production, biofilm formation, fixation of atmospheric N, etc. (Etesami and Glick 2020). Since many genes-controlling exudates have been identified, it is possible to genetically modify plants to redesign the rhizosphere for desired features. However, it is important to consider that redesigning plant to influence rhizosphere could be a very complex process due to little knowledge about exudates composition, degradation of the engineered compound in the soil, low exudation rate, the effect of plant development, and external stimuli on exudation time and levels. Thus, advances in understanding the plant–microbe interaction in the rhizosphere are necessary for the development of improved genotypes for improved sensitivity to the application of specific microbial inoculants (Schlaeppi and Bulgarelli 2015).

14.3.1.2 Introduction of Synthetic Microbial Communities

Building up synthetic microbial communities to impact rhizosphere requires vast knowledge about the naturally occurring microbiota. Many microbial genera are known that colonize the rhizosphere, have publically available genome sequences, and are amenable to genetic engineering efforts. These genera include *Pseudomonas, Paenibacillus, Bacillus, Streptomyces*, and *Rhizobium* (Abd El-Daim et al. 2014). However, designing artificial microbial communities represents a huge challenge and raises many questions which need to be answered first. There are many types of ecological interactions that operate between microbial strains. The interactions can be positive (cooperation, commensalism, mutualism), negative (ammensalism, competition, and predation), or no interaction and the complexity of these probable interactions will scale linearly with the addition of extra strains (Großkopf and Soyer 2014). The main challenge in this approach is to minimize parasitism and competition and maximize cooperation. Minimizing competition is particularly challenging as even in two strain co-cultures competition tends to dominate rather quickly (Foster and Bell 2012). Further, environmental factors, such as root exudates, pH, temperature, nutrient availability, also affect stabilization, growth rates, susceptibility to pathogens, and sustainability of the applied synthetic microbial community.

PGPR have been reported to alleviate extreme temperature effects in various crops and promoted growth under stressful environments (Abd El-Daim et al. 2014; Mukhtar et al. 2020). Hence, exploiting such PGPR via a multi-omics approach for the development of the synthetic microbial community or as a source for novel genes can be a feasible option for improving crop productivity under the changing climate scenario.

14.3.2 Drought

Global water scarcity is a major threat to agriculture and the changing climate will further aggravate the problem affecting plant growth and development. An impressive number of scientific reports on plant drought tolerance mechanisms are available in the public domain but the complexity of the trait has slowed down the progress in this field. The rhizospheric bacteria coevolved with plant roots play an important role in the coping strategy of plants to drought. PGPR impart drought tolerance in plants by releasing exopolysaccharides, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, volatiles, phytohormones, osmolytes and antioxidants, regulation of stress-induced genes, and alteration in root morphology (Vurukonda et al. 2016). For example, inoculation of wheat seedlings with *Bacillus thuringiensis* AZP2 and *Paenibacillus polymyxa* B resulted in significantly higher survival rate of drought-stressed plants and increased photosynthesis and biomass production which was reflected in alteration in volatile profiles and total emission rates (Timmusk et al. 2014). Phenazines are heterocyclic compounds known to inhibit plant diseases and help in biofilm formation (Thomashow and Weller 1988; Maddula et al. 2006). To understand the specific role of phenazines in drought stress tolerance in plants, phenazines producing rhizosphere-colonizing *Pseudomonas chlororaphis* 30–84 and isogenic derivatives deficient or enhanced in phenazine production and wild type was used to inoculate in wheat seedlings. After 7-day water deficit, seedlings treated with water or by the phenazine mutant showed wilting symptoms whereas seedlings treated with phenazine producers displayed less severe symptoms. After a 7-day recovery period, the survival rate of wheat seedlings treated with phenazine-producing strains was higher as compared to the water control (Mahmoudi et al. 2019). These studies suggest that the use of rhizospheric microbiota can be an important and cheap strategy to alleviate the effects of drought stress in plants. There are evidences which suggest that plants influence rhizospheric microbes to cope with the drought situation. For example, during drought, maize forms symbiotic association with arbuscular mycorrhizal fungi which modulate water loss by downregulating aquaporin-related genes in the root (Quiroga et al. 2017). Furthermore, in trifoliate orange, mycorrhizal root encourages H_2O_2 efflux to lessen the effect of oxidative damage during drought stress (Huang et al. 2017). There are also reports which suggest that plant exudates can specifically select bacteria to get protection from drought. For example, drought stress in maize increased the malic acid exudation rate (Henry et al. 2007), which is an effective chemoattractant for *Bacillus subtilis* (Allard-Massicotte et al. 2016). These scientific evidences hint that to cope with drought situations, rhizosphere engineering can be a good option. Rhizosphere can be engineered by (i) redesigning the plant metabolism where root exudates properties can be altered to select for the desired microbial community, (ii) employing synthetic microbial communities consisting of natural or genetically engineered microbes to rhizosphere. However, the knowledge gap regarding the complex communication in the rhizospheric region during drought needs to be addressed first which will further expedite research on rhizosphere engineering.

14.3.3 Rhizosphere Engineering for Tolerance to Salinity

Salinity refers to a high concentration of soluble salts in the soil and or water, with an electrical conductivity (EC) of the range 4–<16 dS/m to <15 ESR (Singh et al. 2018). Natural causes of soil salinity are rock weathering, seawater inundation, and atmospheric deposition. Several anthropogenic processes like the use of poor quality of irrigation water, inadequate drainage, rise of groundwater, etc. are also leading to the accumulation of salt in soil. Approximately 0.3–1.5 million hectares of cultivated land is lost to salinity every year and a productivity decrease is observed to the tune of 20 to 46 million hectares per year (FAO 2015). In arid and semi-arid regions salinity is the most limiting factor among all the biotic and abiotic stresses (Nadeem et al. 2014). Salinity affects more than 20 percent of the world's cultivated land. Though some plants are halophiles, well adapted to live under saline soil, most of the crop plants are sensitive to saline conditions (Glycophytes) (Gupta and Huang 2014).

Salt stress induces osmotic stress, ionic stress, and secondary stress. Salinity-induced osmotic pressure in rhizosphere causes closure of stomata-creating water deficit in plants and affects leaf expansion. If continued for long, ions like Na+, B, Cl- accumulate in toxic levels affecting plant growth, yield and cause the death of plant (Munns and Annie 1986; Ahkami et al. 2017). Increased osmolarity and toxic levels of ions damage the architecture of root and thereby hindering water uptake and nutrition intake. Secondary stress induced by oxidative stress denatures DNA and proteins, affects the stability of membrane by lipid peroxidation leading to programmed cell death and subsequent death of plant (Nawaz et al. 2020).

The tolerance mechanisms to salt stress responses by plants are to exclude ions from the shoot; tissue tolerance by compartmentalizing ions to specific tissues, cells, or organelles; keeping water balance by maintaining transpiration use efficiency and growth of plant despite accumulated Na + (Chen et al. 2018). Plants use mechanisms like osmolyte accumulation, synthesizing antioxidants, employing defense mechanisms against reactive oxygen species, compartmentalization and transport of ions, etc. (Ahkami et al. 2017).

General practices followed are leaching of salts, use of salt-tolerant varieties, bioremediation, chemical amelioration, and organic amendments (FAO 2015). The traditional practice of adding Gypsum, Sulfur and fertilizers reduce exchangeable sodium ratio and reduces soil pH by subsequent production of acid in the soil. Conversion of S from soil amendments to sulfuric acid is mediated by microbes and the time required for it depends on the temperature and moisture availability in soil. Sustainable approaches are necessary to improve crop productivity as traditional practices are not enough to improve productivity owing to the continuing losses of agricultural produce (Egamberdieva et al. 2019).

14.3.3.1 Halophiles in Salinity Stress Tolerance

Halophilic or halotolerant microbes, which can survive under high saline habitats, use mechanisms like the synthesis of Osmoprotectants or uptake of osmolytes, ion transporters, etc. (Talaat 2018; Egamberdieva et al. 2019). Common genera of bacteria used to be inoculated in plants under high saline conditions are *Pseudomonas, Agrobacterium, Bacillus, Streptomyces, Enterobacter, Ochromobacter, Klebsiella*, etc. (Sharma et al. 2016; Sarkar et al. 2018; Singh and Jha 2016). Major features of halophilic bacteria/rhizobacteria involved in providing stress tolerance are synthesis of osmolytes- amino acids, sugars, proline, polyols and detoxification ROS by antioxidants, Reduction in Malondialdehyde in plants, up-regulation of plant genes involved in tolerance to Salinity (Bharti et al. 2016, Nawaz et al. 2020).

14.3.3.2 PGPR in Salinity Stress Tolerance

Plant growth under saline soil can be aided by inducing systemic tolerance by inoculating microbes adapted to saline ecosystem, i.e., halophiles (Grover et al. 2011; Nawaz et al. 2020). Microbes synthesize stress coping chemicals and induce plant stress genes, and this molecular response is found to be strain-specific in response to rhizobacterial inoculation (Bharti et al. 2016). PGPR promote plant growth in several ways improving plant growth both under normal growth conditions and under stress. By involving in synthesis of phytohormones, siderophores, volatile compounds, osmotically active metabolites, antioxidants, exopolysaccharides; assisting in nitrogen fixation; producing antifungal and antibacterial metabolites (Nawaz et al. 2020), PGPR mediate salt tolerance via signal transduction, ion transport, transcriptional regulation and influencing metabolic pathways and maintain high k+/Na+ ratio in plants during salt stress.

PGPR with ACC deaminase increase growth and yield of plants (Nadeem et al. 2014). Inoculation of *Klebsiella oxytoca* with the enzyme improved absorption of major nutrients under salt stress by reducing the negative effects (Liu et al. 2013). According to Fu et al. (2010), the modulation of mineral absorption and increase in antioxidant enzymes may be key factors in salinity tolerance in eggplant inoculated with *Pseudomonas* spp. under saline conditions. The ability and success of PGPR colonization in the rhizosphere depends on the compatibility of host plant genotype, microbial strain, and the developmental stage of the plant, etc. (Delaplace et al. 2015; Poli et al. 2016; Wintermans et al. 2016). Salt-tolerant plant varieties and salt-sensitive plant varieties of the same species perform differently to inoculated microbes in wheat. Inoculation of *B. pumilus* and *E. aurantiacum* augmented growth and yield of salt-sensitive tolerant varieties and that of *P. fluorescens* improved growth in salt-sensitive varieties. The modification in morpho-physiological and biochemical attributes in wheat varied with inoculant species, genotype, and developmental stage of inoculation with the consortium (Nawaz et al. 2020).

Case Study 1

Dietzia natrolimneae is a carotenoid-producing halotolerant endophyte in plants that helps in salt tolerance by modifying transcription and with ability to bioremediate hydrocarbons. *Dietzia* induces synthesis of proline and lowers malondialdehyde production thereby reduces lipid peroxide production resulting in improved stress tolerance. Induction of ABA-mediated and SOS-mediated signal transduction by *Dietzia natronolimneae* STR1 modifies the expression of many genes associated with salt tolerance such as SOS1, SOS4, WRKY10, MYB in wheat followed by expression of genes such as salt stress-induced ST; ion transporters NHX1, HAK and HKT1; antioxidant enzymes APX, MnSOD, CAT, POD, GPX and GR; and osmoprotectants like proline leading to enhanced tolerance to salt stress (Bharti et al. 2016).

14.3.3.3 Fungi in Salinity Stress Tolerance

Mycorrhiza is the symbiotic association between fungi and plant roots. The soil zone surrounding plant roots and associated fungal hyphae is called the mycorrhizosphere (Johansson et al. 2004). Mycorrhiza helps in nutrition, water uptake, modifies enzyme actions and enables changes in root architecture (Nadeem et al. 2014). Arbuscular mycorrhizal fungi (AMF) enable water transport and improve salinity tolerance (Ouziad et al. 2005). Mycorrhizae increase soluble sugar, electrolytes, antioxidant enzymes, polyamines, nitrogenase, nodulation, etc. under saline conditions (Feng et al. 2002; Gamalero et al. 2009). The ability of AM fungi to sustain a high k+/Na+ ratio in plants is an indicator of salinity tolerance. *Montagnulaceae* sp., dark septate endophyte (DSE), increases plant tolerance to salinity maybe by the high levels of melanin in its hyphae (Yuan et al. 2016).

14.3.3.4 Synthetic Consortia of Microbes in Salinity Stress Tolerance

In 2016, Yuan et al. reported a study highlighting the ecosystem-designed rhizosphere interaction is associated with seepweed *Suaeda salsa.* Phylogenetic studies on the consortia of rhizospheric bacteria obtained were found to be enriched with the genes associated with salinity adaptation, nutrient dissolution, and root colonization. *Pseudomonas* spp. were found to be enhancing plant growth, especially under salt stress. Synthetic consortia of bacterial communities can be constructed by mixing many strains in uniform and can be utilized as potential rhizosphere microbes for non-host plants for adapting plants to salt stress.

14.3.3.5 “Plant-Fungal-Bacterial” Symbiosis in Salinity Stress Tolerance

Taking advantage of culturable rhizospheric bacterial consortia either synthetic or natural will help in improving agriculture in salt-affected lands, utilizing “Plant-fungal-Bacterial” symbiosis along will enhance the effect (Yuan et al. 2016; Nadeem et al. 2014). Substances required to increase the permeability of cells are synthesized by bacteria and hence increase root exudation and thereby helping root penetration by hyphae of mycorrhizae and PGPR thus enhancing colonization and symbiosis (Jeffries et al. 2003, Hildebrandt et al. 2002; Jäderlund et al. 2008). Stress-induced difficulty in colonization can be mitigated by combined inoculation (Nadeem et al. 2014). Combined inoculation of *P. mendocina* and *Glomus mosseae* increased biomass of lettuce even if the stability of the aggregate was reduced under salinity (Kohler et al. 2010). Shirmardi et al. (2010) found dual inoculation of PGPR and mycorrhizae on sunflower-enhanced nutrient uptake in saline environment.

Constraints in utilizing PGP microbes are needed for specific strain for a particular plant genotype for salinity stress, incompatibility of different strains, ecosystem, and colonization stage. Further studies on the culturability of halophilic microbes, genes involved in the regulation, host–microbe combination studies in a naturally saline environment, etc. are necessary to utilize this sustainable approach to improve crop productivity (Nadeem et al. 2014).

14.3.4 Rhizosphere Engineering for Tolerance to Xenobiotic Compounds

Industrialization and input-intensive agriculture have led to contamination of soil with chemicals like polyaromatic compounds, polychlorinated biphenyls (PCBs), petroleum hydrocarbons, phenols, and pesticides (Singh et al. 2018; Hussain et al. 2018a). Many of the organic contaminants are detrimental to plants, environment and human health, and compounds like POPs and chlorinated solvents, etc. are mutagens, carcinogenic, and neurotoxic (Hussain et al. 2018a). Major xenobiotic compounds to soil are chlorinated solvents like trichloroethylene, chloroform, carbon tetrachloride, and perchloroethylene; persistent organic pollutants (POPs) like organochlorines, industrial chemicals, and byproducts; pesticides; explosives like TNT, RDX, octogen, etc.

14.3.4.1 What Are the Mechanisms of Tolerance by Plants against Xenobiotics?

Among the remediation measures available, eco-friendly and sustainable technologies like biotechnology and rhizosphere engineering using plants and microbes are being developed for effective management of organic xenobiotic compounds (Kuppusamy et al. 2017). Bioremediation using microbes that are capable of degrading contaminants and phytoremediation using certain plant species to accumulate contaminants and using both plants and microbes together to combine the advantages and making more effective are being researched (Cheng et al. 2016; Vergani et al. 2017). Phytoremediation has been applied to ameliorate contaminated soil and water for decades. Phytoextraction, phytoaccumulation, rhizofiltration, phytostabilization, phytomobilization, phytovolatilization, phytodesalinilization, phytotransformation, rhizodegradation, etc. are the mechanisms used by plants for phytoremediation (Dzantor 2007; Hussain et al. 2018a). Table 14.3 is showing some recent examples of rhizosphere engineering for tolerance to xenobiotic compounds.

14.3.4.2 What Rhizosphere Engineering Can Do Extra to the Tolerance—Either by Bioremediation and/or by Phytoremediation

Specific microbes can degrade contaminants under aerobic or anaerobic conditions. Bacteria such as *Pseudomonas*, *Sphingomonas*, *Alcaligenes*, *Mycobacterium*, and *Rhodococcus* metabolize pesticides and hydrocarbons under aerobic conditions (Vergani et al. 2017). In the case of pollutants that are recalcitrant, combined use of plants and microbes called rhizoremediation is more effective (Thijs et al. 2016). Even though there are reports on genetically engineered microbes for bioremediation, none of them are commercialized due to regulatory issues, public acceptance, and safety issues (Hussain et al. 2018a).

Table 14.3 Some recent examples of rhizosphere engineering for tolerance to xenobiotic compounds

Plant	Microorganism	Xenobiotic compound	Mechanism	Reference
Jatropacarcas	*P. aeruginosa*	PAH (poly aromatic hydrocarbon)	Glutathione s transferase	Singha and Pandey (2017)
Wheat	Proteobacteria, actinobacteria, firmicutes, bacteroidetes, and cyanobacteria	Aromatic compounds	Metabolism by multiple enzymes	Singh et al. (2018)
Maize	Consortia of *Bacillus, Comamonas,* and *Stenotrophomonas maltophilia*	Oily sludge-petroleum hydrocarbons	Superoxide dismutase (SOD) and peroxidase (POD)	Shahzad et al. (2016)

14.4 Rhizosphere Engineering to Confer Biotic Stress Tolerance/Systemic Resistance to Plants

Biotic stress is another prominent curse to agriculture which causes up to 30% yield loss in agriculture around the world (Kumar and Verma 2018). Indiscriminate use of pesticides potentially calls for trouble to the health of human and other animals when entering into the food chain. This issue has been encouraging scientists to explore more sustainable solutions to combat pathogenic attacks on plants. As a part of this venture, rhizosphere microbes are proved to extend the benefit to serve plants against biotic stress, too, like that of abiotic stress.

Plant growth-promoting microbes facilitate defense response in plants by exerting a diverse range of benefits. PGPR enhance nutrient availability from soil to plant root (Vardharajula et al. 2011) and thereby strengthening nutrient balance which is crucial for plant health and plant to cover up the damage in terms of yield caused by pathogen attack and other environmental stress. They can also adjust the hormone balance in plants eliciting the downward signaling event in turn inducing resistance in plants against biotic stress (Bukhat et al. 2020). Plants also gain from rhizobium through biological nitrogen fixation which promotes plant growth and indirectly helps to combat stresses.

These facts correctly suggest that plant–microbe interactions in their native environment profoundly contribute toward fostering resilience of plants to biotic stresses (Shoebitz et al. 2009). Beneficial microbiomes in the rhizosphere upon interaction with plant root interfaces incite signaling events leading to the resistance response in plants (Hussain et al. 2018b). Nonpathogenic microbes activate induced systemic response (ISR) in plants (Tiwari et al. 2017) which immunize the plant well before pathogen attack using JA and ET pathway. Another pathway of inducing defense response is systemic acquired resistance (SAR) which, unlike ISR, uses the SA pathway to sensitize the plant about stress (Pieterse et al. 1998). Both the

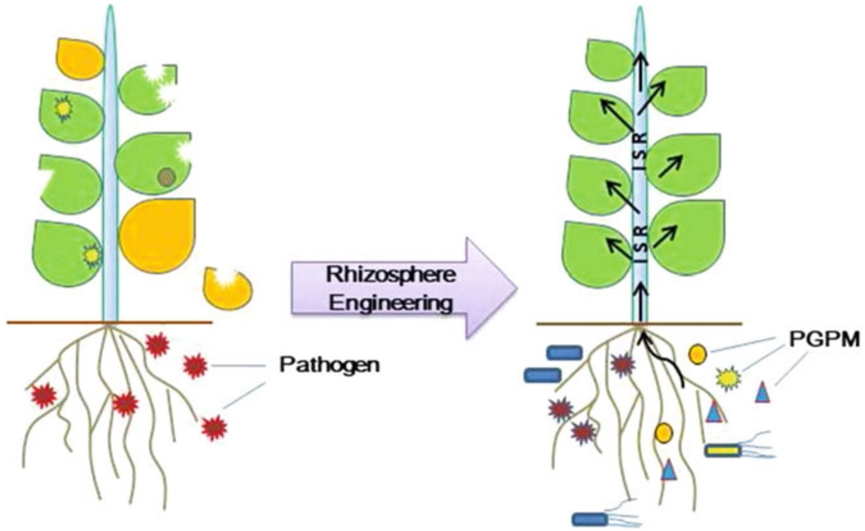

Fig. 14.2 Application of nonpathogenic microbes/PGPM to plants activates induced systemic response (ISR) in plants

pathways can extend tolerance even up to the distal parts of the host plant. NPR is the gene which connects, coordinates, and fine-tunes between ISR and SAR pathway which ultimately determines whether defense-related genes (in case of ISR) or PR genes (in case of SAR) to be induced (Choudhary et al. 2016). The best part about ISR is that while SAR comes into action only after pathogen attack or other biotic stress, ISR is completely independent of such an event which enables them to prime host plant before the stress being imposed. The components of the defense barrier in a plant which encompasses a range of signaling molecules get accumulated in this process causing the establishment of non-specific resistance against the biotic stress. ISR is brought about when elicitors and other chemicals secreted by PGPM come into contact with the root surface triggering an immune response in the plant (De Zelicourt et al. 2013) (Fig. 14.2).

Plants also derive benefits from PGPB particularly when it comes to extracting micronutrients in a nutrient-deprived soil. Many PGPB release “siderophore,” an organic substance which is known for binding Fe3+ and by the virtue of it, they make Fe3+ available for the host plant. Not only that, by sequestering Fe3+, they render a kind of difficult survival to the pathogenic fungi dwelling in the rhizosphere (Choudhary et al. 2016).

The approach of making plants sturdy exploiting rhizobiome engineering and to help them fight against biotic stress has got growing attention all over the world in the past few years. Some successful endeavors also have been reported in this regard (Table 14.4).

Mycorrhizae and actinomycetes also contribute significantly toward the augmentation of plants’ ability to adapt to the effect of stresses. Some reports have been

Table 14.4 Examples of PGPM ameliorating biotic stress in different crops

Crops	Particular of biotic stress	PGPM	Reference
Cabbage (*Brassica oleracea*)	Black rot (*Xanthomonas Campestris*)	*Paenibacillus* sp.	Ghazalibiglar et al. (2016)
Cucumber	Cucumber mosaic Cucumovirus (CMV)	*Pseudomonas fluorescens*, *Bacillus subtilis*, *Azotobacter chroococcum*	El-Borollosy and Oraby (2012)
Panax ginseng	Root diseases (*Phytophthora cactorum*)	*Bacillus amyloliquefaciens* HK34	Lee et al. (2015)
Rice	Bacterial leaf blight (*Xanthomonas oryzae*)	*Bacillus* sp.	Udayashankar et al. (2011)
Pepper	Gray leaf spot disease (*Stemphylium lycopersici*)	*Brevibacteriumiodinum* KUDC1716	Son et al. (2014)
Wheat	*Rhizoctonia* and *Pythium* root rot	*Pseudomonas sp.*	Mavrodi et al. (2012)
French bean	Root-rot (*Rhizoctonia solani*)	Arbuscular mycorrhizal fungi and *Pseudomonas fluorescens*	Neeraj and Singh et al. (2011)
Rice	*Xanthomonas oryzae*pv. *oryzae*, *Rhizoctonia solani* and *Pyriculariaoryzae*	*Delftiatsuruhatensis* HR4	Han et al. (2005)
Arabidopsis	Cucumber mosaic virus (CMV)	*Serattia marcescens* 90–166 and *Bacillus pumilus* SE34	Ryu et al. (2004)
Red pepper	*Myzuspersicae*	*Bacillus cereus* MJ-1	Joo et al. (2005)
Soybean	Wilt (*FUSARIUM oxysporum*)	*Carnobacterium* sp. SJ-5	Jain and Choudhary (2014)
Maize	*Colletotrichum Gloeosporioides, Fusarium culmorum, Fusarium oxysporum* and *Sclerotium rolfsii*	*Burkholderia tropica*	Tenorio-Salgado et al. (2013)
Bell pepper	*Myzuspersicae* (Sulzer)	*Bacillus amyloliquefaciens*	Herman et al. (2008)
Common bean	Bacterial wilt *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*	*Bacillus subtilis* UFLA285 and ALB629	Martins et al. (2013)

documented which reveal the role played by certain fungi for betterment of their host plants (Rodriguez et al. 2008). For example, the plant, *Dichanthelium lanuginosum,* acquires heat tolerance with the help of its associated microbe *Curvularia protuberata* (De Zelicourt et al. 2013). A non-pathogenic Colletotrichum strain is also known to impart disease resistance to host plants but resistance is localized in nature in contrast to *Piriformospora indica* attributed resistance which protects the host plant from disease systematically (De Zelicourt et al. 2013).

14.5 Conclusions and Future Prospects

Plants being sessile are extremely exposed to various sorts of stresses which pose a great threat to plant productivity and sustainability. Chemicals are broadly in use to mitigate these problems but they prompt alarming ecological imbalance. So it appears tremendously important to come up with a solution which can sensitively balance both ecology and economy.

Exploring microbial communities in soil best fits this demand. They can assure a handsome yield as well as good quality even when the host plants are under environmental stress and improve plant health. Stress-tolerant PGPM and AM fungi impact on plants by reinstating physiological and biological set up as per the need to impart a nonspecific and sustainable tolerance in host plants.

Hence, the approach of reshaping the rhizosphere with stress-resistant beneficial microbes and fungi to drive the future agriculture research programs to ensure global food security and sustainability as well as enriched soil health is going to be vigorously embraced in very shortly by the world's scientific community. To formulate this approach, characteristics of a diverse range of microbes and their relationship with the host plant needs to be thoroughly investigated in order to help agriculture achieve a boom.

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Understanding the Microbiome Interactions Across the Cropping System

15

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Abstract

Our planet and its inhabitants have been shaped by micro organisms for over 3.5 billion years. Various studies have been carried out on interactions between these microbes and plants but traditionally emphasized on pathogenic interactions. After numerous depictions and attempts to evaluate the incident and their impersonation to boost microbial diversity linked with flora, it is pretended that somewhat a dinky portion of plant-interacting microbes are pathogenic or harmful comparable to the beneficial one. Mostly microbes inhabiting plant-related alcoves have impartial or advantageous roles in plant buildup. Soil microbiome symbolizes the immense reservoir of biological diversity acknowledged in the world so far. In the present chapter, we counsel the perception that for sustainable development of agriculture we have to understand the mechanisms by which plants interact with their microbiome. This interaction may directly affect the plant health and development which ultimately leads to the organization of novel

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microbiome-driven strategies. In addition, for the benefit of agriculture and food production, we also discussthe potential for root microbiome modification. So, in the above-mentioned context this article will help the researchers to uncover the critical areas of the microbiome that many researchers were not able to explore earlier.

15.1 Introduction

Microbes are considered a prime requisite for the prolongation of life on Earth, yet we figure out the little bit about the plurality of beneficial microbes present in the environments such as soils, oceans, in the atmosphere and even those living on and in our own bodies (Turner et al. 2013). Nowadays, understanding the plant-associated microbial communities and their impact on plant health and development is gaining much interest for sustainable agriculture (Beirn et al. 2017). Soil is referred to as the greatest reservoir of microbial diversity consisting of both beneficial and harmful microbes and plant roots are in a close association to these microbial communities (Gams 2007; Bue'e et al. 2009; Berendsen et al. 2012). This narrow soil zone around the plant root is known as rhizosphere and known as the most active zone for soil microbial activities. Root microbiome or microbial community associated with the plant root is also mentioned as the plant's second genome since composite genome size of the communities of microbes is much larger compared to the plant genome (Lareen et al. 2016). The structure of the rhizosphere communities of microbes is influenced by root exudates in a very coordinated way. However, regarding this connection between microbiome assemblage, root exudates, and plant development our knowledge is still limited (Chaparro et al. 2014).

A blooming recognition of biodiversity and its function in combination with advances in data analytics technologies and omics is needed for sustaining the brisk advances in microbiome exploration and research (Srivastava et al. 2015). One propulsive motivation regarding harnessing beneficial microbes and reducing impacts of detrimental microbes in both humans and crop plants is nearly common (Busby et al. 2017). Joshua Lederberg was the first who antecedently used the word "microbiome" and defined it as the "*ecological community of commensal microorganisms, symbionts or pathogens, which literally occupy a space in our body*" (Lederberg and McCray 2001). Recently, in the presence of specific sets of collective microbes, it was found that most of the features of the host have been linked with the human body which contributes as a great reservoir of microbes proved during the Human Microbiome Project (Turnbaugh et al. 2006; Djikeng et al. 2011; The Human Microbiome Project Consortium 2012). Just a while ago, the utilization of this idiom has been widely tested with the contrasting sets of microbes found in particular hosts or populates of present surroundings (Boon et al. 2014; Ofek et al. 2014). Boon et al. (2014) projected that the most excellent ever description of "microbiome" would relate to the set of genes encountered in partnership of a specific environment with the host, thus showing the less influence of the link among taxonomy and range of capabilities of the microbial community members. The conceptual importance of the microbiome to plants is made doable only by using detected presumptions, which carry out various imperative activities in association with significant microbial faction. This issue has been addressed by some authors, viz. Bulgarelli et al. (2013), Turner et al. (2013), and Rout (2014).

In the present chapter, special emphasis has been put on the characterization of phyto-microbiomes by not only depicting the sub-divisions where the microbes live (phyllosphere, endosphere, and rhizosphere) but also by discussing the need of interactions among plants and microorganisms (Srivastava et al. 2011). The efforts related to microbiome will improve the prognosis of ecosystem response and will assist the progress of the development of new, robust, microbiome-based solutions against significant challenges of our time. So, in the present article, the perception is that for more sustainable development of agriculture we have to understand the mechanisms by which plants interact with the beneficial microbiome.

15.2 Plant-Associated Microbial Communities in Cropping System

Being represented as the most biodiverse ecosystem on Earth, soil characteristics in the form of matrix and texture are crucial for the soil to serve as the reservoir of microbes that interact with flora and fauna of the particular surroundings (Vogel et al. 2009). The soil microbiome is culpable for their signature series of actions to achieve a significant result which are directly related to plant health occurring under this environment (Attwood et al. 2019; Compant et al. 2019). For instance, the soil microbiome has the capability to suppress various phytopathogenic diseases by impressing some restrictions on physiological functions of the related pathogens vital in infecting and colonizing plant tissues (Weller et al. 2002; Mendes et al. 2011). Evenly, the soil microbiomes also pass on a certain degree of resistance to the system against “invaders,” thereby connecting the microbiome diversity to its peculiar ability to limit the situation or prohibit the survival and dissemination of exogenic microorganisms (van Elsas et al. 2012).

Astounding potential has been shown by plant-associated microbiomes during the improvement of plant yields in farming/cropping systems (Lyu et al. 2020). There are many pieces of evidence which prove that the biological technologies using microbes or their metabolites are beneficial in the enhancement of crop yield by nutrient uptake, by managing pests/pathogens and also by mitigating the plant abiotic stress responses. However, for utter realization of the technological potential of microbes, their consistency and efficacy under the wide-ranging extent of the real-world environment need to be upgraded. While the use of biopesticides and biofertilizers is rapidly expanding to cover a wide range of soils, crop varieties, and environments, crop breeding programs have yet to incorporate the selection of beneficial plant-microbe interactions in order to breed “microbe-optimized plants.” Developing attempts to delve into microbiome engineering could lead to microbial

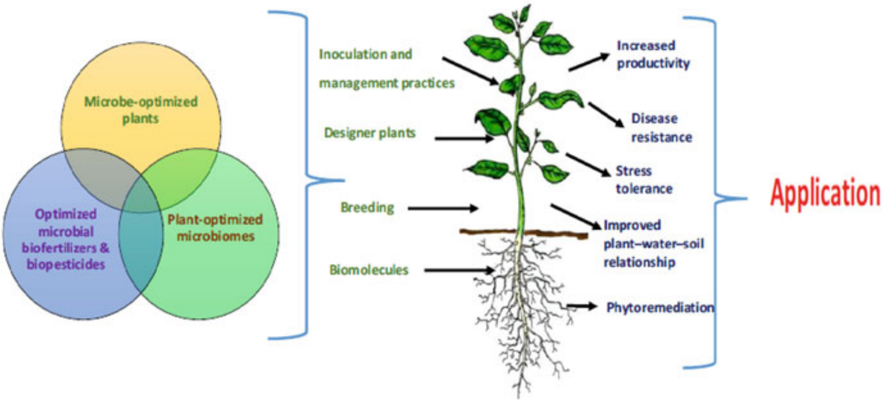

Fig. 15.1 Microbiome interaction fulfilling multiple sustainable development goals (SDGs)

consortia that are exceptionally adapted to hold the plants. The combination of all the three approaches should be unified for the enormous benefits and also to boost agricultural production significantly (Trivedi et al. 2017).

Undoubtedly, development of some traditional agricultural practices has not so far been able to meet the future requirements because they are neither economical nor environmentally feasible. So, there is an urgent need for complimentary feasible approaches to meet the global food demands of the booming population. Another way to develop advanced and improved sustainable crop production methods is to embellish the beneficial plant-associated microbiome having the potential to increase crop growth and vigor, biotic/abiotic stress tolerance, nutrient use efficiency, and disease resistance (Fig. 15.1). If this potentiality of microbes could be harnessed under real-world conditions, it will be very effective in the improvement of farm productivity and produce quality sustainably framing healthy environmental, social, and robust economic outcomes (Trivedi et al. 2017). Because microbe-based formulations can raise the crop yields and also prove promising to or replace, reduce, or at least complement the chemical source of pesticides and fertilizers. Nowadays, many agro-chemical companies have initiated attempts to explore individual microorganisms which can be used as biocontrol or biofertilizer products and also to develop carrier-based inoculants of beneficial strains. An increase of 10–20% in the production of economically important crops has resulted in large-scale field trials (Pérez-Jaramillo et al. 2016).

Considering the microbiome an effective constituent of the host and also responsible for biotic and abiotic changes in the environmental conditions, it is important to understand the composition of the most important diverse plant microbiomes in a better way. Recent studies are more focused to understand the major phylogenetic and functional factors that can influence microbial assembly. The plant microbiome can be separated into three major components, i.e., rhizosphere, endosphere, and phyllosphere (Hardoim et al. 2008; Hirsch and Mauchline 2012) (Fig. 15.2). The rhizospheric zone of soil contributes as a most active narrow zone for soil microbial

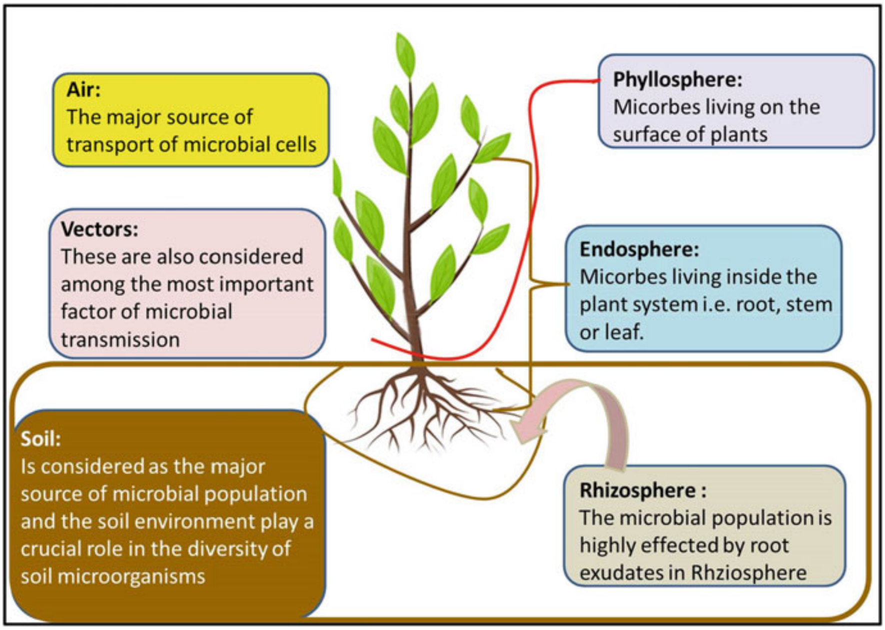

Fig. 15.2 Schematic representation of the major sources for microbes that compose the plant-associated communities: the rhizosphere, endosphere, and phyllosphere

activities beneficial for plant metabolism (Bulgarelli et al. 2013; Philippot et al. 2013). Endosphere is composed of microbial habitats in the plant system the members of which inhabit inner tissues of the plant asymptomatically (Hallmann et al. 1997; Hardoim et al. 2008), and the phyllosphere is inhabited by the microbes capable to colonize plant surfaces (Lindow and Brandl 2003; Lambais et al. 2006).

15.3 Plant Selection for the Rhizosphere Microbial Communities

The soil region under the influence of the roots is known as the rhizosphere zone of a plant (Hiltner 1904; Hartmann et al. 2008; Philippot et al. 2013). Root exudates determine soil microflora by releasing some selective growth substrates thereby selectively influencing the expansion of fungi and bacteria that colonize the rhizosphere zone of a plant by altering the soil chemistry of the environs. In return, microbes also influence the quantity, components, and composition of miscellaneous root exudates by affecting cell metabolism, root cell leakage, and plant nutrition. Microbial communities of the rhizosphere zone can vary on the basis of differences in rhizo-deposition and exudation in structure and species composition of specific root zones at different locations or soil type, plant species, nutritional status, age, and stress, be it biotic or abiotic (De Leij et al. 1994; Mahaffee and Kloepper 1997;

Lupwayi et al. 1998; Griffiths et al. 1999). Some root exudates secreted during the growth of new roots in the elongation zone just following the root tips are beneficial for the growth of colonizers of primary root that can efficiently utilize organic acids and degradable sugars. But in the older root zones, primarily carbon is put in place of safe-keeping as sloughed cells and consisting of more recalcitrant materials, including lignified hemicellulose and cellulose so that bacteria and fungi in these older root zones are probably adapted for oligotrophic conditions. Other nutritionally specific zones include the sites of lateral root emergence and non-growing root tips, distinctly known as nutrient-rich environments colonized by mature communities of microbes (Yang and Crowley 2000). In nutshell, taking all these above-mentioned effects together, plants have evolved to grant the rhizosphere zone to attract specific microbes necessary for the growth and development of plants (Mendes et al. 2011; Prashar et al. 2014).

15.4 Endosphere—A Niche for Intimate Friends

Diverse communities of various microbes consisting of bacteria, archaebacteria, fungi, and other protistic taxa spending some parts of their life cycle by living inside the plants are technically known as endophytes (Hardoim et al. 2015; Pavithra et al. 2020). Further, over the coming years the concept of endophyte is likely to change and expectedly will evolve by some researchers realizing that plant tissues could be colonized by bacteria as much as fungi living inside plants (Hallmann et al. 1997). This concept of endophytes proves that plants are firmly associated with the microbes present in their surroundings particularly with those living inside the plants rather than living alone as lone entities. Recently emerged concept of the "plant microbiome," i.e., the composite genomes of microbes living associated with plants has paved the way for the stimulation of new ideas regarding the evolution of plants where selective forces do not act solely on the plant genome itself but rather on the entire plant, including its microbial community. Hologenome approach for vertical transmission of beneficial traits provided by endophytes to plants is the best example used to explain the Lamarckian concepts of acquired heritable characters (Rosenberg et al. 2009).

While the observation of the microbial cells presents inside the plant tissues, De Bary (1866) was the first person who described the presence of other non-pathogenic organisms inside plants. This observation was explored at the end of the last century until after the emergence of the endophytic concept. Universally, the concept behind endophytes is based on the capacity to identify the microbial cells from formerly surface-sterilized plant tissues (Hallmann et al. 1997); in nutshell this proves that these microorganisms are not epiphytes. A functional definition for endophytes was also provided by Petrini (1991), as "Organisms which colonize plant tissues internally while spending some part of their life cycle without causing any apparent harm." Endophytic communities have been divided into two sub-groups, viz. "obligate" and "facultative" by some authors (Das et al. 2021) (Ref., if any, please). Hardoim et al. (2008) classify the obligate endophytes as those which depend on plant metabolism for their survival, being spread among plants by the activity of vectors or by vertical transmission. The endophytes living outside the host during a certain stage of their life cycle are known as facultative endophytes, which are recruited by the plant from adjacent communities in the form of bulk soil, mainly through the rhizosphere zone (Andreote et al. 2014; Harman and Uphoff 2019).

In nutshell, the endophytic microbes play crucial roles in plant growth, development, and diversification. The increased awareness and information related to endophytes provide intuitiveness into the complexity of the plant microbiome. The basic nature of plant–endophyte interactions ranges from mutualism to pathogenicity depending on the set of biotic and abiotic factors including environmental conditions, microbes, and plant genotypes along with their dynamic connection of interactions within the plant biome. Accordingly, the latest insights into evolution, plant ecosystem functioning, and multipartite interactions are fulfilled by the concept of endophytism (Hardoim et al. 2015).

15.5 Microbial Groups Living in the Phyllosphere

A third component of the plant microbiome, i.e., the phyllosphere is made up of microbes colonizing the aerial plant tissues although this term can be used for any extrinsic plant surfaces (Vorholt 2012). The phyllosphere contributed as an enormous environment on Earth that is supposed to be an area of 6.4×10^8 km^2 fully colonized by microbes (Morris and Kinkel 2002).

Our knowledge regarding the phyllosphere microbiology or the microbiome of aerial parts of plants has historically lagged comparative to the rhizosphere or the below-ground habitat of plants, particularly concerning fundamental investigations such as which microbes are prevalent and what activities do they perform there. However, for the enhancement in this regard recently floated cultivation-independent studies have revealed that a few bacterial phyla predominate in the aerial parts of several distinct plants and the plant factors which are involved in shaping these phyllosphere communities, feature specific adaptations, and exhibit multipartite relationships both with host plants and among community members. Awareness regarding the structural principles of indigenous microbial phyllosphere populations will help us develop an understanding of the phyllosphere microbiota also showing advantageous promotion of plant growth and protection.

Many fungi whether they are filamentous or yeasts-like, bacteria, algae, and at lower densities some protozoa and nematodes are comprised by the phyllosphere community (Lindow and Brandl 2003). Out of all, the bacterial community is the major group presented in the phyllosphere the numbers being between 10^5 and 10^7 cells/cm^2 (Andrews and Harris 2000). Microflora of phyllosphere is characterized to thrive well in an oligotrophic environment, i.e., live under harsh environmental conditions where there is an unavailability of nutrients and under fluctuating conditions of humidity, presence of ultraviolet radiations along with a wide range of temperature and pH (Andrews and Harris 2000).

The microbial communities of the phyllosphere performing an efficient role in the processes related to plant growth, for example, by fixation of nitrogen, biosynthesizing phytohormones along with the protection of plants facing biotic stresses (Jones 1970; Freiberg 1998; Brandl et al. 2001; Kishore et al. 2005). These above-mentioned activities make them the main contributor of many global processes such as the sequestration of carbon (Bulgarelli et al. 2013), and they can also be potentially used for the sustainable development of agriculture. Thus, microbes present in the phyllospheric region play a promising role to offer the eco-friendly protection of plants (Lindow and Brandl 2003).

15.6 Microbial Interaction Across the Cropping Systems

Farming/cropping systems are broadly grouped into (1) those which use chemical or synthetic means of pest control and nutrient fertilization (a.k.a. conventional), and (2) those which favor an integrated system with the goal of sustainability (a.k.a. organic). Within each system, a number of management techniques may be used which collectively alter the above-ground and below-ground biodiversity, including chemical use, fertilization, irrigation, crop rotation or crop-fallow rotations, co-cropping, and livestock grazing. Several studies broadly comparing organic and conventional systems have shown differences in crop production, competition by weeds, pests, or microbial pathogens (Pollnac et al. 2009). Notably, organic farming, and often the increased soil organic matter associated with organic farms, is selected for a higher overall microbial diversity (Flohre et al. 2011; Chaudhry et al. 2012; Pershina et al. 2015; Hartmann et al. 2015; Ishaq et al. 2016).

15.7 Soil Fertilization

Soil fertilization utilizes organic matter (mulching) or chemical supplementation to add nutrients back into the soil. Long-term use of mineral fertilizers has been shown to increase bacterial and fungal diversity, microbial biomass carbon, as well as dehydrogenase and another enzyme activity (Luo et al. 2015). However, these benefits are variable depending on the type and source of minerals. Using only mineral nitrogen (typically ammonium sulfate) does not increase soil microbial diversity (Ramirez et al. 2010; Luo et al. 2015; Zhalnina et al. 2015) and may even reduce it (Campbell et al. 2010). Phosphorous-only supplementation has a similar lack of effect (Zhalnina et al. 2015) except where it was limiting (Su et al. 2015). This reduction may be driven by a shift toward more acidic soil which tends to reduce total microbial diversity and shift toward acid-tolerant species, such as within the bacterial phylum Acidobacteria (Lauber et al. 2009; Rousk et al. 2010; Fierer et al. 2013; Zhalnina et al. 2015). It may also be a function of the relative type and amount of plant residues (Roesch et al. 2007), or a change in nutrient availability and the C:N ratio in soil (Ramirez et al. 2010; Zhalnina et al. 2015).

Animal manure has been shown to be significantly more effective at increasing microbial biomass than mineral fertilization (Hartmann et al. 2015; Luo et al. 015). Integrated livestock grazing has recently re-emerged as an alternative method of crop-residue removal, specifically in organic systems (McKenzie et al. 2016). Its implementation has been slow, especially in large production systems, as the use of grazing livestock can be time- and labor-intensive. Inputs of feces and urine from livestock grazing increases soil organic carbon and nitrogen (Liu et al. 2015), as well as total microbial biomass (Liu et al. 2012, 2015). However, this may only be reflected in bacterial biomass and not an increase in fungal biomass (Taddese et al. 2007). In systems where grazing pressure is high, this effect can be reversed as soil nutrients are lost to erosion caused by a lack of plant cover material (Mofidi et al. 2012; Chen et al. 2015; Liu et al. 2015).

15.8 Cover Crops

Cover crops are grown as an alternative to fallowing or leaving a field unplanted to rest. They provide additional economic benefit (Adusumilli and Fromme 2016; Duzy et al. 2016), feed for livestock (Sulc and Franzluebbers 2014), reduce erosion, and facilitate weed and insect pest management (Dabney et al. 2001; McKenzie et al. 2016; Duzy et al. 2016). Specifically, cover crops can reduce weed seed production via competitive exclusion (Gallandt et al. 1998), or survivability of weed seeds gets decreased by recruiting a microbial community which contributes to seed decay (Dabney et al. 1996; Liebman and Davis 2000). Mineralization of cover crop residues causes an increment in the organic matter of soil (Reeves 1994; Hartwig and Ammon 2002), which can increase cation exchange capacity, and enhance the cycling of macronutrients (Kamh et al. 1999).

Not only do the additional inputs of organic matter from cover crop residues encourage microbial diversity, but they also allow the above-ground biomass to generate more below-ground biomass (Wild 1993; Reeves 1994; Hu et al. 1999; Hartwig and Ammon 2002; Snapp et al. 2004). Crop rotations can also improve soil quality and microbial diversity (Ghimire et al. 2014). The use of legumes as a cover crop or in rotation, or other crops which encourage rhizobial symbiotic bacteria to biologically fix nitrogen, and the subsequent mineralization of those nitrogen-rich plant residues back into the soil can provide usable available nitrogen for other plant species (Snapp et al. 2004; Biederbeck et al. 2005). For example, bacterial liter increased most in response to clover (*Trifolium repens* L.) conditioning compared with wheat (*Triticum aestivum* L.), ryegrass (*Lolium perenne* L.), bentgrass (*Agrostis capillaris* L.), or sucrose conditioning (Grayston et al. 1998). Additionally, microbial communities differed strongly among the four cover crop conditioning species (Grayston et al. 1998).

15.9 Tillage

In both organic and conventional systems, tillage is the most common method of incorporating crop residues back into soil, as well as redistributing weed seeds either further into soil to prevent germination or onto the surface where they may be eaten. Due to the disruptive nature of tillage in the first 30–50 cm of topsoil, significant detriment can be done by physically destroying mycorrhizal root colonization (McGonigle et al. 1990). Moreover, soil microbial diversity and density is highly correlative to soil depth and local factors (e.g., oxygen content, UV light, moisture). Thus, intensive soil tillage can drastically decrease soil microbial diversity and density, specifically bacterial and fungal, through erosion and wind dispersion of microorganisms or nutrients, or through selective culling of sub-surface species brought to the surface (Lupwayi et al. 1998; Castillo et al. 2006; De Quadros et al. 2012; Mathew et al. 2012; Fierer et al. 2013; García-Orenes et al. 2013; Ghimire et al. 2014). However, the addition of soil organic matter through mulching may attenuate some of these adverse effects (García-Orenes et al. 2013; Ghimire et al. 2014). No-till systems typically have more soil carbon (Brevik 2013).

15.10 Chemical Control and Bioremediation of Farmland

Chemical control used for managing agricultural systems has been shown to alter the microbial community, notably in decreasing diversity (elFantroussi et al. 1999; Lupwayi et al. 2004; Lo 2010). However, the persistence of pesticides and other chemical contaminants in soil is also of concern for biological systems in natural and agricultural settings, not only because they may accrue and affect other beneficial organisms and soil health indicators, but many contain heavy metals which are toxic (Hussain et al. 2009). Additionally, the local water sources and runoff may add contaminants from exogenous sources. Phyto, microbial, or combined bioremediation of chemical contamination has been sought to degrade or detoxify pesticides (i.e., herbicides, insecticides, fungicides, rodenticides), heavy metals, and antibiotics.

For the above purpose, bacteria belonging to the genera *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Burkholderia*, *Corynebacterium*, *Flavobacterium*, *Micrococcus*, *Mycobacterium*, *Pseudomonas*, *Sphingomonas*, and *Rhodococcus*, and the fungus *Phanerochaetechrysosporium* are just a few of the microorganisms shown to degrade different types of hydrocarbons from petroleum spills (Kuhad et al. 2004; Hussain et al. 2007a, b, 2009; Das and Chandran 2011). The degradation of chemicals, the sequestration of heavy metals, or the detoxification of heavy metal compounds by microorganisms is dependent on the nature of the compound, as well as on the ambient conditions of the environment (Kuhad et al. 2004; Singh 2008). Endosulfan degradation depends on soil type and oxygen content (Kumar and Philip 2006a, b), as well as on soil texture, organic matter content, inoculum concentration, pH, and specificity of bacterial strains used (Hussain et al. 2007a). Similarly, dichlorodiphenyltrichloroethane (DDT), metoxychlor, and gamma-hexachlorocyclohexane (gamma-HCH) degradation processes are dependent on temperature (Baczynski et al. 2010). HCH degradation was also shown to be dependent on oxygen content and nitrate concentration (Langenhoff et al. 2002). An additional nutrient source, such as molasses, is often needed to increase the rate of chemical degradation in culture (Lamichhane et al. 2012; Hussain et al. 2014).

Field trials have been focused on removing chemical and metal contamination from soil or water runoff, either using direct application of microorganisms or by the use of a "biobed" as a biological filter or retaining system to remove contaminants from farm wastewater (Antonious 2012). The bacterium *Mycobacterium gilvum* was successfully used to degrade polycyclic aromatic hydrocarbons, and increase soil bacterial diversity, on a vegetable farm (Ma et al. 2018). A strain of *Arthrobacter* and another of *Bacillus* were used to reduce metal contamination in soil, improve rice biomass production, and reduce the amount of metal accumulated in rice (Du et al. 2016). Halophilic bacteria were used to remove the salt left behind after the March 2011 tsunami in Japan, as well as green compost to restore organic matter that had been washed away (Azizul and Omine 2013). Furthermore, bacteria that are able to mitigate salt-stress in plants can promote growth into similarly affected areas (Cao et al. 2008; Nabti et al. 2015).

The concept of remediating soil diversity toward a "more natural" community has been slower to take root. A study of pre-agricultural prairie soil reported a very different bacterial community than that found in the human-associated agricultural soil (Fierer et al. 2013). Notably, prairie soils were dominated by the bacterial phylum Verrucomicrobia, whereas agricultural soil shows a dominance of Proteobacteria, Bacteroidetes, or Firmicutes (Lauber et al. 2009; Ishaq et al. 2016). Verrucomicrobia grow more slowly, but survive better in nutrient-limiting soils. Likewise, Acidobacteria are also known to survive under nutrient-limiting (oligotrophic) conditions (Fierer et al. 2012; Koyama et al. 2014; Greening et al. 2015; Kielak et al. 2016). Moreover, Verrucomicrobia from pre-agricultural soil contained more genes for carbohydrate metabolism than nitrogen metabolism (Fierer et al. 2013), suggesting that their abundance in agricultural soil may be negatively selected for by the use of nitrogen fertilizer. And, as Proteobacteria produce the quorum-sensing molecule AHL which triggers beneficial and pathogenic responses from bacteria, selecting for these species under agricultural conditions may be contributing to plant disease dynamics.

15.11 Understanding and Exploiting Plant Beneficial Microbes

We all know about the link between the trillions of microorganisms that exist inside the body related to our health. Earlier studies have already proved that some sort of depressions and food allergies has been prevented by the activities of some healthy microbiome present inside the body. These types of miniatures also perform a favorable role in plant growth and development similar to what they do inside the human body. Various methods are adopted for the addition of growth-promoting bacteria or fungi by using a variety of ways like an addition inside the seed coats, suspended in water and sprinkled on plant or soil surfaces, or mixed into mulches that are added to the soil or placed around plant stems which will be beneficial for the plant growth and development with a robust yield (Ishaq 2017; Srivastava et al. 2020). Nowadays, this microscopic world attracts more attention of researchers looking for some new cost-effective eco-friendly techniques for the betterment of agriculture production (Srivastava et al. 2019).

15.12 Nitrogen Fixation

The entire range of flora present around the natural ecosystem is closely associated with microbes, including bacteria and fungi (Finkel et al. 2017). The symbiotic relationship between the plants and microbes has been proved by evidence of fossils since the last four million years. To cope with the numerous challenges of the environment, plants rely on microbes for growth since their evolution. For example, various growth-promoting microorganisms such as fungi and bacteria can fix nitrogen from the air and then make it available to plants for growth and development. Most of these microbes inhabit the surface of roots often inside special structures known as root nodules (Gage 2004).

The first formal description of the enhancive role of soil microbes on agricultural plant growth was given by Lorenz Hiltner in 1904, though agricultural treatments anticipated to customize the microbial activities present in the soil have been utilized since Roman times. For example, the Romans used to plant alfalfa and clover that form close associations with nitrogen-fixing bacteria during the cultural practices like crop rotations which were helpful in improving soil fertility. They didn’t exactly know the scientific reason of this practice but now we do know that these plants can elevate the nitrogen content of the soil.

There are also enormous fungal species that are able to build a symbiotic association with the roots of different higher plants by forming some structures known as mycorrhizae (Denison and Kiers 2011). Mycorrhizae spread into the root system of the plant in association and the fungal mycelium intermingles or passes through the plant cells/tissues with the help of some specific structures like Hartig net, vesicles, and arbuscules. by which nutrient uptake or exchanges occur for enhancing plant growth and development. By using these specific structures fungi enable the plant to scavenge water from the surrounding soil and also allow the plant to exchange sugars for nutrients. Microbes which are able to establish a mutually beneficial partnership by forming specialized structures with the plants are scientifically recognized as symbiotes (Skriabin 1923) (Table 15.1).

Table 15.1 Types of nitrogen fixation along with their examples

Type of nitrogen fixation	Important examples
Symbiotic	*Rhizobium* and *Azospirillum spp*
Asymbiotic or free living	Classified into three types:
(a) Aerobic	*Azotobacter, Beijerinckia, Nostoc, Anabaena, Tolypothrix, Aulosira*
(b) Anaerobic	*Clostridium, Desulfovibrio, Rhodospirillum, Rhodopseudomonas, Desulfotomaculum, Chromatium, Chlorobium*
(c) Free living	*Klebsiella pneumoniae, Bacillus polymyxa*
Associative symbionts	*Azospirillum, Herbaspirillum, Acetobacter diazotrophicus, Azoarcus*

15.13 Balancing Action of Lodgepole Pine

Lodgepole pine is a perfect plant host example of beneficial non-symbiotic or free-living bacteria and fungi (Beirn et al. 2017) which can live in the soil, plant roots, on plant surfaces, or even within the plant tissues. Beneficial free-living microbes, especially endophytes that live within plant tissues, whether they are acting as symbiotes or not, are able to suppress the phytopathogens along with other harmful organisms thereby affecting the plant metabolism and health. Additionally, endophytes can also facilitate plant growth directly by regulating plant hormones, activating plant immune responses, and also by providing supplementary nutrients to the plant (Timmusk et al. 2017).

Despite the fact that endophytes have shown enormous potential needed for the betterment of the plant health, discovering some non-symbiotic microbes that usually produce significant positive growth responses under extremely variable field conditions still remain a challenging task. Addition of any beneficial microbe that can improve the growth of plants may not constantly be sufficient because some other group of microorganisms present in the surroundings as a part of the plant's environment will also be affecting the interaction between the beneficial microbe and the plant. For example, a bacterium being necessary for the growth of lodgepole pine can be thwarted by the occurrence of another contending bacterium (Bent et al. 2001). According to the study this effect was shown by both, i.e., by endophytic plant-beneficial bacterium as well as in case of those simply living on the root surface of the plant.

15.14 Microbial Cocktail

The sole meaning of the term is that if we isolate a phyto-beneficial microbe under a protected environment, then it is not certain to be compatible under field conditions unless it is scientifically tested exhaustively. During deciding the specific conditions necessary for the optimum activities of plant-beneficial microorganisms it may also be mandatory to consider the complete composition of the microbiome to which the particular flora is supposed to be exposed either it is soil, root, or plant surface. Exploring the microbes that can be exploited in agriculture for crop production is a prime quest having a long debate (Schloter et al. 2018). During the study about these arduous interactions of plants-microbiomes along with the favorable environmental conditions, we also have to reveal that in what way or manner these age-old traditional know-how and practices can be refined and utilized in the future.

15.15 Advancement Required for Improving Microbiome in Future

Treasured information regarding the genome desperately augments our knowledge related to the diversity of microbial metabolic pathways used to access the novel and innovative traits (Trivedi et al. 2017). These recent discoveries of novel genes evolved some genetically engineered plants for disease resistance, stress, and herbicide tolerance, and last but the ultimate is for crop improvement program (Macdonald and Singh 2014). Nevertheless, most of these breakthroughs were brought with the successful conclusion by inserting some minor or along with the combo of many targeted genes (multiplexing). Forthcoming and imminent researches have to focus on integrating the distinct plan of action, for example, more than one gene of importance simultaneously be incorporated in transgenic plants by using the multigenic approach. Other than this, we have to apply new tools and resources to initiate intricate heterologous pathways into plants (Shih et al. 2016) which possess the key to frame the useful clusters of synthetic genomes from microbiomes, enable the shuffling and stacking of stress-tolerance and disease resistance traits between the crop plants. Further intensification of the rate of novel gene discovery will be fueled up by using the novel efficiency developed in the trait discovery. For example, forward genetic screening based on the CRISPR–Cas9 will be helpful in the future learning of plant–microbiome interactions to surpass particular genes and evolve as a best holistic strategy while explaining the process behind the plant–microbiome interactions along with the uncovering of novel genes needed for biotechnological applications and innovations (Barakate and Stephens 2016).

The integration of microbe-optimized crops for distinct types of soil, microbial biofertilizers, optimized microbiomes, biocontrol microbes, and soil amendments would be the ultimate purpose of action behind the enhancement of plant–microbe interactions. Undoubtedly, being principally untapped, this area should be entitled to considerable research attempts which will prove promising to address the issue of food security by improving crop yields in a sustainable and eco-friendly manner. Globally, the emerging microbiome along with existing microbial technologies and correlated overtures offer advance and most sustainable methods for use to enhance agriculture productivity. Furthermore, if the existing scientific and technological challenges in this area can be planned out along with advanced work strategies (e.g., product registration, safety requirements) emerging microbial-based solutions can potentially reconstruct the field of agriculture sustainably. Above all, it is universally proven that the aforementioned approach can enable us to achieve the multiple

Table 15.2 Important microbes and their role in crop production

Microorganism	Role in crop production
Rhizobium	Acts as biofertilizer for all the leguminous crops
Azotobacter	Biofertilizer for wheat, maize, mustard, cotton, potato, etc.
Azospirillum	Biofertilizer for sorghum, millets, maize, sugarcane, and wheat
Blue green algae, *Nostoc* or *Anabaena*	Biofertilizer for paddy
Pseudomonas putida	Phosphate solubilizing bacteria
Pantoea agglomerans	Phosphate solubilizing bacteria
Nitrobacter	Converts nitrite to nitrate (nitrogen fixation)
Mycorrhiza	Symbiotic association between fungus and roots of higher plants

sustainable development goals (SDGs) if put into action in a truly systematic way (Table 15.2).

15.16 Conclusions

Integration of the beneficial plant–microbe and microbiome interactions is the need of the hour which may prove as a promising sustainable solution for the enhancement of our agricultural production. Holistic ecological studies and reductionist mechanistic discoveries both form a beneficial tactic for the study of plant microbiomes during their interactions. Both schools of thought are substantiating reflective awareness into the ecological operations that take command over plant–microbe interactions likewise the specific molecular mechanisms work behind them. The induction of enormous microbial isolates and of synthetic microbial communities if blended with genetic resources of plants will significantly grant us to tide over the chasm lying in between and to conduct reductionist hypothesis-driven studies in increasingly complex ecological contexts up to field tests. These significant advances will contribute to the next green revolution by potentially revolutionizing knowledge regarding the interactions of plants and microbes occurring in natural ecosystem being utilized in agriculture. This chapter notifies the role of microbial diversity that can be beneficial for flora and will help the researchers to uncover the critical areas of microbiome that many researchers had not been able to explore earlier.

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Role of Rhizosphere Microorganisms in Endorsing Overall Plant Growth and Development

16

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Abstract

The rhizosphere, the abutting soil region under living root proximity, is the abode for diverse microorganisms which are distinct in their ecosystem services. Rhizosphere, being a water and nutrient uptake portal, provides a forum for interaction between soil, microorganisms, and living root which brings desirable changes in soil physicochemical properties impacting plant growth, nutrition, and health. Rhizosphere inhabiting microbes have been known for their nitrogen fixation and mineral solubilizing properties since long. In addition to these conventional roles, microbes have been demonstrated to execute multifarious chores. Bacteriogenic phytohormones, exudates, and volatile compounds act as signals for phytostimulation, activating immunity, morphogenesis, and efficiently modulating root system architecture. Novel agricultural applications offered by the rhizosphere microbes could certainly endow manipulation of phenology anticipating changing climatic conditions. Coping up with the adverse effects of environmental stresses on crop development and safeguarding against pests and diseases are the most important agricultural issues being faced by the farming community. Long-term use of chemicals to achieve targeted agro-based demands cannot be an everlasting approach. The use of rhizosphere microbes can circumvent extreme environments limiting crop production. Exploiting microbial potential as biofertilizers, biocontrol agents, and in rhizoremediation can benefit crop growth with lesser environmental hazards. Utilizing rhizosphere microbial potential could be a promising strategy over existing agricultural practices to overcome the challenges and enable sustainable agricultural production.

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16.1 Introduction

Diverse soil-inhabiting organisms are in continuous interaction with field crops. These organisms belong to micro- to macroscopic world comprising of bacteria, fungi, algae, protozoa, nematodes, earthworms, and insects. These organisms exert beneficial, neutral, or harmful effects on the overall growth and physiology of crop plants. Among all soil-inhabiting organisms, a plethora of microbes is present at root–soil interface forming a close association with roots. The microbial association is established on the root surface (rhizoplane), within the root tissue and/or along the immediately adhered root–soil interface (rhizosphere). Microbes colonizing root tissue are called endophytes. These endophytes play a major role in nitrogen fixation by forming root nodules, of which *Rhizobium* spp. in legumes are the most common example. Many of the non-rhizobia endophytes (*Paenibacillus, polymyxa,* and *Paenibacillus* sp.) also induce nodulation and nitrogen fixation in leguminous crops (Ahmad et al. 2019). Some of the endophytes belong to non-nodulating strains like *Endobacter medicaginis, Brevibacillus choshinensis,* and *Micromonospora* spp. (Ramírez-Bahena et al. 2013; Trujillo et al. 2010). Endophytes play important role in amelioration of biotic and abiotic stresses (Waqas et al. 2015; Herrera et al. 2016; de Zélicourt et al. 2018; Sandhya et al. 2017). Endophytic bacteria comprise a much less diverse yet distinct community than the rhizosphere or bulk soil (Dastogeer et al. 2020; Lundberg et al. 2012; Bulgarelli et al. 2012). Some of the endophytic fungi play substantial roles in modification of ecosystem productivity. Endophytic fungus, *Phomopsis liquidambaris,* increased yield, nodulation, and N_2 fixation in peanut (Xie et al. 2019). Soil inoculation with endophytes (Serratia PRE01 or Arthrobacter PRE05) influences several ecological factors such as root morphology, rhizosphere soil properties, bioavailability of heavy metals, composition of endophytic bacterial communities, and phytoremediation (Wang et al. 2020).

Rhizosphere is the home to an enormous diversity of microbiota which offers a variety of ecosystem services to crop plants. Rhizosphere circumscribes nitrogen-fixing bacteria, plant growth-promoting rhizobacteria (PGPR), mycorrhizal fungi, plant growth-promoting fungi (PGPF), biocontrol microbes and protozoa, in addition to soil-borne phytopathogens. Rhizosphere and root microbiota composition is substantially driven by edaphic factors (pH, nutrient content, root exudates, soil texture, temperature, water availability), crop species, crop genotype, stages of crop development (Dastogeer et al. 2020). Cordero et al. (2020) investigated rhizosphere and root interior associated bacterial communities belonging to cereal (wheat), pulses (field pea and lentil), and oilseed (canola) grown at different locations. Distinct rhizosphere bacterial communities were found associated with each crop. The phyla *Acidobacteria, Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Gemmatimonadetes,* and *Proteobacteria* were the dominant rhizosphere bacteria in the crops studied. Rhizosphere, being colonized by similar bacterial communities, viz. *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Acidobacteria*, and *Gemmatimonadetes,* has been reported previously in winter wheat and barley (Mahoney et al. 2017; Bulgarelli et al. 2015). Some studies contextualized the effect of soil type to be more pronounced on rhizosphere microbial communities than plant genotype (Dombrowski et al. 2017; Bulgarelli et al. 2012).

Rhizoshpere, through intricate interactions between plant roots, soil, and soil microbiota, endorsees plant growth by facilitating nutrient acquisition and transport portal, nitrogen fixation, siderophore production, phytostimulation through released exudates, volatile organic compounds and plant growth regulators, ameliorating biotic and abiotic stresses, signal transduction and shaping plant microbiome for sustainable crop production. Intrinsic biological properties of rhizospheric microorganisms such as nitrogen fixation, maintaining soil phosphorus dynamics through phosphate solubilization, hydrogen cyanide, phytohormones and siderophores production, 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity are appraised as plant growth promotion (PGP) traits. Several rhizosphere microorganisms have been reported exhibiting various PGP traits (Table 16.1). PGPRs are used as biofertilizer inoculants, bioremediation, and for competitive suppression of pathogens/antibiosis. PGP microbes sequester soil minerals and facilitate their efficient uptake by growing plants. Owing to their multi-faceted role in crop growth, the rhizosphere microbial community makes up the main component of integrated farm/crop management. Rhizosphere microbiota can be harnessed in defining root system architecture and manipulating phonological traits. The utilization of PGP microbes in agriculture offers tremendous potential as a cost-effective, eco-friendly, and sustainable alternative. Understanding the factors promoting diversity and richness of beneficial microbes in the rhizospheric region can supplement conventional agriculture transforming it into a sustainable venture. This chapter provides insight into the role of rhizosphere inhabiting microbes in furnishing overall plant growth through direct (nutrient acquisition and assimilation, phytostimulation through exudation and hormones) and indirect (amelioration of biotic and abiotic stresses) mechanisms and their use as an alternative strategy (biofertilizer, modulating root architecture, manipulating phonological traits) to meet environmental standards besides food security (Summarized in Fig. 16.1).

16.2 Microbial-Based Fertilizers

Rhizosphere microorganisms belonging to several bacterial/fungal genera or kingdom protozoa can be potentially used for the production of microbial-based fertilizers. Microbialfertilizers assist in efficient soil nutrient uptake when delivered through seed or soil. Microbialfertilizers contribute to PGP traits, higher-yielding ability as well as improved soil fertility supporting sustainable agriculture. Several studies indicate the potential of rhizosphere microorganisms as biofertilizers for boosting the growth and nutrient content of crops under field conditions (Majeed et al. 2015; Lally et al. 2017). Wheat seeds inoculated with *Streptomyces nobilis* significantly increased shoot and root length, fresh and dry weight, number of leaves and roots (Anwar et al. 2016) as the rhizobacterial isolates efficiently produce IAA, siderophores, ammonia, and hydrogen cyanide along with possessing phosphate solubilization ability. Plant inoculation in wheat using rhizosphere and root-endosphere bacterial isolates *Stenotrophomonas* spp. and *Acetobacter pasteurianus*

Table 16.1 List of rhizosphere microorganisms with their plant growth promoting (PGP) traits

Crops	Microorganisms	PGP traits	References
Wheat	*Streptomyces* spp.	IAA production, phosphate solubilization, siderophore, ammonia and HCN production, ACC deaminase production	Anwar et al. (2016)
	Stenotrophomonas spp., *Stenotrophomonas rhizophila, Acetobacter pasteurianus*	Phosphate solubilization, production of IAA, enzymatic activity	Majeed et al. (2015)
	Bacillus siamensis (PM13), *Bacillus sp.* (PM15) *Bacillus methylotrophicus* (PM19)	Phosphate and zinc solubilization, production of IAA, ammonia, siderophore, exopolysaccharide, enzymatic activities (ACC-deaminase, catalase, protease)	Amna et al. (2019)
	Pseudomonas libanensis	Solubilization of potassium and zinc, production of siderophores, hydrogen cyanide, ammonia and ACC deaminase	Kour et al. (2019)
Rice	*Pseudomonas* sp. *Bacillus* sp.	Production of IAA, siderophores, ACC deaminase and phosphate-solubilization ability	Xiao et al. (2020)
	Pseudomonas aeruginosa, *Ralstonia* picketti, *Burkholderia cepacia, Klebsiella pneumoniae*	ACC utilization, exopolysaccharide production, P and K solubilization	Gontia-Mishra et al. (2017)
Maize	*Bacillus, Halobacillus Pseudomonas*	P-solubilization activity and IAA production	Mukhtar et al. (2020)
	Bacillus polymyxa, B. pantothenticus, B. anthracis, B. thuringiensis, *B. circulans*, *Pseudomonas cichorii, P. putida*, *P. syringae* and *Serratia marcescens*	Production of ammonia and hydrogen cyanide	Agbodjato et al. (2015)
Chickpea	*Cedecea davisae* RS3	Production of ammonia, IAA, phytase, and solubilization of inorganic phosphate and zinc	Mazumdara et al. (2019)
	Rhizobium leguminosarum, Pseudomonas aeruginosa	Production of phytohormone (IAA) and ammonia, phosphate-solubilization, siderophore and HCN (by P. Aeruginosa)	Yadav and Verma (2014)
Mung bean	*Pseudomonas aeruginosa* BHU B13–398, *Bacillus subtilis* BHU M	P solubilization, production of siderophore, HCN and ammonia	Kumari et al. (2018a)
	Pseudomonas spp., *Bacillus* sp., *Acinetobacter* sp.	IAA production, P solubilization, ammonia production, catalase	Kumari et al. (2018b)

(continued)

Table 16.1 (continued)

Crops	Microorganisms	PGP traits	References
		production, siderophore production, and antagonistic activity against phytopathogenic *Rhizoctonia solani*	
French bean	*Aneurinibacillus aneurinilyticus*, *Paenibacillus* sp.	Production of IAA, siderophore, ammonia, HCN and P and Zn solubilization	Gupta and Pandey (2020)
Soybean	*Streptomyces*	Production of siderophores, ACC deaminase, IAA and phenazines	Horstmann et al. (2020)
Sunflower	*Bacillus licheniformis* AP6 and *Pseudomonas plecoglossicida* PB5	P solubilization, IAA and ACC-deaminase activity	Yasmeen et al. (2020)

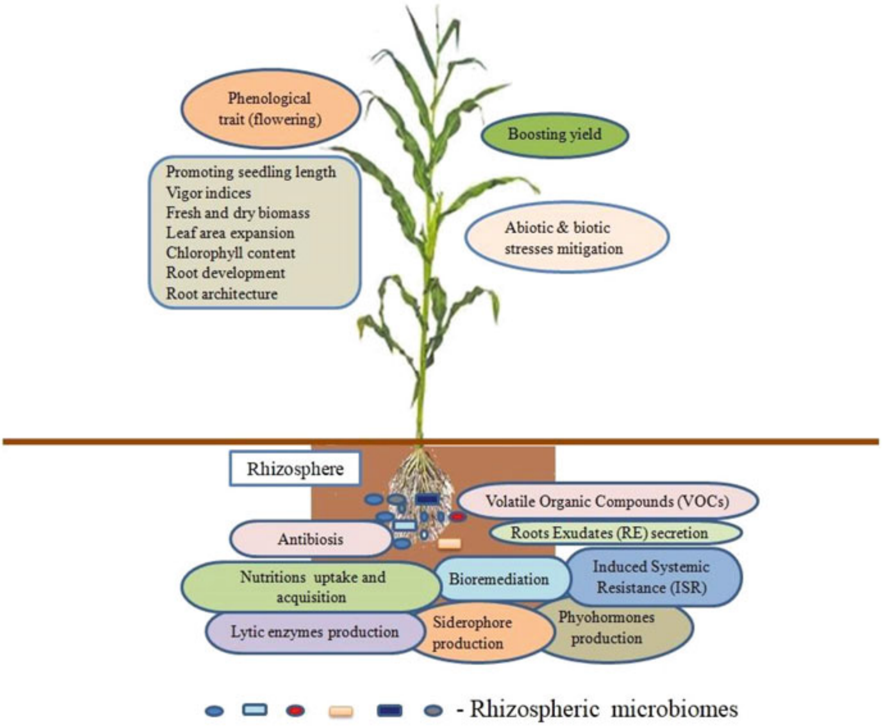

Fig. 16.1 Diverse roles performed by rhizosphere-inhabiting microbes

significantly increased shoot and root length, and shoot and root dry weight, N contents of wheat seedlings in vitro through their role in nitrogen fixation, P-solubilization and IAA-production (Majeed et al. 2015). Seed inoculation with

PGPR strains, viz. *Bacillus licheniformis*, *Bacillus*, *Paenibacillus polymyxa*, *Pseudomonas putida*, significantly increased root and shoot weight in barley (Canbolat et al. 2006). PGPB *Bacillus cereus* and *Klebsiella variicola,* isolated from rhizosphere and root endosphere, respectively increased plant growth and nutrient (iron, zinc, calcium, magnesium, copper, sodium, and potassium) contents significantly in tomato and mungbean. These two PGPB produced IAA, gibberellic acid, and kinetin resulting inenhanced shoot length in tomato and shoot length and dry weight in mung bean (Sunera et al. 2020). Endospore-forming diazotrophic bacilli isolated from the sunflower rhizosphere produced indolic compounds while a few of them exhibited nitrogenase activity as well. *B. mycoides* species significantly promoted N content and shoot dry weights of sunflower (Ambrosini et al. 2016). Application of biofertilizers modulates rhizosphere bacterial communities through changing soil physicochemical properties along with alleviation of mineral phytotoxicity and thus facilitates plant growth (Wang et al. 2019). Besides nutrient supplement to the soil, microbial biofertilizers suppress disease abundance. Fu et al. (2017) reported banana Fusarium wilt to be suppressed by biocontrol inoculant, *Bacillus amyloliquefaciens*. Biofertilizer-amended rhizosphere soils exhibited increased abundances of *Cryptococcus, Dyadobacter,* and *Sphingobium* while lowered abundances of *Burkholderia Fusarium* and *Ralstonia* (Fu et al. 2017).

Microbial-based fertilizers/biofertilizers consist of microorganism formulations developed using a single or cocktail of potential microbial strains. Dal Cortivo et al. (2020) demonstrated effects of bacterial (*Azospirillum* spp., *Azoarcus* spp., and *Azorhizobium* spp.) and mycorrhizal fungal-bacterial consortia on rhizosphere bacterial biomass and on plant growth and grain yield in wheat field trial. Wheat seed application with biofertilizers significantly enhanced plant growth and nitrogen accumulation with small gain in grain yield, upregulated two high-quality glutenin subunits, and increased rhizosphere microbial biomass. Endophytic and rhizospheric *Pseudomonas fluorescens* strains and endophytic microbial consortium were tested under glasshouse and field conditions in *Brassica napus*. PGP determinants (crop height, stem/leaf, pod biomass, seed, and oil yield) were enhanced in field as a result of biofertilizer application (Lally et al. 2017). Biofertilizers, posing low environmental threats, can play a key role in the development of integrated nutrient management systems sustaining agricultural productivity. A better understanding of rhizospheric microbes, their colonizing ability, functional diversity, mode of actions, and strategic application would facilitate their use for far-sighted sustainable agricultural systems.

16.3 Role of Rhizosphere Microorganisms in Nutrient Acquisition and Assimilation (N, P, K, Zn)

16.3.1 Biological Nitrogen Fixers

Nitrogen is an indispensable element required for normal physiological functioning of plants. Atmospheric nitrogen is not accessible to plants directly. Soil inorganic nitrogen available in the form of ammonium and nitrates is absorbed and utilized by plants. Plants procure nitrogen in utilizable form either through manure and/or nitrogen fertilizer or biologically fixed nitrogen. Soil nitrogen fixers (bacteria and *Archaea*) significantly contribute to atmospheric nitrogen fixation by the nitrogenase complex. Nitrogen-fixing prokaryotes are called diazotrophs. These diazotrophs exist either free-living or symbiotically associated with plants. Application of these microbes in the form of bio-inoculant or biofertilizer can reduce dependency on nitrogenous fertilizers. Seed treatment or soil inoculation of endophyte symbiotic microbes such as *Rhizobium* helps in fixation of biological nitrogen in legumes via root nodulation and in non-leguminous crops such as wheat (Yanni et al. 2016) and sorghum (Hara et al. 2019). *Azotobacter, Azospirillum, Clostridium, Gluconacetobacter diazotrophicus,* and *Beijerinckia* are some of the commonly known nonsymbiotic nitrogen fixing bacteria. Dent and Cocking (2017) reviewed the importance of nonrhizobial, nonnodulating BNF bacteria *Gluconacetobacter diazotrophicus* which significantly improves the yields in cereals and oilseed. Saha et al. (2016) applied a consortium of non-rhizobial endophytic microorganisms from *Typha angustifolia* and suggested the consortium to be effective in improving nitrogen uptake, assimilation, nitrogen use efficiency, and plant growth promotion in rice. Bacterial isolates closely related to *Azospirillum, Brevundimonas, Herbaspirillum, Pantoea, Pseudomonas, Rhanella, and Rhizobium* isolated from maize depicted nitrogen-fixing ability by reducing acetylene to ethylene (Montañez et al. 2009). Several studies have been reported where engineering of nitrogenase expression and *nif* gene clusters of endophytic bacteria can deliver higher nitrogen to cereals (Ryu et al. 2020; Li et al. 2016). Apart from bacteria, future research may unfold genetically engineered nitrogen-fixing cereals amenable for nodule organogenesis and infection by nitrogen-fixing bacteria.

16.4 Phosphorus-Solubilizing Microorganisms

Excessive use of inorganic fertilizers to augment agricultural productivity causes water pollution, waterway eutrophication, dwindled soil fertility, and accumulation of toxic elements in the soil (Alori et al. 2017). Phosphate-solubilizing bacteria and fungi facilitate phosphate solubilization and acquisition through conversion of insoluble organic and inorganic phosphates into readily utilizable form. Production of various enzymes (phosphatases, phytases, and lyases), organic/inorganic acids, and ion excretion by these microorganisms facilitate phosphate mineralization in soil (Sindhu et al. 2014). *Bacillus*, *Pseudomonas*, *Rhizobium, Azospirillum, Azotobacter, Enterobacter, Penicillium*, *Aspergillus*, actinomycetes, and arbuscular mycorrhizae are notable PSM genera (Kalayu 2019; Saleemi et al. 2017; Sharma et al. 2017). Srinivasan et al. (2012b) isolated 23 PSB and 35 PSF from salt-affected soil and investigated their phosphate solubilization potential under stress conditions. PSB were identified belonging to genera *Aerococcus, Alteromonas, Bacillus, Enterobacter, Erwinia, Pseudomonas,* and *Xanthomonas,* whereas PSF isolates were identified as *Aspergillus* and *Penicillium*. Fungal isolates were more efficient in terms of P solubilization than bacterial isolates (Srinivasan et al. 2012a).

Inoculation of PSB and vesicular-arbuscular mycorrhizal fungj reduced dependency on phosphorus in soybean–wheat cropping system providing better root property and higher grain yield (Mahanta et al. 2014). Inoculation with arbuscular mycorrhizal fungi (AMF) as biofertilizer improved plant growth and production by facilitating nutrient acquisition, modifying the abundance and diversity of rhizosphere microorganisms and by suppressing soil-borne pathogens acting as a probiotic agent (Liu et al. 2020). Wan et al. (2020) characterized 8 genera of PSB (*Acinetobacter, Arthrobacter, Bacillus, Cupriavidus, Massilia, Ochrobactrum*, *Pseudomonas,* and *Stenotrophomonas*) and their potential in immobilizing soil lead and identified *Acinetobacter pittii* gp-1 as good candidate. Mukhtar et al. (2017) assessed effects of biogas sludge and six phosphate solubilizing strains *Bacillus endophyticus, B. sphaericus, Enterobacter aerogenes* isolated from sugarcane rhizosphere, *B. megaterium,* and *B. safensis* and isolated from wheat rhizosphere and one halophilic strain *Virgibacillus* sp. isolated from *Atriplex amnicola* rhizosphere as biofertilizers on growth and yield of wheat. Under field conditions, enhanced root and shoot dry weights and seed weights were reported by *B. megaterium*, *B. safensis,* and *E. aerogenes* strain using biogas sludge as carrier.

16.5 Potassium-Solubilizing Microorganisms

Potassium is one of the essential macronutrients required by plants for enzyme activation, regulation of stomatal aperture, cell elongation, absorption, and utilization of other plant nutrients and maintaining osmotic balance (Xu et al. 2020). Potassium, like phosphorus, is slowly decomposed in soil matrices thereby its supply to growing crops is relatively low. In the soil matrices potassium is present as: water-soluble, exchangeable, non-exchangeable, and mineral forms (Bahadur et al. 2016). Potassium-solubilizing microorganisms (KSM) solubilize the fixed potassium forms to enhance their availability to crops. KSM are capable of solubilizing unavailable forms of potassium-containing minerals (micas, feldspar, illite, and orthoclases). Production of organic acids (citric acid, ferulic acid, coumaric acid, tartaric acid, oxalic acid, succinic acid, and α-ketogluconic acid), and protons supply by these microorganisms facilitate potassium mineralization in soil (Bakhshandeh et al. 2017; Setiawati and Mutmainnah 2016). *Arthrobacter, Agrobacterium, Acidithiobacillus, Aspergillus, Azospirillium, Bacillus, Burkholderia, Enterobacter, Erwinia, Pantoea, Flectobaccilus, Flavobacterium, Klebsiella, Microbacterium, Myroides, Paenibacillus, Pseudomonas, Rhizobium, Serratia,* and *Stenotrophomonas* are some of the well-known KSM in rhizopshere region (Bakhshandeh et al. 2017; Kour et al. 2020). Inoculation of rice with KSB *Pantoea agglomerans*, *Rahnella aquatilis,* and *Pseudomonas orientalis* enhanced grainyield and K uptake in addition to IAA production and tolerance to different environmental stresses (Khanghahi et al. 2018). *Bacillus* spp. effectively enhanced potassium uptake in the rhizosphere region by production of organic and inorganic acids eventually improving the root architecture system (Yasin et al. 2016). Despite their great relevance in replenishing soil fertility, phosphate−/potassium-solubilizing microorganisms are yet to be incorporated as an integral component of sustainable agriculture.

16.6 Zinc-Solubilizing Microorganisms

Compared to other soil nutrients zinc deficiency is not associated with availability rather solubility, which decreases at higher pH, high organic matter, Mg:Ca ratio, and P, Fe, Mn content in the soil (Nadeem and Farooq 2019). Zinc sulphate, the soluble form of zinc, which is generally applied to soil in agiculture gets converted to different insoluble forms like Zn(OH)2 at higher soil pH, $ZnCO_3$ in calcium-rich alkali soils, $ZnPO_4$ in alkali soils with heavy dose of P fertilizers application (Sarathambal et al. 2010). Plant growth promoting rhizobacteria helps in increasing the bioavailability of native zinc by solubilizing insoluble form (ZnO, $ZnCO_3$) to soluble form. Sindhu et al. (2019) suggested that seed/soil inoculation with ZSB enhances the bioavailability of not only Zn but also other nutrients including P and K, thereby maintaining the plant enzyme functions, oxidative stress, metabolic processes which get affected under deficiency and thus increases the crop yield. Gontia-Mishra et al. (2017) demonstrated ZSB from 4 different genera, namely *Burkholderia cepacia, Klebsiella pneumonia, Pseudomonas aeruginosa, and Ralstonia picketti* to efficiently solubilize ZnO and $ZnCO_3$. Zn solubilization and reduction in pH were positively correlated. Bhatt and Maheshwari (2020) characterized *Bacillus megaterium*, a zinc solubilizing bacteria for its potential in transforming ZnO to available zinc form along with phosphate solubilization and plant growth promotion attributes. *Pseudomonas* sp. and *Bacillus* sp. inoculation increased grain yield and plant growth parameters in chickpea through transforming insoluble forms of P and Zn to available form (Zaheer et al. 2019). Eshaghi et al. (2019) demonstrated with inoculation of siderophore-producing rhizobacterial strains of *Pseudomonas japonica in maize that these strains* can be used as a bio-fertilizer to combat iron and zinc deficiency in crops. The contribution of ZSB *Pantoea, Enterobacter cloacae,* and *Pseudomonas fragi* has been indicated in promoting growth and zinc content of wheat (Kamran et al. 2017). *Bacillus* sp., *Bacillus aryabhattai,* and *Bacillus subtilis* have been identified as ZSB strains with potential to promote maize growth and to be used as bio-inoculants for biofortification (Mumtaz et al. 2017).

16.7 Role of Rhizosphere in Root Development and Defining Root System Architecture

Roots are vital plant organs providing physical and nutritional support. Root system starts developing in the embryo favoring anchoring the plant system, nutrient uptake, and acquisition. The spatial arrangement of root system (primary root, lateral root, root hairs, root tips, crown roots, seminal roots) in the soil matrix is defined as root system architecture (RSA). Adaptation of the RSA helps plants cope with extreme conditions along with nutrient uptake and acquisition. Proper root/rhizosphere management could fortify nutrient use efficiency and crop productivity intensifying sustainable agriculture. Rhizosphere microbiota can modulate root system architecture by promoting root growth and distribution. Several PGPR have been documented modulating RSA through altered cell division and differentiation in primary root affecting lateral root development and root hair formation (Zhao et al. 2018; Ambreetha et al. 2018; Ortiz-Castro et al. 2014). Rhizosphere inhabiting fungi *Trichoderma atroviride* increases root hair length and density (Contreras-Cornejo et al. 2015). Similarly *T. virens* promotes lateral roots by altering auxin-inducible marker *DR5:uidA* in *Arabidopsis* (Contreras-Cornejo et al. 2009). Rice inoculation with PGPR *Bacillus altitudinis* strain FD48 affected root morphogenesis and RSA. Expression of auxin-responsive genes inhibited primary root elongation while promoting formation of lateral roots (Ambreetha et al. 2018). *Rhizobium* sp. IRBG74 suppresses elongation of main root and promotes formation of lateral roots through modulating auxin signaling in *Arabidopsis*. Transcriptome analysis revealed induced expression of auxin responsive reporter *DR5:GUS* in *Arabidopsis* roots (Zhao et al. 2018). Ortiz-Castro et al. (2020) reported *Pseudomonas putida* and *P. fluorescens* stimulated lateral root and root hair formation and increased plant biomass through activation of auxin signaling in *Arabidopsis thaliana*. Auxin signaling modulated auxin-responsive gene expression in roots through production of cyclodipeptides. Similarly, root colonization with *Achromobacter* sp. 5B1 has been demonstrated to influence growth and root architecture in *Arabidopsis thaliana* through auxin perception and redistribution resisting salt stress (Jimenez-Vazquez et al. 2020). Soil protozoa *Acanthamoeba castellanii* has been shown to increase root branching via modulation of auxin IAA metabolism and to foster auxin-producing soil bacteria (Krome et al. 2010).

16.8 Rhizosphere-Mediated Amelioration of Biotic and Abiotic Stresses

Boosting crop yield to meet global food, feed, and fuel demands under continuously changing climate is often threatened by several stresses imposing new challenges for sustainable agriculture. Crop plants are exposed to various biotic (soil pathogens and pests) and abiotic (drought, salinity, heavy metals, acidity, sodicity, temperature extremes, water-logging, submergence) stresses which severely affect crop survival, fitness, and productivity. Crop growth is ceased depending upon the intensity of stress, duration of stress, crop growth stage, crop physiology, and cultivar susceptibility. Mitigation of abiotic stresses employing rhizosphere microbiota has emerged as a potential strategy offering minimal ecosystem disturbance.

16.9 Plant Health and Biocontrol

Synthetic pesticides and fungicides applications have been under common agronomical practices leading to enhanced food production in the last few decades. However, injudicious application of these chemicals has a negative effect on non-target organisms, with future threat of development of resistant pathogen races. Hence microbe-based plant health management and biocontrol agents are gaining attention being a non-chemical alternative. Several rhizosphere dwelling microorganisms have been reported as biological control agents (BCAs) against various soil pathogens in different crops (Table 16.2). Seed coating with biocontrol agents benefits crops by effectively providing disease resilience along with stimulating plant growth. *Pythium*-infested soils challenged with biocontrol agents *Paenibacillus peoriae* and *Streptomyces fulvissimus* strains coated wheat seeds significantly promoted plant height, root weight, and number of heads. Microbial diversity in wheat root and rhizosphere soil was notably influenced as per stages of crop growth under field and greenhouse trials (Araujo et al. 2020). Araujo et al. (2019) employed *Streptomyces* strain as a biocontrol agent in wheat seeds against *Rhizoctonia solani* infested soil in the glasshouse. Biocontrol application promoted plant growth and head maturation along with modulating root endosphere and rhizosphere soil microbiomes. Operational taxonomic units (OTUs) of *Balneimonas, Massilia, Pseudomonas,* and unclassified Micrococcaceae responded as potential protectors against *Rhizoctonia* infestation. The application of biocontrol agents can reduce fungal infestations by promoting a beneficial microbiome contributing to sustainable agriculture. Bio-inoculation with strains belonging to genera *Bacillus*, *Pseudomonas,* and *Rhizobium* promoted plant height, number of nods, fresh and dry weight in bean along with checking growth of *Fusarium solani* f. sp. *phaseoli* (Kalantari et al. 2018). Phagotrophic protists have been reported to protect plants by pathogen suppression and predicting pathogen dynamics through predation-induced shifts (Xiong et al. 2020). These microbiome predators can potentially promote plant performance through microbiome engineering.

16.10 Drought

Drought stress severely affects crop plants by deteriorating their morphological, physiological, and biochemical integrity. Drought hampers water and nutrient uptake, photosynthetic efficiency, stomatal conductance, membrane stability, and transpirational rate resulting in stunted growth, poor biomass, and dropped yield. Drought tolerance is a complex physiological phenomenon involving hormone-regulated modulation of root system architecture, signaling through ROS, osmoregulation, induction of systemic tolerance, and transcriptional regulation of host stress response genes. PGPR application mediates growth promotion as well as alleviation of drought stress in plants. PGPR-mediated stress tolerance in plants includes phytohormone production, enzyme stimulation (nitrogenase, phosphatase, anti-oxidant enzymes), siderophore and HCN production and regulating endogenous

Table 16.2 Biocontrol agents against soil-borne pathogens

Crop	Biocontrol agents	Source of occurrence	Targeting pathogens	Modes of action	Reference
Bacteria as BCAs					
Wheat	*Lactobacillus plantarum* SLG17 and *Bacillus amyloliquefaciens* FLN13	Rhizophere	*Fusarium culmorum*	Damaged hyphae through digestive enzymes	Baffoni et al. (2015)
	Stenotrophomonas maltophilia *Bacillus cereus* *Trichoderma harzianum*	Rhizophere	*Stenotrophomonas maltophilia* *Bacillus cereus* *Trichoderma harzianum*	Inhibit the mycelial growth	Dal Bello et al. (2002)
Potato	*Serratia plymuthica strain A294, Enterobacter amnigenus strain A167, Rahnella aquatilis strain H145, Serratia rubidaea strain H440, and S. rubidaea strain H469*	Rhizophere	*Pectobacterium spp., Dickeya spp.*	Production of antibiotic compounds, bio-surfactants and siderophores	Maciag et al. (2020)
Tomato	*Bacillus subtilis, Burkholderia cepacia, Bacillus atrophaeus*	Rhizosphere	*Fusarium oxysporum* f. sp. *lycospersici*	Secretion of chitinase and β-1,3-glucanase	Shanmugam and Kanoujia (2011)
	Pseudomonas segetis, P. syringae	Rhizophere	*Dickeya solani, Pectobacterium atrosepticum, P. carotovorum*	Enzymatic deterioration of signal molecules in quorum sensing interaction	Rodríguez et al. (2020)
	Streptomyces atrovirens N23 and *Trichoderma lixii* NAIMCC-F-01760	Rhizophere	*Rhizoctonia solani*	Chitinase, β-1,3-glucanase	Solanki et al. (2019)
	Pseudomonas sp. CHA0	Rhizophere	*Ralstonia solanacearum*	Competition for resources and interference with the pathogen	Hu et al. (2016)

Banana	*Pseudomonas putida* *Achromobacter sp.* *Rhizobium sp.* *Bacillus flexus*	Endophyte and Rhizophere	*Fusarium oxysporum* f. sp. *Cubense*	Inhibition of spore germination and mycelial growth due to antibiosis and antifungal metabolites production	Thangavelu and Gopi (2015)
	Bacillus sp. EPB10, *Bacillus* sp. EPB56, *Pseudomonas fluorescens* Pf1	Rhizosphere	*Fusarium oxysporum* f. sp. *cubense*	Enhances activity of cell wall degrading enzyme	Mathiyazhagan et al. (2014)
Ground nut	Bacillus amyloliquefaciens	Endophyte	*Ralstonia solanacearum*	Release of antimicrobial substances of Surfactin and Fengycin A	Wang and Liang (2014)
Soybean	*Pseudomonas aeruginosa, Bacillus cereus, Bacillus amyloliquefaciens*	Rhizosphere	*Sclerotinia sclerotiorum* *Macrophomina phaseolina*	β-1, 3 glucanase, chitinase, cellulase	Thakkar and Saraf (2014)
Pea	*Pseudomonas* sp., *Bacillus* sp., *Azotobacter* sp., *Azospirillum* sp., *Pseudomonas*	Rhizosphere	*Sclerotinia sclerotiorum*	Induced systemic resistance	Jain et al. (2015)
Chilli	*Bacillus cereus* *Bacillus subtilis*	Rhizosphere	*Phytophthora Capsici*	Alternation of the soil bacterial community	Zhang et al. (2019)
Fungi as BCAs					
Wheat	*Purpureocillium lilacinum*	Rhizophere	*Tribolium confusum*	Secretion of digestive extracellular enzymes that degrade cuticle	Barra et al. (2015)
	Beauveria bassiana	Rhizophere	*Heterodera filipjevi*	Secrete of chitinases, proteases	Zhang et al. (2020)
Maize	*Trichoderma harzianum*	Rhizophere	*Fusarium graminearum*	Disintegration of the hyphae by lytic enzymes	Saravanakumar et al. (2017)
Tomato	*Purpureocillium lilacinum*	Rhizophere/ egg of *M. incognita*	*Meloidogyne incognita*	Colonize the egg and degrade through lytic enzymes	Singh et al. (2013)
Bean	*Metarhizium* Sp.	Rhizophere	*Fusarium solani* f. sp. *phaseoli*	Secretion of enzyme	Barelli et al. (2020)

(continued)

Table 16.2 (continued)

Crop	Biocontrol agents	Source of occurrence	Targeting pathogens	Modes of action	Reference
Physalis peruviana	*Trichoderma virens* GI006 and *Bacillus velezensis* Bs006	Rhizophere	*Fusarium oxysporum* f. sp. *phaseoli*	Formation of biofilms and production of antimicrobial compounds	Izquierdo-García et al. (2020)
Citrus orchards	*Beauveria bassiana* *Metarhizium anisopliae*	Rhizophere	*Thaumatotibia leucotreta*, *Scirtothrips aurantii* *Planococcus citri*	Secrete of digestive enzyme	Acheampong et al. (2020)

ethylene levels through ACC-deaminase. A consortium consisting of ACC-deaminase-producing rhizobacteria (*Ochrobactrum pseudogrignonense*, *Pseudomonas* sp., and *Bacillus subtilis*) alleviated drought stress along with improved seed germination, dry weight, root, and shoot length in black gram and pea through elevated levels of ROS scavenging enzymes and osmolytes in conjugation with down-regulated ACC-oxidase gene expression (Saikia et al. 2018). ACC-deaminase-producing rhizobacteria *Achromobacter xylosoxidans* in combination with biochar enhanced grain yield, stomatal conductance, photosynthesis, chlorophyll, and carotenoids content in maize under drought stress (Danish et al. 2020).

Selvakumar et al. (2018) evaluated osmotolerant rhizospheric microbial strains toward growth responses and water stress alleviation in tomato for pot culture conditions. Stomatal conductivity, transpiration rate, photosynthesis, relative water contents, biomass, and fruit yield were significantly enhanced as a result of microbial inoculation. *Actinobacterium*, *Citrococcus zhacaiensis*, *Bacillus amyloliquefaciens* were reported as promising strains boosting crop yield. *Bacillus subtilis* inoculation alleviated drought stress and promoted shoot growth of *Platycladus orientalis* seedlings through cytokinin production. Bacterial inoculation significantly increased root exudates such as sugars, amino acids, and organic acids (Liu et al. 2013). *Bacillus cereus*, *B. subtilis*, and *Serratia* sp. were reported to induce drought tolerance in cucumber (Wang et al. 2012). In a similar study by Bresson et al. (2014), *Phyllobacterium brassicacearum* strain has been reported to alleviate drought stress in *Arabidopsis thaliana* through improved water status. Rhizosphere management strategy and rhizosphere engineering/enrichment by addition of drought-tolerant PGPR should be integrated for drought stress mitigation.

16.11 Temperature Stress

Heat stress often occurs in conjugation with other stresses under field conditions, among which drought stress is most common. Sensitivity of crops to heat stress is more during the reproductive phase than the vegetative phase. Seed germination, seedling growth, tillering, pollen fertility, grain filling, grain quality, and yield are hampered under elevated temperature. Bacterial strain *Pseudomonas brassicacearum* along with other PGPRs has been elucidated to reduce heat stress in wheat when applied as bioinoculant as the seedling growth has been promoted due to catalase, oxidases, antioxidant enzymes, proline, and protein content (Ashraf et al. 2019). Seed treatment with strains of *Bacillus amyloliquefacien*s or *Azospirillum brasilense* has been demonstrated effective for heat stress tolerance in wheat corresponding to lesser reactive oxygen species generation and preactivation of certain heat shock transcription factors (Abd El-Daim et al. 2014). Heat tolerant PGPR *Bacillus cereus* has been demonstrated to significantly reduce heat stress in tomato through ACC-deaminase and exopolysaccharide production in the growth chamber resulting in augmented plant growth (Mukhtar et al. 2020). Thermotolerant PGPR *Bacillus tequilensis* improved growth of Chinese cabbage seedlings and soybean under heat stress through elevated levels of jasmonic acid and salicylic acid while down-regulating ABA production (Kang et al. 2019). Thermotolerant *Bacillus cereus* SA1 inoculation has been reported to augment heat stress in soybean through overexpression of stress responsive genes and HSPs along with modulation of auxin, ABA and SA (Khan et al. 2020).

Effect of PGPR *Serratia nematodiphila* was assessed on physio-hormonal attributes of *Capsicum annuum* plants subjected to low-temperature stress. Inoculated plants exhibited higher endogenous GA_4 and ABA levels whereas endogenous jasmonic acid and salicylic acid contents were down-regulated (Kang et al. 2015).

16.12 Salinity

Higher concentration of salt present in soil is one of the major environmental stresses drastically affecting crop productivity. Physiological processes such as respiration, photosynthesis, reproduction, nitrogen fixation, water, and nutrient uptake are impaired under salt stress. Soil salinity leads to osmotic stress, nutrient deficiency/ imbalance, ion toxicity, and oxidative stress on plants. PGPRs and several fungi have been demonstrated for their potential toward alleviation of salt stress in crops (Yasmeen et al. 2020; Hamayun et al. 2017). Inoculation of rice with salt-tolerant PGPRs *Bacillus tequilensis* and *Bacillus aryabhattai* demonstrated improved photosynthesis, transpiration, and stomatal conductance resulting into higher yield corresponding to exopolysaccharide production and sodium ion chelation (Shultana et al. 2020). Abdelmoteleb and Gonzalez-Mendoza (2020) demonstrated that inoculation with two *Tamarix ramosissima* rhizosphere PGPR strains, *Bacillus megaterium* and *Bacillus cereus* significantly increased plant height, root length, root and shoot dry weight, P content and photosynthetic pigments in *Phaseolus vulgaris* under salt stress condition in vivo. Amna et al. (2019) elucidated PGPR *Bacillus siamensis* PM13, *Bacillus* sp. PM15 and *B. methylotrophicus* PM19 strains alleviated the effects of salt stress on wheat seedling through sodium sequestration and ACC degradation. Nawaz et al. (2020) suggested bioinoculation with *Bacillus pumilus*, *Pseudomonas fluorescens,* and *Exiguobacterium aurantiacum* both singly and in consortium can augment yield, nutrient acquisition and plant growth parameters in wheat genotypes. Endophytic fungus, *Aspergillus flavus* (isolated from *Chenopodium album* roots) ameliorated adverse effects of salt stress by down-regulation of ABA and JA synthesis and elevated activities of various antioxidant enzymes (catalase, peroxidase, polyphenoloxidase, and superoxide dismutase) in soybean plant apart from growth promoting activities (Asaf et al. 2018). Another endophytic fungus *Porostereum spadiceum* has been reported mitigating salt stress by modulation of endogenous phytohormones (GAs, JA, and ABA) and isoflavones in soybean seedlings (Hamayun et al. 2017).

16.13 Metal Toxicity

Heavy metals such as arsenic, lead, copper, cadmium, mercury, aluminum are soil pollutants having phytotoxic effects. These heavy metals get accumulated in plant tissues hampering crop productivity. Heavy metal accumulation interrupts enzymatic activity and causes chlorosis, stunted growth and ROS generation in plant system (Tiwari and Lata 2018). PGPRs can effectively immobilize heavy metals and reduce their translocation in plants via precipitation, chelation, complex formation, and adsorption. Rhizobia possess phytoremediation and plant growth-promoting mechanisms besides nitrogen fixation. Brígido et al. (2017) characterized a collection of native Portuguese chickpea *Mesorhizobium* isolates to evaluate their potential toward PGP traits and tolerance to different metals. Most of the bacterial isolates exhibited IAA synthesis, siderophore production, P solubilization, acid phosphatase and cytokinin activity and tolerance to Zn or Pb indicating their potential as PGP rhizobacteria as well as in phytoremediation. *Pseudomonas aeruginosa* and *Burkholderia gladioli* enabled tomato seedlings to reduce cadmium stress (Khanna et al. 2019). PGPR *Bacillus toyonensis* strain Bt04 enhanced growth and root development under aluminum toxicity through production of IAA, a non-indole phenylacetic acid cytokinins and increasing protection against oxidative stress in maize (Zerrouk et al. 2020). Auxin transport pathway has been demonstrated crucial for aluminum-induced stress response post PGPR inoculation (Zerrouk et al. 2020). Kang et al. (2017) explored the ameliorative capacity of PGPR *Leifsonia xyli* against copper stress in tomato. Detrimental effects of copper stress were relieved through modulation of endogenous amino acids contents (arginine, glutamic acid, glycine, threonine, phenylalanine, and proline) and phytohormones gibberellic acid and IAA. Bio-inoculation increased P and Fe content, stimulated total polyphenol and flavonoid content whereas reduced superoxide dismutase activity in plants grown under elevated Cu stress. Similarly *Paenibacillus polymyxa* and *Bacillus circulans* inoculation conferred copper toxicity resilience to maize plants through decreased malondialdehyde and copper content in root and shoot tissues due to better antioxidant enzymes activities and osmolyte regulation (Abdel Latef et al. 2020).

16.14 Phytostimulation Through Exudation and Hormones

Rhizosphere microbiome exert phytostimulatory effect through releasing various root exudates, volatile organic compounds (VOCs), and hormones such as auxin, gibberellins, cytokinin, and ethylene. Root exudates modify soil properties, nutrient acquisition and enable crop survival under extreme environmental conditions through mechanisms such as (1) changing soil pH for mineral solubilization, (2) chelating toxic compounds, (3) recruiting rhizosphere microbiota, or (4) releasing toxic substances for pathogens, etc. Root exudation and its composition are affected by physical, chemical, and biological agents. Root exudates include carbohydrates, amino acids, and organic acids (primary metabolites) and flavonoids, glucosinolates, auxins (secondary metabolites) depending up on the quantity of release. Abiotic stresses, nutrient availability, and several other physical, chemical, and biological factors affect root exudation process. Bacteriogenicindole-3-acetic acid (IAA) controls lateral root formation, primary root elongation, and root hair synthesis which ultimately enhances root length and surface area coverage, improving water and nutrient acquisition and overall plant growth. Gibberellic acids (GAs) promote shoot elongation, germination, flowering, and leaf growth. Cytokinin triggers proliferation of root hairs and promotes cell division and acts antagonistically with auxins by inhibiting lateral root formation and primary root elongation.

16.15 Auxin

Seed bacterization with PGPR of the genera *Escherichia*, *Micrococcus*, *Pseudomonas,* and *Staphylococcus* significantly increased shoot length, pod number, biomass, and grain weight of mungbean through IAA production in the presence of L-tryptophan (Ali et al. 2010). Bose et al. (2016) reported bacterial isolate *Enterobacter cloacae* exhibiting maximum IAA production along with other PGP traits. Seed bioinoculation with this siderophorogenic auxin producer enhanced seed germination and plant vigor under in vivo condition of different cereals (rice, wheat, and maize), pulse (mung), and oilseed (groundnut). IAA-producing Bacillus strains have been demonstrated to stimulate growth in wheat (Ravari and Heidarzadeh 2014). Bhattacharyya et al. (2015a, b) reported that volatile indole produced by rhizobacterium *Proteus vulgaris* stimulated plant growth through hormonal interplay of auxin, cytokinin, and brassinosteroid in *Arabidopsis thaliana. Arabidopsis thaliana* Col-0 seedlings exposure to volatile indole for 2 weeks resulted in overexpression of *small auxin up RNA, histidine kinase1*, and *brassinosteroid biosynthetic cytochrome P450* genes. Auxin-producing microorganisms strengthen crops to withstand various abiotic stresses besides stimulating growth. Auxin-producing PGPR of the genera *Bacillus*, *Enterobacter*, *Moraxella*, and *Pseudomonas* have been effective in mitigating drought stress in wheat (Raheem et al. 2017). *Pseudomonas* strains (*P. aureantiaca* TSAU22, *P. extremorientalis* TSAU6, and *P. extremorientalis* TSAU20) effectively alleviated salinity stress on wheat seed dormancy (Egamberdieva 2009). *Pseudomonas fluorescens* Ms-01 and *Azospirillum brasilense* DSM1690 have been reported prominent to neutralize salinity stress in wheat and promoting plant growth due to production of auxin and P solubilization (Kadmiri et al. 2018).

16.16 Ethylene

Crop growth and productivity are often impeded by environmental stress induced ethylene content. Under such circumstances bio-inoculation with bacteria-producing ACC deaminase could be an effective approach to mitigate abiotic stress and promote plant growth with lowered endogenous ethylene level. Bacteria-producing 1-aminocyclopropane-1-carboxylate deaminase enzyme actively modulates ethylene levels and facilitates plant growth under adverse environmental conditions. ACC deaminase-producing bacteria prevent flower wilting, promote rooting, and facilitate nodulation of legumes (Gamalero and Glick 2015). Seed bacterization employing rhizobacterial strains *Aneurinibacillus aneurinilyticus* and *Paenibacillus* sp. isolated from *Allium sativum* rhizosphere significantly alleviated salt stress stimulated ethylene levels and its associated growth inhibition by virtue of their ACC deaminase activity in french bean seedlings. In addition to this, bacterial consortia treatment facilitated PGP parameters (fresh weight, shoot and root biomass and chlorophyll content) through PGP traits (Gupta and Pandey 2019). Similarly, Gupta and Pandey (2020) demonstrated that consortium inoculum formulated with two salt-tolerant strains *Aneurinibacillus aneurinilyticus* and *Paenibacillus* sp. markedly improved seed germination, root/shoot length, fresh and dry weight, water content, chlorophyll and carotenoid content, osmoprotectants (proline and total soluble sugar) levels of salt exposed plants due to down-regulated ethylene levels in common bean under saline conditions. Co-inoculation with halotolerant IAA and ACC deaminase-producing bacterial strains belonging to genera: *Achromobacter, Enterobacter*, *Microbacterium*, *Pseudomonas,* and *Serratia* improved seedling emergence, biomass and SOD activity under saline conditions in wheat (Barra et al. 2016). Rice seedlings inoculated with ACC deaminase-producing salt-tolerant *Burkholderia* sp. improved seedling growth exposed to salt stress through mechanisms involving reduced ethylene levels, scavenging ROS and proline and IAA production (Sarkar et al. 2018). ACC-deaminase-producing PGPR *Achromobacter xylosoxidans* and *Enterobacter cloacae* in combination with biochar help ameliorate drought stress in maize (Danish et al. 2020).

16.17 Gibberellins

Gibberellin-producing microorganisms have been isolated from soil and rhizosphere having a beneficial effect on plant growth. GA secreting rhizobacterium *Leifsonia soli* sp. SE134 culture filtrate prompted biomass and root lengths of cucumber seedlings, growth of rice seedlings, growth promotion in tomato and radish (Kang et al. 2014). Bacterial endophyte *Bacillus amyloliquefaciens* improved growth parameters and physiology of rice plants through GA production. Inoculated rice plants exhibited higher endogenous salicylic acid and lower abscisic and jasmonic acid (Shahzad et al. 2016). Tomato plants inoculated with PGPR *Promicromonospora* sp. SE188 exhibited higher shoot length and biomass due to up-regulated GA biosynthesis pathway. Abscisic acid was decreased whereas salicylic acid was profoundly higher in inoculated tomato plants (Kang et al. 2012). do Amaral et al. (2014) reported that endophytic diazotrophic bacterium, *Herbaspirillum seropedicae,* modulated expression of gibberellin biosynthesis pathway genes and NADPH oxidase transiently in maize root. Inoculated seedlings had a higher number of lateral roots. Quantification of transcript levels of ten maize genes revealed *ent-kaurene oxidase* and *respiratory burst oxidase protein C* to increase in inoculated seedlings.

PGPR *Leifsonia xyli* SE134 effectively ameliorates copper stress on tomato through modulating GAs and IAA metabolism along with endogenous amino acids (arginine, glutamic acid, glycine, threonine, phenylalanine and proline) contents (Kang et al. 2017). GA and ABA released by *Azospirillum* significantly contributes to the alleviation of water stress in maize (Cohen et al. 2009). Gibberellin-producing PGPR *Serratia nematodiphila* helps in mitigating low-temperature stress in pepper plants owing to higher endogenous GA and ABA levels and reduced jasmonic and salicylic acid levels (Kang et al. 2015).

16.18 Cytokinin

Phytohormone-producing PGPR possesses phytostimulatory potential. Phytohormones, especially cytokinin, have been studied for their biocontrol mechanisms and environmental resilience in addition to developmental regulation and governing plant physiology. Großkinsky et al. (2016) demonstrated *Pseudomonas fluorescens* G20–18 in biocontrol of *P. syringae* infection in *Arabidopsis* where cytokinin has been identified as being the key determinant. Inoculation of *Platycladus orientalis* seedlings with *Bacillus subtilis* improved leaf water potential and relative water content while decreased root/shoot ratio under water-stressed conditions due to higher cytokinin and abscisic acid content (Liu et al. 2013). Rhizosphere bacterial strains, belonging to the genera *Pseudomonas*, *Bacillus,* and *Azospirillum* have been screened for their phytostimulatory effect through phytohormones (cytokinins and IAA) production. Bacterial cytokinin stimulated shoot length, fresh and dry weight under axenic conditions while bacterial IAA negatively correlated to root length and positively to the number of roots. Inoculated plants exhibited an increase in spike length, tillering and seed weight under natural conditions (Hussain and Hasnain 2011a). Cyanobacteria possess phytostimulatory and biofertilization ability in addition to nitrogen-fixing ability. Cyanobacteria, viz. *Anabaena*, *Chroococcidiopsis*, *Oscillatoria*, *Phormidium*, and *Synechosystis* have been reported to release cytokinin (Hussain et al. 2010). Rhizospheric and free-living cyanobacteria *Chroococcidiopsis* sp. and *Anabaena* sp. improved seed germination, shoot length, spike length, number of tillers, number of lateral roots, and grain weight of inoculated wheat plants owing to phytohormones cytokinin and IAA (Hussain and Hasnain 2011b). PGPR *Bacillus aryabhattai* strain SRB02 promotes growth and tolerance to heat stress in soybean through phytohormones, viz. abscisic acid, IAA, cytokinin, jasmonic acid, and gibberellic acids (Park et al. 2017).

16.19 VOCs

Rhizospheric microorganisms can alter plant morphology, physiology, and phenology through the emission of volatile organic compounds (VOCs). Emission of VOCs by rhizosphere inhabiting microbes promotes growth and flowering of various crop plants. VOCs emitted by the phytopathogen *Alternaria alternata* triggered photosynthesis and cytokinin and sugars accumulation in *Arabidopsis* through modulating the expression of genes involved in photosynthesis, growth, and flowering (Sánchez-López et al. 2016). Microbial volatiles induce plant growth through phytohormonal regulation. *Bacillus subtilis* strain SYST2 induced growth in tomato through regulation of endogenous auxin, gibberellins, cytokinin, and ethylene levels. Albuterol and 1,3-propanediole were the main VOCs which promoted growth through phytohormonal regulation (Tahir et al. 2017). Venneman et al. (2020) demonstrated that VOCs from roots of endophytic fungus *Serendipita* spp. improve *Arabidopsis* seedling performance in terms of plant biomass, petiole elongation, leaf area expansion, lateral root extension, enhanced quantum efficiency of photosystem II. Fungal respiratory CO_2 along with methyl benzoate and other volatile compounds elicited plant growth promotion.

Microbial VOCs induce tolerance to abiotic stresses and defense against various biotic stresses. Volatiles (hexanedioic acid and butanoic acid) from *Alcaligenes faecalis* strain improved growth performance of *Arabidopsis thaliana* under salt stress through mediating auxin, gibberellins, and brassinosteroid pathways (Bhattacharyya et al. 2015b). *Bacillus amyloliquefaciens* strain emitted VOCs, diacetyl modulates immune system and phosphate-starvation response system in *Arabidopsis thaliana* through salicylic acid- and jasmonic acid-mediated immunity (Morcillo et al. 2020). Despite diverse roles offered by microbial VOCs we are still lagging in implementation of current knowledge of microbial VOCs as phytostimulants to support intensified sustainable agriculture.

16.20 Manipulating Phenological Traits (Flowering) and Heterosis

Though modulation in plant phenology is largely determined by environmental factors, several recent findings reported soil microbial communities to act as possible drivers of flowering time (Wagner et al. 2014; Lu et al. 2018). The role of rhizosphere microbiota in triggering and driving plant phenology has emerged as the latest area of interest to the scientific community. Rhizosphere microbiota can play an important role in modulating flowering time and contributing to phenotypic plasticity equipping crop plants to withstand climatic perturbations and increased crop production. Application of PGPRs consortium (*Azotobacter chroococcum*, *Bacillus subtilis, Pseudomonas florescens,* K-mobilizing bacteria, and AM fungi) in different combinations along with soil solarization significantly improved yield, phenological and pomological attributes of strawberry besides promoting rhizosphere microbial count in calcareous soils (Kumar et al. 2020). Lu et al. (2018) documented the role of rhizosphere microorganisms in delaying the flowering of *Arabidopsis thaliana* through conversion of tryptophan to IAA. Prolonged nitrogen bioavailability stimulated vegetative growth as the flowering genes were downregulated. Wagner et al. (2014) suggested soil microbes contribute to phenotypic plasticity of flowering time and reproductive fitness of *Boechera stricta.* Panke-Buisse et al. (2015) reported microbial inoculation shifted flowering time in

Arabidopsis thaliana and *Brassica rapa*. Host plants inoculated with late-flowering-associated microbiomes showed increased inflorescence biomass correlated with higher soil nitrogen mineralization resulting due to enhanced microbial enzyme activities.

Rhizosphere microbial communities have been reported to be associated with heterosis (Wagner et al. 2020). The rhizosphere of inbred lines and hybrids harbored different bacterial and fungal communities besides leaf-associated fungal communities. Microbiome composition is affected by heterotic host traits. Wagner et al. (2020) pinpointed the role of natural selection behind heritable components of microbiome variation reflecting plant–microbe interactions.

16.21 Conclusion

Augmenting crop yield and production in a sustainable way to meet raised demands of ever-growing global population under climate change scenario is an acquainted concern. Under such circumstances rhizosphere inhabiting microbes render a natural yet potent strategy to confront forthcoming challenges. These invisible forms of life can bring out visible and discernible changes by unlocking the gateway to sustainable agriculture. Advances in the next-generation sequencing platform, genome editing technologies (CRISPR/Cas9), bioinformatics and omics-based approaches are some of the genetic tools that would buttress efficient utilization of rhizosphere microbiome as “bioguards” against stresses and sustainable food production.

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Rhizospheric Microbial Community as Drivers of Soil Ecosystem: Interactive Microbial Communication and Its Impact on Plants

17

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Abstract

Soil-inhabiting rhizospheric microbial communities are interlinked with complex soil microbiomes. Many of these microbial community members are prime regulators of the soil ecosystem and contribute significantly to facilitate nutrient recycling and supply to their host plant along with aiding to overcome the various stresses (abiotic and biotic). It is a widely acknowledged point that various microorganisms present in the rhizosphere promote plant growth. These microbes colonize the rhizosphere and provide defence to plants, promote growth and development by secreting various growth-promoting substances and enhance nutrient uptake efficiency. Soil ecosystem is majorly regulated by nitrogen-fixing bacteria and arbuscular mycorrhizal (AM) symbiosis. To utilize these untapped resources there is a need to unravel the underlying mechanism of beneficial rhizospheric microbes that are critically important for plant health and which could lead to further devising of novel strategies for developing stress resilient and improving agronomic traits. The chapter also focuses on our current knowledge regarding signals of soil microbes towards its association with plants and development of symbiosis.

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17.1 Introduction

Soil microbes determine ecosystem structure and it is important to understand the mechanism operating within and crosstalk among microbes and plants that regulates underground and aboveground community structure. It is a widely accepted fact that mutualistic interactions between microbes and plants play key role in soil ecosystems (Clay and Holah 1999; Rasmann and Turlings 2016). Different types of soil such as sand, desert, rock, thermal, marsh, sediment, semiaquatic harbour microbial flora of various groups (bacteria, fungi, actinomycetes, soil nematodes, protozoans) specific to its niche (Mukerji et al. 2006). Plant rhizosphere is a dynamic environment where plant microbes interact for exchange of micro and macronutrients and other growth-promoting substances (Solanki et al. 2012). Rhizosphere segregates bulk soil from the soil adjacent to roots as soil is the reservoirof a wide variety of compounds; the exchange of nutrient and other compounds continuously takes place between these zones (Belna et al. 2003). Depending upon their capability to fix nitrogen and confer disease tolerance capability can be employed as bioinoculant, biofertilizers and biocontrol agents (Bloemberg and Lugtenberg 2001). Rhizobacteria help plants to withstand various abiotic stresses like drought, metal, salinity (De Zelicourt et al. 2013).

Root exudates from plantsrelease a variety of low molecular weight substances into the soil which help strengthen association between microbes and plants. Plant microbe interactions modulate rhizosphere and maintain soil fertility (Yadav et al. 2015). With regard to nutrient mobilization in soil, root exudates release crucial primary metabolites (Canarini et al. 2019; el Zahar et al. 2014). Microbial mediated decomposition is much dependent upon the primary metabolites released from root exudation (Cheng et al. 2014). However, mineralization is a complex process that involves various parameters. Overall signalling and interactive communication between soil microbes and plants are prime drivers of soil fertility.

17.2 Arbuscular Mycorrhizal Fungi

In terms of microbial symbiosis arbuscular mycorrhizae (AM) form associations with different plants and play crucial roles in shaping landscape regeneration, mitigation of desertification and in improving soil quality (Jeffries et al. 2003). Soil degradation is a major threat for agriculture causing loss of fertility, but apart from physical and chemical characteristic soil biota contributes to its fertility (Doran and Linn 1994). Mycorrhizal association is the most widespread relationship between plant and microbe that is ubiquitous in the plant kingdom, around 80% plants having association with AM (Remy et al. 1994; Dodd 2000). AM fungi have been widely acknowledged to suppress fungal pathogens causing damage to plants (Azcón-Aguilar and Barea 1997). Within the soil AM fungal hyphae form an intricate network for mineral nutrient and water acquisition from the soil (Smith and Read 1997). The plant provides photosynthetic carbon to the fungal partner which is provided to the soil via hyphae (Linderman 2000). The ubiquitous presence of AM in a variety of habitats renders various ecological services, specially conferring resistance to the plant and maintaining soil fertility and structure (Chen et al. 2018). Providing phosphorusto the host is an important benefit provided by AM fungi (Karandashov and Bucher 2005) findings suggest fungal arbuscular transfer phosphate to plant (MacLean et al. 2017). Various nutrient transporters operate in mycorrhizal roots (Wang et al. 2017) and enrich hosts with increased minerals (nitrogen, sulphur, zinc) content via the arbuscules (Clark and Zeto 2000; George 2000). The 3-D matrix formed by a hyphal network with soil stabilizes soil aggregates without compacting the soil (Rillig 2004; Singh et al. 2013). The soil aggregates formed by AM fungi are termed as glomalin, and it improves quality of soil and forms a stable carbon sink which lastsfor a longer time (Rillig et al. 2001). The hyphal network contributes to plant growth through various plant-promoting roles (Gutjahr and Parniske 2013) and makes soil more stable by reducing its erosion. Agriculture soil achieves high amounts of chemical fertilizers but they are not retained by the soil due to the unavailability of a nutrient retention system (Cameron et al. 2013). AM fungi contribute to reduced leaching of nutrients and make soil more stable (Cavagnaro et al. 2015). Thus, it is acknowledged that AM fungi through various strategies make soil more stable and help promote plant growth and development.

17.3 Nitrogen-Fixing Microbes

Nitrogen deficiency is a major hurdle in plant growth. Nitrogen fixation refers to reduction of nitrogen into ammonia, where various nitrogen-fixing bacteria inhabit the rhizosphere and interact with plants (Franche et al. 2009). The symbiotic association of nitrogen-fixing bacteria within root nodules are formed by two groups, namely *Alpha-proteobacteria* that form association with leguminous plants (Sprent and Platzmann 2001) and *Actinobacteria* that form association with broader class of plants of different families (Huss-Danell 1997; Vessey et al. 2005). Non-symbiotic free-living microbes have shown ability to fix nitrogen; common examples include *Azotobacter, Azospirillum, Bacillus, Enterobacteriaceae* and *Herbaspirillum* (McGill and Cole 1981). Apart from nitrogen fixing, *Azotobacter* bears potential of producing plant growth-promoting substances such as cytokines and gibberellins which promote plant growth (Rao 1995). Free-living bacteria and nitrogen fixers are recognized to modulate rhizosphere to boost crop productivity by supporting increased biomass thus it can be harnessed to fulfil agricultural demands (Igiehon and Babalola 2018). In the paddy rhizosphere under organic management abundance and community structure of nitrogen-fixing bacteria were influenced by carbon/nitrogen (C/N) and nitrogen (N) content (Shu et al. 2012). Although various diazotroph associate with plant roots but *Rhizobium,* which shows symbiotic association, is prime supplier of biologically fixed nitrogen than other nitrogen-fixing bacteria that associate non-symbiotically (Herridge et al. 2008; Kennedy et al. 2004; Kennedy and Islam 2001). Non-symbiotic free-living nitrogen-fixing bacteria affect plant growth directly and indirectly. The synthesis of phytohormones like indole acetic acid (IAA) stimulates root growth, production of siderophores and biological nitrogen fixation affect plant growth (Dobbelaere et al. 2003; Garvin and Lindemann 1986). Microbes-based biological nitrogen fixation has enormous potential to lessen the use of chemical fertilizers and such a formulation can be helpful in boosting agricultural productivity by its application as biofertilizer (Singh et al. 2017). To support plant growth in metal-inflicted area co-inoculation of rhizobia and plant growth-promoting rhizobacteria (PGPR) have shown promising results by showing enhanced enzyme activities and C and N concentrations. Co inoculation further showed to affect the bacterial community of the rhizosphere (Ju et al. 2020). In hydrocarbon-contaminated soil from the rhizosphere of *Paspalum vaginatum* Sw, three nitrogen-fixing strains namely *Brevundimonas diminuta* C4B, *Alcaligenes faecalis* B5 and *Alcaligenes faecalis* D4A were isolated that showed to bioaugment the growth showing potential application of nitrogen fixers in bioremediation (Omotayo et al. 2017). From soil of tin mining *Azospirillum lipoferum* CBT4 was isolated displaying potent PGP traits such as IAA production, Ca-P solubilizing ability and PME-ase activity to stimulate growth of plants *S. bicolor* on post mining soil (Widawati and Suliasih 2019).

17.4 Symbiosis Between Plant and Rhizospheric Microbes

Symbiosis is a phenomenon that involves dynamic alterations in metabolism, signalling network and genome (Kawaguchi and Minamisawa 2010). Arbuscular mycorrhizal (AM) and plant symbiosis are well-studied interactions between plants and microbes with respect to phylogeny and ecology (Kistner and Parniske 2002; Bonfante and Genre 2010) (Fig. 17.1). The arbuscular mycorrhiza inhabits cortical cells of plant root and derives carbon from plants while it aids in transmission of mineral nutrients from the soil to root cells. In addition, it helps in plant systemic signalling and root development (Harrison 2005). AM fungi through their strong hyphal network absorb minerals and phosphates and make them available to the associated plants through specialized structures called arbuscules (Parniske 2008). To facilitate phosphorus to plants, phosphate transporter gene MtPT4 expression is upregulated in plant cells consisting of arbuscules (Javot et al. 2007).

On advent of symbiosis, discrete responses are shown by host and partner where rhizobia nod gene which is part of NodD regulatory protein gets activated by flavonoids contents being released by plants in the form of root exudates. On activation, nod gene triggers a plant organogenic system by excretion of lipoligosaccharides that ultimately leads to formation of root nodules (van Rhijn and Vanderleyden 1995). To establish symbiosis between host plant and bacteria, factors like Nod, lipo and exopolysaccharides play critical roles (Jones et al. 2007). In this regard, phytohormones signalling mediated by auxin Cytokinin, and strigolactones (SLs) are critical regulators of nodule formation, whereas abscisic acid (ABA), Ethylene, gibberellic acid (GA) and jasmonic acid (JA) regulate nodule development negatively (Liu et al. 2018). To establish legume symbiosis signalling and organogenesis is primarily regulated by Nod factors (Kouchi et al. 2010). Plants

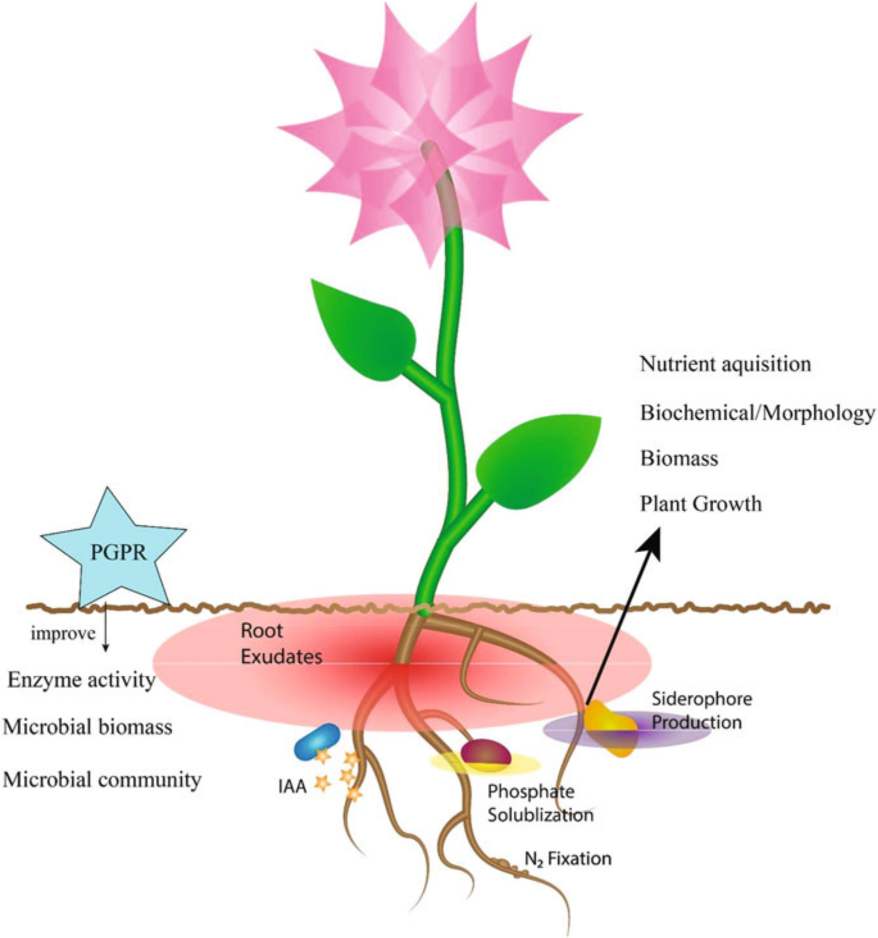

Fig. 17.1 Highlights the symbiotic association between rhizospheric microbes and plant

regulate nodule number and to maintain symbiosis with rhizobia plants have developed autoregulation of nodulation (AON) which is a negative feedback system mediated by CLAVATA1-like receptor kinases (Oka-Kira and Kawaguchi 2006; Ferguson et al. 2010). Rhizobia derive essential nutrients and reduced carbon required for metabolism from plants (Udvardi and Poole 2013). Kempel et al. (2009) have shown that in *Spodoptera littoralis* a nodulating strain *T. repens* rhizobia enhanced plant growth and development.

17.5 Signalling Between AM and Plants

The mutualistic microbe and plant association is largely facilitated by root exudates. The most prominent among these microbes are nitrogen-fixing bacteria, mycorrhizal fungi and plant growth-promoting bacteria (Azcón-Aguilar and Barea 1997). AM fungi spores persist in the soil and upon germination lead to development of a hyphal germ tube that associates with the host plant root. On contact with root, the fungus forms an appressorium which penetrates the root (Cavagnaro et al. 2001; Smith and Smith 1997). In most symbiotic events earlier detection of signals occurs before direct association, while in some cases molecular events set the onset of physical interaction. In case of plant–oomycete association, root exudates release a flavonoid compound called cocliophilin which onsets the chemo attraction and germination of zoospores (Islam et al. 2003; Wesley et al. 2001). Even for association with parasitic plants, the plant releases phenolic compounds from roots that leads to initiation of haustoria development (Estabrook and Yoder 1998; Keyes et al. 2001). But out of all these the best studied interaction operates in rhizobium-legume symbiosis in which the bacterial Nod factor detects the flavonoid molecules released by plant roots. Nod factor is detected by host plant roots, which triggers a series of events for physical association that ultimately leads to nodule formation (Denarie et al. 1996; Fisher and Long 1992; Long 1996).

17.6 Rhizobacteria-Mediated Nutrient Cycling in Soil and Soil Health

PGPRs flourish in the soil ecosystem and play a significant role in determining soil health by breakdown of soil organic matter, crop residue in association with other microbes (Chaparro et al. 2012). Soil moisture is an important parameter in PGPR colonization in the rhizosphere (Vargas et al. 2019). This association leads to biosynthesis of various growth-promoting substances and in turn enhances soil fertility (Raheem et al. 2019). The broken organic compounds enhance soil quality, through nitrogen fixation PGPR boosts soil nutrients (Ijaz et al. 2019). Phosphate solubilizing bacteria enhance phosphate level in soil which can easily be taken by plants (Liu et al. 2019). PGPRs aid in soil enrichment by producing different metabolites such as antibiotics, enzymes, proteins (Prasad et al. 2019). The abundance of bacteria in soil forms an important part of nutrient recycling and maintenance of soil fertility. The zone encircling 1–2 mm around the roots is abundant in nutrients and provides habitat to bacteria (Van Loon and Bakker 2003). Microbial interaction of rhizospheric bacteria and arbuscular mycorrhizal fungi enrich physicochemical properties of soil by aggregate development (Barea et al. 2005). Organic amendment in the soil is shown to be a result of interaction between mycorrhiza and phosphate-solubilizing-bacteria in rhizosphere soil of alfalfa (Barea et al. 2002). Rhizospheric microbes play an essential role in biofixation and biosolubilization contributing towards soil fertilization and enrichment with essential nutrients thus luring a special attention to agricultural application (Compant et al. 2019). PGPR utilizes different direct and indirect modes of action to regulate plant growth and soil nutrition (Harish et al. 2019). Their competence towards rhizosphere, rapid proliferation, contribution in plant growth, disease suppression, green approach makes them suitable for wider application (Santoyo et al. 2012). In a diverse range of soil, PGPRs have shown to metabolize xenobiotic and natural substances by following different modes of action. The combined role of PGPRs exerts more effective response in checking pathogen growth (Kumar and Verma 2019). Overall soil health gets improved by PGPRs and contribution towards replenishment of micro- and macronutrients leads to better crop yield and soil health (Summuna et al. 2019).

17.7 Engineered Nanoparticle Effect on PGPR

Nanomaterials promise a completely new domain which enhances nutrient uptake efficiency in comparison to the conventional methods. Nanoparticles can significantly enhance plant growth at physiological levels and improve crop yield (Siddiqui et al. 2015). Nanoparticles (NPs) role in plant growth promotion in combination with plant growth-promoting rhizobacteria (PGPR) has been widely reported (Khan and Bano 2016; Jahangir and Javed 2020). The PGPRs along with NPs application can help to improve agricultural yield and enhance disease tolerance capability in crops (Nayana et al. 2020). Titania NPs inoculated with double PGPR have stimulated root colonization by PGPR by 25% via leading to formation of micro niches that promote bacterial units for crop improvement (Timmusk et al. 2018). Titania NPs have been shown to act as an interface between the bacterium, *Bacillus amyloliquefaciens* UCMB5113 and roots of *Brassica napus* which conferred disease resistance against the fungal pathogen *Alternaria brassicae* (Palmqvist et al. 2015). Similarly, gold NPs have enormous application in the agricultural sector owing to its lower toxicity (Rashid et al. 2014). Formulations based on Gold NPs may have huge role to play in agricultural sector (Pestovsky and Martinez-Antonio 2017). The presence of 6.25 μg/mL gold nanoparticles showed to enhance growth of *Bacillus* and *Pseudomonas* (Shukla et al. 2015). Likewise, gold NPs enhanced indole acetic acid (IAA) production in *P. monteilii* (Panichikkal et al. 2019). Siderophore production in bacteria was enhanced by zinc oxide nanoparticles (ZnONPs) in dose-dependent way and zinc solubilizer bacteria like *Pseudomonas*, *Bacillus* and *Azospirillum* are capable of forming ZnONPs (Haris and Ahmad 2017; Sultana et al. 2019). Report confirms that the production of solubilizing zinc is produced by zinc solubilizing bacteria and affect plant growth (Kamran et al. 2017). The PGPR in combination with ZnONPs may revolutionize the agricultural domain. Nano-fertilizers has enormous potential to enhance nutrient uptake efficiency, boost crop productivity and incur minimum negative impact on soil (Qureshi et al. 2018). PGPR in combination with NPs can be a new area for environmentally sustainable technology for management of better yield and disease management.

17.8 Microbial Signals in Plant Growth and Development

The rhizosphere defines that soil volume which is greatly affected by plant roots and their exudation (Andrade et al. 1997, Mahaffee and Kloepper 1997, Bringhurst et al. 2001). The coordinated effect of cell proliferation, expansion and differentiation regulates plant growth and development which are regulated by various signals under biotic and abiotic stresses (Table 17.1). The microbes and plant relationship is intricate where plants provide microbes with organic compounds and other nutrients whereas in return microbes release volatile molecules, phytohormones that confer immunity to plants and support their growth and development (Ortíz-Castro et al. 2009). Molecular dialogues from microbes and plants play an important role to decide the nature of partnership from symbiosis to pathogenesis.

Plant growth stimulation by rhizobacteria indulges various exchanges of signals between bacteria and plant roots. The first step towards the onset of signalling cascade includes the release of phenolic compounds, flavonoids, isoflavonoids by roots of plants that induce nod genes (Bais et al. 2004). In return, rhizobia secrete various host-specific signalling compounds. Soybean plants secrete genistein that stimulates the expression of nod gene in *B. japonicum* (Ip et al. 2001). Bacterial association with plants depends on bacterial proximity and level of intimacy with roots. Based on it PGPR association may be defined as extracellular or intracellular (Gray and Smith 2005). Soil microbes are capable of detecting host plants and accordingly they initiate producing phytohormones such as auxins and cytokinins and determine their colonization strategy (Bais et al. 2004). During biotic and abiotic stresses, crosstalk among bacteria and phytohormones modulates endogenous hormone levels and induces systemic resistance to support plant growth and development (Khan et al. 2020). The growth regulators produced by microbes modulate a plant's hormone level that imparts a similar function as induced by exogenous phytohormones (Egamberdieva 2009; Turan et al. 2014). Rudrappa et al. (2008) showed root exudates like malic acid acts as signalling molecules that attract *Bacillus subtilis* towards root which provides tolerance against pathogen *Pseudomonas syringae* to plants. Increased colonization by rhizobacteria and mycorrhizal fungi has been observed in response to organic acids such as citric acid or malic acid by genetically engineered tobacco and alfalfa plants (Tesfaye et al. 2003; Lopez-Bucio et al. 2000). These studies suggest that bringing about changes in root exudation transgenetically may alter the rhizosphere for application in agriculture.

17.9 Important Research Gaps and Future Challenges

The beneficial rhizospheric microbial communities are drivers of soil geochemical cycles and have enormous potential to boost plant growth-promoting environmental sustainability. However, beneficial roles of these microbes are well known but unfortunately they have not been exploited to their potential as commercial products, but with growing concern towards organic agriculture and seeing long-term

Table 17.1 Interactive role of PGPR inoculant in boosting plant growth and development

S. no.	PGPR inoculant	Mode of action	Plant	Effect on plant	References
1.	*Providencia rettgeri* P2, *Advenella incenata* P4, *Acinetobacter calcoaceticus* P19 and *Serratia plymuthica* P35	IAA production, nitrogen fixation, phosphate solubilization	Alfalfa, cucumber, oat	Improved root/shoot weight, root/shoot length, chlorophyll content	Li et al. (2020)
2.	*Azotobacter chroococcum* (Ac), *Azospirillum brasilense*	PGP traits	Pennyroyal	Increased flavanoid, oxygenated monoterpenes phenolic, proteins and soluble sugars	Asghari et al. (2020)
3.	*B. amyloliquefaciens*, *B. brevis*, *B. circulans*, *B. coagulans*, *B. firmus*, *B. halodenitrificans*, *B. laterosporus*, *B. licheniformis*, *B. megaterium*, *B. mycoides*, *B. pasteurii*, *Paenibacillus polymyxa*, *B. subtilis*	PGP traits	Basil	Significantly improved plant height, water use efficiency, stomatal conductance and yield	Moncada et al. (2020)
4.	*Bacillus xiamenensis PM14*	ACC deaminase	Sugarcane	Disease suppression	Xia et al. (2020)
5.	*Azospirillum brasilense* SP7, *Bacillus Sphaericus* UPMB10, *Erwinia sp.* UPMSP10, *Klebsiella sp.* UPMSP9	Nitrogen fixation	Sweet potato	Improved storage root yield	Yasmin et al. (2020)
6.	*Paenibacillus mucilaginosus Sinorhizobium meliloti*	Increased the nutrient (N, P and K) contents	Alfalfa	Improved biochemical response in plant	Ju et al. (2019)
7.	*Bacillus subtilis*, *Bacillus thuringiensis* and *Bacillus megaterium*	Phosphate solubilization, antibacterial and antifungal activity	Chickpea	Enhanced chlorophyll, protein and sugar contents	Khan et al. (2018)
8.	*Klebsiella* sp.	PGP traits, higher root colonization	Oat	Increased shoot/root length, shoot/root dry weight and relative water content (RWC)	Sapre et al. (2018)

(continued)

Table 17.1 (continued)

S. no.	PGPR inoculant	Mode of action	Plant	Effect on plant	References
9.	*Pseudomonas aeruginosa*	Phosphate solubilization, siderophore production, chitinase production	Sunflower	Higher seed oil concentration, fatty acid composition and harvest index	Arif et al. (2017)
10.	*Bacillus* sp.	IAA, ammonia production, phosphate solubilization, nitrogen fixation	Tomato	Root growth, root length, total chlorophyll	Moustaine et al. (2017)
11.	*Stenotrophomonas maltophilia* SBP-9	ACC, gibberellic acid, IAA, siderophore, phosphate solubilization	Wheat	Increased plant growth, fresh weight, dry weight, chlorophyll content	Singh and Jha (2017)
12.	*Arthrobacter protophormiae* (SA3) and *Dietzia natronolimnaea* (STR1)	IAA, ACC production	Wheat	Enhanced photosynthetic efficiency	Barnawal et al. (2017)
13.	*Bacillus megaterium, B. subtilis, B. subtilis* subsp. *subtilis* and *pseudomonas* sp., *Bradyrhizobium* sp.	IAA production	Peanut	Root morphology, disease suppression	Yuttavanichakul et al. (2012)
14.	*Bacillus* sp. (AW1), *Providencia* sp. (AW5) and *Brevundimonas diminuta* (AW7)	HCN, ammonia production, siderophore production, ACC deaminase, antifungal activity	Wheat	Enhanced plant height, root weight, panicle weight	Rana et al. (2011)
15.	Rhizospheric bacteria PGB1, PGB2, PGB3, PGB4, PGB5, PGT1, PGT2, PGT3, PGG1 and PGG2	IAA, phosphate solubilization	Rice	Improved plant height, root length and dry matter production of shoot rice seedlings	Ashrafuzzaman et al. (2009)

ACC 1-Aminocyclopropane-1-carboxylate deaminase, *IAA* indole-3-acetic acid, *HCN* hydrogen cyanide, *N* nitrogen, *P* phosphorous, *K* potassium, *PGP* plant-growth promoting ill-effects of using chemical fertilizers, a major group of farmers is attracted to the use of these microbes. Currently, studies have remained focused mostly on the beneficial role of rhizospheric microbes but their application in the form of a product is limited. However, the development of bio inoculants, plant-growth-promoting rhizobacteria (PGPRs) formulation based upon crop species and environmental stress could change the game.

17.10 Conclusion

Excessive use of chemicals in agricultural soils is a threat to the environment and identifying an eco-friendly approach towards sustainable practice is on priority. Application of PGPR is one approach that could change the game but its wide-scale application in the field is still in its beginning stage. Rhizospheric microorganisms perform multiple functions towards improving plant growth and conferring disease tolerance in plants. The complex signalling between soil microbes and plants helps both to survive and contribute towards replenishment of soil fertility. The potential role of PGPR has enormous power to help achieve the goal of sustainable agricultural practice if utilized at the field level. However, stable PGPR-based formulations need to be developed further for their wider applicability in the agricultural domain.

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Rhizospheric Microbes and Plant Health

18

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Abstract

The microflora of rhizosperic microorganisms at different richness levels have shown to contribute in the composition and plant biomass with complementary relationship among the microbial diversity. In the pursuit of demanding food production, tonnes of synthetic fertilizers have been applied in agroecosystems all over the world, resulting into deterioration and degradation of physicochemical properties of soil, leaving behind the toxic residues. However, the participation of rhizospheric microorganisms in organic matter decomposition, enhanced plant growth and nutrient cycling can be the alternative source of nutrient management. The rhizospheric microorganisms as biocontrol agents have a great role in improving the plant health by playing antagonistic against other plant pathogens. Hence, exploring these beneficial rhizospheric microorganism towards the sustainable development of agro-ecosystems is a major concern.

18.1 Introduction

Post green revolution the production and productivity of food grains has become a blessing to prevent food shortage worldwide, but to meet the goal the increased chemical input has led to nutritional imbalance, soil acidification and poor structure of the rhizosphere micro-ecological environment, consequently leading to the activity of heavy metal ions in soil. Xiang de Yang et al. (2017) have shown that long-term application of nitrogen fertilizers in tea cultivation has significantly decreased soil pH and microbial metabolic activity hence altering the bacterial composition of

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soil. The global issue in trend currently is sustainable agriculture. The alternative tool to replace the harmful impact of synthetic fertilizers is to switch to organic fertilizers. Another factor is that organically bound nutrients are more stable than synthetic fertilizers. In addition, organic fertilizers are ecofriendly, pollution-free and cost-effective being affordable to the farmers. A better option is to restore the soil fertility in a sustainable way and when it comes to the positive plant-soil feedback, it reflects on the beneficial interactions between plant roots and microorganisms for nutrient acquisition, disease suppression and growth promotion. Zhang et al. (2016) showed that the use of compost @3000 kg ha^{-1}, which was equal to 60 kg N ha^{-1} replacing 30% total N fertilizer (250 kg N ha^{-1}), was an effective method of increasing the maize yield, N-uptake and soil fertility and reducing N loss. The potential of organic fertilizers in combination with biofertilizers has been documented in a number of studies that have shown increased growth of foliage, root matrix and rhizospheric microbial community resulting into yield enhancement.

The underground world, which is the treasure of minerals and nutrients that provides all the essential elements to the plants for better growth and development, has its own peripheral dimension within which the complex interactions between the roots and microorganisms are found. The root exudates or the chemicals released by the plant roots into the soil encourage in attracting beneficial microbes such as fungi, mycorrhiza and rhizobacteria etc. towards the rhizospheric zone which ultimately form complex relationship with each other. These beneficial microbes are believed to play important roles in the enhancement of the innate immunity of the plant against the phytopathogens by triggering the genes responsible for inducing the resistance. The noble reason behind this theory is that there are phenotypic and genotypic variations in plant traits that guide the specific microbial group that can enhance growth by a variety of ways. Apart from it their concentration, movement and distribution of the exudates around the roots may also be affected by the biotic and abiotic processes. During the last decade, the perception of root biology, composition, molecular and genetic approaches have been drastically improved.

18.2 Composition, Structure and Function of the Rhizosphere

The diversity and ubiquitous nature of microorganisms are well known but their distribution is not even. Rhizosphere is the core habitation of these microbes, where the population density is significantly higher as compared to the non-rhizospheric soil. Also, the population structure is influenced by the biotic and abiotic factors. Some of the features such as plant age, cultivar genotype, soil and root structures etc. have key roles to play in determining the population (Smalla et al. 2001; MacDonald et al. 2004; Schmidt et al. 2019). This has been reported that species of *Bacillus, Chloroflexi, Microcoleus Clostridium, Caldilinea* were found dominant in the rhizosphere of Para grass (*Urochloa mutica*) (Mukhtar et al. 2016). Similarly, in soybean (*Glycine max*) the microbial community consisted of *Bacillus, Bradyrhizobium rhizobium, Stenotrophomonas, Streptomyces* (Sugiyama et al. 2014). In rice (*Oryza sativa*) the dominant species reported by Mahyarudin et al. (2015) were

Geoderma tophilus, Actinokineospora, Actinoplanes and *Streptomyces.* In wheat (*Triticum aestivum)* rhizosphere the dominant microorganism recorded were *Azoarcus, Balneimonas, Bradyrhizobium, Gemmatimonas, Lysobacter, Methylobacterium, Mesorhizobium, Microvirga, Rubellimicrobium, Rhodoplanes, Skermanella* (Naz et al. 2014). *Acidobacteria, Gemmatimonas* and *Rhodoferax* were found to be associated with Maize (*Zea mays* L.) (Correa-galeote et al. 2016). This selective microbial population is governed by the biochemical and molecular determinants around the rhizosphere.

The term “rhizosphere” derived from “rhiza” a word used in Greek for roots. Rhizosphere denotes the narrow zone or medium near root consists of a gradient of physical, chemical and biological properties which interface between root and soil (Dubey et al. 2019) inhabited by a population of microorganism. The hostspot for the microbial community in a nutrient-rich niche helps in their colonization and biochemical activity in exchange influences the host plant in several ways. Rhizodeposition is one of the key factors for an initial substrate-driven community shift, which has the impression on rhizospheric microorganisms, that influence in connecting the genotype of the host-dependent microbiome in colonizing various parts of the root. Plant roots release exudates or secondary metabolites to regulate the rhizosphere that acts as a messenger to play either symbiotic or defensive roles in which plant will ultimately act in negative or positive communication, depending on the elements present in its rhizosphere. The rhizosphere has been broadly classified into three functional compartments or zones, viz. Ectorhizosphere, Endorhizosphere and Rhizoplane (Pinton and Varanini 2001). Ectorhizosphere is the outer most zone or the area of soil adjacent to the root. Endorhizosphere is the zone ranging from root tissues, endodermis and cortical layers. Rhizoplane is the root surface where soil particles and microorganisms adhere. Hence, it is the unique region where all the chemical and biochemical exchange reactions are performed in host–microbe interactions. Depending upon the overall soil environment, defensive mechanism of the plant, nutrient availability and proliferating microorganism, various relationships like symbiotic, parasitic, associative etc. may develop as a result of the colonization of the zone by the microorganisms (Parmar and Dufresne 2011).

So, as a result it has been seen that the association of microbes with plant roots leads to the various favourable influences on the physiological processes of the latter leading to plant growth promotion through nitrogen fixation, solubilizing ferric ions, conversion of insoluble forms of phosphorus to the soluble form, induced systemic resistance (ISR) in plants against pathogens and cell lysis of soil-borne fungal pathogens of plants. Association of these beneficial microorganisms has been reported by several authors and those belonging to genera *Bacillus, Pseudomonas, Rhizobium, Serratia, Kushneria, Rhodococcus, Arthrobacter* were found associated with phosphate solubilization (Igual et al. 2001; Chen et al. 2006; Zhu et al. 2011), while *Pseudomonas, Bacillus, Serratia, Rhodococcus, Acinetobacter* have been reported to produce siderophores (Chaiharn et al. 2009; Koo and Cho 2009; Rokhbakhsh-Zamin et al. 2011; Sahu and Sindhu 2011). Antagonistic behaviour in plants induced by *Pseudomonas, Bacillus, Serratia* and streptomyces through the production of antibiotic compounds such as phenazines, 2,4-diacetylphloroglucinol, pyoluteorin, pyrrolnitrin and lipopeptides etc (Thomashow and Weller 1988; Hwang et al. 1994; Maurhofer et al. 1994; Kamensky et al. 2003; Kim et al. 2004; Jayaprakashvel et al. 2010). It is also evident that salicylic acid and elicitors are produced by some bacteria viz; *Pseudomonas, Bacillus* and *Serratia* (de Meyer and and Hofte 1997; Bargabus-Larson and Jacobsen 2007). The microbes associated with rhizosphere also contributes to disease management through antagonism such as root rot caused by *Macrophomina phaseolina* in brinjal which is controlled by *Trichoderma harziznum* NBRI-1055 and *Trichoderma harzianum* BHU-99 (Singh and Singh 2014a), and the immune response observed in *Solanum melongena* after inoculation with *Trichoderma* isolates (BHU51 and BHU105) against the pathogen *Sclerotium rolfsii* (Bisen et al. 2019).

18.3 Plant Health and Rhizosphere Microorganisms

As discussed above, the role of microbes in releasing metabolites essential for the growth and development of plants and acting as biocontrol agents against the disease-causing pathogens is of paramount importance. So, the question arises how plants recruit their microbes for their selective functions. Hongwei Liu et al. (2020) confirmed that in the presence of the pathogen, the beneficial bacterium SR80 was able to significantly enhance plant growth both in roots and shoots as well as it induced resistance against the Crown Rot disease by upregulating plant defence signalling (e.g. JA and SA) in shoots with the presence of the pathogen. These findings provided a novel mechanism of tripartite interactions between the devastating pathogen, the plant host and plant-associated microbiota. It can also put the point that for plant–microbiome assembly disease infection can be a critical driver. However, this change in the microbial community does not happen with all the diseases as it has been mentioned that healthy plant equally acts on recruiting microbial population into the rhizosphere (Qiao et al. 2017; Imchen et al. 2019). So this theory supports the co-evolution theory of mutualism between plants and microbes as well as it is likely a survival strategy conserved across the plant kingdom (Liu et al. 2019; Liu and Brettell 2019) and the mechanism underlying is still unclear in many aspects. The composition of the exudates from plant roots that lure the microbial community towards the rhizosphere in forming colonies includes water-soluble sugars (Zhang et al. 2015), amino acids (Feng et al. 2018) and organic acids (Kandaswamy et al. 2017). In addition, some other chemical substances like phenolics, vitamins, sugar-phosphate esters, amino substances and hormones too are released as exudates. The microbial assembly in the rhizosphere is mostly influenced by these exudated or rhizodeposits that can act as major carbon sources for microbes, antimicrobial agents and signalling molecules.

Reciprocally, the beneficial microbes residing in the rhizosphere trigger signal transduction and important functional physiochemical processes leading to root enhancement, disease suppression and improved vegetative growth. A list of some rhizospheric microbes in disease suppression is given in Table 18.1).

Table 18.1 Different rhizospheric microbes as biocontrol agents against disease causing pathogen

Sl. no.	Rhizospheric microbe	Pathogen suppressed	Host plant	References
1.	*Trichoderma asperellum* *T. harzianum*	*Sclerotinia sclerotiorum*	Soybean	Macena et al. (2020)
2.	*Trichoderma asperellum*	*Fusarium graminearum* *Fusarium verticillioides*	Maize	He et al. (2019)
3.	*Penicillium* spp. (GP15–1)	*Colletotrichum orbiculare, Rhizoctonia solani*	Cucumber	Hossain et al. (2014)
4.	*Trichoderma asperellum* *T. asperellum* (T-203)	*Fusarium oxysporum* f.sp. *lycopersici* *Pseudomonas syringae* pv. *lachrymans*	Tomato Cucumber	Segarra et al. (2010) Yedida et al. (2003)
5.	*T. harzianum* T-22	*Alternaria solani*	Tomato	Seaman (2003)
6.	*T. harzianum*	*Rhizoctonia solani*	Tomato	Singh et al. (2014)
7.	*Trichoderma koningii*	*Gaeumannomyces graminis* var. *avenae* and *tritici*	Wheat	Duffy et al. (1996)
8.	*Pseudomonas putida*	*Ralstonia solanacearum*	Tomato	Sun et al. (2017)
9.	*Pseudomonas fluorescens*	*Botrytis cinerea*	Pepper	Dutta et al. (2020)
10.	*Pseudomonas* sp. DF41	*Sclerotinia sclerotiorum*	Canola	Berry et al. (2010)
11.	*Pseudomonas aeruginosa*	*Cucumber mosaic virus CMV*	Tomato	Dashti et al. (2012)
12.	*Pseudomonas aeruginosa*	*Heterodera cajani*	Sesame	Kumar et al. (2009)
13.	*Pseudomonas pseudoalcaligenes* AVO110	*Rosellinia necatrix*	Avocado	Pliego et al. (2019)
14.	*Bacillus siamensis* LZ88	*Alternaria alternata*	Tobacco	Xieab et al. (2020)
15.	*Bacillus brevis*	*B. cinerea*	Chinese cabbage	Seddon and Edwards (1993)
16.	*Bacillus amyloliquefaciens*	*Huanglongbing*	Citrus	Tang et al. (2018)
17.	*B. subtilis*	*Curtobacterium flaccumfaciens*	Common bean	Martins et al. (2013)
18.	*Bacillus subtilis* B47	*Bipolaris maydis*	Corn	Ye et al. (2012)
19.	*Bacillus subtilis* PTS-394	*Fusarium oxysporum* and *Ralstonia solanacearum*	Tomato	Qiao et al. (2014)
20.	*Bacillus subtilis* HJ5	*Verticillium dahlia*	Cotton	Li et al. (2013)
21.	*Bacillus amyloliquefaciens* FZB42	*Rhizoctonia solani*	Lettuce	Chowdhury et al. (2015)
22.	*Streptomyces* spp.	*Phytophthora capsici*	Pepper	Abbasi et al. (2021)

18.4 Role of Different Rhizospheric Microorganismsin Plant Growth and Development

18.4.1 *Trichoderma* Spp.

Trichoderma is an ascomycete widely used as biocontrol agent in sustainable agriculture owing to its effective antagonistic mechanism against plant pathogens through mycoparasitism, competition or antibiosis. *Trichoderma* spp. are the most frequently isolated soil microorganism that colonizes endophytically the roots, can survive under unfavourable conditions, has strong aggressiveness against plant pathogens and high reproductive capacity apart from releasing several secondary metabolites such as volatile and non-volatile terpenes, peptaibols, NRPs, siderophores, pyrones and nitrogen-containing compounds. *Trichoderma* spp. have symbiotic association with a broad range of plant hosts which consequently results into a change in the metabolism, phenolic compounds, photosynthetic rate, transpiration etc. This recognition and attachment process between the fungus and the host plant is mediated by hydrophobins. During the course of interaction between the plant and *Trichoderma* spp. the latter has been found to induce ethylene (ET) which regulates cell differentiation and defence responses as reported on *T. atroviride* (Contreras-Cornejo et al. 2015b), whereas both *T. atroviride* and *T. virens* produce Indole −3-acetic acid (IAA) that controls a number of growth and development processes in plants (Contreras-Cornejo and Garnica-Vergara 2015a). Hence, *Trichoderma* spp. help in enhancing the nutritional quality in many crops such as brinjal (Singh and Singh 2014b), chickpea (Mishra et al., 2018), tomato (Molla et al. 2012) and other leafy vegetables (Fiorentino et al. 2018).

18.5 Mycoparasitism-Related Secondary Metabolites

Mycoparasitism is a complex process that involves growth of the biocontrol agent towards the pathogenic fungi, through lectin-mediated coiling of hyphae to the pathogen (Harman 2000), followed by the secretion of antibiotic metabolites, resulting in attack and killing of the pathogen (Omann et al. 2012). Another mode of action includes the specific inhibitors of mitochondrial metabolism in the pathogen. Apart from these, the activity of pathogens in colonizing the plant tissues is also inhibited by the biocontrol agents through the degradation of pectinases (Harman et al. 2004). *Trichoderma harzianum* releases mycoparasitic metabolites anthraquinone, a compound called pachybasin which inhibits the growth of *Rhizoctonia solani* by increasing the number of coils against the pathogen (Lin et al. 2012). Another chemical component of bisorbicillinoid compound is bisvertinolone containing antifungal properties through inhibition of β-(1–6)- glucan biosynthesis released by *T. longibrachiatum* (Kontani et al. 1994). Similarly hydrolytic enzymes such as chitinases and ß-(1-6) glucanaseshydrolyse secreted extracellularly by *Trichoderma sp.* that inhibit the pathogens such as *Rhizoctonia solani, Botrytis cinerea* and *Phytopthora citrophthora* degrading chitin which is the structural component of fungal cell walls (Druzhinina et al. 2011). Siderophore-producing fungi such as *T. atroviride, T. reesei* and *T. virens* release the compound ferricrocin which is the key metabolite for the competition of iron in the rhizosphere. Several other antimicrobial secondary metabolites such asterpenes, cyclonerane sesquiterpenoids, trichocitrin and trichosordarin A have been reported to be released by different species of *Trichoderma,* namely *T. virens*, *T. harzianum* P1–4, *T. citrinoviride* cf-27 and *T. harzianum* R5 (Ramírez-Valdespino et al. 2018; Fang et al. 2019; Liang et al. 2019).

18.6 Plant Growth Regulators

Plant growth regulation by *Trichoderma* has been observed mostly through the production of plant growth hormones such as auxins. Involvement of *T. atroviride* in the production of ethylene was found which enhances formation of a number of root hairs and lateral roots. The plant roots are very complex in nature both morphologically and physiologically. In *A. thaliana* the signalling mechanism created by *T. virens* was found to enhance shoot growth and lateral root development (Contreras-Cornejo et al. 2009). Another mechanism of *Trichoderma* spp. in enhancing the plant growth and productivity involves both chelation and reduction as well as solubilization of mineral nutrients such as rock phosphate, Cu^{2+}, Mn^{4+}, Fe^{3+} and ZnO in the rhizosphere. *T. harzianum* T22 produces some diffusible metabolites that are capable of reducing Cu(II) and Fe(III) as determined by the formation of Cu(I)-Na2–2, 9-dimethyl-4,7-diphenyl-1,10-phenanthroline disulfonic acid and Fe(II)-Na2-bathophenanthroline disulfonic acid complexes (Altomore et al. 1999). Plants can only utilize the solubilized elements as a source of nutrition from the rhizosphere, hence chelation and solubilization by the beneficial microorganism improve the growth and health condition of the host plants.

18.6.1 Plant Growth-Promoting Rhizobacteria (PGPR)

The free-living soil bacteria beneficial for the growth and development of plants are referred to as plant growth-promoting rhizobacteria (PGPR). Some of the known bacteria such as symbiotic nitrogen-fixing bacteria including *Rhizobium*, *Allorhizobium*, *Azorhizobium*, *Sinorhizobium*, and *Mesorhizobium* and free-living nitrogen fixers like *Azospirillum*, *Enterobacter*, *Klebsiella* and *Pseudomonas* have been found to colonize the root surfaces of their host plants. A rhizobacterium will be marked as PGPR when it exhibits growth-promoting properties like production of phytohormones such as Gibberellic acid (GA), indole acetic acid (IAA) and cytokinins, ammonia and HCN, in addition to solubilization of mineral phosphate and the antagonistic behaviour towards plant pathogens. The presence of PGPR in the rhizosphere induces physical and chemical changes in plants including the rhizosphere. Several nutrients that remain in the soil in their insoluble forms are required to be solubilized to be available for the plants. Strains of P-solubilizing

Pseudomonas striata, B. Polymyxa and *B. megaterium* have been commercialized in India by AgriLife company (Mehnaz 2016).

18.7 Abiotic Stress Tolerance

During the course of abiotic stresses in the plant, a significant increase in the concentration of EPS, total soluble sugar content, total free amino acids and proline can be observed. The PGPR strain *Paenibacillus polymyxa* enhanced the drought tolerance of *Arabidopsis thaliana* by stimulation of the transcription of a drought-response gene. It has also been demonstrated that some of the volatile organic compounds (VOCs) that are released from *Bacillus subtilis* GB03 (Ryu et al. 2004) have been marked as determinants involved in 'induced systemic tolerance' (IST), a term proposed for PGPR-induced chemical and physical changes during the abiotic stress that result in enhanced tolerance of plants (Yang et al. 2009).

18.8 Siderophore Production

Iron nutrition in plants serves as the first line of defence against root invading pathogens and helps in the removal of toxic substances from the polluted soil. Several reports suggest the involvement of microbial siderophores in the conversion of insoluble iron into a soluble form. Regulation and uptake of iron by concomitant oxidation of Fe^{2+} to Fe^{3+}, and precipitation of the latter as ferric hydroxide is the mechanism through which PGPR make iron available to the plants in its soluble form. Based on their chelating groups they are categorized into two groups: (1) hydroxamate, viz. ferribactin, francobactin, aerobactinand Schizokinen and (2) catecholates or carboxylate, viz. enterochelin, agrobactin and parabactin. Pyoverdin types of siderophore is produced by the PGPR *Pseudomonas flurescense, P. aeruginosa* and *P. putida.* However, Hydroxamate type of siderophores are released by *P. syringae.* Sayyed and Patel (2011) have reported that along with metal-resistant siderophore production PGPR have also been seen as a potent biocontrol agent over synthetic fungicides.

18.9 Major PGPR Involved in Plant Health

18.9.1 *Bacillus* spp.

Bacillus spp. are one of the frequently studied PGPR due to their potential to act as biocontrol agents by the production of a wide range of antibiotics, toxins, lipopeptides and hydrolases (Lamsal et al. 2012). In addition, this species is more attractive and effective due to its resistance to adverse environmental conditions. These are mechanisms by which *bacilli* can enhance plant growth: either by increasing nutrients through the production of phytohormones, organic acids involved in

P-solubilization, siderophores and nitrogen fixation or by the production of inhibitory substances or by stimulation of the plant resistance against pathogens. As it has been specified earlier that the production of protease by the microorganism has very significant use in the industry such as cheese making, meat tenderization, detergents and baking. *Bacillus* sp. however, produces alkaline proteases that are important in the detergent industry due to their high thermal and pH stability. Chakraborty et al. (2009) reported that *Bacillus subtilis* under in vitro conditions cause structural deformities in pathogenic fungi due to the production of diffusible and volatile antifungal compounds.

18.9.2 *Pseudomonas* spp.

Pseudomonas spp. are ubiquitous PGPR and a diverse genus of the *c-Proteobacteria*, the cultural characteristics comprising of rod shape with polar flagella and gram negative. The term is derived from two Greek words, 'Pseudo' meaning 'false' and 'monad' meaning 'a single unit.' The species of *Pseudomonas* comprises *P. fluorescens* with four biotypes, *P. putida* with two biotypes, *P. syringe*, *P. chlororaphis, P. aureofaciens* and *P. aeruginosa* (Schippers et al. 1987). Unlike the *Bacillus* spp. PGPR *Pseudomonas* spp. also have antagonistic activity against phytopathogenic microorganisms by synthesis of antibiotics, enzymes, anti-fungal compounds, siderophore production, nutrients, competition for binding sites etc. This PGPR is one of the extensively studied PGPR in agricultural research. Reports by Antoine Desrut et al. (2020) suggest that the species *Pseudomonas simiae* WCS417 has plant growth-promoting effects on the model crop *Arabidopsis thaliana* by inducing major transcriptional changes in plant sugar transport possibly by controlling the amount of sugar transported from the shoot to the root and to the rhizobacteria. *Pseudomonas* spp. exert a direct inhibitory effect on the growth of fungal hyphae through the production of cell wall degrading enzymes such as ß-1,3 glucanase, protease and chitenase, therefore plays effective role against soil-borne invaders or pathogens (Masood and Bano 2016). Hence, the structural intrigrity of the phytopathogen affected by these cell wall degrading enzyme resulting into osmotic lysis of the cell (Budi et al. 2000).

18.9.3 *Rhizobium* spp.

Rhizobium spp. are well-known biofertilizer, the symbiotic fixer of nitrogen and some of them are endophytic bacteria. They can solubilize soluble organic and inorganic phosphates unlike other PGPR and produce siderophores, HCN, phytohormones and can also colonize the roots of non-leguminous plants (Sessitsch et al. 2002). In agriculture, *Rhizobium* spp. are the most common type of biofertilizer developed commercially. These rhizobacteria bear unique ability to produce root nodules through which nitrogen fixation takes place. The formation of nodules on the host plants is the consequence of infection caused by the bacterium. In leguminous plants this typical mutualism for nitrogen fixation has been studied widely and exploited as a means of enhancing yield on crop plants (Sharma et al. 1993). After the host plant dies, the nitrogen becomes available to the other plants. In several studies on crop plants such as rice, wheat, maize and many other crops, a significant increase in yield has been reported after inoculating with *Rhizobium* spp. (Bhattacharjee and Singh 2008; Hilali et al. 2001; and Riggs et al. 2001).

18.9.4 Azotobacter

Azotobacter in contrast with the *Rhizobium spp*. are free-living, nitrogen-fixing PGPR. They are oval or spherical in shape, the size ranging from 2 to 10 mm in length and 1 to 2 mm in width. Under unfavourable environmental conditions, these structures form thick-walled cysts. These PGPR are known to accelerate many functions such as production of phytohormone like Indole-3-Acetic Acid, fix nitrogen from the atmosphere for their protein synthesis which leads to mineralization in the soil making the availability of nitrogen to the plant with the efficiency of fixing about 20 kg N/ha/per year (Kizilkaya 2009; Esmailpour et al. 2013), in addition plays significant role in the degradation of pesticides and bioremediation of heavy metals. This PGPR is also found resistant to oxygen due to respiration protection of the enzyme nitrogenase during the process of nitrogen fixation. *Azotobacter chroococcum* as bioinoculants have shown significantly greater plant dry weight of different crops like tomato, maize and chickpea (Baral and Adhikari 2013; Akram et al. 2016).

18.10 Omics Approaches to Unravel the Rhizosphere Interactions and Function

A series of genomic approaches have been applied till date to study the genomic, taxonomic and functional information of the entire community of microorganisms. The classical method used to study the composition and diversity of microbes is the amplicon sequencing approach where the variation among microorganisms in the soil can be analyzed (Xu et al. 2018). However, due to primer bias, some of the microbes may remain undetectable through this approach. Now researchers are more into studying microbe–microbe and plant–microbe interactions through advanced approaches. In addition, the unculturable microorganisms mostly bacteria residing in the rhizospheric zone and their qualitative characteristic can be analyzed through independent approaches such as metabolomics, metagenomics, proteomics and transcriptomics (Yergeau et al. 2014). Through the metabolomics approaches like gas chromatography–mass spectrometry (GC–MS) and liquid chromatography–mass spectrometry (LC–MS) the chemical constituent of the rhizosphere can be analyzed. Nuclear magnetic resonances (NMR) apart from quantification of chemical compounds also helps in decoding the chemical structure. The complex interactions between microbes and the plant represent the metabolic state of the

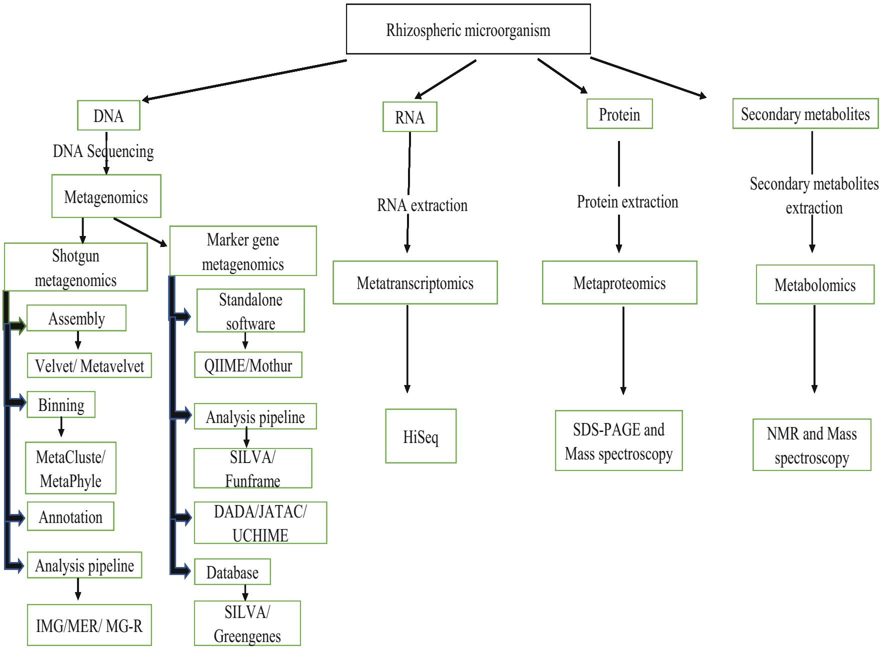

Fig. 18.1 Omics approach development strategies for the analysis of rhizospheric microbiome

organism and yet more things to learn about the mechanism and biological functions of the metabolites released in situ or rhizospheric zone. Also, this is challenging to solve the interaction network of the microbial community hence there is a need for new algorithms and solvers.

Many approaches have been develop so far to know the gene expression in plant-host relationship studies such as connection up-and down-regulation expression analysis of microarrays to know the up- and down-regulation of specific genes as well as their expression studies in different micro-environments. Transcriptomic approaches involve understanding the response of microbial communities toward changing environments or their association with different plants through the comparative analysis of the transcriptome. Metatranscriptomic approaches reveal active community members including various metabolic pathways involved in it. In this approach, the total RNA from the samples is sequenced and it is one of the robust approaches as samples are enriched with the rRNA so without the selection of taxonomic group the entire microbiome can be analysed. Also, these can be carried out on multiple samples excluding the PCR based steps (Urich et al. 2008). However, due to its relatively difficult data storage and high cost for transcriptional profiling, microarray is one of such alternative commonly used tools.

Genomic-based technology in revealing the actual function of microbial communities in situ has been less than successful, whereas metaproteomics helps identify the metabolic activity and functional expression of the metagenome or the whole community proteomics. It is the large-scale study of differentially expressed proteins by the organism, while metaproteogenomics links the combinatorial study of metagenome and metaproteome of the same sample. It allows one to overcome the limitations of metaproteomic approach in protein identifications due to non-availability of closely related reference genomes (Fig. 18.1).

18.11 Conclusion

The rhizospheric microbial flora studied in the past decades were based on the cultivable microbial diversity, in contrast to the uncultivable microbes, which have rarely been explored. The cultivable microorganism in the earth is around 1–5% whereas uncultivable are 95–99% (Malla et al. 2019). Hence, there is an urgent need to explore the potential of this microbial diversity. Future research direction can be focused on developing new optimistic methods, analyzing various parameters and factors affecting root development. To know more about the properties and functions of enzymes or chemicals released by both plants and the microbes is the need of the hour.

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Omics Approaches to Unravel the Features of Rhizospheric Microbiome

19

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Abstract

Over the past few centuries, the rhizosphere has been drawn the attention of the scientific community because of its dynamic structure and function. It is a narrow-zoned vital space that holds the complex ecosystem comprising diverse microbes including fungi, bacteria, protists, nematodes and invertebrates. It is one of the most dynamic interfaces between the plants and its surrounding soil ecosystem. But still there are many knowledge gaps present in the mechanistic understandings of the rhizosphere functioning. Therefore, to untangle the complexity of the rhizo-microbiome interface, an integrated 'multi-omics' approach can be applied to demystify the complex 'rhizosphere-specific data.' The concomitant advent of omics technologies has a great impact on agriculture for the development of crops with the help of their rhizo-microbiome. Thus, the regime of omics technologies with the tandem of computer-aided approaches will render the opportunity of understanding the true sovereignty of the rhizosphere and its microbiome.

19.1 Introduction

The rhizosphere is a highly complex interfacial ecosystem of the plant roots and its surrounding soil. It is the most complex microbial domain on Earth, with an estimated 10^{11} microbial cells per gram of root representing over 10^{12} functional genes per gram of soil (Egamberdieva et al. 2008; Prosser 2015). The term was given by Lorenz Hiltner in 1904, who showed that the synchrony of the rhizospheric

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microbiome significantly influences the plant nutrition (Morgan and Whipps 2000). It consists of all domains of microbial life, including archaea, bacteria, fungi, picoeukaryotes and viruses. Some microbes are associated with the plant roots in terms of mutualistic association to promote each other, while some microbes render parasitic or pathogenic functions. Over the past few centuries, it has drawn the attention of the scientific community because of its dynamic structure and function. The microbial community associated with the rhizosphere of the plants constitutes their microbiome and is involved in promoting their growth by eliminating the pathogens from the system and producing the chemicals that help in their development. The microbiome infers the totality of the genomes of a microbiota of a specific place that renders the entity of the microbial traits encoded by the microbiota. The behaviours of the rhizospheric microbiota are believed to change as per the nature and features of the plant root exudates because they grow and reproduce under the strict influence of the plant-root system (Hartmann et al. 2008; Doornbos and van Loon 2012; Burns 2010). The plant root secretes a miscellany of primary metabolites (sugars, amino acids and organic acids) and secondary metabolites (phenolics, flavonoids and terpenoids) to interfere, shape and communicate with their rhizospheric microbiome (Philippot et al. 2013; Doornbos et al. 2012). In turn, the structural and functional characteristics of rhizosphere microbiome have a reciprocating impact on plant growth and vitality (Morgan and Whipps 2000). In the root micro-environment, microbes build up and convert organic matter into inorganic compounds and provide nutrients to the plants; also they secrete various stimulants for the plant-growth development.

19.1.1 Present Scenario of the Rhizospheric Science

A PubMed search between 1947 and 2020 shows a total of 10,486 published manuscripts containing the ‘rhizosphere’ keyword, of which almost 75% have appeared in the last 10 years. The early publications, studies carried out without using computers, demonstrated the significance and need of technological upliftment to understand the essence and characteristics of the rhizosphere at micro-level. The growth in published rhizosphere studies over the last decade can be correlated with many advancements in high-performance technology, improving computing skills, web sophistication as a medium to disseminate data and the emergence of new algorithms for complex, multi-omics datasets study (Berg and Smalla 2009; Mendes et al. 2011; Bulgarelli et al. 2012; Lundberg et al. 2012). The new methodologies seek to determine the microbial composition, specificity and functionality of the community scale of rhizosphere microbiome and diverse network of molecular plant-microbe interactions (Crick 1958; Weston and Mathesius 2013; Moe 2013; Hartmann and Schikora 2012; Ryu et al. 2003).

19.1.2 Multi-Omics Approach

Multi-omics is a biology-based interdisciplinary approach to the study of the biological systems with the association of multiple -omes viz. genome, proteomes, transcriptome and metabolome at holistic level. The given multiple -omes datasets were comprised of the individual organisms as well as multi-organism communities and consortia. The integrative multi-omics approach directly gathers the data from various equipment and integrate them with multivariate statistics to make them applicable for the study of 'central dogma' of molecular biology: that is, DNA, mRNA and proteins (White et al. 2017).

The goal of this study is to provide a top-view on how the rhizospheric field could emerge as an evolving area for integrative multi-omics. In this context, the difficulties in obtaining rhizosphericomics results, the lack of rhizosphere-direct multi-omics studies, the implementation of multi-omics in substitute areas (e.g. land and plant systems) and the potential of integrated multi-omics research have been addressed.

19.2 Integration of Omics Approaches in Rhizosphere Studies: An Overview

Rhizosphere analysis currently yields few integrated multi-omics findings, but numerous illustrative studies have applied diverse omics methods for the elucidation of structural and functional aspects of the rhizosphere. For example, the community structure of the microbiome has been directly revealed by NGS, 16S rRNA, PCR amplicons, which measured organism diversity in the rhizosphere of *Arabidopsis thaliana*, common annual grass (i.e. *Avenafatua*), soybeans, maize, coffee and white lupins. Furthermore, outstanding metagenomic experiments in soybeans and rice have shown potential metabolic capacity of the rhizosphere (Mendes et al. 2014; Peiffer et al. 2013; Caldwell et al. 2015; Shi et al. 2015; Bulgarelli et al. 2012; Marschner et al. 2002; Knief et al. 2012).

A cereal (soybeans and maize) meta-transcriptome analysis found that glyphosate formulations influence nutrients, carbohydrates and amino acid metabolism of bacterial cell. In a further meta-transcriptomic analysis comparing cereal grain rhizospheres (i.e. oat, wheat) to that of legumes (i.e. peas), it was found that the rhizosphere microbiome is greater in peas and demonstrates very different rhizospheric microbiomes because of the 'rhizosphere impact' (Newman et al. 2016; Turner et al. 2013).

While no studies in the rhizosphere or in any other ecosystem of the meta-epigenomes are currently available, such a study will represent an imminent opportunity to understand the regulation of a diverse gene microbial community. In the landmark research by Murray et al. 2012 bacterium epigenomics is employed as a method for the examination of the rhizosphere meta-epigenome. While no experiments on the rhizosphere or soil meta-epi-proteome are still available, meta-epi-proteomics have been successfully applied to a wide variety of less diverse microbial ecosystems such as deep vents and acid mining waste (Zhang et al. 2016; Li et al. 2014). It represents another potential opportunity of calculating downstream regulation and role of rhizospheric proteins of the rhizosphere.

Meta-proteomic rhizosphere studies include that of leaf litter decomposition, rhizosphere/root tissue methanotrophy of field-grown rice plants and Black truffle. 4600 proteins with methanogenesis and methanotrophy as the dominant functions were seen by the metaproteomic of rice phyllo-sphere, the above-ground portion of the plant and rhizosphere (Schneider et al. 2012; Bao et al. 2014; Zampieri et al. 2016).

Rhizosphere metabolomics research was based on general exudates and was reviewed in greater detail in van Dam and Bouwmeester et al. (2016). An analysis of 103 total metabolites with *Arabidopsis thaliana* showed that 42 of these were authenticated by established compound criteria. While these experiments have provided a wealth of information, individual observations only scratch the surface information accessible inside a single rhizosphere study. In the meantime, the incorporation of omics will connect various datasets on the community structure to microbial activity, translation, expression and end product metabolites with a holistic approach.

19.3 Omics Techniques to Study the Rhizo-Microbiome Interface

The study of microbial diversity and plant microbe interaction in rhizospheres has experienced considerable developments in non-cultural methods focused on molecular biology and omics in recent years. In rhizospheric study, DGGE, T-RFLP and ARDRA, cloning of DNA and Sanger sequencing continue to be useful, while different omics tools, such as FISH, SIP, microarray, and NGS. grow rapidly for greater insight into the microbiota and microbiome of the rhizosphere. Amplicon sequencing, metagenomic sequencing and whole genome sequencing, which address the biology and biotechnology of the rhizosphere plant microbiome, are demonstrated here for versatile applications of NGS technologies.

19.3.1 Denaturing Gradient Gel Electrophoresis (DGGE) and Temperature Gradient Gel Electrophoresis (TGGE)

The DGGE was developed in the late 1980s to identify the point mutation of genomic DNA fragments and was soon applied in 1993 to investigate the structure of microbial culture. Then TGGE was derived from it (Smit et al. 1999). From then, these methods were commonly used in extensively every field of microbial molecular ecology (García-Gonzalo et al. 2017; Jorquera et al. 2016). The molecular weight and charge influence the migration of the DNA double strand in the common PAGE. The various length DNAs can be divided, but the same length DNAs cannot be separated. DNAs with different sequences of the same length with denaturing agent (urea and formamide) gradient could be distinguished in DGGE/TGGE.

19.3.2 Terminal Restriction Fragment Length Polymorphism (T-RFLP)

T-RFLP was designed to quantify the polymorphism length of the selected DNA fragments, and to analyse the structure and function of the microbial communities based on molecular systematics, PCR, restriction digestion, fluorescent labelling and DNA sequence analysis (Kong et al. 2016). In terms of comparative genomics, a DNA sequence with phylogenetic marker characteristics should be picked and used as the target sequence. Either the ribosomal small subunit 16S rRNA (prokaryote), 18S rRNA (eukaryote) and some functional gene sequences and any specific DNA of the microbiota can be used as the target sequence, potentially. The dynamic change of rhizosphere microbiota and its functions may be dissected by T-RFLP.

19.3.3 Amplified rDNA Restriction Analysis (ARDRA) and Random Amplified Polymorphic DNA (RAPD)

ARDRA selectively amplifies rDNA fragments (e.g16S rDNA), and then rDNA is evaluated for polymorphism of the restriction fragment length (Lee et al. 2006; Verma et al. 2016). Isolation and further characterization of isolates by ARDRA and 16S rRNA sequence were used to determine the diversity of the culturable microbial community (Andreote et al. 2008). (Fairly common rhizosphere species have been identifiable, including α-Proteobacteria, β-Proteobacteria, Actinobacteria and bacilli). The PCR-DGGE showed that with early plant development changes in the bacterial communities occurred, but the initial community composition was observed over time. Transgenics have less impacts in the rhizosphere than in rhizoplane and after a single plant cycle, the soil resistance will recover the original bacterial diversity. RAPD was used to detect the haplotypes of the rhizosphere soil, roots and stem/leaves of *Echinacea purpurea* and *E. angustifolia* (Chiellini et al. 2014). In various plant species and compartments of the same plant species, the presence of distinct bacterial communities may reflect the difference in medicinal properties.

19.3.4 DNA Cloning and Sanger Sequencing

The conventional Sanger clone library sequence of gene cloning is an effective method in the detection of rhizospheric microbes before the invention of high throughput DNA sequencing. Three large 16S rRNA libraries were sequenced from the gene of *Taxus* rhizosphere to explain the geographical variations in the structure and diversity of the bacteria in the *Taxus* rhizosphere (Hao et al. 2008). Just

146 clones were sequenced for three libraries because of the high cost of Sanger sequencing. Rhizosphere fungi can also be used for cloning and Sanger sequencing procedures. The PCR-amplified 18S rRNA genes were used as clones in soil fungal populations of willow canopies at the forefront of a receding glacier (Jumpponen 2007). The four above methods are representative traditional PCR methods which can amplify the trace amount of DNA for gene sequence and rhizosphere microbial diversity comparative analysis. In the rhizosphere experiments, the DNA clone library and Sanger sequencing were obsoleted and replaced with the increasingly high-performance sequencing.

19.3.5 NGS in Crop Improvement

With population and consumption set to rise over the coming decades, achieving food security will require action in many fronts. In order to feed the rising population in 2050, around 3.1 billion tones enhancement in cereal production will be required (FAO). Breeders and scientist are working in many facades to increase the crop productivity in limited arable land. In this era of crop improvement Next Generation Sequencing (NGS) serves as a boon for researchers. The high throughput NGS technologies provide largescale insight for detailed investigation of various crops. Additionally, it also offers the tools for analysing genetic changes occurs during domestication processes.

Sequencing started long back ago when two group of scientists viz. Sanger and Coulson and Maxam and Gilbert developed first ever sequencing method (Morey et al. 2013). Sanger was considered as the ‘first generation sequencing platform’ which was based on chain termination method (Schuster 2008). However, at that time sequencing a genome was very tedious, inefficient and time taking process. Unremitting demand and advancement in sequencing technologies give birth to ‘NGS era’ of sequencing. Roche 454 is the first ever NGS platform which relies upon pyrophosphates for decoding the nucleotides (Pettersson et al. 2009). Swiftly these revolution in sequencing changed the scenario and now we are able to sequence even complex polyploid plant genomes. Presently, Illumina, Pacific Bioscience (Pac-Bio) and Nanopore are among the most widely used sequencing techniques. Pac-Bio and Nanopore allows long read and repetitive region sequencing in low cost and time (Goodwin et al. 2016). These upliftment in sequencing approaches allows long read sequencing, overrules GC biases, high accuracy, low cost and no pre-requirement of PCR amplification (Goodwin et al. 2016). The progression in sequencing created a milestone in the field of crop improvement, as this facilitates sequencing of dozens of crops, even the large and complex genomes. Rice was the first crop plant whose genome was sequenced (Goff et al. 2002; Matsumoto et al. 2005; Yu et al. 2002). Further, the sequencing of maize, sorghum, soybean and various other crops as well as other plants are also achieved. The International Wheat Genome Sequencing Consortium (IWGSC) uncovers the polyploid genome of wheat (Appels et al. 2018). These sequencing projects unearthed various genes and allow us to understand the domestication and selection processes of various plants across the globe.

Current advancement in sequencing led us the identification of various novel markers, which are highly convenient to be utilized in the breeding purposes. The use of marker surpassed the problem of phenotype-based selection which is very tedious and time-consuming process (Varshney et al. 2009). Here populations of large number of individuals can be screened rapidly making easy and fast to determine the genetic basis of phenotypic difference in plants (Henry 2012). Previously, restriction fragment length polymorphism (RFLP), random amplification of polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and single sequence repeat (SSR) were widely used markers in breeding. However, these conventional markers were mostly located in non- regulatory regions, thus maximum times gives false positive during marker assisted selection (Thottathil et al. 2016). Furthermore, these markers were gel-based marker, thus require heavy physical work and is time taking. Reduction in sequencing cost popularized single nucleotide variation (SNP) based markers in marker assisted breeding programmes. SNPs are powerful and highly reliable marker used widely in various genomics studies for quantitative trait loci (QTL)/gene identification (Mammadov et al. 2012). Presently, these markers are widely used in agriculture for incorporating beneficial traits. Marker assisted breeding (MAB) accelerates the pace of breeding and allow development of higher yielding resistant varieties.

Crop improvement is done by harnessing the gene pools of species with large number of beneficiary genes for delivering superior plant performance in agriculture, food and energy production. Upliftment in sequencing and genomics technologies enhances the gene reservoir via uncovering vast number of genes from wild species. Moreover, it is also necessary to understand the traits that are beneficial for human and altered during domestication event. As all traits selected during domestication are not useful, they may often reduce the fitness or adaptation of the plant in wild condition. By using NGS its easy and fast to explore these traits in much larger scale than earlier technologies (Bevan et al. 2017). Along with identifying the genetic diversity of potential value for crop improvement, NGS also help in clarifying phylogenetic relationship (Henry 2012). These all together results in rapid fulfilling the need of fastidious human demands through crop improvement and domestication of new crops.

19.4 Challenges Ahead for Themulti-Omics in the Field of Rhizospheric Science

In several fields of science such as geology, pedology, physics, genetics, bioinformatics, mathematics, statistics and computers science, rhizosphere and soil sciences are soon undertaking a transaction into interdisciplinary approaches. Effective holistic studies of the rhizosphere, along with robust data processing and applications, would focus on advances in both technologies throughout the future.

In our study, we discuss advances in omics technology and strategies as used in rhizosphere or environmental ecosystems.

As previously described, rhizosphere soil is one of those habitats which is extraordinarily complex due to the amount of microbes only. The high diversity of the rhizosphere microbes is demonstrated by a rhizosphere analysis using 16SrRNA PCR amplicon in *Arabidopsis thaliana* sequences across 613 samples. It found greater than 2000 species per gram of rhizosphere soil as operational taxonomic units (OTUs) (Bulgarelli et al. 2012). If the microbial genome is believed to contain ~3000 protein coding genes for ~2000 species, that represents 6x106 bacterial proteins at the low end. At the higher end, 9 × 107 proteins are measured at ~30,000 species per gram (Bulgarelli et al. 2012).

The rhizospheric soil contains very high amounts of soil intrusion humic acids, plant polyphenols and other degraded macromolecules, all of which make it difficult to extract biological molecules, such as DNA, RNA, protein and metabolites. This interferes with the co-extraction and inhibits PCR and ionization required for protein and metabolite analysis. For high resolution omics performance, it is imperative to distinguish these biological molecules from interfering compounds.

One big problem is the extraction bias because many of the microbes are lysis-resistant, and thus the view of microbial communities is bias. Extraction bias is an important issue because most of the times extraction of biological molecules such as DNA, RNA, proteins and metabolites of soil microbes is done by using a single commercial kit or tool. To achieve the most detailed downstream comparisons and evaluations with lowest bias extraction, omics require both rigorous extraction and lysis. Later, in the sections of next-generation sequencing (NGS) and meta-proteomics, we address this challenge in greater depth.

19.5 The Future Prospect of Multi-Omics in the Rhizosphere Science

Currently, only a few numbers of studies provide an overview of the potential of an integrated multi-omics approach in the field of rhizospheric science. In fact, the Hultman et al. study on permafrost is currently the only major integrated study available on multi-omics. The integration was done in this analysis using independent care of each omics to then show the additional value of all omics by using ratios, genome visualization and biplots (Hultman et al. 2015). While multi-omics data were not obtained directly from a plant-mycorrhizal fungal association, previous genome-related information, protein-protein interactions, DNA binding motifs and proteome were used in synchronization with lab-based co-cultures and transcriptomics to obtain multi-omics (Larsen et al. 2016). The plant-mycorrhizal relationship provides an excellent framework for the simulation of the different -omics in a multi-omics approach in a wholesome manner. Larsen et al. (2016) used *k-means* to integrate different omics and a simplified predictive model of sensory networks for further experimentation, which will be associations between plants and mycorrhizal fungi. This is the first step and is an asset in modelling dynamic relationships using many -omics. The most popular approaches for integration of multiple -omics from the mathematical viewpoint and in the case of a single microbial genome are network-based and clustering-based methods (Bersanelli et al. 2016). In order to fully incorporate the rhizosphere's multi-omics, we emphasize that more models, framework and computational technology are need of the hour (De Keersmaecker et al. 2006).

19.6 Conclusion

The rhizosphere microbiome is a fascinating domain for research to understand the potential aspect of plant-microbe interaction in basic and applied microbiology. The integration of omics approaches at the multi-regime level led to greater understanding of the plant growth, crop production and ecosystem health. However, the equipment module associated with the research in rhizosphere microbiome has outpaced its associated software component by generating huge volume of data that can't be analysed with the present set of omics approaches. Therefore, this study tried to answer the possible questions that came with the integration of multi-omics approaches to the rhizosphere microbiome research. The application of multi-omics approach in rhizosphere science offers great potential in fabricating the rhizosphere as a source of improved plant growth and development with increased soil carbon storage in presence of different environmental stresses.

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20

Rhizo-Deposit and Their Role in Rhizosphere Interactions Among the Plant, Microbe and Other Ecological Components for Crop Management

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Abstract

Rhizosphere is the most important ecological niche, which plays a vital role by acting as a connecting link between plant, soil and microbes. Rhizo-deposits are the secretions released by the plant roots in the form of root exudates, border cells of roots and other rhizo-deposits. These secretions mostly include low molecular weight bioactive organic compounds, which may attract various beneficial microbes like PGPR (plant growth promoting rhizobacteria), PGPF (plant growth promoting fungi) for their colonization and some of them also contain antimicrobial defensive compounds that repel various deleterious or plant pathogenic microorganisms. Among several forms of benefits derived from rhizo-deposits, root exudates that help in attracting and harbouring various beneficial microbes like rhizobacteria and fungal bio-agents, play a crucial role in the suppression of various soil borne plant pathogens, insect pests, nematodes and harmful microbes apart from their growth promoting activities. These PGPR, PGPF, and various microorganisms with bio-control potential exhibit excellent colonizing ability with the roots and even some of them establish symbiotic and endophytic relationship with plants. These beneficial microbes colonizing the rhizosphere zone exhibit various types of mechanisms like nutrient solubilization by supplying water and inorganic mineral nutrients in the available form to the plant. They

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exhibit various defence mechanisms like hyper-parasitism, competition, antibiosis and induced systemic resistance response in plants against plant pathogenic and harmful microbes. In addition to these bio-control activities, these beneficial microbes like PGPR's and PGPF's present in the rhizosphere region help in the enhancement of plant growth parameters like shoot and root length, shoot and root biomass, chlorophyll content, vigour, flowering, fruiting and ultimately yield of plants. They also help in alleviating several abiotic stress in plants such as water scarcity, submergence and salinity. This chapter provides the insights into, how bacterial and fungal biocontrol agents help the crop plants in combating several biotic and abiotic stresses through several morphological and phsiological changes in the crop due to application of biocontrol agents and also that how these microorganisms help plants in increasing their growth and vigour. How root exudates help in colonization of various rhizosphere-inhabiting microorganisms and how application of various organic substrates and de-oiled cakes in the rhizosphere zone help in the population build-up and establishment of these beneficial microbes in the rhizosphere zone.

20.1 Introduction

Agriculture in general and crop production in particular during twenty-first century have become more productive and remunerative because of many modern agricultural technologies. These technologies include utilization of agro-chemicals such as pesticides to tackle the losses due to plant pathogens and insect pests and also the application of fertilizers. Basic purpose of using these technologies is to increase crop productivity and yield. Application of chemical pesticides has resulted in tremendous increase in crop productivity and yield but has left some adverse effect on ecosystem and environment due to toxic nature of these agro-chemicals. Loss of beneficial microorganisms and declining population of earth worms due to agrochemicals application have also been noticed during recent past. Prevalence of these toxic side effects has diverted the scientists and visionary to think on some alternate options for crop production. Here natural farming and other methods of cropping/agriculture with lesser or no dependence on chemical input will be a suitable option for future. Some soil micro-floras have been identified, isolated and characterized exhibiting promising characteristics of plant disease suppression and plant growth promotions, once they are applied to rhizosphere. In addition to myco-parasitism, these micro-floras also exhibit the activities of nutrient solubilization. Under these changed scenario, use of microorganisms for plant protection and also for crop growth enhancement is now being adopted as an alternative method of agriculture where no or lesser use of chemicals will be required (Olga and Marcelo 2011). Some indigenous microorganisms are capable of inhabiting the specific crop rhizospheric soils with a strong ability to inhibit the growth and pathogenesis of some important plant pathogens. Their disease management activities are as good as a chemical pesticide. Since these antagonistic microorganisms are highly beneficial for crop growth and vigour apart from their greater potential of suppressing the growth of certain phytopathogens/parasites without use of chemical pesticides, the soils holding them are referred as 'suppressive soils' (Weller et al. 2002). These suppressive soils are charged with a very high population dynamics of crop beneficial and antagonist microflora.

These plant beneficial microorganisms persisting in these suppressive soils are known to perform the biological control of soil borne plant pathogens through competition, parasitism and also through induced systemic resistance. The antagonist generally competes with plant pathogen for space and essential nutrients and by virtue of its fast growth and over exploitation of space and nutrients, the competing pathogen gets inhibited and suppressed. Activity of parasitism is performed by production of hydrolytic enzymes, like chitinase, glucanase, protease, and cellulase, that can lyse pathogen cell walls and kill them; inhibition of the pathogens by antimicrobial compounds (antibiosis) is another way of suppressing the pathogen. Induction of systemic resistance in host plants is performed due to altered metabolism in the plant and rhizosphere to enable the plant to overcome the pathogenesis and harmful effect of plant pathogens (Dennis and Webster 1971; Compant et al. 2005; Bae et al. 2009, 2011; Shoresh et al. 2010). Tremendous research has been carried out during last 50 years on the line of beneficial microorganisms harboring crop rhizosphere. These microorganisms have been found to be competent enough to manage the plant diseases and also to enhance the crop growth and productivity. In spite of the fact that plenty of microorganisms have been potentially identified as biocontrol agents and also as growth promoters, farming community is in strong need for more competent and reliable and consistent antagonists so that the fast-growing demand of toxic residue-free food products can be meeted out. Care should be taken to identify, isolate and improve the beneficial microorganisms from the geographical location, where they are targeted to be applied.

The objectives, 'identification and isolation of beneficial microorganisms with potential biocontrol ability from the intended locality' may be fulfilled by following two steps:

1. The antagonist should be isolated from such an area where soil is naturally suppressive to multiple pathogen.
2. The crop rhizosphere soil, seed or roots are mainly targeted niche where the biocontrol agents are generally applied; hence, one should isolate the antagonists from these niche areas.

During last several years, a number of micro-organisms are being floated in the market as biocontrol agents and multiple low-cost materials are being used as carrier (inert materials), but the problem is that they do not perform in the field as effectively as they performed during lab and glasshouse experiments and are seem to be less effective. They also have many limitations especially the loss of their viability and decreased shelf-life during storage and transportation. So, there is a need for such materials which are of low cost, contain nutrients, degradable and compatible with other ingredients along with support to bio-control agent (BCA). These materials can be used as substrates for mass multiplication and maintain the BCAs population during storage and transportation to a considerable level, also provide nutrition, if it is applied to the soil (field & pots), it will help to sustain the viability and survival for a longer period of time.

Since last few decades, some microorganisms are being used as biocontrol agents. The biocontrol agents are capable of adversely affecting the growth and reproduction of targeted pathogenic microorganisms if they are in closer association either by chance or deliberately. In general, antagonists possess the capacity to interfere with different physiological and biological activities of targeted plant pathogens. All type of microorganisms such as fungi, bacteria, nematodes, protozoa, viruses and phanerogamic plant parasites can be used as potential suppressive agents.

20.2 Microorganisms as Biocontrol Agents

The definition of biological control has been spelled as- *any condition under which, or practice whereby, survival and activity of a pathogen is reduced through the agency of any other living organism (except man himself) with the result that there is a reduction in the incidence of disease caused by the pathogen* (Garret 1965). Basically, biological control is a practice which is an integral component of the method, ‘cultural control’ of plant pathogens where we normally do some alteration and manipulation of favourable ecosystem for disease/pathogen to make the same unfavourable for plant pathogen in such a way that there should be discouragement or reduction in the accumulation of infective propagules (Inoculum density) thereby reducing the disease-causing ability of the pathogen. Biological control was defined by Baker and Cook (1974) as the ‘reduction of inoculum density or disease producing activities of a pathogen or parasite in its active or dormant state, by one or more organisms, accomplished naturally or through manipulation of the environment, host, or antagonists, or by mass introduction of one or more antagonists’. Subsequently, in 1982, the definition was reviewed, revised and redefined as ‘biological control is the process where there is reduction in the amount of inoculum (Inoculum density) or disease producing activity of a pathogen (Inoculum capacity) which is brought about by either one or more organisms (Consortia of antagonist) other than man’.

20.3 Phenomenon of Antagonism (Mechanism of Action)

In the process of biological control, the bioagents protect the plants against plant pathogens or their harmful effects, i.e. diseases, either through direct action against the pathogen (i.e. antagonism) and/or indirectly through induced systemic resistance (ISR).

Trichoderma spp. have been widely used as antagonist for managing several diseases and as plant growth promoters as well. Faster metabolic rates of antagonists, the anti-microbial secondary metabolites produced by them, and some other physiological activities are the key factors which chiefly contribute any biocontrol agents to make them most competent. One of the most important activities is the development of systemic resistance in the plants where they are administered. ***Trichoderma*** spp. has recently become so important that in addition to biological control of plant pathogens and plant growth promotions they are also being exploited for several other use for commercial and industrial purposes, namely production of wide range of commercial enzymes, namely, cellulases, hemicellulases, proteases, 1,6-glucanase and 1,3-glucanase.

20.4 Direct Action of Biocontrol Agents

20.4.1 Hyper-Parasitism

In the process of biological control, mycoparasitism is the main action or mechanism of action which is imparted by the antagonists. Mycoparasitism mainly occurs in biocontrol agents of fungal nature where the antagonist can grow and attack the targeted organism and finally by producing the cell wall degrading enzymes; they dissolve the cells of a target pathogen and utilize the same as its food/nutrient (Weindling 1932; Chet 1987; Elad 1995; Mukherjee et al. 1995a; b; Sharon et al. 2001; Eziashi et al. 2007; Jayalakshmi et al. 2009). Several findings revealed that antagonistic action of *Trichoderma* takes place through the process of mycoparasitism. (Mukhopadhyay 1994; Howell 1987; Mukherjee et al. 1995a; b; Sharon et al. 2001). The process of mycoparasitism actually includes chemotropic growth of antagonist, recognition of the host by the mycoparasite, coiling of host hyphae by the hyphae of mycoparasite, excretion of intracellular enzymes, lyses and colonization of the host hyphae Different isolates of *Trichoderma harzianum* and *T. virens* show different mechanisms of action. Excellent mycoparasitism of *Trichoderma harzianum* has been noticed against hyphae of *R. solani*, whereas strong antibiosis activity was noticed in the case of *T. virens* against hyphae of the same (Roy 1977). *T. virens* was found to be able to colonize and degrade the sclerotia of *R. solani* by utilizing them as a source of its food and nutrients. Type and nature of target pathogen may affect the nature of antagonism in addition to the antagonist which is performing the biocontrol. It has been noticed that in case of biological control of *R. solani*, mycoparasitism is most predominant mechanism of action, while in case of *Fusarium* spp., antibiosis is the most predominant mechanism of antagonism. Some nematodes have also been found to be parasitized and killed by some strains of *T. harzianum* (Sharon et al. 2001). Chief mechanism of action by *Trichoderma harzianum* against nematode is that it gets coiled around the second stage juveniles of *Meloidogyne javanica.* After coiling around the nematode body, the Trichoderma penetrates it by its haustorial action. Egg masses were also noticed to be parasitized by *T. harzianum* hyphae. A common mechanism of biocontrol has been revealed by Diby et al. (2005) through which *P. fluorescens* and *Trichoderma* spp. could effectively suppress the root rot disease in black pepper (*Piper nigrum* L.). All five strains of *Pseudomonas fluorescens* and *Trichoderma* spp. were found to produce fungal cell wall degrading enzymes, namely 1,3-glucanases, 1,4-glucanases and lipases in vitro and these are the enzymes which make both the antagonist capable of lysing the cell wall of the pathogen, *P. capsica* the incitant of root rot in black pepper. Each five strains of *Pseudomonas fluorescens* and *Trichoderma* spp. exhibited considerable degree of variations in the production of the enzymes mentioned above.

Antagonistic potentiality of *Trichoderma viride* mutants were screened by Nakkeeran et al. (2005) using antagonism index which is the indicator of competitive saprophytic ability, colonization behaviour, per cent inhibition of pathogen, propagule lysis, speed of overgrowth on pathogen and inhibition zone (AI = CSA × CB × PI × PL × SOOP × IZ). The *Trichoderma* strains MG3, MG6, UV10 and MNT7 showed highest antagonism index which ranged between 192 and 480 with comparatively higher enzymatic activity of cellulase, chitinase, b-1, 3 glucanase and IAA. Mycoparasitic activity of *Trichoderma viride*was noticed by Eziashi et al. (2006) against *Ceratocystis paradoxa* while growing near the pathogen. *T. viride* was seen twisting around the mycelium of *Ceratocystis paradoxa. Trichoderma viride* found to have stimulated to produce branches that grew directly up-to the pathogen mycelium. These branches get firmly attached with the pathogen conidia which resulted in the penetration of mycelium and conidia of *C. paradoxa* by *T. viride.* This further facilitated the successful growth of *T. viride* and some of the impregnated *C. paradoxa* were found dead.

20.4.2 Antibiosis

Through the action of antibiosis, the antagonists produce some secondary metabolites containing antibiotics, hormone and enzymes. These metabolites are harmful to the pathogen and suppress their growth. Majority of these metabolites including some important antibiotics have been isolated, identified and characterized. Okuda et al. (1982), Sivan and Chet (1989) and Sharma and Dohroo (1991) identified and characterized some most important metabolites from the antagonist fungus *T. virens* which include iso-nitrile, gliotoxin and glioviridin. Similarly, some other metabolites, namely viridin, alkyl pyrones, iso-nitriles, polyketides, peptaibols, diketopiperazines, sesquiterpenes and some steroids were identified and characterized from *Trichoderma* spp. by Upadhyay and Mukhopadhyay (1986) and Howell (1998).

Culture filtrates from four isolates of *Trichoderma harzianum,* i.e. T9, T10, T15 and T19 were studied by Küçük and Kivanç (2003) and all these filtrates were quite effective against *Fusarium culmorum*, *F. oxysporum*, *F. moniliforme*, *Rhizoctonia solani*, *Sclerotium rolfsii*, *Gaeumannomyc*es *gramini*s var. *tritici* and *Drechslera sorokiniana*. However, out of all the four isolates, *T. harzianum* T19 exhibited wide inhibitory effects on plant pathogenic microorganisms. A similar study was conducted by Ûdem (2003) as done by Küçük and Kivanç (2003) and similar findings were noticed by them too.

A team of scientists led by Abbas El-Hasan in 2009 noticed the production of secondary metabolites, i.e. viridiofungin A (VFA) from *T. harzianum.* Two isolates T16 and T23 were most potent in the production of these metabolites (Abbas et al.

2017). They also reported elucidation and antifungal potential of VFA and some other secondary metabolites isolated and purified from *T. harzianum* cultures against *Fusarium moniliforme*. Several secondary metabolites with antifungal activity were confirmed using bioautography assay.

20.4.3 Competition

It is very difficult to observe the mechanism of competition through experiment, although it is chiefly prevalent in any natural ecosystem. It is assumed that if there is no evidence of biological control through mycoparasitism or antibiosis then certainly competition must be going on and resulting in inhibition/ management of disease in a given situation (Alexander 1982; Cook and Baker 1983).

There are evidences of *Trichoderma* being antagonized by some other soil microorganisms and thus the effective inoculum density of this biocontrol agent must have been checked. In this way, the effectivity of *Trichoderma* can be drastically reduced and lower level of biocontrol efficacy may be resulted (Papavizas 1985).

A detailed knowledge of biocontrol through competition for food and nutrients and infection sites on root surface has been provided by Paulitz (1990) after a thorough review of available literature on ‘biochemical and ecological aspects of competition’.

Competition for food and nutrient may vary depending upon diverse range of rhizosphere and also on the basis of available sources of carbon nitrogen, sulfur, phosphorus and many other essential micronutrients (O’Sullivan and O’Gara 1992).

Trichoderma has been found to be an efficient competitor as its presence has been noticed and documented in diverse environment throughout the world including agricultural and natural soils. Trichoderma can compete effectively for space and nutritional resources. A good account of information has been compiled by Elad and Kapat (1999) regarding inhibition of a pathogen, *B. cinerea* by T-39 strain of *T. harzianum*. They also concluded that *B. cinerea* conidia needs a supply of nutrients from external sources for its successful germination and infection and when conidia of Trichoderma strain T-39 were placed on leaves; there must have been a natural competition between *Trichoderma conidia* and *B. cinerea* conidia for external source of nutrients; hence as a result germination of conidia of the pathogen got slowed down. This evidence is a clear indication of competition (Elad 2000).

20.5 Indirect Action of Biocontrol Agents

20.5.1 Induced Systemic Resistance (ISR)

A well-researched subject has been discussed, and has given a good overview of the scientific literatures on it (Bailey and Lumsden 1998). Diverse morphological, biochemical and molecular changes in the plant inoculated with some specific strains of the genus *Trichoderma* have been noticed. Once inoculated or applied to seed or rhizosphere, *Trichoderma* colonizes and penetrates plant root tissues and initiates these diverse reactions. These reactions are considered to be part of the plant defence mechanism, which ultimately result in induced systemic resistance (ISR) response in whole plant. Initiation of induced resistance like responses has been observed due to some *Trichoderma* strains. When applied to rhizosphere, *Trichoderma* spp. releases the xylanase as a result of interaction in root zone which is chiefly responsible for induction of resistance response in the system of cotton, tobacco, grapevine etc. (Yedidia et al. 1999; Elad 2000).

Similarly, synthesis of terpenoids in the cotton roots has been observed after seed treatment with *T. virens* and these terpenoids ultimately stimulate defence responses. Increased peroxidase activity and terpenoid synthesis have also been observed in seedling roots of cotton seedlings inoculated with *T. virens* which can check the problem of cotton seedling damping off. These alterations were noticed in advance of pathogen attack to the cotton seedlings (Howell 1998).

In the recent scenario, biological control-mediated induced systemic resistance is now being considered to be the primary method of biological control (Elad and Kapat 1999) T-39 strain of *T. harzianum* has been found to act through the path of induced systemic resistance in the management of powdery mildews as it is well documented that neither mycoparasitism nor production of pathogen inhibitory compounds has been noticed by T-39 that can inhibit powdery mildew fungi.

T-203strain of *T. harzianum* was found to have a growth promoting effect in the plant where it was applied. The cucumber plants treated with this strain had larger fruits than those where none of the Trichoderma or some other strain of Trichoderma was applied to the rhizosphere. This experiment was done in the presence or absence of strain T-203of *T. harzianum* in an axenic hydroponic condition (Yedidia et al. 1999). They also noticed the penetration of root cortical tissues in cucumber by T-203strain of *T. harzianum* whereas the T-22 strain of *T. harzianum*, could not show such activity. The compound gliotoxin and gliovirin may have a crucial role to play in ISR as Howell (1998) noticed the presence of these compounds in the plants showing induced systemic resistance followed by treatment with *T. virens*, which usually produce gliotoxin or gliovirin.

Similarly, induction of phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO)new trypsin and chymotrypsin inhibitors in the trichoderma-treated plants system may be attributed to ISR. This thought was well proven with experiment on the induction of plant defence response by inoculating the roots of chickpea cv JG62 with *T. harzianum* strain L1. The root extract of chickpea inoculated with the strain of trichoderma mentioned above showed greater activities of PAL and PPO and new trypsin and chymotrypsin inhibitors as well in the chickpea plants. Protease-2 of *Fusarium oxysporum* was noticed to be completely inhibited by root extract of *T. harzianum*L1 treated chickpea and with a greater resistance against root rot of caused by wilt disease in chickpea (Jayalakshmi et al. 2009). Gene modulation and their enhanced expression due to some strains of *Trichoderma harzianum* has also been noticed and experimentally it has been justified also.

Perazzolli et al. (2011) noticed reduced level of severity of downy mildew in susceptible genotypes of grapevines after T39 strain of *Trichoderma harzianum* treatment under green-house. A direct modulation of defence-related genes and the activation of priming for enhanced expression of these genes were also observed in the T39 strain of *Trichoderma harzianum* treated grape plants after challenged inoculation with downy mildew pathogen. There was a stronger local effect than systemic modulation of defence-related genes in T39-treated plants. The activation of a priming state was confirmed by the absence of any negative effect of T39 on grapevine growth, shoot and root weight, leaf dimension and chlorophyll content, in contrast to benzothiadiazole (BTH) applications.

20.5.2 Growth Promotion

Mycoparasitism's ability to compete fiercely against the pathogen that causes observable loss in plants, Harman et al. (2004b) after some thorough investigations, realized that this antagonist is chiefly available in rhizosphere and rhizoplane which is a site of biologically active soil ecosystem. The fungus is basically of free-living nature which can survive without any specific host or substrate in soil specially in root zone. Potential *Trichoderma* strains with beneficial effects on plant growth etc. were earlier being selected on the basis of production of antibiotic compounds and their ability of mycoparasitism against some important plant pathogens but nowadays their effects on vegetative growth and productivity of crop have also become key parameters to be considered for selection of potential strains (Harman 2006). Chang et al. (1986), Yedidia et al. (2001) and Adams et al. (2007) have also sustainably noticed the increased crop vigour and yield in the crops due to application of *Trichoderma*.

Both fungal and bacterial type of biocontrol agents has been found to enhance the growth and productivity of the crop where they were applied. Plant growth promotion due to these fungal and bacterial bio-agents are mainly due to inhibition of unfriendly micro-flora inhabiting root zone soils with no pathogenic activity. Release of growth promoting compounds by trichoderma like plant growth regulators and hormones and/or trichoderma also results into increased absorption of nutrients because of its enzymatic action that leads to solubilization and sequestration of nutrients, which further result into greater root growth to deeper depth of soils (enhanced root development is also helpful in tolerating the biotic and abiotic stresses by the plants). Wu (1977) did some intensive and exhaustive research work on the effect of *Trichoderma* on oat and further he noticed increase emergence of oat seedlings with high vigour. Similar type of effect of this fungus was noticed in germination of radish crop by Henis et al. (1978). Seed coating of broad bean with *T. viride* yielded increased plant vigour and increased rhizobium nodulation (Yehia et al. 1994). Chang et al. (1986) tested *T. harzianum* multiplied on wheat bran to different crop, namely tomato, pepper, cucumber, petunia, chrysanthemum and periwinkle. They observed enhanced vegetative growth of tomato, pepper and cucumber in addition to increased height and flowering of petunias, chrysanthemum and periwinkle due to application of *T. harzianum*. Growth enhancement type of effect was also noticed by Conway and Khan (1990) who observed increased plant weight in transplanted broccoli due to application of *T. harzianum* chlamydospores @ 5 kg mixture per hectare. Chet (1987) and Kleifeld and Chet (1992) have also reported induced growth in different crops due to application of *Trichoderma* spp. Experiments on seed treatment with *T. harzianum* and *T. virens* were also conducted by Mukhopadhyay (1996) and an increase vegetative growth of many different crops were observed.

Bjorkman et al. (1998) and Harman et al. (2004a) noticed some tremendous changes in root architectural system of maize due to application of *Trichoderma*. They observed enhanced root biomass production and excessive root hair development which resulted in greater nutrient absorption, increased plant's growth and development. Bal and Altintas (2006) mixed the *Trichoderma harzianum* to seedling growing media at the time of sowing which consists of a peat and soil-based mixture. The rate of *T, harzianum* application to seedling growing media was 4 g/m^2, 10 g/m^2 and 24 g/m^2. It was found that combination of Peat x Belleveu F1 × 10 g/m^2 resulted in the highest total yield, marketable yield and early yield with 1552, 1373 and 681 g/plant, respectively.

Cornejo et al. (2009) assumed and investigated the role of auxin in growth promotion of *Arabidopsis thaliana* seedlings as a result of applying *Trichoderma virens* and *Trichoderma atroviride*. Application of *T. virens* and *T. atroviride* to seedlings of Arabidopsis resulted in an increased activity of growth regulators like auxin which ultimately yielded higher biomass and stimulated secondary root development. Presence of indole-3- acetic acid, indole-3-acetaldehyde and indole-3-ethanol were noticed in the culture filtrates of *T. virens* which are auxin-related compounds and perform major functions of the growth regulator auxin.

Nosir (2016) studied the capacity of two antagonist fungi, i.e. *Trichoderma harzianum* and *Aneurinibacillus* spp. for managing the corm rot of gladiolus in addition they also observed some other growth attributes like vegetative and root growth along with enhancement in flowering. It was noticed that *T. harzianum* was comparatively more effective in minimizing the disease and enhancing the growth and vigour of plant which manifested in increased number of flowers with better quality. It was also noticed that in mixed application of two antagonists, the efficiency of *T. harzianum* get reduced. It was observed that number of CFUs of *T. harzianum* get increased in the substrate and on corms also when it was applied solo whereas the CFUs get decreased when consortia of two antagonists was applied and it was interesting to note that no *T. harzianum* CFUs could be observed in the substrate by 120 days after planting. SEM and results of Glasshouse experiments clearly indicated that *T. harzianum* performed the activity of biological control through joint action of antibiosis and competition for substrate.

Kashem et al. (2011) tested the efficacy of 14 *Trichoderma* spp. isolates (*T. harzianum* and*T. viride*) for managing the of foot and root rot in lentil (*Lens culinaris* Medik) caused by *Fusarium oxysporum* Schlecht. The range of growth inhibition of *F. oxysporum* by *Trichoderma* isolates was between 45.87 to 92.07% at 7 days after inoculation on agar plates. Highest level of growth inhibition of

Fusarium oxysporum Schlecht was exhibited by the isolate TG-2 of *T. harzianum* in field condition. This isolate (TG-2) also exhibited lowest incidence of foot and root rot (6.9%), highest germination (82.08%), maximum population of plants (93.12%) and the highest grain yield (3726.67 kg/ha).

20.5.3 Solubilization and Sequestration of Inorganic Plant Nutrients

Soil is a chief source of nutrients and essential minerals required by plants as their food but majority of these minerals are in such a form which is not get readily available to plants because of their insoluble estate. Under the influence of several biological and biochemical activity of rhizosphere microflora and microfauna, these insoluble plant nutrients and essential minerals undergo complex transition from insoluble to soluble forms which effectively affect their accessibility and absorption by plant roots because in the soluble form they become available to plants and easily get absorbed by plant roots and metabolized. The microorganisms harbouring the plant rhizosphere may strongly influence the transitions of insoluble nutrients and minerals to get converted to soluble one (Altomare et al. 1999; Sen 2000).

Graham and Webb (1991) thoroughly studied the iron and manganese specially, with regard to microbial solubilization of their oxidized forms and their influence on the occurrence and spread of plant diseases.

Sen (2000), reported that several fungal antagonists like *Aspergillus* spp., *Trichoderma* spp. etc. produce a kind of biochemical compound known as siderophores. Harman et al. (2000) found some strains of *Trichoderma* with an ability of solubilizing rock phosphate, Zn-metal, Mn^{4+}, Fe^{3+} and Cu^{2+} by producing a large number of biochemical compounds.

20.5.4 Inactivation of Pathogen's Enzymes

Wall degrading enzymes are major weapons of plant pathogens which they use for breaking the outer wall, i.e. cell wall of plants and thus these pathogens enter the living host system. Majority of biocontrol agents are known to produce secondary metabolites and proteolytic enzymes, these chemicals are capable of inactivating the wall degrading enzymes produced by plant pathogens which results in reduced ability of the pathogen to infect the plant (Geremia et al. 1993; Rodriguez-Kabana et al. 1978; Bertagnolli et al. 1996).

Serine protease produced by conidia of *T. harzianum* (T39 and NCIM 1185 strains) have been found to be capable of inactivating the pathogen's plant cell wall degrading enzymes on the leaves, thus the ability of the pathogen to infect the plant get drastically reduced (Zimand et al. 1996; Kapat et al. 1998; Elad and Kapat 1999; Elad 2000).

20.6 *T. harzianum* Grown on Different De-Oiled Cakes and Composts May Have a Better Rhizosphere Competence

20.6.1 Effect on Vigour and Yield of Tomato

Since effect of different substrates, namely, de-oiled cakes and two composts on general growth, vigour and induced systemic resistance had to be assessed, hence, application of *Trichoderma* directly harvested from PDA was treated as check for pot experiments. It was noticed that all the substrates where *Trichoderma* was initially grown for 30 days and then applied to either sterilized or unsterilized soil planted with tomato, in general there was increase in the greenness of leaves. However, application of Jatropha cake grown *Trichoderma* to the pot soil resulted in highest increase of tomato plants height followed by Mahua, Karanja and Neem cakes. The application of FYM grown *Trichoderma* resulted in lowest increase of tomato plant height. Similarly, the highest fruit yield of tomato was also recorded due to application of *T. harzianum* grown on Jatropha cake followed by Mahua cake, Karanja cake, Neem cake and vermicompost. Application of *T. harzianum* grown on FYM, resulted in lowest fruit yield of tomato. As already discussed, that, after soil application, the Jatropha cake resulted in highest recovery of *Trichoderma* CFUs, thus the growth promoting activity of *Trichoderma* along with the nutritional supply due to Jatropha cake might have resulted in the increased plant growth as compared to other substrates. Since there was lowest recovery of *Trichoderma* CFUs after FYM grown *Trichoderma* application, hence it might have resulted in least increase of tomato plant height, since direct application of PDA grown *T. harzianum* resulted in comparatively inferior plant height, thus it can be interpreted that, the increase in plant height of tomato is due to joint action of plant nutrition supplied by different cakes and growth promoting activity of *T. harzianum*.

Several workers such as Chang et al. (1986), Yedidia et al. (2001) and Adams et al. (2007) have already reported the plant growth enhancement activity of *T. harzianum* due to suppression of deleterious root microflora, production of growth stimulating factors and increase nutrient uptake through solubilization and sequestration of nutrition and also by enhancing the root growth. Cornejo et al. (2009) also noticed auxins activity in *T. virens* and *T. atroviride* treated *Arabidopsis thaliana* which resulted in increased growth and development of treated plants. Interestingly, Neem cake and FYM which were little inferior than other substrates with regard to growth enhancement were found to be superior in enhancing the chlorophyll content (denoted by greenness of the leaves) in tomato plant as compared to other substrates. This may due to some unknown reason which needs further confirmation.

20.7 Application of Bioagents for Suppressing the Diseases in Plants

Wherever intensive tomato cropping is followed, Fusarium wilt often result in severe damage to the crop. In the coming future, progress of agriculture is expected to be achieved through sustainable use of natural resources and less use of agrochemicals like pesticides and fertilizers. This goal can only be achieved to some extent through adoption of organic agriculture and use of beneficial microorganisms as bio-pesticides and bio-fertilizers (Bidellaoui et al. (2019). Did some comparison between a mycorrhizal fungus *Rhizophagus irregularis* and the biological control agent *Trichoderma asperellum* strain T34 that how these two microorganisms are affecting the fusarium wilt incidence and plant growth in tomato. Both the organisms were at par in lowering down the incidence of tomato wilt and also in increasing plant height. Chlorophyll content was higher in the tomato plants treated with *R. irregularis* as compared to those treated with *Trichoderma asperellum* strain T34.Both the microorganisms were again at par with regard to uptake of micronutrients like Ca, Mg, S, Mn, B and Si in tomato plants. However, *R. irregularis* treated tomato plants had a greater P, K, Zn, Cu and Mo accumulation than T34.

Wilt disease caused by *Fusarium oxysporum*f. sp. *lycopersicae* is a major disease of tomato and result in heavy loss at vegetative and reproductive stage. Srivastava et al. (2010) tried to develop a management strategy for wilt disease in tomato which should be ecofriendly with no use of agrochemicals. Fluorescent Pseudomonas, *Trichoderma harzianum* and *Glomus intraradices* were the major biological components which were slotted to be used for managing the disease. As it is well established that in addition to suppressing the plant pathogens through mycoparasitism and antibiosis they also induce the systemic resistance in plants where they are applied. A study was conducted using, a huge number of *Trichoderma* spp. and pseudomonad isolates to test that up to how much extent they can be successful in suppressing the *Fusarium* wilt of tomato. Out of six different substrates which were evaluated for *T. harzianum* multiplication, an underutilized grain crop, i.e. Jhangora of Kumaun and Garhwal divisions of Uttarakhand was found to be a better substrate. Seed bio-priming of tomato with *T. harzianum* and fluorescent *Pseudomonas* resulted in significant increase in germination (22–48%) and drastic reduction in germination periods (2.0–2.5 days) required for seed germination. All antagonists be it fungal or bacterial resulted in significant reduction of tomato wilt incidence in both trials, i.e. pot and field. It was also noticed that consortia of bioagents were more effective as compared to single bioagent in reducing the incidence and severity. Consortia of bioagents were also effective in increasing the crop yield by 20%. Cow dung compost (CDC) mixing with either single bioagent or their consortia had some synergistic effect as level of disease further get reduced and yield was improved wherever it was mixed.

Khare et al. (2010) reported that control of plant diseases has always remained a challenge as diseases affecting plant health are a major and chronic threat not only to food production, but also to ecosystem stability worldwide. As agricultural production intensified over the past three decades, producers became dependent on agrochemicals as a relatively reliable method of crop protection. However, growing concerns regarding continued use of agrochemicals, posing adverse effects on human health besides posing the threat of environmental deterioration, has driven search for novel environment friendly methods to control plant diseases that in turn can contribute to the goal of sustainability in agriculture. Mitigation of plant diseases by naturally inhabiting antagonistic micro-organisms such as plant growth-promoting rhizobacteria has gained much importance as biocontrol agents seem to be the best possible measures for saving plants from phytopathogenic organisms without causing any harmful effect to mankind as well as to the environment. Mechanisms of microbial antagonism toward phytopathogenic organisms include competition for nutrients and space, production of siderophores, hydrogen cyanide, antibiotics, and/or production of fungal cell wall-degrading lytic enzymes.

Raju et al. (1999) applied biocontrol agents to five different cultivars of sorghum seeds infected with a varied degree of *Fusarium moniliforme*. Pure cultures of *Pseudomonas fluorescens*, *Trichoderma harzianum* and *Chaetomium globosum* at the rate of 1×10^8 cfu $g^{-1}$and talc-based formulations of (28×10^7 cfu g^{-1}), (19×10^7 cfu g^{-1}) and (4×10^6 cfu g^{-1}) at the rate of 6 g kg^{-1} and 10 g kg^{-1} of seeds were used, respectively. The treated seeds were evaluated for per cent reduction of *F. moniliforme*, seed germination, vigour index and field emergence. It was found that the pure culture of *P. fluorescens* was more effective in reducing the *F. moniliforme* infection followed by *T. harzianum* and *C. globosum* than the Bavistin treated and untreated seeds. Formulations of *P. fluorescens* were effective in reducing the *F. moniliforme* infection and also in increasing the seed germination, vigour index and field emergence, followed by *T. harzianum* and *C. globosum* treatments in comparison with control.

20.8 Application of Bioagents for Tolerance to Water Stress in Crops

Boat et al. (2018) reported that drought and salinity are major environmental stresses resulting in secondary stresses such as osmotic and oxidative stress (common to both stresses) as well as ionic stress (during salinity) causing alterations in physiological, biochemical and molecular processes in plants resulting in substantial loss to crop productivity. The major physiological parameters studied in plants during stressed conditions are malondialdehyde (MDA) content and relative electrical conductivity in leaves, relative water content (RWC), stomatal conductance (gs), Chl content and Chl-fluorescence. Plants inoculated with plant growth promoting rhizobacteria (PGPR) induce morphological and biochemical modifications resulting in enhanced tolerance to abiotic stresses defined as induced systemic tolerance (IST). Molecular approaches such as RNA differential display (RNA-DD), reverse transcriptase PCR (RT-PCR) microarray analysis, real time PCR, differential display PCR (DD-PCR) and illumina sequencing revealed PGPR inoculation caused upregulation of drought stress related genes such as ERD15 (Early Response to Dehydration 15)and

ABA-responsive gene, RAB18 in Arabidopsis genes, APX1 (ascorbate peroxidise), SAMS1 (S-adenosyl-methionine synthetase), and HSP17.8 (heat shock protein) in leaves of wheat, Cadhn (dehydrin-like protein), VA (Vacuolar ATPase), sHSP (Plant small heat shock proteins) and CaPR-10 (Pathogenesis-related proteins) in pepper, dehydration responsive element binding protein (DREB2A), catalase (CAT1) and dehydrin (DHN) in mung, salt stress responsive genes such as RAB18 (LEA), RD29A, RD29B regulons of ABRE (ABA-responsive elements) and DRE (dehydration responsive element) in Arabidopsis.

Shukla et al. (2015) noticed an enhancement in drought tolerance of wheat as a result of seed biopriming due to some selected strains of *T. harzianum*. Seed biopriming as induced several physiological changes in wheat, namely delayed stomatal conductance, delayed net photosynthesis and also delayed chlorophyll fluorescence. Drought exposure of 4–13 days of withholding water induced an increase in the concentration of stress induced metabolites in leaves, whereas, colonization of rhizosphere by Trichoderma resulted in lowering the content of proline, malondialdehyde (MDA) and hydrogen peroxide (H_2O_2), but total phenolics get increased. It has been observed that whenever a plant faces the drought stress conditions, a greater accumulation of toxic reactive oxygen species (ROS) has been observed. Seed biopriming with *Trichoderma harzianum* has been found to reduce the damages resulting from accumulation of ROS in stressed plants. Trichoderma treated plants have been found to exhibit a higher l-phenylalanine ammonia-lyase (PAL) activity in leaves after 13 days of exposure to drought stress in wheat. However, similar such activity was noticed in untreated plants also after a drought exposure but comparatively of lower magnitude. Thus, it can be concluded that seed biopriming in wheat with drought tolerant *T. harzianum* strains may increase root vigour in addition to performing the process of osmoregulation.

Bhattacharyya and Jha (2012) reported that plant growth-promoting rhizobacteria (PGPR) are the bacterial microorganisms which are capable of surviving in plant rhizosphere. These PGPR are capable of enhancing plant growth by a wide variety of mechanisms like phosphate solubilization, siderophore production, biological nitrogen fixation, rhizosphere engineering, production of 1-Aminocyclopropane-1-carboxylate deaminase (ACC), quorum sensing (QS) signal interference and inhibition of biofilm formation, phytohormone production, exhibiting antifungal activity, production of volatile organic compounds (VOCs), induction of systemic resistance, promoting beneficial plant-microbe symbioses, interference with pathogen toxin production etc. Plant growth promoting rhizobacteria has recently become very important because of its diverse potential. It is assumed that PGPR can be an alternative of chemical fertilizers, pesticides and other agricultural inputs for increasing the productivity of crops in near future. These rhizospheric microorganisms can be widely exploited for large scale and commercial scale production of growth promoting substances to be used in indirectly manipulating overall morphology of the plants. In future with more progress in the level of understanding the diversity of PGPR in the rhizosphere along with their colonization ability and mechanism of action will be able to facilitate their use in sustainable agriculture system.

Harman (2011) discovered that some plant-symbiotic strains of the Genus *Trichoderma* colonize roots and induce profound changes in plant gene expression that led to enhanced growth, especially under biotic and abiotic stresses. They tested the hypothesis that one of the protective mechanisms enhanced by *T. harzianum* T22 colonization is the antioxidant defence mechanism. Having established that strain T22 modulates the expression of the genes encoding antioxidant enzymes, the status of antioxidant defence of tomato seedlings in response to colonization by T22 and water deficit was investigated. Total ascorbate or glutathione levels were not affected by either stimuli, but under water deficit, antioxidant pools became more oxidized (lower ratios of reduced to oxidized forms), whereas colonized plants maintained redox state as high as or higher than those plant which are without any stress and without any treatment also. Why treated plants show higher redox state could be explained by their higher activity of ascorbate and glutathione-recycling enzymes, higher activity of superoxide dismutase, catalase, and ascorbate peroxidase, in both root and shoot throughout the experiment. Similar induced enzymatic activities were noticed in untreated plants also under water-deficit condition but comparatively little lower than those plants which were not provided with any seed biopriming treatment. This orchestrated enhancement in activity of reactive oxygen species (ROS)-scavenging pathways in colonized plants in response to stress supports the hypothesis that enhanced resistance of colonized plants to water deficit is at least partly due to higher capacity to scavenge ROS and recycle oxidized ascorbate and glutathione, a mechanism that is expected to enhance tolerance to abiotic and biotic stresses.

Mayak et al. (2004) evaluated whether bacteria populating arid and salty environments can confer resistance in tomato and pepper plants to water stress or not. Plant growth-promoting bacteria that have ACC deaminase activity were isolated from soil samples taken from the Arava region of southern Israel. One of these strains, *Achromobacter piechaudii* ARV8 significantly increased the fresh and dry weights of both tomato and pepper seedlings exposed to transient water stress. In addition, the bacterium reduced the production of ethylene by tomato seedlings, following water stress. During water deprivation the bacterium did not influence the reduction in relative water content; however, it significantly improved the recovery of plants when watering was resumed. Inoculation of tomato plants with the bacterium resulted in continued plant growth during both the water stress and after watering was resumed. Based on the results of the experiments reported/ cited here, the use of plant growth promoting bacteria such as *A. piechaudii* ARV8 may facilitate plant growth in arid environments.

Subramanian et al. (2006) studied and reported the effects of root colonization by the arbuscular mycorrhizal (AM) fungus *Glomus intraradices* on growth, flower and fruit production, and fruit quality in field-grown tomato plants exposed to varying intensities of drought stress. Inoculated (M+) and non-inoculated (M−) tomato seedlings were exposed to varying intensities of drought stress by adjusting irrigation intervals. Mycorrhizal plants had significantly higher uptake of N and P in both roots and shoots regardless of intensities of drought stress. AM inoculation also significantly increased shoot dry matter and the number of flowers and fruits. The fruit yields of M+ plants under severe, moderate, mild drought-stressed conditions were higher than M− plants by 24.7%, 23.1%, 16.2% and 12.3%, respectively. Furthermore, M+ plants produced tomato fruits that contain significantly higher quantities of ascorbic acid and total soluble solids (TSS) than M− plants. Mycorrhizal effects increased with increasing intensity of drought. The overall results suggest that mycorrhizal colonization affects host plant nutritional status, water status and growth under field conditions and thereby alters reproductive behaviour, fruit production and quality of fruits under both well-watered and drought-stressed conditions.

20.9 Rhizosphere Application of Bioagents for Growth Promotion and Disease Management

Several studies conducted at Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut under the guidance of senior author of this chapter has also concluded some interesting facts about rhizosphere competence of several fungal and bacterial bioagents and their ultimate effect on different crops. Pandey (2020) concluded that Sorghum grains+ rice husk may be used for mass multiplication of *Trichoderma harzianum* and *Pseudomonas fluorescens*. Multiplication of these bioagents required a minimum of 30% substrate moisture, while they grew better up to 70% maximum level of substrate moisture. Since *Trichoderma harzianum* and *Pseudomonas fluorescens* are well compatible with a ‘Pencycuron’ fungicide, he also noticed that integrated use of both bioagent and Pencycuron had a positive effect on root and shoot growth of the wheat along with increasing relative water content, increasing chlorophyll content, flag leaf area, leaf area index, membrane stability index and ultimately wheat yield besides preventing rhizoctonia root rot of wheat. It was also noticed that colonization of wheat seed by drought tolerant strains of *Trichoderma harzianum* and *Pseudomonas fluorescens* enhanced growth and delayed the drought response in wheat. Thus, these findings may help to chalk-out the strategy of growing wheat crop under water deficit conditions. Similarly, Singh (2018) noticed a significant delay in response to drought when wheat plants were 75 days old and raised from the seed bioprimed with drought tolerant *Trichoderma* and *Pseudomonas* strains. A simple explanation of drought tolerance in wheat due to seed biopriming with *Trichoderma* and *Pseudomonas* is that colonization of rhizosphere with these two bioagents resulted in enhanced root growth which performed improved water acquisition hence increase in water content in treated plants tissues which later withstand the water deficit due to drought. An increased in free proline content was also noticed in the wheat plants raised from bioprimed seed. Drought tolerance in wheat may also be correlated to increasing catalase and peroxidase activity as noticed during the study conducted by Singh (2018). Singh (2016) concluded that among the several strains of *Trichoderma harzianum*, few strains have been found to enhance the root and shoot growth (Length and biomass both) in rice crop, when these strains were applied to the rice crop using the delivery system of seed biopriming. Application of *Trichoderma harzianum* as seed biopriming in rice were quite effective in minimizing the leaf rolling due to drought exposure clearly because of enhanced root growth in the rice treated with *Trichoderma harzianum* using seed biopriming method. As noticed by Singh (2018) in wheat crop, similar attributes were found to be increased in rice crop also due to seed biopriming with *Trichoderma harzianum*. An increase in total chlorophyll content, leaf area index, relative water content, membrane stability index along with reduction in free proline content were noticed in rice crop due to seed biopriming with *Trichoderma harzianum*. Singh (2015) also noticed and concluded that *Trichoderma harzianum* can be used for enhancing drought tolerance in rice because almost all strains of this fungus collected from across the state of Uttar-Pradesh were found to be growth enhancer of root in rice crop. Enhancement in root length and increased secondary root development can be penetrated to deeper level of soil acquiring more water from remote depth of soil sub surface. Tomar (2015) also noticed and accordingly concluded that application of *Trichoderma harzianum* and *Pseudomonas fluorescens* seed biopriming and soil application of Neem, Jatropha, Mahua and Karanja cake may help in inducing systemic resistance in the rice crop and may protect the crop against diseases like bakanae, blast, sheath blight and brown spot in field condition. Tomar (2012) also concluded that mixed application of *Trichoderma harzianum* and *Pseudomonas fluorescens* to the soil was effective in enhancing root growth, shoot growth and fruit yield in tomato. Application of de-oiled cakes to the cropping field soil has been in practice by the farmers for growth promotion of the crops grown afterward without knowing that these soil amendments impart a very positive effect on the beneficial soil microflora and ultimately disease suppression through either direct action (Hyper parasitism) or through induced systemic resistance in the plants grown in such amended soils. De-oiled cakes available in the local market, namely Neem, jatropha, mahua, karanja, castor, groundnut and mustard have been applied by the farmers in different season and crops. It was noticed by Tomar et al. (2014) that deoiled cakes of jatropha was comparatively better than deoiled cakes of neem for supporting fast multiplication of *Pseudomonas fluorescens,* enhancement of plant growth, vigour and also fruit yield in tomato.

Chandel (2017) noticed that different inoculum loads of *Trichoderma harzianum* and *Pseudomonas fluorescens* as seed biopriming resulted in maximizing the vegetative and reproductive ability of tomato, in vitro and in vivo both. However, seed biopriming with consortium of *Trichoderma harzianum* strain IRRI-1 @ 1 g + *Pseudomonasflourescens*pf-6 @ 0.5 g was found to be far better than the recommended dose of *Trichoderma harzianum* IRRI-1 @ 2 g and *Pseudomonas fluorescens* strain pf-6@1 g. In addition, application of this consortium also resulted in enhancing germination, root growth, shoot growth, root weight, shoot weight, flowering ability and colour of leaves also in tomato.

Ali (2015) collected and maintained twenty isolates of *Pseudomonas fluorescens.* From 20different districts of Uttar Pradesh and concluded that all twenty isolates were quite effective in inhibiting the radial growth of *Sclerotium rolfsii* and *Rhizoctonia solani* in vitro with varying degree of inhibition percent. During this study it was also noticed that several deoiled cakes specially neem and jatropha can be used as solid substrate for mass multiplication and longer self-life of *Pseudomonas* *fluorescens*. However, the jatropha cake was found to be better substrate than neem cakes with regard to population dynamics. Root dipping of chilli in the suspension of *Pseudomonas fluorescens* resulted in significant reduction of foot and collar rot in chilli caused by *Sclerotium rolfsii* and enhancing vegetative growth and fruit yield as well.

20.10 Conclusions

Rhizosphere is the area surrounding the root zone of any crop. This zone is a dynamic area where all sorts of biological, biochemical and molecular activity take place and play a key role in the growth and overall health of plant. Rhizosphere is the area where all plant nutrients get absorbed by the plant roots which are applied either as basal application or as top dressing. Pesticides applied for the management of soil borne plant pathogens and insects pests are also absorbed through rootlets/ secondary roots in the rhizosphere. Different biological and biochemical activities in the rhizosphere are highly influenced by the physical, chemical and biological stresses faced by the related plant and of significant importance. Rhizosphere is also a site for biological activities which ultimately result in either biological control of plant pathogens or growth promotion of plant. The biochemical environment of the rhizosphere is highly influenced by the taxonomic nature of the plant and thus variation in the rhizospheric microflora in different taxonomic group of plant has been noticed and documented. The root exudates released in the rhizosphere may contain diverse biochemicals and greatly vary in different plants of diverse taxonomic group. It can be assumed that biological activity in the rhizosphere may highly be influenced by the chemical property of the root exudates. If a plant is under stress, it may release some different biochemical compounds through root exudates as compared to the situation which is normal for that plant. Biological activity in the rhizosphere is highly dependent on the biochemicals released in the zone and accordingly the dominance of a particular microorganisms may depend upon the biochemical properties of root exudates. The microorganisms in the rhizosphere are also highly interdependent on the chemicals that are released. Ultimately these microorganisms and different types of chemicals released that are present in the root exudates may enhance or bring different changes in plant related biological activities, namely growth promotion, growth inhibition, tolerance to biotic and abiotic stress. Rhizosphere also provides a platform to antagonistic microflora to perform the activity of competition, predation and parasitism (An integral component of biological control). Interaction between plant roots and rhizosphere microflora results in the release of several beneficial biochemical compounds including some growth regulators and phenols and these released chemicals further get absorbed by plant roots and may result in induced systemic resistance (ISR) and acquired systemic resistance (ASR). Manipulation in rhizospheric environment may lead to solution of several bottlenecks related to growth, yield and diseases and pest management. The microbial bioagents need some delivery system for their application to the plant. Rhizosphere is an important zone around the plant roots for delivery of microbial bioagents, as the bioagent applied has to follow the rhizospheric path for their acquisition by the plant roots. The bioagents may either be applied as soil application, seed biopriming or through root dipping of seedling. Application of bioagents in the rhizosphere zone requires a huge amount which can be achieved by mass multiplication on several agro-industrial by-products, namely de-oiled cakes and cereal's grain. Thus, in future the rhizosphere is going to be a focal area/point for research and development. Accordingly, there should be greater emphasis in the study of rhizosphere and related technology will have to be developed so that favourable manipulation in rhizosphere and also in the inputs to be applied can be done for the benefit of crops and maximum benefit can be harnessed using rhizosphere-based technology.

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Effects of Irrigation with Municipal Wastewater on the Microbiome of the Rhizosphere of Agricultural Lands

21

Theodore C. Crusberg

Abstract

Irrigation using municipal wastewater has been in limited use, especially when potable water sources have already been taxed to their limits. However, as populations increase and food security become more of an issue, reclaimed wastewater could play a significant role in enhancing productivity of certain food crops. Municipal wastewater is rich in many plant nutrients but also in heavy metals, a myriad of biologically recalcitrant organics, as well as numerous viral and microbial pathogens and helminths. If irrigation using municipal wastewater is chosen as a supplement or especially a primary method of providing moisture to consumable crops, then it is important not only to deal with the related public health issues, but also to understand how the components contained in the water affect the microbiome of the rhizosphere. This discussion centers on evaluating how using such wastewater and its contents affects perhaps the most important microbial populations in the rhizosphere, the arbuscular mycorrhizal (AM) fungi. Associated with about 80% of all agriculturally important plants, these fungi form symbiotic unions with the roots, providing an increase in the plant's ability to acquire water, an array of soluble nutrients, and specially phosphate from the soil. Methods for identifying AM fungi have evolved from classical microscopy and analysis of the fungal protein glomalin, to molecular methods based on species-specific/unique sequences of DNA found in the fungal genomes. Notably, polymerase chain reaction (PCR) is the preferred way of identifying AM fungi to the species level, and all materials and equipment needed to use this method are now available commercially. Longitudinal studies dealing with how AM fungi respond to the presence of wastewater irrigation have been

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limited, but those that have investigated the issue have found some minor changes in species associated with certain plant roots, but the changes seem to have little consequence on the productivity of the crop under study. Increased use in the future of municipal wastewater for irrigation of consumable crops should drive the need for additional studies on how AM fungi respond to this new resource.

21.1 Introduction

Population growth almost everywhere on the planet coupled with climate change has put stress on water resources, and almost all sources of high-quality potable fresh water have already been exploited. Direct human use of the world's available water accounts for between 20 and 30% and this includes water for domestic, commercial, institutional, and industrial needs. The remaining 70–80% is culled by agriculture for growing food crops and a large variety of ornamental plants, and raising livestock and other animals for human use. Drought also takes a significant toll because, although water managers know that it is always there on the horizon, planning for drought is difficult. When drought becomes a reality there are few options available to water managers except to curtail use, which puts stress on those needing the water to live or for business and commerce to thrive. Nevertheless, municipal potable water already having been put to use but now highly contaminated with a myriad of waste products, which we know as sewage, still accounts for an enormous volume of potential usable water if it can be treated properly and reclaimed. If chosen to augment agricultural needs as an optional supply, properly treated municipal wastewater has great potential to help solve problems of food insecurity by adding an optional, albeit potentially troublesome, enhancement of needed irrigation water (Hamdy 1992; Valipour and Singh 2016; Tchobanoglous and Angelikis 1996). Zavadil (2009) reported that in a study on several crops treated with municipal sewage, those crops irrigated with wastewater after only primary settling demonstrated substantial growth, while those subject to irrigation with biologically treated wastewater following secondary treatment benefited far less. A high bacterial contamination of all crops was found, yet no pathogens or intestinal nematodes were detected. Indeed, raw municipal wastewater is well endowed with many plant nutrients and its use should be expected to enhance growth, but secondary biological treatment, if done properly, does effectively remove most of the nutrients and its use should not improve plant growth that much.

There are many issues needing assessment if municipal wastewater is chosen as a supplement for agricultural use, especially public health considerations of viral, bacterial, and helminth pathogens commonly found in municipal wastewater as well as the presence of heavy metals and organic chemicals commonly found in the same wastewaters. The chief concerns of course are to protect both crop health and ultimately human health. However, still another consideration that should be addressed is the health of the organisms making up the microbiome of the soil subject to irrigation using treated municipal wastewater, especially those fungi associated with the roots of crop plants, the ectomycorrhizal and endomycorrhizal fungi, the main theme of this chapter. The role of fungi in nutrient cycling in the biosphere has already been reviewed (Crusberg 2008). Those plant-root-associated higher fungi are termed ectomycorrhizal if they occupy and carry out nutrient exchange mechanisms only on the external surfaces of root cells of certain families of Gymnosperms and Angiosperms creating a mutualistic association. Symbiotic fungi whose hyphae are able to penetrate and internally occupy root cells are termed endomycorrhizal fungi. Arbuscular mycorrhizae are a type of endomycorrhizae of the phylum Glomeromycota in which the symbiont fungus hyphae penetrate the cortical cells of the roots of a vascular plant forming arbuscules and intracellular vesicles. Arbuscular mycorrhizae are characterized by branching hyphal tree-like structural aggregates growing within the cortical root cells themselves while spores are produced in packets at the termini of external hyphae. Nearly 90% of plant species including flowering plants, bryophytes, and ferns have been shown to develop interdependent connections with arbuscular mycorrhizal (AM) fungi (Zhu et al. 2010a; Ahanger et al. 2014) and represent the most ecologically important symbiosis found in terrestrial ecosystems (Fitter et al. 2011). Arbuscular mycorrhizal fungi (AM fungi) in general enhance the nutrient uptake of host plants (particularly P and also N) and increase the tolerance and resistance of their host plants to environmental stresses such as drought (Gilmore 1971; Augé 2004; Hodge and Fitter 2010; Bowles et al. 2016; Yang et al. 2017; Zou et al. 2017).

This chapter will begin by briefly discussing the components of municipal sewage, the most common treatment methods that render it safe for use in irrigation, and how changes in the subsurface microbiome, the rhizosphere, were seen when raw (untreated) municipal wastewater, sludge from treated wastewater, and finally biologically treated municipal wastewater were used to enhance the growth of various ornamental and food crops.

21.2 Municipal Sewage and Treatment

Potable water is delivered from surface and subsurface supplies and is used by households, businesses, industries, and institutions for their various needs. Sewers carry the used water and its contents (called contaminants) through piping or sewerage, usually by gravity, to treatment plants where a considerable amount of contaminant removal takes place before the treated water is released into a receiving body or even into the subsurface. First, the wastewater enters a settling basin where its flow is reduced to a velocity allowing solids to sink to the bottom forming primary sludge. After primary settling, the water enters a tank containing a mix of organisms that have been naturally selected to decompose the dissolved and some suspended carbonaceous materials oxidatively. Air and sometimes even pure oxygen is added to the waste liquid and in short order (actually several hours) a good deal of the fixed carbon is converted to carbon dioxide before the effluent is discharged into a receiving body (lake, river, or ocean). The secondary treatment facility can be designed to function both aerobically and with some anaerobic regions where dissolved fixed nitrogen (ammonia and nitrate anion) can be biologically converted into nitrogen gas. The effluent is then disinfected using chlorine gas, hypochlorite salts, ozone, or even sodium chlorate to remove pathogens, but not necessarily all harmless organisms that are present, before release (Crusberg 2014). During secondary treatment more sludge forms as the organisms that constitute the mass multiply, and the gelatinous excess is removed, combined with the primary sludge, and disposed off by incineration or land filling. Sometimes the effluent requires excess phosphate and nitrogen removal before its release, in a process termed tertiary treatment (Henze and Comeau 2008; Ostad-Ali-Askari et al. 2017; Tchobanoglous et al. 2014).

21.3 Results of Biological Treatment

Biological treatment of this kind is designed to remove fixed carbon and nitrogen compounds, phosphorous, and heavy metals, but dissolved salts such as sodium chloride (NaCl) pass through unaffected. The efficiency of carbon removal in the treatment process is measured by a laboratory test known as biochemical oxygen demand (BOD), which measures the conversion of much of the fixed carbon present to carbon dioxide, by actually measuring the depletion of dissolved oxygen in the sample obtained from the waste stream (American Public Health Association 2001; U.S.E.P.A. https://archive.epa.gov/water/archive/web/html/vms52.html). However, since the test involves action by microbes, it is incapable of oxidizing many biologically recalcitrant carbon-based chemicals, especially those of anthropogenic origins. Microorganisms that are a component of the assay system are responsible for the oxidation. The assay measures the change in dissolved oxygen in a 300 mL flask at 25°C over a 5-day period, and the result is known as the 5-day BOD or BOD5. Table 21.1 relates values of BOD and other chemicals found in raw municipal wastewater and the water subsequent to secondary treatment. Another laboratory assay known as the chemical oxygen demand (COD) uses a strong oxidizing agent that completely converts fixed carbon and some other reduced chemicals that may also be present in the water, into carbon dioxide and other oxidized species, but it has limited importance in assessing the actual safety of wastewater destined for reuse. The COD assay does indicate just how much oxygen would be needed to oxidize every chemical present that could react with dichromate anion. Examples of using raw municipal wastewater, sludge, and biologically treated municipal wastewater for agricultural purposes will be discussed, with the understanding that the latter is ultimately safer than the others when considering crops used for human consumption. Criteria for the safe use of treated municipal wastewater for the irrigation of crops used for human consumption have been detailed by Alobaidy et al. (2010). They do note that proper water management and water quality analyses be routine and that reclaimed wastewater is now a viable supplement to meeting the water needs especially in semi-arid areas of the world.

Table 21.1 Selected pollutants of concern and their concentrations found in untreated (raw) municipal wastewater (Henze and Comeau 2008) and the weekly average concentrations permitted in effluents from secondary treatment at the Nogales International Wastewater Treatment Plant (Arizona, USA) and expected removal efficiencies in activated sludge as assessed by CH2MHill (2009).

Selected pollutant of concern	Concentration in raw sewage	Concentration in secondary effluent	Percent removal by activated sludge
Five Day Biochemical Oxygen Demand (BOD5)	350 ± 54% mg/L	40.0 mg/L	
Chemical Oxygen Demand (COD)	750 ± 33% mg/L	Not designated	Not designated
Total Suspended Solids (TSS).	400 ± 44% mg/L	45.0 mg/L	
Cadmium	2.0 ± 75% μg/L	2.42 μg/L	67
Chromium (total)	25.0 ± 60% μg/L	100.00 μg/L	
Copper	70.0 ± 50% μg/L	0.57 μg/L	86
Cyanide	5.5 μg/L		69
Lead	3.13 μg/L		61
Mercury	0.141 μg/L		60
Nickel	58.6 μg/L		42
Selenium	1.64 μg/L		50
Silver	3.29 μg/L		75

21.4 The Fungi of the Rhizosphere Microbiome

As already stated, this chapter deals with an assessment of primarily how the mycorrhizal fungi can tolerate and respond to irrigation with municipal wastewater before and especially after treatment. In order to accomplish this, a variety of assays are used. Most simply, light microscopy can be applied to roots extracted from soils of interest, observing hyphae and the mycelia they form, spores, vesicles, and arbuscules using classic staining methods. A soil protein composite known as glomalin, a glycoprotein produced on hyphae and spores of arbuscular mycorrhizal fungi, can also be used as an indicator of certain fungi. Nevertheless, species identification using these techniques is at best questionable. Organisms can resemble one another under the microscope. Rather, new sophisticated molecular methods based upon DNA sequences and sequencing can identify fungi to the species level.

Fungi are eukaryotic microorganisms and their DNA is linear and double stranded. Genes reside on DNA that is made up of four nucleotide bases linked to one another by phosphodigester bonds forming strands that in some cases number millions of bases in length. The two complementary strands of DNA are associated with one another through hydrogen bonds of the complementary bases, A = T and G ≡ C base pairs (A, adenine; T, thymine; C, cytosine; G, guanine). The = and ≡ symbols denote the number of hydrogen bonds that stabilize the two types of base pairs on opposite strands. The double strands form a helix with ten base pairs per turn. A description of the nature of the eukaryotic genome is certainly not the purpose of this chapter and is a well-known aspect in every introductory biology book nowadays. Only aspects related to the arbuscular micorrhizal (AR) fungi will be of concern.

An organism's genome is a unique sequence of DNA that encodes the genes needed for the species to survive in a specific ecosystem. The human double-stranded genome embedded on 23 chromosomes contains over 30,000 genes and is three billion base pairs in overall length. Although a mammoth undertaking, the Human Genome Project provided a rather well-defined sequence of the vast majority of genes, although to this day the purpose of every gene is still not known with absolute certainty. Fungal genomes should be, and are, much smaller and most range less than 30 Mbps (million or mega-base pairs) in length starting with just less than 9 Mbps but overall with an average of 42.3 Mbps (Mohanta and Bae 2015), but the genomes of AM fungi are much larger. The genome of the AR fungus *Rhizophagus irregularis* (formerly *Glomus intraradices*) was found to have 28,232 presumptive genes and is 153 Mbps in overall length (Tisserant et al. 2013). The term "presumptive" is used because certain features of the sequence characterize regions that allow the assumption that such a region is indeed a gene, but some gene sequences are simply not functional for one reason or another. That the sequencing and analysis of this unusually large fungal genome required the assistance of a total of 44 co-authors speaks to the overall difficulty of such a venture in 2013. The genome of *Rhizophagus clarus* HR1 was just recently sequenced and found to be 146.4 Mbps in length with 27,753 presumptive genes (Kobayashi et al. 2018).

Pure DNA must be prepared before DNA sequencing can be carried out. Most fungi are easily cultured for this purpose, but not for those that form symbiosis with plant roots. Strains of viable fungi are usually obtained from a gene or cell culture bank and are grown for as long as two months monoxenically with *Agrobacterium rhizogenes*-induced hairy roots in carrot (*Daucus carota*) cell culture growing on a special medium. The *Agrobacterium* bacterial species causes the carrot cells to form very complex roots with a "hairy" appearance. After two months of incubation, hairy carrot roots are removed and the fungal mass collected using citrate-mediated lysis of the fungal cells to release the genome (Tsuzuki et al. 2016; Bécard and Fortin 1988; Doner and Bécard 1991). Most AR fungi have now been sequenced, and the sequences entered into the appropriate free-access databases, e.g., the National Center for Biotechnology Information (NCBI), Bethesda, MD, USA; available at https://www.ncbi.nlm.nih.gov/. It has to be mentioned that the chief problem with these so-called DNA methods is that they are unable to determine the viability of the organism present in a root or soil sample or even if there are viable fungal cells present. DNA is not readily degraded in nature so any genetic material released from non-living fungi in a soil sample may be detected by these methods.

21.5 Polymerase Chain Reaction (PCR)

A newer, more simple, very specific, and much less expensive method is now available for species-level identification of microorganisms, known as the polymerase chain reaction (PCR) outlined in Fig. 21.1 (Saiki et al. 1985). In the first step

(**a**) *3′-........AATGGCAATTACGGC~600nucleotides-TAAACTCCCTTAACGGGAT..........-5′*
5′-.......TTACCGTTAATGCCG~600nucleotides-ATTTGAGGGAATTGCCGTA..........-3′
Example of a long double stranded genomic DNA sequence (in *italics).* The ....
indicate long stretches of genomic DNA beyond unique regions to the species being studied.

(**b**) *3′-......AATGGCAATTACGGC~600nucleotides-TAAACTCCCTTAACGGGAT.........-5′*
After heating to 95 °C (Tm) the strands dissociate
5′-......TTACCGTTAATGCCG~600nucleotides-ATTTGAGGGAATTTGCCCTA........-3′

(**c**) *3′-..........-AATGGCAATTACGGC~600nucleotides-TAAACTCCCTTAACGGGAT..........-5′*
5'-CGTTAATGCCG-3' DNA polymerase action ------->
The temperature is reduced and two different 11-base single strand oligonucleotide "**primers**"
are added allowing the complementary regions to base pair with the genomic DNA.
5′..........-TTACCGTTAATGCCG~600nucleotides-ATTTGAGGGAATTGCCCTA..........3′
<--------DNA polymerase action **3'-TCCCTTAACGGG-5'**

(**d**) 3′-..........*-AATGGCAATTACGGC~600nucleotides-TAAACTCCCTTAACGGGAT..........5′*
5'-CGTTAATGCCG~600nucleotides-ATTTGAGGGAATTGCC-3'*

5'-TTACCGTTAATGCCG~600nucleotides-ATTTGAGGGAATTGCCCTA-3'
3'CAATTACGGC~600nucleotides-**TCCCTTAACGGG-5'***
* 2 newly synthesized strands usually 600-700 bases in length

(**e**) The temperature is raised again to 95 °C and strands again separate.

3′..........-AATGGCAATTACGGC~600nucleotides-TAAACTCCCTTAACGGGAT..........-5′

5'-CGTTAATGCCG~600nucleotides-ATTTGAGGGAATTGCC-3'*

5′..........′-TTACCGTTAATGCCG~600nucleotides-ATTTGAGGGAATTGCCCTA..........3′

3'-CAATTACGGC~600nucleotides-**TCCCTTAACGGG-5'***

(**f**) The temperature is lowered again allowing the two primers to bind to their respective
sequences and DNA polymerase to elongate the strands once more.

3′..........-AATGGCAATTACGGC~600nucleotides-TAAACTCCCTTAACGGGAT..........5′
5'-CGTTAATGCCG-3'. DNA polymerase action -->

5'-CGTTAATGCCG~600nucleotides-ATTTGAGGGAATTGCC-3'*
<-- DNA polymerase action **3'-TCCCTTAACGGG-5**'

5′..........-TTACCGTTAATGCCG~600 nucleotides-ATTTGAGGGAATTGCCCTA..........3′
<-- DNA polymerase action 3'-**TCCCTTAACGGG-5**'

3'-CAATTACGGC~600 nucleotides-**TCCCTTAACGGG-5'***
5'-CGTTAATGCCG-3' DNA polymerase action -->

Fig. 21.1 Schematic of the PCR methodology

(Fig. 21.1a), a double-stranded DNA sample from roots or soils believed to contain AM fungal DNA is heated in a 1.5 mL or less volume to about 95°C (called the "melt" temperature or Tm), which causes the hydrogen bonds holding the strands together to break, forming two single DNA strands (Fig. 21.1b). The temperature is then reduced to 74°C. Two different DNA single-stranded "primers" known to be specific only to the genome of the species of concern, a heat-stable DNA polymerase, all four nucleotide triphosphates (NTPs), and about 1 mM Mg^{2+} is added. Primer sequences are determined using databases that enable the user to select sequences unique only to the genome of interest (Fig. 21.1c). Often the primers chosen contain sequences of the large ribosomal subunit DNA gene. The DNA polymerase finds the $3'$ end of the primer sequences and using the NTPs adds bases according to the information on the genomic strand until the reaction is stopped by raising the temperature to the Tm. A 600–700 base long single strand is synthesized on each genomic strand (Fig. 21.1d), and each is released at the Tm. In the next step (Fig. 21.1e), the short primers bind to the newly synthesized replicons and synthesize another complementary 600–700 base strand.

In Fig. 21.1f, there is one double-stranded genome or genomic fragment and two replicons undergoing DNA replication. The heating/cooling cycle is then repeated 25–50 times, each time doubling the number of replicon DNA strands in an exponential progression, mn, where $m =$ the number of original strands of fungal DNA present in a sample, and $n =$ the number of cycles performed. The heat-tolerant DNA polymerase survives each cycle. After m cycles, the solution is assayed for DNA, and, if shown to be present, proves the presence of the organism of interest. Actually, after all cycles have been completed the genomic fragments are of no consequence in the assay. Their large size and small number ($2\ m$) prevents them from even being seen in the assay. After the final treatment at the reduced temperature, the replicons are all double stranded and all are about the same size. A small aliquot of 25–50 μL is electrophoresed in a gel made up of 2% agarose in the presence of the dye ethidium bromide, and after a given time period in the electric field the gel is observed under ultraviolet light. The replicons have a large negative charge and travel toward the positive electrode. If bands in the gel are observed visually their presence is proof that the species of fungus of interest is present, and the gel is photographed. Commercialization of every step in the protocol makes this technology available to any laboratory as long as funds and well-trained technicians are available.

21.6 AM Fungi and Raw Sewage Used in Irrigation

Elhindi et al. (2018) studied how using raw sewage water might affect the growth of ornamental marigold (*Tagetes erecta L.*) in an experimental field when inoculated with the AM fungus *Glomus constrictum*. There was no doubt about the value of the AM fungal inoculant because compared with uninoculated controls, marigolds in the inoculated plots accumulated more P, N, K, and Mg, grew larger, and exhibited much less heavy metal (Zn, Co, Mn, Cu) content than plants with no fungal inoculant. These results supported the work of Shen et al. (2006) who reported a similar study for maize. In fact, the partition of heavy metals in root tissues in symbiosis with AM fungi further suggests that the beneficial use of wastewater is made even safer as long as the fungus is present in the soil to colonize the roots. Mishra et al. (2019) suggest how AM fungi are reliable partners with their symbiont in carrying out heavy metal bioremediation of soils. Probably the greatest concern over using raw domestic wastewater for irrigation for crops is the likelihood of finding heavy metals in the plant tissues. In a recent report from China, Meng et al. (2016) clearly warns those considering the use of raw municipal wastewater that heavy metals do indeed accumulate in crops such as wheat irrigated with untreated sewage. Roots are especially susceptible to heavy metal uptake. Dhalaria et al. (2020) clearly make a case for the role of AM fungi in accumulating heavy metals found in soils, but also in the mechanisms by which they take up their residence in some of the structures found in the fungi, such as their vacuoles and hyphae. In another study, Wang et al. (2010) found that when raw sewage was allowed to pass through a 100 m^2 mangrove plot, the effluent concentrations of pollutants such as BOD5, COD, fixed nitrogen, and several others were substantially reduced over a three-day period (residence time) until the effluent was released. They assayed AM fungi microscopically in mangrove roots and found that fewer fungi grew near the entry to the 33 m long plots, but near the exit the roots exhibited greater fungal colonization.

Longitudinal studies in science are difficult because researchers need funding for several years to ensure the completion of a project. Fortunately, Calheiros et al. (2019) were able to investigate changes in AM fungal populations and species in a constructed wetland over a three-year period, using raw sewage derived from a septic system from a vacation guest house in southern Portugal. A 45 m^2 artificial wetland was populated with *Canna flaccida*, *Canna indica*, and *Watsonia borbonica* and roots were collected during the three cold and hot seasons, and DNA prepared using a commercial kit. First, using PCR they amplified the region of all 18S RNA genes using the primer NS31 (5′-TTGGAGGGCAAGTCTGGTGCC-3), followed by the AM fungus-specific *primer AM1* (*5′*-GTTTCCCGTAAGGCGCCGAA-*3′*). The 18S genes code for the 18S eukaryotic ribosomal RNA molecule, of which there are many per individual cell. A second PCR reaction, called a nested reaction, was then carried out using the different primers (NS31-GC (5-′-CGCCCGGGGCGCGCCCCGGGCGGGGCGGGGGCACGGGGGTTGGAGGGCAAGT CTGGTGCC-3′) and Glo1 (5′-GCCTGCTTTAAACACTCTA-3′), which amplify a second specific genomic sequence within that targeted during the first round of replication, and is more specific in selecting desired sequences. The DNA products were separated on a 1.5% agarose gel and visualized using the commercial dye Sybr Safe, which replaces ethidium bromide, a suspected carcinogen. Still, another separation method was carried out to purify sequences, denaturing gradient gel electrophoresis (DGGE). DGGE uses a polyacrylamide gel of about 8% w/v made up such that there is a gradient of concentration of urea and formamide through the gel, which denatures the DNA making it linear in conformation, and the loci (called bands) of certain DNA strands separated within the gel were observed using still another dye. Finally, the separated DNA bands were eluted from the gel, and sequenced, which now allowed the positive identification of AM fungi present to the species level. What was found were a total of nine species belonging to the *Acaulospora*, *Glomus*, and *Rhizophagus* genera, which were able to colonize and persist in this inhospitable environment.

Even the thought of carrying out an experiment over the course of a century would be out of the question for most scientists, but given just the right situation, Ortega-Larrocea et al. (2007) managed to do so, kind of anyway. Having access to various large plots of land outside of Mexico City that had been irrigated with raw sewage for periods up to almost a century proved beneficial for that study. Examining the two types of top soils known as vertisols and leptosols in raw sewage treated fields used for agriculture for 5, 35, 65, and 95 years, the levels of Zn, Pb, Cu, and Cd were found to increase linearly over time. They also extracted spores of AM fungi by wet-sieving, and used trypan blue to stain spores, vesicles, arbuscules, and hyphae from root segments mounted in glycerol and viewed by light microscopy. AM fungi were shown to decrease in abundance following long periods of irrigation, with *Glomus mosseae* found to be the most abundant species, but over the longer time AM fungi decreased in population.

Another problematic issue when using raw water for irrigation is the likelihood of accumulation of salts in the soils, especially in semi-arid areas. Gómez-Bellota et al. (2015) approached this issue by inoculating soils with *Glomus iranicum* var. *tenuihypharum*, which improved plant physiology by enhancing both water and nutrient uptake, and they proposed that this fungus could protect Laurustine plants against salinity by alleviating salt-induced oxidative stress.

21.7 Effect of Secondary Sludge Amendments to the Soil on AM Fungi

Raw water is rather rife in many plant nutrients as well as in heavy metals and other substances, which may negatively affect crop productivity. On the other hand biological or what is called secondary sewage treatment removes a great deal of both beneficial and harmful materials forming a sludge. Often that sludge is first "dewatered" and then either incinerated to reduce mass or landfilled directly. In any case, there is an interest in recovering the nutrients in sludge as an agricultural fertilizer, and studies have shown its value in this regard. Recently, Zielonka et al. (2021) reported the results from Poland involving the use of secondary sludge as an amendment to soils growing hemp (*Cannabis sativa*), which provides fiber used in a variety of ropes and twines, grown in experimental plots over a three-year period. AM fungi associated with roots of the plants were observed using microscopy and identified to the genus and even species level using molecular analysis. Roots bearing AM fungi were prepared for microscopy by treatment with 10% KOH or NaOH followed by acidification with lactic acid, and then stained with either carbol fuchsin (pink) or aniline (blue). DNA was prepared from roots and soils using a commercial kit. Nested PCR reactions were performed, and DNA was cloned into

Escherichia coli, amplified again using PCR, and then sequenced. Sequences were compared with those already entered in the NCBI database to obtain genetic assignments of AM fungi colonizing the roots of the hemp plants. DNA analysis showed that the most frequently occurring AM fungi in both the soil and hemp roots were the species *Funneliformis mosseae*, *Funneliformis caledonium*, and *Funneliformis geosporum*, with a minor contribution from *Glomus occultum*. Their data did not justify stating that the sludge amendment did in fact prove substantially beneficial to overall plant growth. Results were variable depending on variety of *Cannabis sativa* and length of time in the field. The purpose of cloning DNA sequences into the bacterium *Escherichia coli* is to enhance the amount of fungal genetic material needed to carry out the sequencing reaction, which avoids performing another nested PCR reaction.

Another recent report from Brazil (de Figueiredoa et al. 2019) treated sewage sludge in a pyrolysis furnace in the absence of air, at 300°C and 500°C forming a char, before applying it to a field planted with corn. The study was conducted over two years with a crop produced each year in a soil classified as Red-Yellow Latosol (Typic Haplustox), with clayey texture. Sewage sludge is known to have higher phosphorous and potassium content so its choice as a soil supplement is not surprising, but it is usually rife with heavy metals making it a complicated solution as a soil fertilizer amendment. In this study corn grain yield in the presence of either type of char was equal to the yield of the crop subject to the application of mineral fertilizers (NPK—nitrogen, phosphorus, and potassium). The presence of AM fungi produced glomalin was determined using the spectrophotometric Bradford assay for protein, after extraction of the material from soil by autoclaving with sodium citrate, followed by separation of the supernatant by centrifugation at 3500 rpm. Microscopy to identify fungi associated with the fine corn roots was carried out after treatment with 10% KOH at 60°C and staining with trypan blue. Easily extractable glomalin and corn fine root colonization were both shown to be statistically enhanced with the application of pyrolyzed sewage sludge compared with plants in the control group.

In addition to identifying AM fungi using microscopy, a glycoprotein found in soil can also be correlated with the presence of those fungi. Janos et al. (2008) reviewed the various ways to prepare and quantify glomalin, as mentioned briefly already, a proteinaceous brown-colored material produced by AM fungal spores and hyphae, which causes soil particles to adhere to one another. The brown color is probably due to iron, which can make up from 1 to 9% of the material. Extraction from soil requires treatment with citrate and an hour of autoclaving at 121°C. Its presence in soil can be determined using the classic Bradford spectrophotometric assay or an enzyme-linked immunosorbent assay (ELISA), and if it is present then so can AM fungi be assumed to be present. However, it is highly resistant to bacterial decomposition, capable of surviving in soil for 40–50 years. True, its presence in soils may mean that AM fungi might be present, or perhaps, they were present, sometime in the past.

Although many propose to employ land application of sewage sludge as a source of phosphate, whether raw, dried, or pyrolyzed, the nutrient is chemically bound and much of it is rather inert. Mackay et al. (2017) found that in their study that dealt with the value of adding dried and incinerated sludge as amendments to soil, that only 6.84% was available for wheat plants growing in pots in the former (air-dried sludge) but much less, 0.54%, was available in the latter (incinerated sludge). AM fungi were prepared from soils extracted from a wheat crop and used to colonize roots of single wheat plants growing in pots fertilized with either dried or incinerated sludge. As probably expected, based upon the poor level of soluble and hence available phosphate in incinerated sludge, phosphate uptake by plants was much less than in experiments using dried sludge. The same was true for AM hyphae observed by trypan blue staining and light microscopy. The high-temperature incineration and pyrolysis processes most likely allow insoluble phosphate salts to form as C–O–P bonds of organophosphates, which are broken and reformed in other ways.

Amir et al. (2019) took a rather sophisticated approach to investigating the role of very specific AM fungal species in the growth of the Myrtaceae shrub *Metrosideros laurifolia* in heavy metal-contaminated ultramafic topsoils, characteristic of mining area in New Caledonia. First, they grew up three different isolates of AM fungi, one of *Claroideoglomus etunicatum* and two of *Acaulospora rugosa*, all three having been isolated from local soils and identified by sequencing of the genes coding for their small ribosomal subunit RNAs. Roots of the plant were harvested after 365 days of growth, DNA was prepared using a commercial kit, and nested PCR was used to isolate stretches of AM fungal DNA that were cloned into the pGEM-T *E. coli* vector (Promega) following the manufacturer's instructions. Ligated plasmids (plasmids that were first cut with a restriction enzyme, the DNA inserted into the plasmid, the plasmid-DNA breaks sealed with DNA ligase) were transformed into $CaCl_2$ competent *Escherichia coli* DH5α cells using a heat shock protocol. The bacteria were grown on Luria-Bertani (LB) agar plates containing ampicillin at 37°C. Two universal primers were used to PCR the clones, and DNA of the selected size (in gels) was sequenced and the results were submitted to GenBank. AM fungi inoculated plants combined with an added sewage sludge amendment to soil were ultimately shown to improve the ecological restoration of ultramafic mine-degraded areas.

21.8 AM Fungi and Irrigation Using Biologically Treated Municipal Wastewater

Contaminants of a chemical nature found in municipal wastewater subject to secondary treatment are much reduced compared to those in raw sewage (Table 21.1), with the sludge accounting for those of greatest concern, the heavy metals for example. Certainly, the public health and safety that is always first priority in the application of treated wastewater in crop irrigation is important. Also of importance is how the long-term use of such wastewaters affects the subsurface microbiome, especially those organisms in the rhizosphere that are known to improve plant fitness and crop productivity. One extremely comprehensive study reported out of Spain on soil AM fungal populations found in experimental plots in an orange grove is very interesting. For 43 years, plots of *Citrus aurantium L.* have been drip irrigated, some only with fresh water, and others using water using effluent from a secondary sewage treatment process (del Mar Alquacil et al. 2012). The treated wastewater exhibited both a BOD5 and COD seven times higher than the fresh water used in irrigation, and phosphate levels in wastewater averaged about 2.5 mg/L vs. near 0 mg/L for fresh water. Fungal identification was accomplished using molecular methodology. DNA was extracted from ten soil samples using a commercial kit, and several PCR steps were used to amplify sequences belonging to the large ribosomal subunit RNA genomic region. The PCR products were separated in a 1.2% agarose gel, specific bands were removed from the gel, transformed into *Escherichia coli* pGEM-T (commercially available), and further amplified prior to sequencing. They found and deposited the sequences of 44 clones into the NCBI library. They were able to identify four *Glomus* species using molecular methods, a number of species related to that genus, and several totally unidentified species of AM fungi. They concluded however that although AM fungal diversity was higher and biological activity as measured by assaying several enzymes was lower in soils receiving fresh water irrigation, crop productivity seem unaffected by biologically treated wastewater irrigation. This suggested to them that ecosystem resilience was modified but not reduced by fewer species of AM fungi or by an increase in overall biological activity, which they assumed led to greater soil fertility. In fact, Ibekwe et al. (2018) using similar molecular methods compared bacterial populations in soils subject to treatment with fresh water and wastewater, and found that there was no significant differences in microbial diversity, but that wastewater-treated soils exhibited a greater number of nitrifying bacteria and bacteria considered human pathogens.

Although only reporting a short-term study, El-Nashar et al. (2021) grew *Nemesia* plants from seed sterilized to remove any exogenous bacteria or AM fungi in pots, but then inoculated the same pots with soil containing AM fungi and irrigated the pots 3 times weekly for 160 days with fresh water, well water, and treated municipal wastewater. There was no doubt that the plants grown using municipal wastewater thrived more so than ones irrigated with uncontaminated water, and AM fungi were found colonizing roots of wastewater-treated plants using microscopic methods as described previously.

21.9 Risks Associated with Irrigation with Biologically Treated Municipal Wastewater

Release of municipal wastewater, even that subject to secondary treatment, comes with a variety of risks, the obvious being microbial and viral pathogens as well as some helminths. Recently, however, it has become clear that the myriad of chemicals used routinely for pharmaceutical, personal care, “life-style,” and selected industrial compounds are found in trace amounts in wastewater discharges. Vigneswaran and Sundaravadive (2004) provided disturbing evidence dealing with the survivability of human pathogens in domestic wastewater, measured sometimes in many weeks or even months after irrigation. Lapworth et al. (2012) reviewed 143 studies and identified 63 chemicals found in municipal wastewater with a mean total concentration of 100 ng/L, and such compounds will surely enter the groundwater and moisten the soil of fields irrigated with reclaimed wastewater. Even though Foster and Chilton (2004) considered many issues related to enhancing groundwater recharge with treated municipal wastewater, their many concerns should be addressed for irrigation with reclaimed wastewater as well. Perhaps one specific example of how the release of biologically treated municipal wastewater has been evaluated, not for irrigation but from a public perception viewpoint, is that described by Sprouse and Atondo (2004). They describe the management of the International Wastewater Treatment Plant (IWWTP) serving about 300,000 inhabitants of both Nogales, Sonora, Mexico and Nogales, Arizona, USA that releases about 14.3 million gallons (54.1 million L) into the usually dry Santa Cruz River. The river flows northward into Arizona where the effluent/water gradually disappears into the river bed, becoming part of the local groundwater resource. During the planning stage for IWWTP, an engineering review estimated the expected minimal release of pollutants including heavy metals into the Santa Cruz River (CH2MHill 2009). The authors note that a good deal of the effluent is used in Mexico for irrigation of public spaces as well as for electricity generation, and that another unrelated source of treated wastewater released upstream in the city of Tucson is used for irrigation of many parks and golf courses. In this case contact between water and the public has not been of a public health concern, but the effluents have not been put to use for irrigating food crops. At this time there are no reports of how treated wastewater from the IWWTP affects the local groundwater along the Santa Cruz River in Arizona.

21.10 Conclusions

Although arid and semi-arid regions are the most likely beneficiaries of using wastewater for crop irrigation, many urban areas with once plentiful water supplies often suffer from occasional drought. Reclaimed wastewater can reduce the dependency on having to rely on expensive alternate and unpopular solutions to water shortages. One calculation for a city of 500,000 residents for example with a potable water consumption of 200L/day per person would produce approximately 85,000 m^3/d (30 Mm^3/year) of wastewater, assuming 85% inflow to the public sewerage system. If treated wastewater effluent is used in carefully controlled irrigation at an application rate of 5000 m^3/ha.year, an area of some 6000 ha could be irrigated. In addition to the economic benefit of the water, the fertilizer value of the effluent is of importance (National Academy of Sciences 1996). Already, cities along the Mexico–USA border are finding beneficial use of treated municipal wastewater for irrigation of public recreational lands and for use in electricity generation. For use in irrigation of food crops in the field, however, there is still great reluctance. Indeed, wastewater can be, and in fact is, reused in certain settings, for human consumption, but achieving that level of purification necessary and safety is very expensive (Becerra-Castro et al. 2015). Perhaps when feasible there will/should be an opportunity for a tradeoff by diverting agricultural water to public municipal use while supplying an equal volume of treated wastewater for agriculture. Of course, irrigation with municipal wastewater has its drawbacks. Since most plant roots colonize AM fungi, planters of commercially important crops may have questions when asked to choose wastewater as a supplemental, or maybe even a permanent substitute for their regular supply of irrigation water. They realize the importance of AM fungi to their livelihood. The information at hand right now suggests that long-term use of effluent from secondary treatment of municipal wastewater can slightly alter AM fungal populations compared to crops irrigated with a better quality of water. However, over time it appears that changes in the rhizosphere microbiome are inconsequential to crop productivity, even after decades of application of treated wastewater (i.e., sewage), based upon but a very few scientific articles. More needs to be done, of course, but the type of studies needed will require years to complete! The use of raw sewage for irrigation is not at all popular and presents many problems. Also, sewage sludge is not without its issues. All in all though, a good case can be made for using biologically treated secondary effluent in specific cases as a supplement to irrigating certain food crops, such as fruits or nuts, growing where the irrigation water rarely contacts the product. Until then, more research hopefully will become available to answer these questions.

21.11 Future Perspectives

Together, water and food security will likely encourage the acceptance of irrigating agricultural crops using biologically treated municipal wastewater in many parts of the world. Public health of consumers of the agricultural products grown using that reclaimed wastewater will of course be the primary consideration. The health of the soils and their microscopic inhabitants receiving that same irrigation water, the microbiome of the rhizosphere, should also be taken into account. Of special concern are the AM fungi, their composition and their population, largely because those organisms, in symbiotic union with the plant roots they colonize, play a most important role in assuring that the plants themselves obtain sufficient water as well as a variety of essential nutrients to attain optimal growth and product yield. Molecular methods are now applicable for identifying specific AM fungal species in plant root and soil samples. A few longitudinal studies will be most useful in following any changes in AM fungal species richness in the fields irrigated with municipal wastewater, and if possible a correlation hopefully will be made between agricultural productivity and microbiome composition.

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Plant–Rhizospheric Microbe Interactions: Enhancing Plant Growth and Improving Soil Biota

22

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Abstract

Microbes present in the rhizosphere change the soil environment. Rhizosphere microorganisms use mucilage and exudates secreted by plant roots and these plant roots influence the microbial diversity and their function. Roots release various flavonoids, organic acids, and auxin monomers that are involved in the regulation of plant–microbe interactions. Methyl salicylate produced by the plant roots triggers colonization of *Bacillus subtilis*. Beneficial microbes in the rhizosphere respond to the root exudates by tuning their transcriptional machinery toward traits associated with mobility, chemotaxis, biofilm formation, and polysaccharide degradation. Once beneficial microbes are established in the rhizosphere, they stimulate the biofilm formation on the root surface. Researches on below-ground microbial community unveil various important interactions occurring between plants and microbes. These interactions can be harnessed for the betterment of agriculture to enhance crop productivity in stressed areas.

22.1 Introduction

There are several evidences that reveal the potential of a range of microbes to improve plant productivity and yield in cropping systems. Realizing the potential of beneficial microbes needs an understanding of the role of microbes in growth promotion, mainly in terms of fertilization and disease control, the underlying

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mechanisms, and the challenges in application and commercialization of plant growth-promoting (PGP) microbes.

It is well known that microbial communities play a significant role in upholding soil health, ecosystem functions, and production. An extensive variety of species will survive better with threats than a restricted number of those in large populations. The escalating demand for food, fiber, and fuel will escort to rising losses of biodiversity and ecosystem services. Deforestation, soil degradation, fragmentation, inappropriate fire regimes, and unsustainable use of natural resources are manual threats to biodiversity whereas spreading of invasive species, change in climate, and changes within aquatic environment are natural threats to biodiversity. Globally, most of the natural habitats including forests, wetlands, and coral reefs are in a state of decline. Microorganisms interact with one another in the ecosystem, forming a complex web of biological activity. However, the soil biota is being placed under increasing threat due to the increase in anthropogenic pressures such as loss of soil through erosion, chemicalization, pollution, and climate change. There is a lack of information on the current levels of below-ground biodiversity and it is difficult to assess the exact limits of the same. However, there are many tools and management techniques already available for the sustainable management of soil, although uptake of these tools and techniques could be improved. The complex ecological interactions between soil organisms and agriculture are not yet fully understood—there is a need for continued research into soil biodiversity if we are to measure the effect of the many threats to this essential and critical resource. Therefore, it is an immediate need of the hour to quantify the effect that human activities are having on the soil biota and put a limit over it to ensure that the next generation gets resources for their sustenance too.

22.2 The Rhizosphere

The rhizosphere is the expanse of soil on the brink of plant roots that holds large number of diverse organisms. The instance of microbial entrants that are found in the rhizosphere consists of the arbuscular mycorrhizal fungi (AMF) and rhizobacteria. These rhizosphere microorganisms exploit plant root secretions (for instance, mucilage and flavonoids), which are capable of influencing their assortment and role and also improve their potential to colonize plant’s roots.

22.3 Natural Interactions Between Microorganisms and Plant

In the natural environments, plants live in communications with diverse microorganisms, which flourish below ground in the rhizosphere and above ground in the phyllosphere. Some of the plant–microbe interactions (which can be in the form of antagonism, symbiosis, parasitism, and amensalism) protect the host plants against unfavorable microbial and non-microbial intruders and supply nutrients to the plant at the same time as others negatively influence it. These interactions can

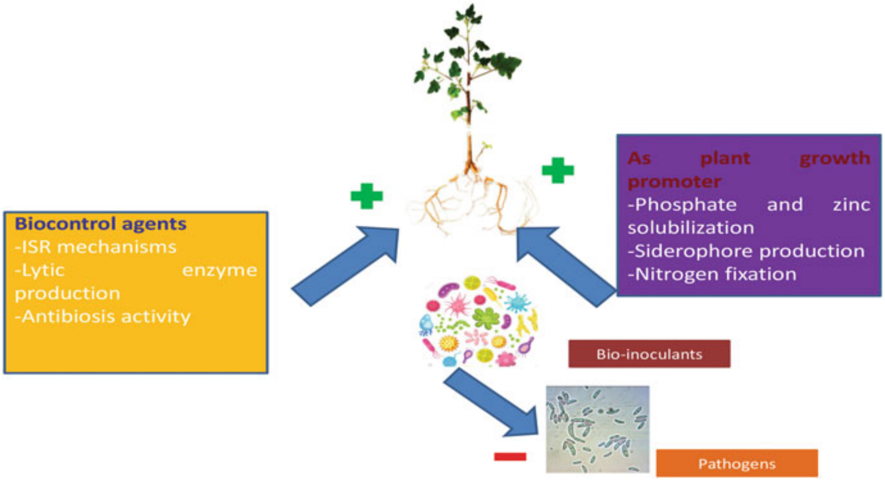

Fig. 22.1 Various mechanisms imposed by microbes on plants

influence below- and above-ground plants' biomass development thus playing momentous role in sustaining plants. Therefore, understanding microbial interactions within the rhizosphere and phyllosphere is urgent toward farming practices that are less reliant on conventional chemical fertilizers, which have known downbeat force on the environments (Fig. 22.1).

22.4 Biocontrol Potential

Plant diseases caused by soil pathogens are difficult to predict, detect, identify, diagnose, and manage. Investigations and management of these pathogens are further limited by the complex soil environment, heterogeneous incidence, biotic and abiotic factors, and lack of knowledge on epidemiological aspects of soil pathogens. Chemical pesticides applied to manage pests in soil adversely affect non-target organisms, leave pesticide residues, and accumulate in the underground water, which are potential hazards to human health and environment. Biological control is one of the potential, cheap, ecofriendly, and alternative approach for the management of soil pests and combats the above-listed problems. A large number of *Trichoderma* spp. are effective biocontrol agents that are being exploited commercially for management of soil pathogenic fungi, viz. *Fusarium oxysporum*, *Fusarium roseum*, *Fusarium solani*, *Phytophthora colocasiae*, *Pythium*, *Rhizoctonia*, *and Sclerotium rolfsii*. Besides inhibition of growth of soil-borne plant pathogenic fungi, it has potential to enhance yield along with the quality of produce by enhancing shoot/root growth, solubilization of phosphorus and micronutrients, and augmentation of nitrogen fixing. It releases biochemical elicitors of disease and can induce disease resistance in plants. It has been found effective on potatoes, radish, and cucumbers. It has great potential to be used to develop transgenic plants by introducing endochitinase gene into plants to increase their resistance against fungal pathogens. It can play an important role in the bioremediation of soil that is contaminated with chemical pesticides; e.g., *Trichoderma* has the ability to degrade a wide range of insecticides, viz. organophosphates, carbonates, and organochlorines.

In today's world, with booming population and continuously deteriorating arable land, the pressure on agriculture for enhancing crop yield is soaring. The popularity of the concept of plants being no longer considered an individual entity, but being a component of a "holobiont" stresses on the equal importance to the microbiome it harbors. Plant selects a specific microbiome in its rhizosphere, which is beneficial for it. The sensitivity of these microorganisms toward different stresses makes them excellent markers for eco-toxicological studies and as indicators for soil status.

22.5 Disease-Suppressive Soil Microbes

Microorganisms that exist in the rhizosphere of soil and participate in active plant growth by inducing root exudation, enhancing the accessibility of nutrients to plant, and releasing growth regulators, and also assist in controlling soil-borne infection, are referred to as rhizospheric microbes. Beneficial rhizospheric microorganisms are broadly classified into two groups (on the basis of their major effects):

22.5.1 Biocontrol Agents

They circuitously assist with plant productivity all the way through the control of plant pathogens. For example, *Trichoderma* spp. and *Pseudomonas* spp.

22.5.2 Plant Growth-Promoting Microorganisms (PGPM)

They apply direct effect on plant growth promotion, e.g., *Rhizobium* and *Glomus* spp. Bacteria that have the propensity to colonize roots vigorously (Schroth and Hancock 1982) are called as plant growth-promoting rhizobacteria (PGPR). In order to enhance population of these capable microbes in soil, they are applied as inoculants. This has brought to the forefront a new promising technology in the formulation of biocontrol agents. Soil strength and crop form the foundation for the population of rhizobacteria in soil and it fluctuates from species to species (Tilak et al. 2006). For soil-borne pathogens or disease management, rhizospheric microbes come out as a biological weapon that triggers the mechanism of disease reduction through systemic acquired resistance (SAR) and induced systemic resistance (ISR). Favorable microbes or disease-suppressive soil microbes are those that are constructive for plant growth and advancement by improving the soil health and quality, and providing necessary nutrients and minerals from soils, which are normally not available to the plant; e.g., *Bacillus*, *Trichoderma*, *Pseudomonas*, and *Rhizobium*.

These bacteria compete with surrounding microflora, multiply, and colonize plant roots at different stages of plant growth (Antoun and Kloepper 2001). Plant growth promotion engrosses siderophore production, antibiosis, phytohormones like indole acetic acid (IAA), solubilization of phosphate, inhibition of plant ethylene synthesis, production of volatile compounds such as HCN, and induction of plant systemic resistance to pathogens (Richardson et al. 2009).

The modern agricultural system, being based on agrochemicals, forces the shift of agricultural practices to large-scale, intensive, and specialized cultivation, which though increases crop production significantly but poses threat to environment and human health, besides enhancing the cost of crop production. Over past decades, this has disrupted the co-evolutionary dynamics between host plants and pathogens. Water and air pollution resulting from agricultural wastes, and overuse of chemicals to nurse plants and manage pests and weeds have generated many near-irreversible changes reducing farmland quality through heavy metal and pesticide residue contamination, with reduced organic material causing mineral imbalances and soil compaction. Additionally, this worsening in farmland excellence will possibly further reduce host plant immunity against pathogen infection. Healthy soils are the key to sustainable agriculture as well as plant disease management through their impact on pathogen density predominantly of soil-borne diseases and the availability of organic and inorganic nutrition for plant growth and development. Mycorrhizae and rhizobium application in agricultural production have been known since long back. *Glomus fasciculatum* association with grass species increases fodder yield to the extent of 19–24%. Enhanced emphasis on applications are given to the microbial communities in agriculture, directly or through their produced metabolites, for combating various plant diseases because of being environmentally viable or ecofriendly and safer for human health. These microbes help plants in fitting to several biotic and abiotic factors, besides making the availability of different macro and micro nutrients to the plant. The indigenous rhizospheric microbial population of agricultural soils is greatly influenced by agricultural practices, crop plant species, cultivar and genotype, as well as soil type. Plant exudates may cause changes to soil characteristics such as pH and carbon availability, impacting the diversity and activity of microbial populations. Plant-associated microorganisms have tremendous possibilities to enhance soil productivity and provide protection to the plant from many biotic stresses. Microbial products can increase crop yields and have the potential to complement or replace agricultural chemicals and fertilizers. *Trichoderma*-based bioformulations are available as biopesticides along with other microbial species, such as *Rhizobium*-based bioformulations as biofertilizers. Several species of *Aspergillus* and *Penicillium* are being used for nutrient mobilization in soil. Grass leaf litter decomposition rate was 12% higher with the association of fungal species. A number of bacterial and fungal endophytes have attracted considerable attention for their capacity to promote plant growth and protection from biotic and abiotic stresses. Plant microbiome has been considered one of the key determinants of crop health and productivity. Advanced understanding of microbial technology, especially gene sequencing, metabolomics, and proteomics, helps us to monitor different microbial diversity with respect to the different host plants and soil conditions. Now, these technologies help in the application of selective microbes and their colonization in the rhizosphere for specific traits. The limitation in microbes' application in agriculture is that microbes cannot be compared with chemical alternatives for their effects in all the terms. Application of multifarious organisms in agriculture can be an alternative to hazardous chemicals with at par results, and its only benefit is that it is ecofriendly and cost-effective technology for sustainable agriculture.

22.6 Effect of Below-Ground Microbial Interactions on Above-Ground Microbes

Interactions of below-ground microbes have a significant impact on above-ground microbial world. Rhizospheric microbes trigger the systemic plant defense response, which helps the plant in combating the pathogens and pests from the above-ground plant biomass. Induced defense response in the phyllosphere could affect the rhizosphere microorganisms. Rhizospheric microbes trigger systemic resistance in plants by activating defense response. The plant immune responses are modulated by plant-derived jasmonic acid, ethylene, and salicylic acid leading to secondary metabolite production. Rhizobacteria not only promote the production of defense metabolites like glucosamine, they also promote plant metabolite production (van de Mortel JE et al. 2012).

AMF interactions have been found to trigger protection of the phyllosphere. *Bacillus*, AMF, and *Pseudomonas* are able to induce a systemic resistance in plants that helps plant species against various disease-causing agents (Philippot et al. 2013; Zamioudis and Pieterse 2012). Studies have shown that these above- and below-ground associations have resulted in the increased level of resistance against diseases, protective enzymes, and expression of genes that are effective against pathogens and help plant in growth promotion. It has been assumed that the increased rate of resistance is due to the transfer of fungal disease protective signals from mycelia to the plants (Babikova et al. 2013a, b). This above-ground plant protection by the below-ground microbe interaction is an interesting area to be investigated further (Babikova et al. 2013a, b) (Table 22.1).

Microbial interaction with plants is important for ecological functioning. These interactions provide nutrients to plants and protect them against pathogens enhancing agricultural crops' production. Therefore, there is an urgent need to understand the interactions within the rhizosphere. Some of the important interactions that occur in the rhizosphere are as follows.

22.7 Antagonism

In this interaction one organism antagonizes the other. Antagonist secretes enzymes such as cellulase, chitinase, proteinase, and lipases that degrade the cell wall of fungal pathogens. Rhizobacteria are able to reduce the losses caused by pathogens.

Table 22.1 Microbe-mediated biotic stress tolerance in crops

Disease-causing agents	Crop	Biocontrol agents	References
Alternaria alternata	Chickpea (*Cicer arietinum*) Lentil (*Lens culinaris*) Pea (*Pisum sativum*) Faba bean (*Vicia faba* L.)	*Trichoderma viride* *Trichoderma harzianum*	Surekha et al. (2013) Kayim et al. (2018)
Rhizoctonia solani	Bean (*Phaseolus vulgaris* L.)	*T. harzianum* T019	Mayo et al. (2015)
Fusarium oxysporum f. sp. *pisi* *Fusarium oxysporum* f. sp. *lentis* *Fusarium oxysporum* f. sp. *Ciceris*	Pea (*Pisum sativum*) Lentil (*Lens culinaris*) Chickpea (*Cicer arietinum*)	*Bacillus cereus* *Streptomyces* spp.	Corrêa et al. (2014) Anusha et al. (2019)
Stemphylium botryosum	Lentil (*Lens culinaris*)	*Trichoderma viride* *Trichoderma harzianum*	Subedi et al. (2015)
Pseudomonas syringae pv. *lachrymans* *Pseudocercospora griseola*	Common bean (*Phaseolus vulgaris* L.) Cucumber (*Cucumis sativus* L.)	*Ochrobactrum pseudintermedium* *Pantoea agglomerans*	Akbaba and Ozaktan (2018)
Sclerotinia sclerotiorum *Sclerotinia trifoliorum* *Sclerotinia minor*	Common bean (*Phaseolus vulgaris* L.)	*Bacillus subtilis* *Pseudomonas fluorescens*	Khater (2010) Sabaté et al. (2018) Zhang and Xue (2010)
Xanthomonas fragariae *Xanthomonas axonopodis* pv. *Vignicola* *Xanthomonas arboricola* *Xanthomonas campestris* *Pseudomonas syringae*	Chickpea (*Cicer arietinum*) Cowpea (*Vigna unguiculata*)	*Pseudomonas aeruginosa* *Pseudomonas fluorescens* *Lactobacillus plantarum*	Daranas et al. (2019) Kanthaiah and Velu (2019) Corrêa et al. (2014)
Aspergillus niger	Peanut (*Arachis* sp.)	*Trichoderma viride*	Sayiprathap et al. (2020)
Sclerotium rolfsii	Lentil (*Lens culinaris*)	*Trichoderma viride* *Trichoderma virens* *Trichoderma harzianum*	Kushwaha et al. (2018)
Erysiphe flexuosa	Cowpea (*Vigna unguiculata*)	*Glomus versiforme* *Trichoderma harzianum*	Omomowo et al. (2018)

(continued)

Table 22.1 (continued)

Disease-causing agents	Crop	Biocontrol agents	References
Myrothecium *Anthracnose* *Rhizoctonia*	Soybean (*Glycine max* L.)	*Trichoderma viride*	Kuchlan et al. (2017)
Ascochyta lentis *Ascochyta rabiei*	Lentil (*Lens culinaris*) Chickpea (*Cicer arietinum* L.)	*Bacillus* sp. *Pantoea agglomerans*	Liu et al. (2016)
Fusarium oxysporum f. sp. *ciceri*	Chickpea (*Cicer arietinum*)	*Trichoderma viride* *Trichoderma harzianum*	Patole et al. (2017)
Fusarium oxysporum f. sp. *lentis*	Lentil (*Lens culinaris Medikus* subsp. *Culinaris* L.)	*Trichoderma viride* *Trichoderma koningii* *Trichoderma harzianum*	Tiwari et al. (2018)
Fusarium oxysporum f. sp. *ciceri*	Chickpea (*Cicer arietinum* L.)	*Trichoderma harzianum*	Nirmalkar et al. (2017)
Fusarium solani	Faba bean (*Vicia faba* L.)	*Trichoderma harzianum*	Habtegebriel and Boydom (2017)

Actinobacteria, Firmicutes, and Proteobacteria play an important role in inhibiting the disease-causing fungal pathogens. It has been observed that antibiotic compounds secreted by beneficial bacteria do not harm Arbuscular mycorrhizal fungi (AMF) fungi (Barea et al. 1998). There are some fungal species also that enhance the establishment of mycorrhiza. However, there is a need to understand which microbes are specific for mycorrhizal establishment.

Many plant growth-promoting microbes secrete siderophores, which deprive pathogens from iron acquisition from the environment, which inhibits their growth and enhances plant productivity and growth.

22.8 Amensalism

Amensalism is also known as antibiosis. It is an interaction in which one organism is benefited while the other is inhibited. Chemicals released by one organism inhibit or kill the other organism. Release of antibiotics is the most common mechanism employed in amensalism. *Bacillus*, *Pseudomonas,* etc. produce bioactive lipopeptides that can distort the cell membrane of pathogens.

22.9 Parasitism

In this association, one organism is benefited at the cost of another. For example, metabolites secreted by *Sagartia elegans* are harmful to *Rhizoctonia solani*. The impacts of AMF and bacteria on nematode have also been studied. *Pasteuria penetrans* is able to inhibit the root-knot nematode by parasitic interactions. This multiplies within the nematodes causing their death or resulting in infertility. Plants also have their own defense system against pathogens. RNA sequencing is a recently developed technique that is used to detect the genes that are involved against pathogens during defense response.

22.10 Symbiotic Interaction

Symbiotic relationships are the associations in which both organisms are benefited. The best example of symbiotic relationship is leguminous plants and rhizobia. The atmospheric nitrogen that is not assimilable to the plants is converted into such a form that can be easily utilized by the plants. Rhizobacteria help not only in nitrogen fixation but also in nutrient acquisition. These bacteria also release some compounds (2,3-butanediol [volatile organic chemical], diffusible signal factor diketopiperazines, antibiotics produced by rhizosphere-associated pseudomonads, polyketides, and lipopeptides) that elicit induced systemic resistance in plants. Root-zone-associated microbes secrete di methyl sulfoxide (DMSO) and pyocyanin, which regulate root development and modulate the auxin signaling process. Arbuscular mycorrhizal fungi are found to have above-ground influence, which leads to plant protection. Below-ground interaction not only occurs between microbes and plants but also among microbes, which leads to the protection of plants from their pathogens. Symbiotic relationship has also been shown to exist between the bacterium *Burkholderia* and *Rhizopus* (fungus) and it was reported that in the absence of *Burkholderia* species, the fungus *Rhizopus* was unable to sporulate indicating that the fungus relies on compounds produced by the bacterium to survive (Table 22.2).

22.11 Negative and Positive Interactions and Their Influence on Microbial Diversity

The association between plant and rhizosphere is a little bit complicated process. The below-ground interactions occurring between plants and microbes increase the above-ground activity of microorganisms. The immune defense system of plants plays a key role in modulating microbial structure. Plant hormones such as IAA, gibberellins, and common growth enzymes are secreted by rhizobacteria. There are many compounds secreted by plants that enhance the interactions among diverse microbial groups.

Table 22.2 Different types of interactions between microbes and plants

Sl. no.	Name of interacting organism/plants	Type of relationship
1.	*Pseudomonas putida* and soya bean	Mutualistic
2.	*Rhizobium etli* and *Phaseolus vulgaris*	Mutualistic
3.	*Bradyrhizobium diazoefficiens* and *Aeschynomene afraspera*	Symbiotic
4.	*Rhizobium* spp. and soya bean	Symbiotic
5.	AMF and host plant	Symbiotic
6.	*Bacillus licheniformis* and pepper plant	Mutualistic
7.	Rice and *Burkholderia* and *Rhizopus* spp.	Symbiotic
8.	*Rhizoctonia solani* and *Sagartia elegans*	Parasitic
9.	*Glomus mosseae* and soya bean	Mutualistic
10.	*Erysiphe pisi* and *Pisum sativum*	Pathogenic
11.	*Azospirillum* and wheat plant	Mutualistic
12.	*Blumeria graminis and Pisum sativum*	Pathogenic
13.	*Mesorhizobium ciceri*, *Rhizobium phaseoli*, and *Rhizobium leguminosarum* and wheat plant	Mutualistic
14.	*Bacillus subtilis* QST713	Amensalistic
15.	AMF and *Bacillus thuringiensis*/*Pseudomonas* and *Trifolium repens*	Mutualistic
16.	*Pantoea agglomerans* C9–1	Amensalistic
17.	*Glomus* spp. and the plant *Juniperus oxycedrus*	Mutualistic
18.	*Pseudomonas aeruginosa* and tomato plants	Mutualistic
19.	*Heteroconium chaetospira* and Chinese cabbage	Mutualistic
20.	*Azospirillum lipoferum* and maize	Mutualistic

22.12 Effect of Environment on Plant–Microbe Interaction

Plant–microbe interaction chiefly depends upon the soil and climatic conditions. Legay et al. (2018) showed that the inheritance impact of a past dry spell supported the resistance in *Lolium perenne* to the dry season. Zemunik et al. (2016) showed that non-mycorrhizal species chiefly expand on the phosphorus-deprived soils. In phosphorus-deprived soil, phosphorus is provided by the influx of carboxylates. Gang et al. (2018) found that *Klebsiella* have a prominent effect on the root hair development. Plant and soil microbe interrelationship is chiefly dependent upon the climate and soil texture (Rutten and Gómez-Aparicio 2018).

22.13 Effect of Agricultural Practices on Soil Microbiota

There are many studies that have shown that agricultural practices not only affect plants but also have a profound effect on soil microbes. The agricultural practices affect the microbes, which in turn affect the plant species (Schmidt et al. 2019). Agricultural practices like intercropping, and crop diversification have been used for sustainable agriculture. There are only very few studies that have shown the impact of agricultural practices on the plant–microbe interactions. Therefore, there is a need to understand the impact of agricultural practices on plant–microbe interactions.

In a study conducted by Campisano et al. in 2014, it has been observed that organic pest management causes a different build-up of plant–soil microbiota. In viticulture also, there is difference in the soil microbe build-up. Soils under organic management practices have a different microbial construction as compared to the field under non-organic management practice. In a study published by Hendgen et al. (2018), a vineyard field was assessed for 10 years and it was found that organically treated field has a much enhanced microflora as compared to the non-treated soil.

22.14 Below-Ground Microbes and Agricultural Sustainability

Little information is available about the specific mechanisms among plants and microbes that promote the use of microbes in agriculture. To increase the microbial biomass in soil use, organic farming is being promoted. Beneficial soil microbes are commonly known as plant probiotics (Yadav et al. 2017). Use of microbes to manage fungal pathogens has gained popularity over chemical-based fungicides due to increasing environmental concern (Alori et al. 2017). Seed inoculation by microbes has the advantage of direct delivery of microbes in the rhizosphere where they can form relationships with the plants. Microbial inoculation improves nutrient availability in plants. In leguminous plants, rhizobia form nodules in the roots, which help the plant nutrition by nitrogen fixation. In a study conducted by Ciccillo et al. (2002), it was found that *Burkholderia ambifaria* when used for seed treatment has a positive impact on maize plants but when the same species is applied directly on the plants, it showed negative effect on plant growth.

The high cost of phosphorus (P) fertilizers is a rising issue these days. That may explain why microbe-based P fertilizers are becoming popular. JumpStart is a P-based biofertilizer that is commonly used for Canola and wheat (Monsanto BioAg 2016). This biofertilizer has the fungus *Penicillium bilaii* as its active microbial ingredient. Use of this product toward P nutrition in the crop showed 66% increase in yield. *Pseudomonas* species are also well known for their plant growth potential. In 2019, Passera et al. (2019) conducted an experiment and observed that *Pseudomonas syringae* strain has a positive impact on the growth of tomato plant and inhibits the pathogenic fungus *Botrytis cinerea* and virus *Cymbidium* ringspot. When *Pseudomonas putida* culture was applied through root inoculation, it showed decreased leaf necrosis and low fungal load in the treated sample (Planchamp et al. 2015). Other bacterial species like *Bacillus* have emerged as a potential biocontrol agent against plant pathogens. Inoculation of *Pseudomonas* and *Coronaria* and *Rhizobium* and *Pseudomonas* has been found to increase the root and shoot length and dry weight in rice plants (Deshwal et al. 2011).

Mycorrhiza is a symbiotic association between root-colonizing fungi (Sylvia et al. 2005). The mycorrhizal relationship begins with the exchange of signals between both the partners. Plant roots release branching factors for initiating hyphal exchange with roots. Fungi release myc factors that give molecular as well as cellular signals for the root colonization. By associating with mycorrhizal fungi, plant immunity can be increased. *Piriformospora indica* is a root endophyte that has been isolated from Rajasthan, India (Verma et al. 2018). This fungus has been reported to increase the plant growth in many plant species (Achatz et al. 2010; Gill et al. 2016). *Trichoderma* is a well-known biocontrol agent used widely all over the world. Biocontrol potential of *Trichoderma* isolates against wilt pathogen of pulse has been tested by Mishra et al. (2020). Commercial talc-based formulations of *Trichoderma* are commonly used for the management of phytopathogens (Mishra et al. 2020b). *Talaromyces* is a fungal genus in which heat-resistant fungi are listed. The heat-resistant fungi have the ability to resist high temperatures (90–95 °C). This fungus produces compounds like actofunicone, deoxyfunicone, and vermistatin (Proksa 2010). These compounds help the plant in nutrient competition and plant growth promotion.

22.15 Future Endeavors

Interactions among plants and microbial species are very complicated. Hence, the need for more studies on plant–microbe interactions is being observed. Knowledge of these interactions is useful in developing microbial inoculants. Formation of mycelial network by AMF is an emerging area for research. AMF have the insecticidal activity also. Use of AMF can reduce the risks arising from chemical fungicides, by reducing their use. Studies on plant–microbe relationships have revealed that the interactions among these two diverse groups of organisms could play a significant role in sustainable agriculture, by enhancing crop health, productivity, and yield, and boosting global food security. However, intense knowledge regarding microbial physiology, their interactions, delivery techniques, etc. is required for a better understanding of the system to ensure its optimal use in favor of agricultural production.

Researchers have indicated that microbial ecological interactions have a significant outcome biotechnologically. Thus, there is a need to develop techniques that can help study the plant–microbe interactions in the ecosystem with more depth so that more knowledge regarding the interaction could be obtained (Schenk et al. 2012).

Plant roots are important for nutrient uptake and thus there is a need to intensify our understanding through further studies on the plant–microbe interactions. More studies are required to develop the process for field application of these microbes. With the development of biotechnological tools, it has become possible to effectively monitor microbial species that interact with plants. These could guide us to our goal of ensuring sustainability in the ecosystem of agriculture.

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Microbes-Mediated Rhizospheric Engineering for Salinity Stress Mitigation

23

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Abstract

Soil salinity has appeared as a major concern for global food security. According to reports, salinity has covered around 20% of agricultural land in the world and with the passage of time, the magnitude of salinity is increasing due to several anthropological activities including agricultural practices. The accumulation of soluble salts in agricultural land directly affects crop productivity. Therefore, it is essential to develop strategies for the mitigation of salinity to ensure food security. There are several physical and chemical methods to mitigate soil salinity. However, application of salt-tolerant microorganisms to address the issue is widely accepted and sustainable in nature. Salinity-tolerant microbial

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strains specifically function during salinity stress and potentially mitigate the negative effect of salinity stress on the plants through activation of the plant antioxidant defence machinery by controlling it *via* enzyme activity, as well as through the secretion of proteins, polysaccharides and other important phytohormones. Several salt-tolerant rhizobacteria have also been reported to produce the 1-amino-cyclopropane-1-carboxylic acid (ACC) deaminase enzyme, initiating a series of metabolic changes in the plant system and resulting in the alleviation of the effects of abiotic stresses. Further, several studies pertinent to plant–microbe interactions corroborate the significance of employing such microorganisms in developing adaptation to salinity stresses. In the current chapter, recent updates relating to the occurrence of salinity and its impact on the crop productivity have been presented, along with different salt-tolerance mechanisms present in rhizospheric microorganisms which function in the course of salinity mitigation. The chapter has been concluded with the recent challenges, salinity mitigation and their tentative remedies through microbial applications.

23.1 Introduction

In the past few decades, the world has witnessed several technological innovations in agriculture which considerably enhanced food production (Peng et al. 2020). World food production has continuously encountered several threats through abiotic and biotic factors (Chen and Mueller 2018; Kumar et al. 2019a). A large number of abiotic factors such as drought (Hussain et al. 2019), heavy metal contamination (Bhat et al. 2019), salinity (Chen and Mueller 2018), soil pH, increasing temperature (Varjani and Upasani 2019) and anthropological causes like injudicious pesticide and fertiliser application (Kumar et al. 2018, 2021a), deforestation (Bologna and Aquino 2020), excess agricultural practices (Pavlidis et al. 2020), land-use change (Searchinger et al. 2018), low water quality and scarcity for irrigation (Rosa et al. 2020) are some of the key factors which significantly affect crop productivity. Besides biological and physical environmental stresses, growing population with increased urbanisation (Ke et al. 2021), a large portion of food wastage (Bilska et al. 2020), dietary transition (Priyadarshini and Abhilash 2020), poverty and social inequality (Osabohien et al. 2020) are the indirect factors which cause the stress on food production.

The Food and Agriculture Organization and the Intergovernmental Technical Panel on Soils (FAO and ITPS 2015) have pointed out salinity and salinisation as one of the crucial abiotic factors out of nine major threats to agricultural production. Further, proceedings of the Global Agricultural Productivity (GAP) Index had highlighted that the present pace of food production is unsatisfactory compared to the expected agriculture and foodstuff demand of 9.5 billion people in 2050 (GAP Report 2018). Hence, to proportionate the difference between food production and anticipated food demand for the growing population, agricultural production essentially needs to be accelerated by 1.75% annually. Increasing food production in current agricultural ecosystems is intricate and is affected by climatic conditions, adopted farming systems and execution of agricultural techniques (Kamble et al. 2020).

Soil salinisation is the primary process of soil destruction which further leads to threatening the ecosystem. This is accepted as a crucial issue in the current global scenario pertinent to food production and agricultural sustainability in arid and semi-arid agroclimatic zones. For instance, the delta regions of Bangladesh, India, the Philippines and Myanmar, which have a high production rate of paddy, are encountering a serious threat to food production because of coastal soil salinisation (Abedin et al. 2014; Szabo et al. 2016; Mukhopadhyay et al. 2020). Ghassemi et al. (1995) revealed continuous expansion of saline areas in the delta regions of south-east Asia and reported that salinity caused an annual reduction in farmers' income of worth $12 billion. Similarly, Dove (2017) has reported an annual lowering of crop yield and agricultural productivity worth $3.7 billion in the central California zone. Saline soils have severely affected soil functions, leading to a string of consequences such as a noteworthy reduction in food productivity, addition of ion to ground water, decrease in soil biodiversity and soil erosion. Salinity has decreased the buffering ability of soil and the filtering potential of pollutants. Soil salinity also decreases the crops' potential to take up water and micronutrients available in the soil and concentrate ions that are toxic to plants.

High salt in any form is toxic for plant health and adversely hampers prime soil processes like decomposition, denitrification, nitrification, respiration, microbial biodiversity and rhizospheric microbial function (Schirawski and Perlin 2018; Qazi et al. 2020). Rütting et al. (2018) have reported loss of crop yield due to high salinity where the use of chemical fertilisers is above the recommended dose in soil. Herger et al. (2015) has deciphered the adverse impact of high salt fertilisers to the crop and reported that such fertilisers affect the osmotic pressure essential for the extraction of water from the environment required for plant growth. The irrigation water with a high concentration of salts may also increase soil salinity (Rengasamy 2010; Arora et al. 2018).

Elimination of salts from salt-affected soil is an exhaustive procedure and requires a lot of time and expenses (Bessaim et al. 2020). Still, mitigation of soil salinity is mostly carried out by physical and chemical processes. The physical process of salinity mitigation includes scraping, flushing and leaching methods in which soluble salts from the rhizosphere are extracted. Chemical processes of salinity rectification have been carried out through the application of calcium sulphate and calcium carbonate as neutralising agents (Ayyam et al. 2019; Keren 2005). These methods are unsustainable and inefficient at high salt concentrations. In the current scenario, cultivation of salt-tolerant crop varieties of barley and canola in salt-affected soils is a general trend (Fita et al. 2015). Further, the low salt tolerance in these crops (barley and canola) causes restricted global application and these low salt-tolerant crop varieties stop performing efficiently when the soil has moderate or high salinity.

The researchers in last few decades have reported many salt tolerant rhizobacterium which have potential of plant growth promotion in saline agricultural land. They have the capability to enhance the soil fertility as well as crop productivity (Grover et al. 2011; Fita et al. 2015; Al-Maliki and Ebreesum 2020; Kumar et al. 2022). Further, the adaptive responses of salt-tolerant plant growth-promoting microbiomes pertinent to salt stress are linked with the potential to secrete osmoprotectant compounds and specialised transporters. Hence, these salt-tolerant plant growth-promoting rhizobacteria are currently used as bioinoculants for increasing crop productivity, defence from plant pathogens and improving the fitness of agricultural soil.

Wani et al. (2019), Fatima et al. (2020), Litalien and Zeeb (2020) and Kumar et al. (2021b) have reported various remedial actions initiated to mitigate the salt stress in the soil and some salt-tolerant genes were identified from plants as well as from microbes. These genetic manipulations have been applied in saline salt for enhancing crop productivity. Hence, enhancing crop production in salt-affected soils in the current scenario and achieving food security for the future with the application of sustainable approaches is essential. The sustainable approaches involve application of salt-tolerant plant growth-promoting rhizobacteria, development of salt-tolerant varieties, improvement of soil, irrigation of plant with better quality water and chemical neutralisers. Therefore, considering the importance and potential of plant growth-promoting rhizobacteria in salinity stress mitigation, the current chapter has been drafted revealing the recent coverage pertinent to plant growth-promoting rhizobacteria for improving the productivity of crops grown in saline soil. The effect of salinity and its presence in the world has been discussed. Further, plant growth-promoting rhizobacteria-mediated beneficial mechanisms, covering the latest insights and perspectives on crop productivity enhancement fronting salinity stress, have also been considered.

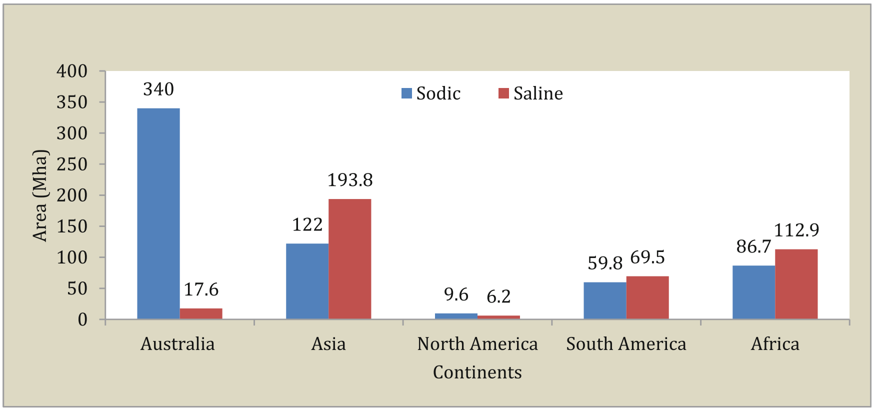

Fig 23.1 Land under (Million Hectare) salinity stress (Egamberdieva et al. 2019)

23.2 Global Distribution of Salinity

Almost all the continents have been affected by the salinity but the distribution of the saline area across the globe has not been mapped till (Shahid et al. 2018). The salinity-affected areas around the world have been presented in Fig. 23.1 according to Egamberdieva et al. (2019). Some researchers around the world have estimated the extent of salinity and reported it to be around 954.83 million hectares (Szabolcs 1989), 932.2 million hectares (Sparks 2003) and 952.2 million hectares (Arora et al. 2017). More recent estimates, however, have come from Mandal et al. (2018), who showed an increase in the total global saline area to 1,128 million hectares and that around 52 million hectares of agricultural soil in South Asia alone is salt affected. According to an estimate proposed by Shrivastava and Kumar (2015), nearly 21% of the total agricultural land worldwide has been affected by high salinity and 33% of the agricultural land has become so due to irrigation with heavy water (Wicke et al. 2011). Recently, Sharma and Kumar (2020) have reported and presented in their report that around 84–85% of the world's agricultural land has been affected by salt in the range of slight to medium, while the remaining 14–15% is affected by high salt dose accumulation, severely restricting crop production.

The International Society of Soil Science (ISSS), the United Nations Educational, Scientific and Cultural Organization–United Nations Environment Programme (UNESCO-UNEP) and the Food and Agriculture Organization (FAO) are the foremost world agencies that have committed their focus on collecting data on land/soil quality in the world. As per the report presented by the FAO on the 'Status of the World's Soil Resources,' an area of one billion hectares across approximately 100 countries in the world has been facing the issue of salinity (FAO and ITPS 2015).

Presently, the soil categorisation system has been adopted by the World Reference Base (WRB) for soil resources, which is certified by the International Union of Soil Sciences (IUSS) and replaced the FAO/UNESCO Legend for the Soil Map of the World (Egamberdieva et al. 2019). The highly accepted and benchmarked map of salt-affected soil was described as solonchak and solonetz. The assessed global area of solonchak is nearly 260 million hectares (IUSS Working Group WRB 2015). Globally, a total of 135 million hectares of solonetz are found in Ukraine, the Russian Federation, Kazakhstan, Hungary, Bulgaria, Romania, China, the United States of America, Canada, South Africa, Argentina and Australia (IUSS Working Group WRB 2015). In India, salt-affected soil covers approximately 5% of the cultivated area, from Jammu & Kashmir (Ladakh region) to Kanyakumari, and the Andaman & Nicobar Islands in the east to Gujarat in the west (Sharma and Kumar 2020).

Solonchaks are distinguished by the deposition of highly soluble salts like NaCl and Na_2SO4. The salic horizon has been considered to start from the soil surface to a depth of about 50cm and is extensively scattered to the arid and semi-arid coastal regions in all agro-climatic zones. On the criteria of salt precipitation, there are two types of solonchaks, viz., external solonchaks, identified by the accumulation on the surface, and internal solonchaks, characterised by deep accumulation

(Egamberdieva et al. 2019). Solonetz has been reported the alkaline and sodic soils for holding a huge portion of accumulated sodium and magnesium ions (Egamberdieva et al. 2019).

23.3 Effect of Soil Salinity on Crop Production

The arid and semi-arid areas of most of the continents are more prone to salinity because of the dryness. Anthropological activities, in addition, exacerbate the situation (Zhu 2000; Etesami and Beattie 2017). The salinity results in salt stress, toxicity, and antioxidant and ROS generation, which damage the plant and may cause a rapid decline in photosynthesis and, subsequently, growth (Etesami and Beattie 2017; Sobhanian et al. 2011). In addition to affecting agriculture, salinity also affects the biodiversity in the region, which may have a severe effect on the environment (Rockström and Falkenmark 2000). Salinity stress might increase in the near future also because of inevitable global climate changes. These changes would exacerbate the degradation of irrigation systems and contamination of groundwater resources by leading to a rise in the seawater level (Joshi et al. 2015).

In the present situation, the area of agricultural soil is decreasing due to increase in salinity. On the other hand, we have a huge demand for agricultural output to feed a vast population as zero hunger is one of the UN’s sustainable development goals (Zhu et al. 2011; Rasool et al. 2013). So the latest demand is the development of salinity-resistant plant varieties with increased salt tolerance (Glenn et al. 1991; Zhu et al. 2011). High-salt concentration in soil disrupts homeostasis, leading to the death of the plant. In these conditions, resistant plants can produce significantly higher yields (Munns and Tester 2008) than those lacking salt tolerance/resistance.

There are several approaches to develop salt resistance in plants. Traditional breeding and genetic engineering are some of the well-established methods which require identification of the salinity-resistance genes. These genetic traits may be integrated to form genetically modified organisms (Dodd and Pérez-Alfocea 2012; Krishna et al. 2015; Joshi et al. 2015; Etesami and Beattie 2017). The alternative approach may be the use of salt-tolerant microbes, which enhances the growth of the plants under such stress. These microorganisms, through plant–microbe interactions, play an important role in the alleviation of salt stress in the plant and protect the plant through several mechanisms (Dodd and Pérez-Alfocea 2012: Grover et al. 2011). In general, microbes have three kinds of plant–microbe interactions, namely epiphytic, endophytic and rhizospheric. There are varieties of bacteria which support plant growth using several mechanisms including making available the otherwise insoluble nutrients to the plant, protecting the plant from external factors of stress like infection from phytopathogenic fungi and phytophagous insects, and protecting the plant from abiotic stresses like salinity (Glick 2012; Hallmann et al. 1997; Yadav et al. 2017; Kumar et al. 2021c).

The bacteria which colonise the roots and promote plant growth are called plant growth-promoting rhizobacteria (PGPR) and are better known for transferring nutrients and increasing plant tolerance to environmental stresses. They are associated with ecological conditions including the climate, weather conditions, soil type (e.g. high salinity) and interactions with other indigenous microbial flora in the soil (Etesami and Beattie 2017; Yoon et al. 2001). These PGPR have properties like phosphate solubilisation, siderophore formation and many more PGP activities. There are several reports on salt-tolerant isolates which have the ability to tolerate salt concentration up to 2–25%, such as *Bacillus pumilus*, *Pseudomonas mendocina*, *Arthrobacter* sp., *Halomonas* sp. and *Nitrinicola lacisaponensis*, with PGP characteristics like P-solubilisation, IAA (indole-3-acetic acid), siderophore and ACC (1-aminocyclopropane-1-carboxylate) and deaminase (Upadhyay et al. 2009; Lucero et al. 2011; Marasco et al. 2012; Kaplan et al. 2013; Etesami and Beattie 2017; Bharati et al. 2020a, b).

23.4 Osmoregulation in Salinity Stress

The osmoadaptation mechanism is referred to the physiological and genetic manifestations which may further leads to the adaptation to low and high salt concentration (Csonka and Epstein 1996; Machado and Serralheiro 2017; Kumar et al. 2022). Salinity is one of the major threats affecting crop production all over the globe. About 20% of the irrigated land in the world, producing one-third of the world's grains, is affected by salinity (Csonka and Epstein 1996; O'Byrne and Booth 2002). Salinity is reported as a major problem in many countries, including China, India, the United States, Australia and Russia (Parihar et al. 2015). In India, most of the states, such as Gujarat, Uttar Pradesh, Maharashtra, West Bengal and Rajasthan, are struggling with this issue (Sharma and Singh 2017; Srivastava et al. 2019a,b). This affects most of the stages of crop development and production attributes like germination, plant growth, flowering, fruiting and seed setting. Because of these problems, prokaryotic osmoadaptation has gained considerable importance these days for its use in agriculture, food and fermentation industries (O'Byrne and Booth 2002).

Intracellular accumulation of the small organic osmolytes is a more common strategy to cope with the osmotic stress produced by the presence of high salt concentration in the extracellular environment. These osmolytes protect the cell from the high salt concentration and function as osmoprotectants. These are also termed as compatible solutes as they provide osmotic balance without interfering with the cell function like metabolism, protein folding etc. The microorganism has evolved with the variety of transporters and efflux systems to maintain osmolarity (Hoffmann and Bremer 2017). There are a variety of compounds present, for example, sugar molecules (sucrose, trehalose), polyols (glycerol, glucosylglycerol, arabitols etc.), amino acids (proline, hydroxyproline, alanine, glycine, glutamate derivatives etc.), quaternary amines (betaine, choline etc.), and ectoine and its derivatives, which act as osmoprotectants (Khatibi et al. 2019; Wiesenthal et al. 2019; Salvador et al. 2018; Srivastava et al. 2020; Srivastava et al. 2022a, b). These organic molecules can either be synthesised in the cell or can be transported from the extracellular environment. Most of the molecules accumulate in the cell because of their *de novo* synthesis by specific biosynthetic pathways, but the uptake of the osmoprotectants from the external environment is energetically preferred over de novo synthesis (Roberts 2005; Vargas et al. 2008; Srivastava et al. 2019a). *Chromohalobacter salexigens* and many other microorganisms have also been reported to synthesise ectoine and β-hydroxyectoine as the main osmoprotectant (Salvador et al. 2018).

23.5 Rhizospheric Microbiome of Salt Tolerance

PGPR act via different pathways to alleviate the salinity stress in plants. It activates the antioxidant defence mechanism by directly affecting the expression of the genes like superoxide dismutase, peroxidase and catalase, which help the plant scavenging reactive oxygen species (ROS) (Goswami et al. 2014; Ji et al. 2014; Kim et al. 2014; Qin et al. 2016; Etesami and Beattie 2017). Besides this, it also supports plant growth by ensuring nutrient availability through nitrogen fixation, phosphate and potassium solubilisation, siderophore formation and altering the soil pH (Dodd and Pérez-Alfocea 2012). They also have the ability to adjust the ion transporter expression to increase the effectiveness of uptake of selective ions (Etesami and Beattie 2017; Chaurasia et al. 2021). Resistance to salinity in plants depends on the ratio of potassium to sodium, so the inoculation of specific isolates from PGPR is probably done by adjusting the potassium-to-sodium ratio and maintaining the balance of nutrients in plants (Hamdia et al. 2004; Maurya et al. 2021).

Another mechanism involves decreasing plant Na^+ accumulation via the excretion of bacterial extracellular polymeric substances (EPS), which comprises hydroxyl, sulfhydryl, carboxyl and phosphoryl function groups. EPS binds to the cations (particularly Na^+) in roots, thus impeding their transfer to leaves and helping attenuate salt stress in the plants (Ashraf and Harris 2004; Nunkaew et al. 2015). Many EPS-producing PGPR facilitate biofilm formation, which better controls the water and nutrient uptake by improving root-adhered soil in non-legume plants (Alami et al. 2000; Han and Lee 2005; Berge et al. 2009; Bharati et al. 2020a).

Besides the abovementioned mechanism, there are several other mechanisms too. Another important enzyme generated in various stress responses is the ACC deaminase, which converts the plant ethylene precursor to ammonia and α-ketobutyrate. It reduces the ethylene in the plant, which in turn leads to improving plant growth even under abiotic stresses like salinity (Glick 2014; Singh et al. 2015). Simultaneously, it helps in root growth and in lateral root formation, which further leads to enhanced nutrient uptake, modifying root architecture and morphology, affecting root hydraulic conductance and altering the root hormone status as well (Arora et al. 2006, 2012). These PGPR emit stress-related volatile compounds, which have some role in the survival of the plant under salinity. In addition, the water homeostasis is maintained by osmolyte accumulation and several other bimolecules like glycine, betaines and other derivatives of amino acids. Sugars like trehalose and sucrose also have a role in osmolarity regulation. In general, researchers have observed an increase in these solutes in response to salinity (Creus et al. 2004; del Amor and

Cuadra-Crespo 2012). Because of these properties, the salt-tolerant PGPR isolated from the halophytes are the better choice to alleviate salt stress to the plant.

23.5.1 Modification in Phytohormonal Content

There is a significant impact of the environment on the production of hormones. There are endogenously produced molecules involved in plant development and growth. They are the key molecules controlling the interaction between the plant and the environment (Raghavan et al. 2006; Chaihar and Lumyong 2011; Ryu and Cho 2015). Because of these abiotic stresses, the plants are either not able to synthesise the phytohormones or there is alteration in their production, which leads to severe changes in the physiology, leading to either disintegration or death of the plant, which further leads to an impact on the yield of the plant (Chaihar and Lumyong 2011). PGPR can alter the levels of plant hormones by producing or preventing the production of these hormones.

23.5.2 Interplay of Ethylene, IAA and ACC Deaminase in Salinity Stress

Ethylene is one of the phytohormones synthesised endogenously by almost every plant. This is gaseous in nature and modulates the salt-stress response by regulating downstream gene expression (Ryu and Cho 2015). Under environmental stress like drought and salinity, the production of ethylene is enhanced, which is called the “stress ethylene”. Ado-L-met (S-Adenosyl Methionine) is the precursor and is converted into 1-amino-cyclopropane-1-carboxylic acid (ACC), which is further converted into ethylene ACC oxidase (Glick et al. 2007a; Glick 2014). The model describing the ethylene stress states the two peaks of ethylene under the stress condition. The first peak is advantageous as it has a role in the transcription of defensive proteins encoding genes. But the second peak has a negative impact on root growth (Glick et al. 2007b; Tao et al. 2015; Glick 2014). On the other hand, the ACC deaminase, as discussed earlier, metabolises the ACC and regulates the production of ethylene. So the PGPR maintain homeostasis by affecting the expression of these genes like ACC deaminase, ACC-synthase and ACC-oxidase (Etesami and Beattie 2017; Tsukanova et al. 2017). The IAA also has a role in the regulation of the ACC synthesis. The IAA stimulates cell proliferation and elongation, and also induces the expression of ACC synthesis. In this case, the IAA stimulates ethylene production. But this is not the true case as whenever ethylene production increases, it acts as a feedback mechanism, which, in turn, lowers the IAA production locally and limits the ACC synthesis. So, basically the ACC deaminase and the IAA have pivotal roles in ethylene production and stress response (Xu et al. 2014; Etesami et al. 2015).

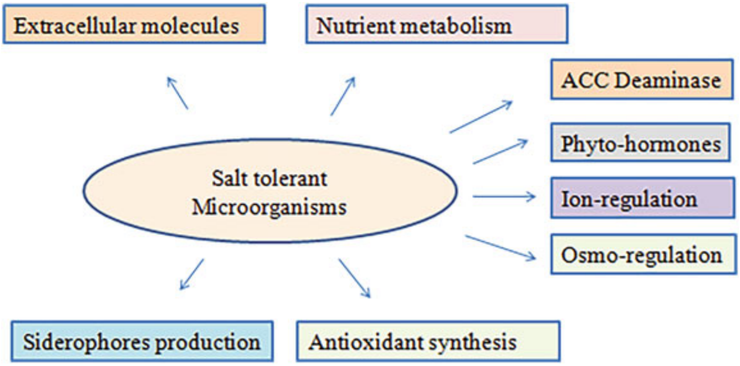

Fig 23.2 Salt-tolerant mechanism under plant–microbe interaction

23.6 Mechanisms of Salt-Tolerant Microbiome

Ecofriendly microbes induce tolerance to many abiotic stresses without adverse effects on plant growth and overall development. This feature of various microorganisms can be explored further for recognising a system to diminish the biotic and abiotic factors of stress to the crop plants. In general, salt tolerance PGPRs are much better than that of any other microbes because they possesses many traits of plant growth promotion along with the traits responsible for amelioration of salt stress. There are distinct molecular mechanisms followed by rhizosphere microorganisms to develop tolerance to salt stress in plants (Fig. 23.2). Some of these mechanisms are listed below:

1. Ion regulation
2. ACC deaminase activity
3. Antioxidant enzymes
4. Osmo-protectants
5. Plant growth regulators
6. Extracellular molecules
7. Siderophore
8. Nutrient Metabolism

The ground-level mechanism followed by microbes is to avoid the higher concentration of salt inside the cytoplasm. The salt-tolerant halophilic microbes maintain the physiological, biochemical and molecular mechanisms such as protein structures and enzyme activity inside the plant system to produce sustainable yield production under salt stress (Ruppel et al. 2013). This plant–microbe interaction plays a key role in sustainable agriculture in the form of microbe-mediated rhizospheric engineering for abiotic stress. This can replace the conventional agricultural practices and promote organic farming. The whole process is environment ecofriendly because it may further reduce the use of chemical fertilizers, which is one of the factors of soil disintegration which further leads to salinity.

The plant–microbe interaction has the ability to mitigate the salinity stress via nutrient acquisition, ACC deaminase, biosynthesis of phytohormone, extracellular biomolecules, and increased osmolytes and antioxidant enzymes (Bhise and Dandge 2019). Acosta-Motos et al. (2015) observed a reduction in leaf surface area to mitigate the water loss in response to salt stress tolerance. But there are studies which are contrasting to the previous reports where greater leaf area was an adaptive strategy to mitigate salinity stress after inoculation of microbes in cucumber and maize (Kang et al. 2014; Naveed et al. 2014). Khalid et al. (2018) observed increased antioxidative enzymes, phytohormones and plant biomass after the application of endosymbiont *Piriformospora indica* into the Pakchoi (*Brassica campestris* ssp. *chinensis*) responsible for tolerance to salinity stress. Jogawat et al. (2013) and Sharma et al. (2017) reported salt tolerance in many plant species by the application of endophytic fungi *Piriformospora indica*. Lubna Asaf et al. (2018) observed increased phytohormone content and antioxidative enzymes such as polyphenol-oxidase, catalase, superoxide dismutase and peroxidase, and downregulation of ABA and jasmonic acid (JA) after treatment with an endophytic fungus, *Aspergillus flavus* CHS1 on host plants. There are previous studies on the usefulness of root inhabitant microbes for the promotion of plant growth under salinity in mung bean, wheat and maize (Ahmad et al. 2012; Kudoyarova et al. 2014, and Piromyou et al. 2011). Mahmood et al. (2016) observed a significant increase in plant growth and seed yield after inoculation of *Bacillus drentensis* in mung bean under salt stress. Evelin et al. (2019) reviewed the importance of microbial fungi to cope up salinity stress in the plant system. Li et al. (2020) observed improvement in the growth of plants under salinity by modulating phytohormone contents, nutrient acquisition, antioxidant levels and so on following the inoculation of *Kocuria rhizophila* Y1 strain of PGPR. Kaushal and Wani (2016) reviewed plant–microbe interactions and suggested the important role of plant growth-promoting bacteria in enhancing tolerance of plants to saline conditions.

Ion regulation: The accumulation of toxic nutrients like Na^+ and Cl^- causes the nutrient imbalance which disrupts the enzyme structure of plants and inhabitant microbial activity under salt stress. This results in alteration in important biological phenomena of plants, such as photosynthesis, respiration and cellular organelles synthesis (Munns and Tester 2008; Ruiz-Lozano et al. 2012). The rhizospheric microbes mitigate the negative effect of salt stress via nutrient acquisition through the root architectural system inside the plants. The Na^+: K^+ ratio gets imbalanced as plants tend to uptake more Na^+ than K^+, which inhibits several physiological processes like stomatal movement, plant metabolism and protein synthesis. Microbes like bacteria and arbuscular mycorrhizal fungi enhance the uptake of K^+ colonisation under salt stress and inhibit the translocation of Na^+ through xylem and phloem during salt stress (Zuccarini and Okurowska 2008; Chen et al. 2017). This results in a higher ratio of K^+: Na^+ inside the plants, which inhibits the metabolic process by efflux of Na^+ or Na^+/H^+ pump transport system from the cytoplasm

(Halfter et al. 2000; Colla et al. 2008). Niu et al. (2016) observed less Na^+ accumulation when *Puccinellia tenuiflora* inoculated with *B. subtilis* GB03. Wheat seed inoculation with *Bacillus aquimaris* strains showed a reduction in the Na^+ level in leaves (Upadhyay and Singh 2015). The microbes reduce the uptake of chloride ions through the root system via xylem loading and compartmentalise inside the vacuole, thereby restricting the ions from interfering with metabolic processes specifically in chloroplast and mitochondria (Gilliham and Tester 2005). The hyperosmotic tension is closely linked to the calcium (Ca^{2+}) channels and interacting proteins such as calmodulin. A rapid rise in cytosolic Ca^{2+} levels occurs within seconds of exposure to salt stress. Increased concentration of Ca^{2+} is the most important messenger during salinity tolerance (Cramer 1992). The Ca^{2+}-mediated signalling regulate the K^+ and Na^+ ions homeostasis leading to salt tolerance in plants. Again, one of the studies suggests that uptake of Mg^{2+} is increased by microorganisms under salinity, which results in more chlorophyll synthesis and ultimately higher photosynthesis production in *Sesbania* sp. (Giri and Mukerji 2004). Zerrouk et al. (2016) observed higher root growth in maize under salinity stress due to the *Pseudomonas fluorescence* strain isolated from date palm. Nautiyal et al. (2013) inoculated rice plants with *Bacillus amyloliquefaciens* SN13 in hydroponic and soil conditions, which showed increased salt tolerance. Navarro et al. (2014) have observed the effect of microbial fungi in the mitigation of salt stress by reducing the uptake of Na^+ and Cl^- in citrus plants.

ACC deaminase activity: Many microbes activate ACC deaminase activity to mitigate salt stress. Salinity upregulates ethylene production, which imparts a negative effect on the growth and development of the plant. The root inhabitant microbes participate in the biosynthesis of 1-amino cyclopropane 1 carboxylate deaminase (ACC deaminase), which convert the plant ethylene precursor into ammonia and α-ketobutyrate by enzymatic hydrolysis (Barnawal et al. 2017). This inhibits the production of ethylene and thereby induces plant growth under salt stress (Glick et al. 2007a). Nadeem et al. (2009) observed significantly higher maize production by the application of the *Pseudomonas fluorescence* and *Enterobacter* spp. Strains, which secrete ACC deaminase. Glick (2012) revealed that plant–microbe interactions result in the secretion of ACC deaminase, which reduces the ethylene level, along with IAA synthesis, which ultimately promotes cell proliferation. Chickpea native bacteria *Pantoea dispers*a PSB3 produces IAA and ACC deaminase, which result in sustainable yield under salt stress (Panwar et al. 2016). Other than this, the plant–microbe interaction has also resulted in an increase in the K^+:Na^+ ratio, nutrient acquisition and relative leaf water content, and decreased electrolyte leakage from the cells. Sapre et al. (2018) and Suarez et al. (2015) showed that the inoculation of the *Klebsiella* sp. strain IG3 and *Hartmannibacter diazotrophicus* E19T to oat (*Avena sativa*) and barley seedlings increased the expression of ACC deaminase activity and IAA production under salt stress, which improved shoot/root length, shoot/root biomass and relative water content. Win et al. (2018) observed that the inoculation of tomato seedlings with an endophytic bacteria, *Pseudomonas* sp., expressed enhanced activity of ACC deaminase, which imparts tolerance to plants and improves plant growth, the photosynthetic rate and ionic balance under salt stress.

Antioxidant enzymes: Salt stress causes oxidative stress by means of accumulation of reactive oxygen species (ROS), which impacts protein degradation, lipid peroxidation, H_2O_2 accumulation, cellular membrane and nucleic acid mutation (Islam et al. 2015; Ahmad et al. 2011). The microorganism upregulates the antioxidative system to control ROS production and the oxidative stress faced by plants during salt stress. This mechanism results in salt tolerance in plants conferred to it by endophytic microbes. There are a number of enzymes like peroxidase, catalase, superoxide dismutase (SOD), glutathione reductase, dehydro-ascorbate reductase and monodehydroascorbate reductases which participate in mitigating reactive oxygen species. In addition to this the non-enzymatic substances accompanied tocopherols, phenols, ascorbic acid, thiols and proline comprised antioxidative properties (Hashem et al. 2016; Cervilla et al. 2012; Miller et al. 2008) in response to mitigate salinity stress and develop salt-tolerant genotypes. Khan et al. (2016) observed that the inoculation of rice plants with *Bacillus pumilus* increased antioxidant activity of peroxidase, SOD and catalase in leaves. Lubna Asaf et al. (2018) inoculated rice and soybean plants with an endosymbiont fungi, *Aspergillus flavus* CHS1. This resulted in increased antioxidative enzymes such as polyphenoloxidase, catalase, superoxide dismutase and peroxidase under salt tolerance. Islam et al. (2015) have also seen enhanced antioxidative contents and nutrient acquisition, and reduced sodium accumulation in mung bean (*Vigna radiata*) after the application of plant growth-promoting bacteria *Bacillus cereus* Pb25, which resulted in an improvement in overall plant growth under salinity stress.

Osmoprotectants: Cellular dehydration followed by lower turgor pressure is physiological osmotic stress observed in the plant system under salt stress. Small biomolecules called osmolytes/osmoprotectants help in maintaining the cell's turgidity and lower water potential to mitigate osmotic stress to promote plant growth. At the time of stress, rhizosphere microbes help in the production and accumulation of these biomolecules at a higher concentration inside the plant cells via direct acquisition from the rhizosphere or *de-novo* biosynthesis without affecting the molecular and metabolic processes and adjust the osmotic difference between the cell's surrounding and the cytoplasm. There are a number of osmolytes such as sugars, alcohols, amino acids, ammonium compounds, glycine, betaine, proline, and glutamate and sulphur compounds the activities of which are increased with the help of microorganisms (Chen and Jiang 2010; Suprasanna et al. 2016). Yuanyuan et al. (2009), Lunn et al. (2014) and Fernandez et al. (2010) reported the role of microorganisms in the synthesis and accumulation of osmoprotectants like sugars such as sucrose, fructose, trehalose, raffinose and fructans to balance the osmotic stress and membrane integrity of plants in response to abiotic stress. Liu et al. (2016) and Zhu et al. (2018) observed higher accumulation of sugar osmolytes after the inoculation of endosymbiont mycorrhizal fungi to the host plants under salinity stress. Akram et al. (2016) reported higher content of proline after the inoculation of plants with rhizobacter. But there are reports on decreased content of proline after microbial treatment of the plants (Rojas-Tapias et al. 2012; Hamdia et al. 2004).

Garg and Bharti (2018) reported higher accumulation of sugar in *Cicer arietinum* cultivar (PBG 5) in an ecofriendly environment of arbuscular mycorrhiza fungi under salt stress. Slama et al. (2015) and Suprasanna et al. (2016) observed a significant role of microbes in inducing osmolytes like pinitol, mannitol, myo-inositol and sorbitol, and sulphur compounds such as dimethyl sulphoniopropionate in plants under salt stress. Garg and Pandey (2016) observed significantly increased accumulation at higher concentration of trehalose in host plants after arbuscular mycorrhizae treatment as compared to the control plants. Redillas et al. (2011) and Chang et al. (2014) reported that trehalose helps maintain a higher K^+: Na^+ ratio, ROS scavenging and increasing soluble sugar concentration, thereby improving the photosynthetic rate and balancing water potential in plants. The accumulation of organic acids such as acetic, citric, fumaric, malic and oxalic acid, and their concentrations get upregulated while organic acids such as formic acid and succinic acid get downregulated in maize plants after fungus inoculation under salt stress (Sheng et al. 2010). Talaat and Shawky (2011) observed increased accumulation of ammonium compounds in mycorrhiza-treated host plants. Etesami et al. (2014) observed a higher seed germination rate after the application of osmotolerant bacteria to rice plants.

Plant growth regulators: Plant–microorganism interaction modulates the plant growth regulators, which may contribute to salinity tolerance in plants. The tryptophan present in root exudates gets converted into indole indole-3-Indole Acetic Acid (IAA) by root bacteria, which is absorbed by the root system and used by plants for cell growth and stimulation during stress (Ilangumaran and Smith 2017). The inoculation of soybean plant with microorganisms enhances IAA production under salt stress (Kumari et al. 2015). Bhattacharyya and Jha (2012) observed the application of plant growth-promoting rhizobacteria significantly upregulates the phytohormones production thereby promoting growth of host plant and alleviating salt stress in an ecofriendly manner. The increment of the IAA has been reported in wheat leaves after the application of *B. cereus* and *Pseudomonas* sp. (Ul-Hassan and Bano 2015). This increased IAA content helps cell growth, cell elongation, seed germination, and others physiological phenomenon associated with the growth and production.

There are very few studies on the role of plant–microbe interaction in terms of ABA content under the salt stress faced by plants. Bharti et al. (2016) observed salinity tolerance in wheat plants by the application of *Dietzia natronolimneae* STR1. Kang et al. (2014) and Yao et al. (2010) showed the downregulation of ABA in cucumber and cotton after inoculation with *Burkholderia cepacia* SE4, *Promicromonospora sp*. SE188, *Acinetobacter calcoceticus* SE370 and *Pseudomonas putida* Rs-198. There are controversies about ABA content due to plant–microbe interaction. Shahzad et al. (2016) and Peskan-Berghöfer et al. (2015) observed increased accumulation of ABA content after the application of *Bacillus amyloliquefaciens* RWL-1 and *Pseudomonas indica* in rice and Arabidopsis. Barnawal et al. (2017) also observed increased ABA and ACC content under salt stress in wheat plants due to the application of *Arthrobacter protophormiae* SA3 and *B. subtilis* LDR2 strains. Khan et al. (2017) identified that other than endogenous phytohormone, rhizospheric microbes also interact with jasmonic acid to overcome salinity stress.

Extracellular molecules: Environmentally free microbes produce a number of extracellular molecules like polyamides, polyesters and polysaccharides, which help plants to overcome the different biotic and abiotic stresses. The extra-polysaccharide composition varies among microbe species but the common ones are glucose, galactose, mannose, rhamnose, fucose, glucuronic and galacturonic acids. Naseem and Bano (2014), Ansari et al. (2019) identified the molecular mechanism of microbes under stress condition for the production of extracellular biomolecules. Microbes secrete extra-polysaccharides in the form of slime material, which gets adhered to the soil via chemical forces such as Van der Waals, hydrogen bonding, and absorption of cations and anions. This slime material, along with soil aggregates, forms a capsule-like structure which gives tolerance to plants under stress conditions. These extracellular biomolecules bind to the cations like Na^+, thereby reducing its acquisition from the salt-affected areas by plants and develop tolerance under salt stress conditions. These biomolecules indirectly help the plant system by increasing the surviving ability of microbes by balancing and regulating water retention capacity along with the movement of organic carbon sources under salt stress. Vurukonda et al. (2016) have identified the role of extra-polysaccharides for the symbiotic plant–microbe relationships under stress condition. Upadhyay et al. (2011) observed improved plant nutrition following the inoculation of sunflower plants with *P. aeruginosa* PF23 due to exopolysaccharide secretion in higher quantity under salt stress.

Siderophore production: The bioavailability of Fe gets downregulated due to the proton pump inhibition under salt stress (Wallender and Tanji 2011). This reduced quantity of Fe causes chlorosis, which is the major issue with saline soils. Siderophore production is one of the main features of microbes. This biomolecule does not help in the improvement of plant growth directly; rather it acts as a chelating agent of Fe. Fe is an essential nutrient of cellular proteins such as dioxygenases, carboxylases, hydroxylases, activator of light reaction (cytochrome P450, cytochrome oxidases), part of electron transporters, storage proteins, receptors like transferrin and transferrin receptor ferritins, sulphur complex enzymes and acts as a catalyst in chlorophyll synthesis (Cassat and Skaar 2013; Cohen 2014; Bogdan et al. 2016; Hu et al. 2017) thereby plant growth in terms of higher rate of photosynthesis. Phyto-siderophores can be used by the plant system to cope up with Fe deficiency. Bacterial strains also produce siderophores which make complexes with free Fe and accumulate in the plant cells, which is used for biosynthesis of chlorophyll (Kumar et al. 2020). Bhise et al. (2017) reported that the inoculation of *Triticum aestivum* with *Chryseobacterium gleum* sp. supports the siderophore formation and the presence of siderophores indirectly helps in plant growth under salt stress. Ferreira et al. (2019) reviewed the role of siderophore-producing rhizobacteria in the improvement of plant growth during salinity stress. Zhou et al. (2017) observed the growth of *Beta vulgaris* L. after inoculation with halotolerant rhizobacter due to ACC deaminase activity and also observed siderophore production which resulted in tolerance to salt stress. Rungin et al.

(2012) showed enhancement in plant growth and root/shoot biomass due to siderophore production after the application of an endophytic *Streptomyces* sp. to the Thai jasmine rice (*Oryza sativa* L. cv. KDML105).

Nutrient metabolism: In general, rhizobacteria play an important role in nitrogen fixation and phosphorus solubilisation. Under salt stress, the availability of these nutrients gets limited to the plant system specially P due to its precipitation with other metal ions such as Ca^{+2}, Mg^{+2}, and Zn^{+2}. The precipitation of the P depends on the pH of the soil. Phosphorus solubilising bacteria (PSB) help in the fixation of soil P and mobilisation of applied P, thereby enhancing crop yield and production. While rhizobacter such as *Nitrosomonas*sp. and *Nitrobacter* sp. participate in mineralisation, ammonification and nitrification of ammonia to the available form of nitrogen to the plant system specially in legumes. The *Acinetobacter*, *Pseudomonas* and *Bacillus* sp. are known as PSB (Phosphorous Solubilizing Bacteria) which help in solubilising phosphorus and make it available to the plants to boost agricultural production in a sustainable manner (Zaidi et al. 2009). Kang et al. (2014) observed increased P concentration in maize after treatment with *Acinetobacter calcoaceticus*. In one of the studies conducted by Dawwam et al. (2013) in potato plants, they observed increased N and P acquisition after inoculation with plant growth-promoting bacteria. Abdel-Fattah (2012) reported the improved availability of P in the wheat plants after inoculation with arbuscular mycorrhizal fungi even under salt stress. Evelin et al. (2019) reviewed plant–microbe interaction under salinity stress and concluded that P acquisition is one of the phenomena followed by mycorrhizal fungi to sustain plant growth. Kadmiri et al. (2018) observed the important role of the solubilising rhizobacterial strain of *Pseudomonas fluorescens* Ms-01 and *Azosprillum brasilense* DSM1690 for plant growth improvement in saline conditions.

23.7 Prospects and Conclusion

Significant progress has been made on the use of PGPR to alleviate the effect of salinity on plants. The ability of plant growth-promoting rhizobacteria to develop salt tolerance in plants can be used for improving crop yield in saline soils. Intellectual capacity of salt-tolerance mechanisms in salt-tolerant plant growth-promoting rhizobacteria is still not deciphered up to a benchmarked, specifically under saline conditions, bacterial genes linked in osmotic regulation and plant-microbe interactions. Pan et al. (2019) reported physiological roles carried out by salt-tolerant plant growth-promoting rhizobacteria which could enhance plant performance under saline conditions and further deep investigation of mechanisms of osmo-adaptation, osmolytes, biosurfactants, precursor of phytohormones and stress enzymes secretion bysalt-tolerant plant growth-promoting microorganims may add durable improvement in agricultural productions under salt-affected ecosystems (Paul 2013). However, deeper elucidation of microbial adaptation and responses to soil salinity are requisite for their better utilisation in the mitigation of saline soils. Kim et al. (2019) showed that biodiversity along with identification and characterisation of the dominant indigenous microbiota of more saline soil and elucidation of their tentative adaptation mechanisms may present a better comprehension for deciphering ecological and evolutionary adaptations in ecosystems. In this regard, metagenomic and metabolomic approaches become very crucial and pertinent for the isolation and identification of novel salt-tolerant plant growth-promoting genes and metabolites engaged in salt tolerance.

Agronomical trials of several crops reported the potential of salt-tolerant plant growth-promoting microorganisms in mitigating salinity stress (Arora and Mishra 2016; Chanratana et al. 2019; Li et al. 2019). Still, repetition of successful results with the same intensity in different agro-climatic regions and for different crops has remained a challenge (Souza et al. 2015; Ambrosini et al. 2016). Kumar et al. (2019b) recommended certain protocols and screening techniques of salt-tolerant PGPRs under salinity stress and suggested the application of indigenous salt-tolerant PGPR strains in bioinoculant construction as indigenous salt-tolerant PGPRs can be adapted in the local field conditions without any difficulty.

Recently, consortia-based bioinoculants have gained popularity as involving diverse salt-tolerant plant growth-promoting rhizobacteria strains not only mitigate salinity stress but also manage phytopathogens and provide nutrients assimilation (Woo and Pepe 2018). Ilangumaran and Smith (2017) have suggested that application of the diverse microbes in consortial formulations holds promising strategy for mitigating stress in plants. In the current scenario, there is insufficient products of salt-tolerant plant growth-promoting rhizobacteria for specific uses in salt-affected agricultural ecosystems. The available bioinoculants are also limited in function under saline conditions. This limitation of microbes in saline conditions prompts us to look for biological alternatives in saline conditions. For instance, successful application of metabolites such as exopolysaccharides (EPS) in saline conditions has been reported in recent years (Tewari and Arora 2014; Arora and Mishra 2016). In recent years, researchers have been trying to develop methods which enhance crop production through the application of biopolymers coupled with microbes (salt-tolerant) in saline conditions (Raj et al. 2011; Sharif et al. 2018). For instance, Chanratana et al. (2019) reported uses of chitosan-immobilised aggregated *Methylobacterium oryzae* CBMB20 as a microbial inoculant for growth enhancement in tomato under salt-stress conditions. Li et al. (2019) also reported synergistic application of a Super Absorbent Polymer (SAP) coupled with *Paenibacillus beijingensis* BJ-18 and *Bacillus* sp. L-56 to promote growth of wheat and cucumber in salinity-affected soil. Improving the productivity of saline soils will prompt in crop production as well as reducing climate change effects (Arora 2019; SDG 2019). Besides the above mentioned mitigation strategies, a major part of research has to be dedicated to the challenges associated with the development of microbial based bio-formulations (Mishra et al. 2018).

The potential of salt tolerance of plant growth-promoting microorganims is because of the presence of different mechanisms to mitigate salt stress such as efflux systems, releasing and deposition of remedial solutes for manipulation of external pressure generated through osmolytes, production of reactive oxygen species, secondary metabolites, salt-related specific gene expression and enzyme secretions to adjust with salinity stress. However, the role of microbes at the molecular level, characterisation of biochemical pathways in regard to salinity and the role of metabolites in salt-stress tolerance are yet to be explored. In-depth studies of the role of microbiomes in salinity mitigation will lead to the preparation of formulations of bioinoculants which could enhance the production of different crops under salinity. Research activities related to salt-tolerant plant growth-promoting rhizobacteria substantiate their widespread ability in the mitigation salt stress and enhancement of crop production under different agro-ecosystems affected by salinity.

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Metatranscriptomics of Plant Rhizosphere: A Promising Tool to Decipher the Role of Microorganisms in Plant Growth and Development

24

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Abstract

The plant rhizosphere is the most living and metabolically active region among all soil habitats. This is mostly because of the multifaceted microbial communities and the metabolic activities they carry on. The advent of metagenomics has revealed the existence and association of microorganisms in the plant rhizosphere. But the dynamic functions carried out by microbes in the rhizosphere under fluctuating environmental conditions have not been explored much. Metatranscriptomics would help unravel the mechanisms and genes expressed by the microbes to combat adverse conditions. In addition, they induce a plant's tolerance to overcome adverse environmental conditions including biotic and abiotic stresses. This chapter deals with the role of metatranscriptomics in understanding the mechanisms involved in biotic and abiotic stress tolerance and plant growth promotion, besides in the identification of novel genes and functions in the rhizosphere.

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24.1 Metatranscriptomics

A complex ecosystem such as soil holds a multitude of significant interactions among the microbial community and hence there is very less or no possibility for a microbe to lead a desolate life in such a habitat (Pace 1997). The plant rhizosphere is the most alive and vibrant component of agricultural soil owing to its multifaceted microbial inhabitants and their functional diversity (Singh et al. 2018). The primary role of the resident microbial population is maintenance and enrichment of soil properties through interactions with other members of the ecosystem (Lavelle et al. 2006). But their unstable nature, diversity and multifaceted functions are yet to be studied using comprehensive approaches despite the fact that physical properties of soil in an ecosystem have been studied extensively. Among the microbial communities, bacterial species have been studied comprehensively but an enormous variety of eukaryotes in soil and the indispensable role played by them are not thoroughly recognised. The cultivation-based method has failed to give reliable outcomes due to the high growth rate of bacteria (Bending et al. 2007). To understand the diversity of both prokaryotes and eukaryotes in a holistic way, a newer approach was required to replace the culture-based method. One such approach is metagenomics, in which nucleic acids are isolated directly from environmental samples, which has been recognised as an effective device for exploring and comparing different soil ecosystems (Biddle et al. 2008). Although metagenomics provides valuable information pertinent to microbial community diversity of the ecosystem, it does not give a picture of the actual activities happening in specific niche or dynamic environmental conditions. To answer this, the metatranscriptomic method links community-based assemblies and their functions in a lone investigation (Bailly et al. 2007). Metatranscriptomics, a subset of metagenomics, provides insights into the complete expression of genes in the complex microbial community of an ecosystem. This is accomplished by the identification of mRNA obtained from the samples of a particular environment. Metatranscriptomics is also known as environmental transcriptomics, whole community transcripts and microbial community RNAs (Morris and Nunn 2013). Metagenomics generally deals with the documentation of microbes existing in the population, whereas metatranscriptomics focuses on the multiplicity of functional genes, their expression patterns in such a population and changes in the level of expression due to fluctuations in environmental conditions (Mukherjee and Reddy 2020). One advantage of using metatranscriptomics is that there is no requirement of either probes or primers, hence the microbial transcripts are sequenced with minimum bias (Moran et al. 2013). Another advantage is that the information about non-coding genes and small RNA can be obtained on the basis of their expression. Also, it helps in the profiling of bacterial endophytes that will endure to augment additional information to the mounting microbial community databases (Wilmes and Bond 2005).

Metatranscriptomics facilitates insights into the active expression of genes in a particular niche at the time of sampling and helps answer questions about the roles and functions of microbial communities in the region adjacent to plant roots, in addition to their interactions with the plant (Moran 2009; Maela and Serepa-Dlamini 2020). The regulation of gene expression at the transcriptional level mediated by post-transcriptional and post-translational gene expression enables organisms to swiftly acclimatise to adverse climatic conditions. This indicates that the direct governing response to the environment is assumed to be well-imitated in the metatranscriptome than in the metaproteome (Moran 2009).

24.2 Methods to Study Metatranscriptomics

In metatranscriptomics, a set of genes that actively get transcribed and show activity in response to the surrounding environmental factors are studied (Moran 2009; Chao-Rong and Zhang 2011). This makes it necessary to study functional metatranscriptomics, which is considered a formidable tool that allows for the description of genes expressed by diverse microorganisms in the habitat. Use of this technique in the soil habitat involves extraction and analysis of mRNA, not DNA, which gives information about the regulation and expression profiles of diverse communities. They are polyadenylated mRNA and are converted into cDNA, which can be cloned into a suitable expression vector such as plasmid for the expression of cloned genes (Yadav et al. 2014). Various methods involved in characterising the functional diversity of microorganisms in a particular habitat have been described in Table 24.1.

Metatranscriptomics sheds light on the profiles of functional genes which are actively expressed by the microbial community and the metatranscriptome affords a picture of expressed genes in a specified sample at a specified point in time under particular conditions (Moran 2009; Poretsky et al. 2005). In metatranscriptomics, the analysis of reads involves two methods: either by mapping the reads to a reference genome or by accomplishing de novo assemblage of reads into contigs followed by super contigs. In the first method, the reads are mapped to databases comprising reference genomes by using various alignment tools such as Bowtie2, BLAST and BWA. The results obtained from alignment are annotated using various means such as KEGG, GO, Swiss-Prot and COG, thus assembling the details to deduce the relative expression of individual genes. The second method also works on similar principle but uses assembled sequences for analysis. Numerous software packages are available for assembling reads into contigs. Tools such as Trinity, MetaVelvet, Oases, AbySS, Trans-Abyss, SOAP de novo, Cufflinks and Scripture were compared and it was found that Trinity has outperformed the other tools and also has sensitivity to expression levels across the range (Celaj et al. 2014; Aguiar-Pulido et al. 2016). A tool named RNA-Seq Expectation Maximisation (RSEM) is a quantitative pipeline for the analysis of transcriptome (Li and Dewey 2011). It takes a reference transcriptome as input accompanied by RNA reads and computes normalised transcript abundance (i.e. no. of RNA-seq reads matching with respective reference transcriptome) (De Bona et al. 2008).

High-throughput sequencing facilities generate huge volumes of data that helps in the characterisation of transcripts that did not have any a priori information. Various tools deployed in analysis of metagenomic and metatranscriptomic data have been described in Table 24.2. A recent technique, stable isotope probing (SIP), has been used in lake sediment to recover precise targeted transcriptomes of aerobic

Table 24.1 Methods employed in metatranscriptomic study of rhizosphere soils obtained from various ecological niches

Metatranscriptome	RNA extraction methods	cDNA preparations	Significant outcomes	Reference
Agriculture soil and organic soil	RNA isolation kit (Mo Bio labs)	IITM double-strand cDNA synthesis kit	Significant expression of genes responsible for nitrification, stress response, and alternate carbon fixation pathways	Sharma et al. (2019)
Agro forestry contaminated soil	RNA isolation kit (Mo Bio labs)	Selective amplification of eukaryotic mRNA by (SMART cDNA kit)	VWD like protein/multi-metal tolerant	Thakur et al. (2019)
Acid sulphate soils	RNA isolation kit (Qiagen)	Illumina Truseq stranded RNA LT kit	Metabolic process and functions from conflicting microbial communities	Högfors-Rönnholm et al. (2019)
Metal contaminated forest soil	RNA isolation kit (Mo bio labs)	Selective amplification of eukaryotic mRNA by Mint2 cDNA kit	Aldehyde dehydrogenase conferring metal tolerance	Mukherjee et al. (2019a)
Metal contaminated forest soil	RNA isolation kit (Mo bio labs)	Selective amplification of eukaryotic mRNA by Mint2 cDNA kit	Serine protease inhibitor conferring metal tolerance	Mukherjee et al. (2019b)
Wheat rhizosphere soil	RNA isolation kit (Mo Bio labs)	Superscript II reverse transcriptase	More expression of a polyketide cyclase, a terpenoid biosynthesis backbone gene (dxs) and cold shock proteins (csp) in suppressive soils than non- suppressive soils	Hayden et al. (2018)
Agro forestry contaminated soil	RNA isolation kit (Mo Bio labs)	Selective amplification of eukaryotic mRNA by (SMART cDNA kit)	Ubiquitin fusion protein/heavy metal tolerance gene	Thakur et al. (2018)
Arctic peat soil	CTAB extraction buffer, phenol, bead-beating	Superscript II double-stranded cDNA(Invitrogen)	Acetoclastic methanogenesis dominating pathway, followed by methanogenesis from formate	Tveit et al. (2014)
Soil near metal Smelter	Guanidine isothiocyanate, bead-beating	Poly-dT primer (SMART) cDNA kit	Metal tolerant genes	Lehembre et al. (2013)

Beech and spruce Forest	Guanidine isothiocyanate, bead-beating	Poly-dT beads for separating eukaryotic polyadenylated RNA	Eukaryotic diversity, novel enzymes for organic matter degradation	Damon et al. (2012)
Spruce forest	Guanidine isothiocyanate, bead-beating	Poly-dT beads for separating eukaryotic polyadenylated RNA	Novel fungal oligopeptide transporter	Damon et al. (2011)
Sugar maple forest Soil	Bead beating,8-hydroxyquinoline, RNA/DNA Midi kit (Qiagen)	Selective amplification of eukaryotic mRNA by (SMART) cDNA kit	Acid phosphatase, imidazole glycerolphosphate dehydratase	Kellner et al. (2011)

Table 24.2 Various tools used in study of metatranscriptomics

Tool	Web address	Comment	Reference
MetaTrans	http://www.metatrans.org/	RNASeq map read and gene expression analysis	Martinez et al. (2016)
COMAN/comprehensive metatranscriptomics analysis	http://sbb.hku.hk/COMAN/	Comprehensive functional analysis of metatranscriptomic data	Ni et al. (2016)
HUMAnN2	http://huttenhower.sph.harvard.edu/humann2	Pathway study from metagenomic and metatranscriptomics data	Franzosa et al. (2018)
SqueezeMeta	https://github.com/jtamames/SqueezeMeta	Highly portable, fully automatic metagenomic analysis pipeline	Tamames and Puente-Sanchez (2019)
FunctionAnnotator	http://fa.cgu.edu.tw/	Determines the structural arrangement of microbial community in environmental samples and support to identify unique proteins by relating RNA-Seq data with proteomics data	Chen et al. (2017)
Leimena-2013		Comparative metatranscriptomic data analysis using RNA-Seq	Leimena et al. (2013)
MetaCLADE	http://www.lcqb.upmc.fr/metaclade	A unique profile-based domain annotation pipeline on the basis of multi-source domain annotation strategy	Ugarte et al. (2018)
SAMSA	https://github.com/transcript/SAMSA	Comprehensive analysis pipeline for metatranscriptomic data and deliver aligned sequences file as input file for MG-RAST	Westreich et al. (2016)
SAMSA2	https://github.com/transcript/samsa2	A quick and effective metatranscriptome pipeline for analysing large RNA-seq datasets in a supercomputing cluster environment	Westreich et al. (2018)
TaxMapper	https://bitbucket.org/dbeisser/taxmapper	Tool for mapping read and provide microeukaryotic database, and also a part of transcriptomics data analysis	Beisser et al. (2017)

microorganisms (Dumont et al. 2013). A key consideration prior to the implementation of the metatranscriptomics approach to study the soil-linked microbial communities is the coverage depth, which is essential to addressing the objective of the study (Carvalhais et al. 2012).

A key limitation in metatranscriptomics study is the shortest average half-life of mRNA, which ranges from a few seconds to a few minutes (Deutscher 2006). Also, the stability of mRNA varies among the species (Bernstein et al. 2002), whereas cells within the species may be determined by the availability of key nutrients (Redon et al. 2005). Hence, it is necessary to freeze the samples into liquid nitrogen or to

RNA preservation solution immediately after sampling in order to minimise changes in transcripts profiling due to loss of RNA. Ideally, the gap between sampling and preservation must be in seconds instead of minutes (Carvalhais et al. 2012). Isolation of RNA from soil samples is really a challenging task due to ineffective cell lysis, presence of RNase and adsorption of RNA to soil particles. The conditions (high salt and low pH) under which RNA is extracted from soil increase the adsorption of RNA to soil. Under high salt conditions, RNase gets deactivated while at low pH, RNA gets separated from DNA (Chomczynski and Sacchi 1987; Carvalhais et al. 2012).

Humic and fulvic acids, complex organic molecules, are co-precipitated with RNA during the extraction of nucleic acid from soil. They limit the availability of templates for oligos binding and thereby impede PCR amplification. To get rid of these acids, the following methods were developed: (a) Activated charcoal-mediated adsorption (Desai and Madamwar 2007); (b) Aluminium sulphate precipitation before cell lysis (Dong et al. 2006); (c) Pretreatment with calcium carbonate (Sagova-Mareckova et al. 2008); (d) Polyvinyl polypyrrolidone (PVPP) (Rajendhran and Gunasekaran 2008); and (e) Extraction and purification with Q-Sepharose column accompanied with cetyl trimethylammonium bromide (CTAB) (Mettel et al. 2010).

The accessibility of a huge count of yeast strains with desired mutations for various enzymatic pathways provides a way to carry out complementation by genes to monitor and discover the gene of concern (Scherens and Goffeau 2004). Additionally, the plasmids have undergone changes in such a way that they can clone environmental cDNA and efficiently express the gene in the host. The transcription of alien genes depends on how well the compatible promoter is placed upstream of the cloning site (Mukherjee and Reddy 2020).

Sequence capture by hybridisation (SCH) can be an effective method for screening of cDNA obtained from mRNA (Bragalini et al. 2014). In this process, pre-determination of the genes to be targeted prior to screening enhances the possibility of discovering new genes by dropping the amount of haphazard cDNA. In the metatranscriptomic library, the sequences from the identified and unidentified members of the target gene family are hybridised and biotinylated with RNA probes (degenerated). Furthermore, they are segregated via paramagnetic beads coated with streptavidin. For example, endo-xylanase (glycoside hydrolase 11) was captured and the probes were able to relate more than 90% of the cDNA to this gene family. Furthermore, the sequence analysis of cDNA revealed that many species are phylogenetically diverse and were not included in public databases (Mukherjee and Reddy 2020).

24.3 Identification of Novel Genes and/or Function in Rhizosphere

Enzymes from fungi have been extensively used as biocatalysts in food industries, pharmaceutical, textile etc. (Guerriero et al. 2016). They help improve soil fertility through litter degradation by involving various enzymatic processes to break down the lignin, cellulose, hemicellulose, polyphenols etc. (Štursová et al. 2012). The forest soil acts as a reservoir of important and novel enzymes that can be tapped extensively by creating a metatranscriptomic library followed by the screening of clones. The collection of industrially important genes from an environmental metagenomic library and screening them through sequence- and function-based approaches to accomplish a constant, commercial-scale enzyme is a lengthy process. Also, it necessitates effective screening and expression systems. Nevertheless, the metatranscriptomics method has shown to be a promising and useful technique for reaping and screening gene-encrypting proteins from a library (Ferrer et al. 2016; Mukherjee and Reddy 2020). Enzymes of industrial importance such as laccase and acid phosphatase have been identified using semi-quantitative PCR and functional screening, respectively (Luis et al. 2005; Kellner et al. 2011). The need to screen a huge number of transformed clones is the only limitation of this process (Mukherjee and Reddy 2020). About 30,000 transformants were screened for the identification of an imidazoleglycerol-phosphate dehydratase and acid phosphatase using the functional complementation approach (Kellner et al. 2011).

Besides prokaryotes, many eukaryotic genes of industrial importance have been explored from forest soil. About 40% genes (out of 9949 eukaryotic genes) involved in carbohydrate and amino acid metabolism are identified by the metatranscriptomic approach. Soils of the forest are the hubs of carbohydrate active enzymes (CAZymes) and about 74,000 active transcripts were identified from the soil metatranscriptome of maple forests (Hesse et al. 2015).

Aldehyde dehydrogenase and a novel serine protease inhibitor gene from phyla *Ciliophora* and *Tardigrada*, respectively, were isolated using the metatranscriptomic approach. In yeasts, which are sensitive to metals, the aldehyde dehydrogenase gene imparts tolerance to several toxic metals when expressed. Similarly, serine protease, which is also a stress response protein, performs as a defence entity against abiotic and biotic stresses (Mukherjee et al. 2019a, b). Metallothioneins (MT) are part of the cysteine-rich proteins (CRP) family and are well known for their ability to bind metal ions via establishment of complex metal-thiolate (Blindauer 2013). Members of the CRP were identified and characterised, which revealed their metal-binding abilities with respect to Cu, Zn and Cd. Novel proteins (environmental metallothioneins) obtained by sub-cloning and expression of soil metatranscriptomes had shown the ability to chelate Cu (II), Zn (II) and Cd (I) (Mukherjee and Reddy 2020). Further, the functional genes identified using the metatranscriptomic approach can potentially be exploited as biomarkers and their expression level may be employed to access the degree of heavy-metal pollution or toxicity in crop fields.

Owing to the injudicious use of chemical inputs in crop production, the soils of agricultural lands are being polluted by chemicals such as polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), phenols, fungicides, herbicides and insecticides. Microbial degradation of these organic pollutants is a continuous process in soil because of the multifaceted metabolic functions and enzymatic competences of the microbial communities associated with soil (Singh et al. 2018). A metatranscriptomic study of pesticide and chemical fertiliser-polluted soil by Sharma and Sharma (2018) identified the transcripts pertinent to the degradation of cypermethrin and related aromatic compounds. In another study, metatranscriptome of the wheat rhizosphere revealed that several kinds of bacteria participated in the detoxification and removal of xenobiotics like polyaromatic compounds and naphthalene (Singh et al. 2018). Sharma et al. (2019) reported numerous transcripts associated with stress-response nitrification and pathways of alternative carbon fixation with high expression in agriculture soil than in organic soil, revealing Archaea's pivotal role in heavy metal- and insecticide-polluted soils. Also, high expression of transcripts for resistance to glyoxalase/bleomycin, 4-hydroxyphenylpyruvate dioxygenase and dioxygenases of pathways in aromatic hydrocarbon degradation in agricultural soil was reported.

Phytoremediation is one of the common methods for rejuvenating the lands polluted by anthropogenic contaminants. The crops employed in phytoremediation like *Salix* spp. to endure and also flourish in those polluted soils mostly depend on the complex and enigmatic interactions of the microbial community (Gonzalez et al. 2018). In the process of phytoremediation using willows, the complication of interactions between willow and its diverse microbial communities impedes the optimisation of phytoremediation. Hence, the metatranscriptome of the rhizosphere and roots of four and two willow species, respectively, from petroleum-polluted and non-polluted soils were studied. Substantial variances in the abundance of transcripts associated with bacterial and fungal taxa were noticed amid willow species, frequently in the petroleum-polluted soils. Significant abundance of transcripts for several microbial taxa and functions were observed in the polluted rhizosphere of *Salix eriocephala*, *S. miyabeana* and *S. purpurea*, while reduced abundance was found in the rhizosphere of *S. caprea*, which indicated its sensitivity to contamination (Yergeau et al. 2018). Gonzalez et al. (2018) sequenced the transcriptome of *S. purpurea* roots grown in petroleum-contaminated or non-contaminated soils to understand the profile of gene expression in different conditions. The abundance of transcripts encodes for abiotic stress tolerance like glutathione S-transferases was also observed. A community shift of genera from Ascomycota to Basidiomycota was observed with increased abundance of transcripts in the contaminated soil and most of them are unique. The expression of genes, required for the formation of biofilm and reduction in contamination stress, by bacterial transcripts and expression of genes, required for amino acid or carbohydrate breakdown, by root and fungal transcripts, showed the role of bacteria in tripartite mutualism with plant root and fungi.

24.4 Metatranscriptomics in Microbial Diversity Analysis

In the rhizosphere, the interactions between the plant and microorganisms play a vital role in the recycling of organic matter and in safeguarding crop health and yield. A metatranscriptomic analysis of the active microbiome of the rhizospheres of wheat, pea, oat and a mutant of oat (*sad1*) found to be lacking in synthesis of avenacin, an anti-fungal compound. The analysis revealed that the microbiome of the rhizosphere differed significantly from bulk soil and between plant species. In the pea and oat rhizospheres, the eukaryotes had fivefold higher relative abundance than in the wheat rhizosphere. All the rhizospheres were enriched for bacterivorous protozoa and nematodes, while the pea rhizosphere was highly enriched for fungi.

Avenacins had negligible influence on communities of prokaryotes but eukaryotes were strongly transformed in the *sad1* mutant, indicating the significant role of avenacins apart from simply defending plants from infection by pathogens (Turner et al. 2013).

An analysis of metatranscriptomes for the profiling of gene expression at specific space and time could give insights into microbial communities pertinent to their structure and function (Sharma et al. 2019). Sharma et al. (2019) performed a comparative study of metatranscriptome between two different ecosystems, that is, agricultural soil vs organic soil. In both the ecosystems, *Proteobacteria* were found to be a pre-dominant phylum with significant changes in the top three abundant phyla. In agricultural soil, the phyla order was *Proteobacteria* > *Ascomycota* > *Firmicutes*, while in organic soil, it was *Proteobacteria* > *Cyanobacteria* > *Actinobacteria*. A comparative metatranscriptomic approach was used by Hayden et al. (2018) to assess the taxonomic and functional characteristics of the microbiome of the wheat rhizosphere in suppressive and non-suppressive soils to *Rhizoctonia solani*. The results showed *Arthrobacter* and *Pseudomonas* as the dominant taxa in non-suppressive soil, and *Stenotrophomonas* and *Buttiauxella* in suppressive soil. In another study, an analysis of the wheat rhizosphere metatranscriptome was performed to unveil the structural and functional microbial communities associated with organic pollutant degradation. The results showed that *Proteobacteria*, *Actinobacteria* and *Firmicutes* were the dominant phyla involved in the degradation of aromatic compounds. The abundance of the transcripts related to aromatic compounds, phenols, biphenyls, benzoates, naphthalene, carbazoles and xenobiotics revealed the profuse degradation competences in the wheat rhizosphere (Singh et al. 2018).

A metatranscriptomic study also reveals the effects of climate change on community shifts in the rhizosphere microbiomes. The probable impact of slightly elevated carbon dioxide (eCO_2) was assessed on rhizosphere microbiomes of grasslands. The results unveiled the substantial impact of eCO_2 on the structural and functional rhizosphere microbiomes of grasslands. Both root-associated and rhizospheric soil microbiomes had significantly higher bacterial abundance with dominant populations of *Actinobacteria* and *Proteobacteria*, while a decline in fungi was observed. In addition, abundance of *Acidobacteria*, *Bacteroidetes*, *Chloroflexi* and *Planctomycetes* was also observed (Bei et al. 2019).

Viruses have influence on nearly all the organisms on earth and greatly impact agriculture, the biogeochemical cycle and human health. Nevertheless, the knowledge on RNA viruses is very negligible in environmental perspective, and indeed, very few are reported with respect to their diversity and interactions in soil, where one of the greatest multifaceted microbial systems exists. Metatranscriptomes from four habitats viz. rhizosphere, detritosphere, rhizosphere with root detritus and unamended soil were assembled and analysed. The results showed huge diversity of *Narnaviridae* and *Leviviridae* and they have been reported to infect fungi or *Proteobacteria*, respectively. The viral and host communities were extremely dynamic and deviated on the basis of presence of root litter. When the host cell dies due to a viral infection, the cell carbon gets mobilised in a process of soil carbon cycling (Starr et al. 2019).

An analysis of the root microbiome of sorghum has shown that the development of the early root microbiome is delayed by drought. This is due to the escalated abundance and function of monoderm bacteria whose outer cell membrane is absent but a thick cell wall is present. These shifts in community composition were correlated with transformed metabolism of plants and the enhanced functions of ATP-binding cassette (ABC) transporter genes of bacteria. A metatranscriptomic analysis of the *Arabidopsis thaliana* (genotype Pna-10) rhizosphere microbiome unravelled the expression of 81 novel transcripts at various stages of plant development. It was deduced that root-exudates comprising compounds and phytochemicals are being released by plants at different developmental stages, which restructure the rhizosphere microbiome (Haldar and Sengupta 2015).

Metatranscriptomics, along with metagenomics, allowed researchers to characterise endophytes from internal tissues of plant in-situ which would help in identification of unique genes that could help in better understanding of significant functions played by microorganisms for plant growth and enhancement of productivity (Maela and Serepa-Dlamini 2020).

24.5 Metatranscriptomics in Bio-Control of Phytopathogens

In the rhizosphere, the plant pathogens tend to develop a parasitic association with plant roots to initiate infection. The pathogen must contest with the rhizosphere inhabitants in order to invade the root tissues for obtaining nutrients. The growth of pathogens is strongly restricted in disease-suppressive soils due to the influence of specific rhizosphere microorganisms (Chapelle et al. 2016). In most of the soils, the disease suppressive is inherent due to their microbiome which is established in soil after many years of high disease incidence. This indicates the requirement for fungal pathogens in soil to activate specific antagonistic microbes or microbial community and thereby exhibiting the suppressiveness. However, the microbes and their mechanisms in most suppressive soils are still not known. Metatranscriptomics plays a major role in getting insights into the active microbes and their traits expressed during fungal invasion into the plant roots. This approach helps reveal the importance of the genes accountable for suppression and could be used to get the complete expression profile of the microbial community. This would unveil the importance of the microbiome in plant-microbe interactions and disease suppression in the rhizosphere (Kothari et al. 2017). The metatranscriptomic investigation of the sugar beet rhizosphere microbiome in disease-suppressive soil displayed significantly higher relative abundance of *Burkholderiaceae*, *Oxalobacteriaceae*, *Sphingobacteriaceae* and *Sphingomonadaceae* in the rhizosphere after fungal infection. Also, an upregulation of genes associated with stress (*ppGpp*) were observed in these families. The invasive pathogens induce the stress responses in rhizosphere microbial communities either directly or via the plant. This leads to a shift in microbiome composition along with the induction of antagonistic characters that limit the microbial infection (Chapelle et al. 2016). A comparative metatranscriptomic investigation was performed to understand the taxonomic and functional characteristics of the rhizosphere microbiome in wheat crop grown in adjacent fields which are suppressive and non-suppressive to *R. solani*. The results revealed that suppressive soils had a higher expression of a terpenoid biosynthesis backbone gene (*dxs*), a polyketide cyclase and several cold shock proteins (*csp*). Non-suppressive soils exhibited a higher expression of antibiotic genes such as phenazine biosynthesis (*phzF*), transcriptional activator of phenazine synthesis (*phzR*) and non-heme chloroperoxidase (*cpo*), which play a major role in the synthesis of pyrrolnitrin. Furthermore, a large number of genes associated with detoxification of reactive oxygen species (ROS) and superoxide radicals were observed in the rhizosphere of non-suppressive soils. This would be a response of wheat roots to the infection by *R. solani* AG8 (Hayden et al. 2018).

24.6 Metatranscriptomics in Plant Growth Promotion

Plant roots are colonised by a group of soil bacteria which promote plant growth either directly by nitrogen fixation, phosphate solubilisation and plant hormone production or indirectly through production of siderophore, ammonia and hydrogen cyanide. These soil bacteria are referred to as plant growth promoting rhizobacteria (PGPR). PGPR have been reported to induce various biochemical changes in plants with respect to their growth and nutrition (Bashan and Holguin 2004). This may be attributed to the complex amalgamations of several PGPR-stimulated machineries that influence both plant growth and nutrition. They include siderophore production for absorption of iron, synthesis and release of plant hormones such as IAA, and solubilisation of phosphates, minerals and nutrients (Mayak et al. 2004; Gamalero et al. 2010; Shariati et al. 2017). They synthesise various secondary metabolites, enzymes and hormones, which aids in the development of plant roots. They also influence the biochemical reactions of roots by shifting the transcription and biosynthesis of metabolite in cells. Furthermore, transcriptomic studies were conducted to elucidate the molecular mechanism variations in plants associated with PGPR-facilitated growth. In order to go further to realise PGPR as an effective device for agricultural crops, the chief mechanisms used by the specified bacteria must be reviewed comprehensively.

Plants in their typical habitat are encircled by a huge variety of microbes, of which a few microbes interact with plants directly through mutualism, while others colonise the plant and exhibit commensalism. Furthermore, the plants are influenced by these microbes to alter their environments (Schenk et al. 2012). The microbiomes associated with plants can enhance plant growth or restrict plant pathogens (Arif et al. 2020). Understanding of plant-microbe interactions may effectively give rise to innovative approaches to improve plant productivity (Schenk et al. 2012). Metatranscriptomics helps discover the potentially interesting (yet unknown) plant-microbe relationships. When plants are under multiple biotic and abiotic stresses, they first prioritise their physiological pathway to cope with stress that can determine the outcome of plant-microbe interactions (Yao et al. 2011; Schenk et al. 2012) and microbial community. In contrast, alteration of the microbiome with PGPR consortia can improve plant growth and alleviate the stress caused by biotic and/or abiotic factors. Manipulation of plants by microbiome engineering either by direct or indirect approaches is an emergent strategy to enhance crop yield and resilience (Arif et al. 2020). Metatranscriptomics helps in rhizosphere microbiome engineering through categorisation and quantification of functionally expressed microbial genes that benefit a wide variety of crop plants (Arif et al. 2020). Malviya et al. (2019) performed a comparative study on sugarcane root transcriptome to understand their response to *Burkholderia anthina* (MYSP113). The results revealed the involvement of many genes in response to strain *B. anthaina* MYSP113 and significantly enriched in terms of quantity. These genes were related to several processes, such as nitrogen metabolism, plant hormone and signal transduction. Furthermore, enzyme actions, such as superoxide dismutase and peroxidase, were substantially enriched in roots after *B. anthaina* MYSP113 inoculation.

24.7 Metatranscriptomics in Abiotic Stress Tolerance

The impact of abiotic stress on plants and its mitigation through microorganisms have been well documented both at the physiological and molecular levels. Among the various abiotic stresses, water deficit, which has affected about 64% of the total area globally, remains a major stress for plants and causes significant loss in crop growth and yield (Mittler 2006; Cramer et al. 2011). Plant-microbe interactions can be well elucidated with the help of methodologies such as microarray and mRNA sequencing, which can generate information at the transcriptome level (Akpinar et al. 2015; Budak and Akpinar 2015; Wang et al. 2016). A comparison of transcripts in biological systems expressed under two different conditions would help in understanding the role of transcripts in combating the negative effects of stresses on plants. A comparative study of the transcription profiles of two *Sinorhizobium meliloti* strains (1021 and RD64) revealed that RpoH1, a sigma factor coding gene, and other stress related genes were found to be induced in strain RD64 (this strain overproduces IAA). The induction of stress-related genes was also reported in IAA overproducing *Sinorhizobium meliloti* by Defez et al. (2016). During a transcriptome analysis of rapeseed inoculated with *Stenotrophomonas rhizophila,* a novel plant growth regulatory molecule, spermidine was identified by Alavi et al. (2013).

MicroRNAs (miRNAs) are single-stranded non-coding RNAs with a nucleotide length of 19–23. Diverse miRNAs are reported to play vital roles in plants such as rice, *Arabidopsis* and *Medicago* by regulating various biochemical processes in response to abiotic stresses like drought, salinity and cold (Trindade et al. 2010; Budak and Akpinar 2015). The importance of miRNA in the regulation of tolerance against salinity in *Arabidopsis* (miR393), and alleviation of drought and salinity in rice (miR169) through modulation of transcription factor (NF-YA) expression has been reported (Zhao et al. 2009; Gao et al. 2011). In tomato, tolerance to drought was conferred owing to miR169c overexpression, which regulates the expression of gene (s) responsible for stomatal activity (Zhang et al. 2011). In cucumber, stress tolerance is mainly mediated by WD-repeat proteins which are regulated by miRNA, Bvu-miR13 (Li et al. 2014). Besides regulating the transcription factors (TFs), miRNAs also control stress-signalling pathways which are involved in the development of roots and leaves (Curaba et al. 2014). The regulation of superoxide dismutases, SOD1 and SOD2, was mediated by miR398 through the reduction of ROS (Kantar et al. 2011). Various kinds of miRNAs mitigate abiotic stress through modulation of diverse cellular reactions and metabolic processes like regulation of transcription, ion transport, apoptosis and auxin homeostasis (Li et al. 2010). They also have been reported for regulation of stress response in plants against aluminium stress. The expression profile of miRNAs in two different subspecies of rice, that is, *japonica* and *indica*, which showed different tolerance to aluminium stress, was compared. The results unveiled 16 unique kinds of miRNA responses that revealed an exhibition of complex responses under aluminium stress (Lima et al. 2011).

In plants, irreversible damages are being caused by flooding and radiation through generation of ROS (Blokhina and Fagerstedt 2010). In *Arabidopsis,* the regulation of miR398 and SOD proteins is vital under oxidative stress (Sunkar et al. 2006). In *Populus tremula,* miR398 induction and miR395 down-regulation were detected during the process of UV-B stress alleviation (Jia et al. 2009). The cold temperature harshly disturbs sugar beet seedlings and recovery of sugar from them after harvest. Transcriptome profiling of leaves and roots from cold-stressed plants unveiled that the CBF3 gene was up-regulated faster in tissues of roots than in leaves (Moliterni et al. 2015). Up-regulation of genes from the AP2/ERF family, which involve in jasmonic-acid-mediated responses, was detected under cold stress (Licausi et al. 2013).

24.8 Challenges

The application of metatranscriptomics may be limited due to the fact that a. predominance of ribosomal RNA significantly reduces the coverage of mRNA, b. the stability of mRNA is very minimum, c. challenges in differentiating the host and microbial RNA despite the availability of enrichments kits (Aguiar-Pulido et al. 2016), and d. the absence of polyA tailed bacterial and archaeal mRNAs which consequently results in reduced recovery of expressed mRNAs after their extraction (Maela and Serepa-Dlamini 2020). Another limitation is that due to the absence of good-quality and assembled reference genomes, the transcripts are seldom assigned to particular microbes (Levy et al. 2018). These challenges have to be taken care of while deciphering the role of rhizosphere metatranscriptomics in plant growth improvement.

24.9 Conclusion

Metatransciptomics plays an imperative role in understanding the dynamics of the rhizosphere microbiome, under various biotic and abiotic stress conditions, to develop a comprehensive catalogue of microbes and their functions in order to develop a blueprint for rhizosphere engineering. It can be concluded from the literature that most of the genes are obtained in dynamic root-associated microorganisms, which is evidenced by the enrichment of gene expression in the root zones as compared to the soil. This is attributed to the production of various metabolites and substances that support the growth and development of rhizosphere microflora, which in return helps mitigate the biotic and abiotic stresses (Table 24.2).

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25

Rhizospheric Engineering for Sustainable Production of Horticultural Crops

Sarita Devi and Poonam Kumari

Abstract

Rhizosphere- and root-associated bacteria are vital components of plant microbiomes and affect the physiology and development of plants. For sustainable production of horticultural crops, it is essential to know about the diversity of the microbes associated with such crops and how the soil factors affect the microbiome. To improve the health and productivity of horticultural crops, various techniques for manipulating plants and their root-associated microorganisms have been studied so far. Some approaches are focused on understanding interactions between roots, microbes and soil, while others are focused on plant mechanisms that affect development. Plants, for example, can be modified to change the rhizosphere's pH or release substances that boost food availability, defend against biotic and abiotic stresses, or promote the production of beneficial microbes. Novel molecular techniques and significant biotechnological advancements will help gain a better understanding of the complex chemical and biological interactions that occur in the rhizosphere, ensuring that rhizosphere engineering approaches are both safe and helpful in enhancing the productivity and quality of horticultural crops. This chapter provides an insight into various rhizospheric microbes associated with horticultural crops and their manipulation for sustainable crop production.

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25.1 Introduction

Life on the earth is sustained with the aid of a small soil volume surrounding the roots, known as the rhizosphere. The soil is where much of our planet's biodiversity occurs, and perhaps the most diverse environment on Earth is provided by the rhizosphere; and it is definitely considered the most significant zone in terms of determining the quantity and quality of human food resource (Hinsinger et al. 2009). Very little information about the functioning of the rhizosphere is available, considering its central significance to all forms of life, and we have an incredible ignorance about how best we can use it to our benefit (Almalak 2018). The rhizosphere acts as a hot spot for various microbial interactions as the plant roots' exudates provide a major food source for microorganisms and an impetus of their populace density and related activities (Hinsinger et al. 2009). There are many rhizospheric microbes with an impartial effect on the plant, but it often attracts microorganisms that have useful or harmful effects on the plant (Mendes et al. 2013). Pathogenic fungi, oomycetes, bacteria and nematodes are the microorganisms which adversely affect plant growth and health (Mendes et al. 2013). Most of the soil inhabitant pathogenic microorganisms are developed in the bulk soil to prosper and grow, but the rhizosphere is the playground and contagion court where the pathogenic microorganisms develop a parasitic relationship with the plant (Fageria 2012; Qu et al. 2020). The rhizosphere is also a battleground where microfauna, microflora and the complex rhizosphere community act together with pathogens and affect the consequence of pathogen infection (Hinsinger et al. 2009). The plant benefits from a wide variety of microorganisms, including nitrogen-fixing bacteria, ecto- and endomycorrhizal fungi, plant growth-promoting bacteria and fungi (McNear Jr 2013). In comparison to synthetic fertilisers, pesticides and insecticides, plant growth-promoting rhizobacteria are known to improve plant performance through various mechanisms, such as production of advantageous hormones, improvement in plant nutritional status and decrease in stress-related damage (Bhattacharyya and Jha 2012; Gouda et al. 2018). The association between plants and plant growth-promoting rhizobacteria (PGPR) is of particular interest as they enhance growth and development of crops in various stress conditions such as high or low temperatures, soil salinity, nutrient deficiency and drought (Schillaci et al. 2019). There are various forms of plant growth-promoting rhizobacteria populations present in the rhizospheric soil, which show beneficial effects on the production of crop. Numerous investigations are done to understand the dynamics diversity and importance of soil plant growth-promoting rhizobacterial communities and their cooperative and beneficial roles in the productivity of various crops (Gupta et al. 2015; Aloo et al. 2019). The most common examples of plant growth-promoting rhizobacterial genera showing plant growth-promoting activity include *Erwinia*, *Azotobacter*, *Pseudomonas*, *Mesorhizobium*, *Azospirillum*, *Bacillus*, *Enterobacter*, *Burkholdaria*, *Mycobacterium*, *Rhizobium* and *Flavobacterium* (Ahemad and Kibret 2014).

In the era of globalisation and modernisation, crop sustainability is a challenge. Agricultural systems and activities aimed at conserving or enriching the health of natural resources are related to sustainable farming. Certainly, horticultural practices have some impact on the natural resources used to grow fruits, vegetables, flowers and other products. Horticultural industries are becoming increasingly concerned with preserving and protecting their resource base and environment as the understanding of sustainability problems increases. With the world population growing at an unprecedented pace, crop production needs to be increased in order to meet global food requirements and, at the same time, boost agricultural sustainability. Feeding the human population, which is estimated to rise from 7.6 billion now to 9.5–10 billion by 2050, will be a huge challenge for scientists around the world. In recent times, crop production is facing severe threats because of numerous abiotic and biotic stresses, as well as reduced land supply. In nature, plants are exposed to trillions of microbes that colonise and occupy different plant chambers or compartments such as the rhizosphere, the rhizoplane, the endosphere and the phyllosphere, therefore considered a secondary plant genome (Kumar and Dubey 2020). The rhizosphere has a large pool of soil microbes and is considered the 'hot spot' for microbial colonisation and activity. It is known as the world's largest ecosystem with tremendous energy flux (Barriuso et al. 2008). The rhizospheric microflora protects against diseases, improves growth by producing phytohormones and can enable plants to withstand environmental disruptions such as irregular changes in temperature, drought and salinity. Knowing the importance of the rhizosphere and its manipulation in sustainable crop production, this chapter aims to discuss the role of the rhizosphere and rhizospheric engineering in horticultural crops.

25.2 Rhizosphere

The soil is the world's most important biodiversity reservoir, and it serves as a vital habitat for prokaryotes and a wide spectrum of eukaryotes, including fungi among other soil microorganisms and a diverse range of invertebrates. The diversity of soil prokaryotes is predicted to be three orders of magnitude larger than the diversity of prokaryotes found in all other ecosystems or ecological sections on the world (Curtis et al. 2002; Hinsinger et al. 2009).

Higher plant roots anchor the terrestrial habitats' above-ground diversity, and make available most of the carbon to power the soil environment. The soil is much more significant from a functional viewpoint, in addition to their role in biodiversity, in maintaining all other aspects of terrestrial biodiversity and providing many ecological unit services (Bach et al. 2020). The temporal and spatial heterogeneities from nanometre to kilometre scales are a significant characteristic of soils (Bach et al. 2020). The environmental heterogeneity and spatial distance's interactions are important factors influencing the abundance and relatedness of the *Burkholderia cepacia* rhizosphere-bacteria complex (Ramette and Tiedje 2007). In addition to species diversity, abundances of species are also significant in soils (Watt et al. 2006). Approximately 10^4 nematodes, 10^4 protozoa, 10^7–10^{12} bacteria and 5–25 km of fungal hyphae are present in 1 g of soil, with an average specific surface area of about 20 $m^2\ g^{-1}$ and the very small size of most of these microorganisms, their

Table 25.1 Composition of root exudates

S. no.	Compositions	Elements identified
1	Amino acid and amide	Serine/homoserine, proline, phenylalanine, methionine, lysine, histidine, glycine, glutamine, cystine/cysteine, aspartic acid, asparagine, arginine and alanine
2	Enzyme	Polygalacturonase, protease, phosphatase, invertase and amylase
3	Growth factor	Thiamine, pyridoxine, p-amino benzoic acid, pantothenate, niacin, n-methyl nicotinic acid, inositol, choline, biotin and auxins
4	Phenolic acid and coumarin	Vanillic acid, syringic acid, salicylic acid, ferulic acid, cinnamic acid, coumarin and caffeic acid
5	Sugar	Xylose, sucrose, ribose, rhamnose, raffinose, oligosaccharide, maltose, glucose, galactose, fucose, fructose and arabinose
6	Organic acid	Valeric, tartaric, succinic, pyruvic, propionic, oxalic, malonic, malic, maleic, lactic, glutaric, citric, butyric and acetic acid
7	Others	Sterols, proteins, lipids, nucleotides, flavanone, fatty acids, carbohydrates, aromatics and aliphatics

surface coverage amounts in total to only 10^{-5} to 10^{-6}% of the total soil surface area (Hinsinger et al. 2009). The soil can be compared to a vast desert, where life is dispersed discreetly, especially when the tendency of many of these bacteria in soil to form colonies and gather, resulting in activity hotspots, is taken into account (Pointing and Belnap 2012; Makhalanyane et al. 2015). One of the most fascinating hotspots of activity and diversity in soils is the rhizosphere (Jones and Hinsinger 2008). The rhizosphere is well-described as the soil amount around living roots, which is affected by root activity (the '*Einflusssphäre der Wurzel'*) according to Hiltner 1904 in Hartmann et al. (2008). To define the soil area under the control of plant roots, Hiltner coined the word 'rhizosphere' in 1904. Our cognizance of the subject has significantly improved over time. The rhizosphere is now better described as the 'field of action or effect of a root'. The rhizosphere is considered a limited area of soil under the influence of live roots, where root exudates can encourage or constrain microbial communities and their activities (details of the same are given in Table 25.1, Pinton et al. 2007; Backer et al. 2018). Because of the release of root exudates that stimulate or inhibit rhizosphere organisms, the rhizospheric zone is characterised by intense biological activity. The dynamics and complexity of this area are further characterised by the interactions between the soil, the plants and the organisms that form the rhizosphere (Pinton et al. 2007). For several bacterial and fungal species, the root surface or rhizoplane also provides a highly favourable nutrient base, and these two zones together are sometimes denoted as the soil-plant interface (Nihorimbere et al. 2011). Although the rhizosphere is an incessant zone, it is useful to define the endorhizosphere as the root's cell layers and the ectorhizosphere as the area around the root. As a result, the rhizosphere is a component of the soil environment in which the plant roots, the soil, and the soil bacteria (biota) interact. In the rhizosphere, plant/microbe and plant/faunal interactions also abound (Pinton et al. 2007). These interactions vary from symbiotic relationships (e.g. mycorrhizal associations and nitrogen fixation) to pathogenic interactions. When the rhizosphere ecology is considered, management strategies such as biological control and bioremediation can also be effective (Igiehon and Babalola 2018). A better understanding of the rhizosphere and its impact on different organisms that occupy this region would allow manipulations of the environs that support the production of plants and the environs.

25.3 Rhizospheric Microbes and Their Interactions with Plants

Numerous investigations have shown that soil-borne microbes interact at the root-soil interface with plant roots and soil constituents. The wide range of root-microbe interactions contributes to the dynamic rhizospheric ecosystem development in which microbial communities interact as well (Barea et al. 2005). When compared to root-free bulk soil, the chemical, physical and biological features of the soil associated with roots are responsible for vicissitudes in various microbial communities as well as growing numbers and activity of microorganisms in the micro-environment of the rhizosphere (Barea et al. 2005; Jacoby et al. 2017). The essential determinants of the functions of the rhizosphere are the carbon fluxes (Toal et al. 2000). For the heterotrophic soil biota, the release of decaying plant material and root exudates as structural material, growth substrates or signals for the microbiomes associated with root provides sources of carbon compounds (Canarini et al. 2019). Microbial activity in the rhizosphere regulates rooting patterns and the delivery of accessible nutrients to plants by modifying the quantity and quality of root exudates in the process (Barea et al. 2005). Furthermore, in the rhizosphere, two kinds of interactions are identified: interactions based on detritus (dead plant material) that affect nutrient and energy flows, and interactions based on living plant roots. Both types of interactions can be used in ecology and agronomy (Barea et al. 2005).

In addition, the rhizosphere is made up of three distinct yet interconnected elements: the rhizoplane, the rhizosphere (soil) and the root itself. Including the firmly adhering soil particles, the rhizoplane is the root surface, while the rhizosphere is the region of the soil impacted by roots by the release of substrates that affect microbial activity. The root, the aforementioned, is part of the rhizosphere system, as some microbes (e.g. the endophytes) are able to colonise root tissues (Rizvi et al. 2009; Nihorimbere et al. 2011). Root colonisation is the microbial colonisation of the rhizoplane and/or root tissue, whereas rhizosphere colonisation is the colonisation of the nearby soil volume, under the effect of the root (Barea et al. 2005; Rizvi et al. 2009). The use of molecular techniques to categorise various microbes is a crucial tool for understanding the ecology of the rhizosphere (Pühler et al. 2004). Because of current public uncertainties about the negative effects of agro-chemicals, there is a growing interest in better understanding the cooperative interactions within rhizosphere microbial communities and how these could be advantageous to diverse crop production (Barea et al. 2005). Some cooperative microbial activities could be used as low-input biotechnology methods, forming the foundation of a technique to promote environmentally benign, sustainable practices that are critical for the productivity and stability of both natural and agricultural ecosystems (Arora et al. 2010). In the rhizosphere, microbial communities and other agents include bacteria, fungi, nematodes, algae, protozoa and microarthropods (Barea et al. 2005). Many members of this rhizospheric group have very little or neutral effect on the plant, but they are important elements of the intricate food web that consumes the massive amounts of carbon that the plant fixes and releases into the rhizosphere (i.e. rhizodeposits). The microbial population present in the rhizosphere often includes members that possess beneficial or deleterious effects on the plant. The pathogenic fungi, oomycetes, bacteria and nematodes adversely affect plant growth and health, while microorganisms that are beneficial include nitrogen-fixation, mycorrhizal interactions, promotion and inhibition of plant growth. Also, biological regulation and bioremediation have been investigated in recent years. Nitrogen-fixing bacteria may make nitrogen available to the plant that is not available otherwise. The nodules on the roots of plants are produced from Rhizobia and associated bacteria (Lindström and Mousavi 2019). As the plant provides protection and nutrients for the bacteria and the bacteria provide nitrogen to the plant, they share mutually beneficial relationship. The associations with plants are also created by other nitrogen-fixing species. This can also include other plant growth-promoting compounds along with plant-available nitrogen (Lindström and Mousavi 2019).

Until now, taxonomists have recognised numerous species in the genus *Azospirillum*, viz. *A. amazonense, A. halopraeferens*, *A. lipoferum, A. irakense* and *A. brasilense* (Srivastava et al. 2015), *A. picis* (Lin et al. 2009), *A. canadense* (Mehnaz et al. 2007a), *A. melinis* (Peng et al. 2006), *A. zeae* (Mehnaz et al. 2007b), *A. doebereinrae* (Eckert et al. 2001) and *A. rugosum* (Young et al. 2008). *Azospirillum* is known to have more effective nitrogenase properties than other nitrogen fixers, among the free-living nitrogen-fixing bacteria. *Azospirillum*-inoculated plants have been demonstrated to absorb solution nutrients faster than uninoculated plants, resulting in higher levels of dry matter, phosphorus, nitrogen and potassium in the leaf foliage (Srivastava et al. 2015). In addition to being one of the indices of soil quality, soil microbial diversity varies on a regular basis in response to management techniques. The effects of 12 years of traditional and sustainable management practices on the metabolic diversity and soil microbial composition of a mature rainfed olive orchard revealed that soil under sustainable practices had more culturable fungi, bacteria, soil enzyme activity and microbial population metabolic diversity indices than that under traditional practices (Sofo et al. 2014). These alterations in soil microbial communities have reacted favourably to improvements in olive fruit production and quality (Sofo et al. 2010). Sofo et al. (2010) observed greater magnitude of quantitative and qualitative vicissitudes in soil microbial communities in kiwi and peach fruit orchards in response to innovative (characterised by minimal tillage, organic matter inputs from composts and cover crops, water, pruning, and adequate irrigation and fertilisation) than in traditional soil management systems (characterised by conventional tillage, zero organic input, empirical pruning, excessive irrigation and strong chemical fertilisation). A great number of microorganisms have shown their usefulness in a variety of fruit crops, both as pure culture (as broth) and as carrier-based cultures (Table 25.2). These results provide strong support for the need to fine-tune such efficient microorganisms in combination with inorganic fertilisers and organic manures so that their multi-dimensional value-added response is seen in perennial fruit crops (Srivastava et al. 2015).

The mycorrhizal fungi, without causing root disease, often create a mutualistic symbiosis with plants and infect roots. In the rhizosphere of most plants, these fungi can be found and form associations with all gymnosperms and more than 79 percent of monocotyledonous and 83 percent of dichotyledonous plants. On the outside (ectomychorrhizae) or inside (endomycorrhizae) of plant roots, the mycorrhizal fungi may also form structures (Warburton et al. 2005). The fungal hyphae cause a greater volume of soil to be contacted by the roots. The solubilisation of nutrients (e.g. phosphorus) is increased by certain types of mycorrhizal fungi. They help the plants/crops increase selective ion uptake and nutrient absorption mainly in stressed environments (e.g. soils with phosphorus and water deficiency), and protect them from environmental extremes (Warburton et al. 2005; Begum et al. 2019). Plant exudation patterns can be modified by these fungi after colonisation, thereby affecting the macrofaunal and microbial populations of the rhizosphere (Bais et al. 2006). Fungi can also shield plant roots from pathogens' invasion. Endomycorrhizal extra radicle hyphae secrete glomalin, a glycoprotein that aggregates soil particles, strengthens water-stable aggregates and enhances the structure of the soil (Warburton et al. 2005). This association may improve plant growth and survival, particularly in low-nutrient or adverse environments, and may have a potential for disturbed sites to re-vegetate. The abundance and diversity of beneficial and harmful bacteria are related to the quantity and kind of rhizodeposits, as well as to the consequences of microbial interactions that occur in the rhizosphere (Somers et al. 2004). It has piqued the interest of scientists from several disciplines who want to learn more about the processes that determine the structure, behaviour and dynamics of the rhizospheric microflora, and how they might be used to develop innovative plant growth and health strategies.

25.4 Plant Growth-Promoting Rhizobacteria

Plant growth-promoting rhizobacteria are basically beneficial microorganisms present in the soil. For such microorganisms, the expression 'plant growth promoting rhizobacteria' is used because they either directly contribute to the growth activities of plants or indirectly enhance plant growth and development (Lugtenberg and Kamilova 2009; Siyar et al. 2019). There are numerous plant growth-promoting rhizobacterial species that improve plant growth and overall efficiency. Plant growth-promoting rhizobacteria may be free-living (different species of *Pseudomonas*, *Agrobacterium*, *Bacillus*, *Burkholderia* etc.) or may be in symbiotic associations with their hosts (various species of *Rhizobium*, *Frankia* etc., Podile and Kishore 2006; Bhattacharyya and Jha 2012; Siyar et al. 2019). To stimulate the growth of plants through a variety of ways, both free-living and endophytic species

Table 25.2 Response of various rhizospheric microbes on yield, nutrient uptake and growth of fruit crops

S. no.	Fruit crop	Response parameters	Microbes involved	Reference
1.	*Malus domestica* Borkh. (Apple)	Yield, growth and plant nutrition	*Microbacterium*, *Bacillus* (M3), *Bacillus* (OSU-143)	Karlidag et al. (2007)
2.	*Malus domestica* Borkh. (Apple)	Fruit yield and tree growth	*Pseudomonas*(BA-8), *Bacillus*(OSU 142, M-3)	Aslantaş et al. (2007)
3.	*Malus domestica* Borkh. (Apple)	Root growth, germination and pest incidence	*Trichoderma viride*, *Pseudomonas striata, Azotobacter chroococcum-*	Raman (2012)
4.	*Prunus armeniaca* L. (Apricot)	Leaf nutrient concentration, shoot length and yield	*Bacillus* (OSU-142)	Esitken et al. (2006)
5.	*Prunus armeniaca* L. (Apricot)	Yield, growth and leaf nutrient composition	*Pseudomonas*(BA-8), *Bacillus* (OSU-142)	Pirlak et al. (2007)
6.	*Musa acuminata* L. (Banana)	Leaf area and yield, girth and height and of pseudo-mostem	*Azospirillium* sp.	Jeeva et al. (1988)
7.	*Musa acuminata* L. (Banana)	Leaf area, bunch weight and number of fingers	*Azospirillum brasilense*, *Azotobacter chroococcum*	Tiwary and Hasan (1999)
8.	*Musa acuminata* L. (Banana)	Fruit weight and finger size	*Pseudomonas fluorescens*, *Azospirillum brasilense*	Suresh and Hasan (2001)
9.	*Mangifera indica* L. (Mango)	Number of leaves and seedling diameter	*Azotobacter chroococcum*	Kerni and Gupta (1986)
10.	*Passiflora edulis* Sims. (Passion fruit)	Improved plantlet growth and yield	*Trichoderma* sp., *Azospirillum* sp., *Azotobacter* sp.	Quiroga-Rojas et al. (2012)
11.	*Citrus sinensis* Osbeck (Sweet orange)	Growth, fruit yield and nutrient uptake	*Glomus fasiculatum*, *Azospirillum brasilense*	Singh and Sharma (1993)
12.	*Vitis vinifera* L. (Grape)	Root development	*Pseudomonas fluorescens*	Wange and Ranawade (1998)
13.	*Pyrus pashia* (Quince)	Yield and fruit properties	*Bacillus T8 and Bacillus OSU-142*	Arıkan et al. (2013)
14.	*Punica granatum* (Pomegranate)	Vigorous plant growth and thrive under stressed soils	N2-fixing bacteria or AM fungi	Aseri et al. (2008)

(continued)

Table 25.2 (continued)

S. no.	Fruit crop	Response parameters	Microbes involved	Reference
15.	*Juglans regia* (Walnut)	Plant height, dry weight, P and N uptake of seedlings	*Pseudomonas chlororaphis* and *Pseudomonas fluorescens*	Yu et al. (2011)

of plant growth-promoting rhizobacteria have been identified. They can yield various types of organic compounds that can be involved in direct plant-growth promotion (Pérez-Montaño et al. 2014). Some plant growth-promoting rhizobacteria are known to solubilise and make available phosphate and iron to plants (Ahemad and Iqbal 2017; Alori et al. 2017; Siyar et al. 2019). Indirectly, by disrupting a broad range of plant pathogens and disease severity, plant growth-promoting rhizobacteria can stimulate the growth potential of host plants (Xiang et al. 2017; Liu et al. 2017). As crops, similar to their wild counterparts, are equally threatened by various stresses, understanding and elucidating the influences of plant growth-promoting rhizobacteria on their growth and development will contribute to the sustainability of crop production.

25.4.1 Types and Underlying Mechanisms of Plant Growth-Promoting Rhizobacteria

Plant growth-promoting rhizobacteria are of different kinds and origin. The microorganisms are copiously present as free-living in the rhizosphere where they do not form any symbiotic association with other species. The species of *Azospirillum, Arthrobacter, Erwinia*, *Flavobacterium, Pseudomonas*, *Agrobacterium*, *Serratia*, *Micrococcous*, *Azotobacter*, *Caulobacter*, *Burkholderia*, *Bacillus* and many others are well-known examples of free-living plant growth-promoting rhizobacteria, while those of symbiotic plant growth-promoting rhizobacteria are *Azorhizobium, Allorhizobium*, *Mesorhizobium*, *Bradyrhizobium*, *Rhizobium*, *Frankia* and so on (Compant et al. 2010; Bhattacharyya and Jha 2012). The dissemination of symbiotic and free-living plant growth-promoting rhizobacteria is ecologically regulated in the rhizosphere, depending on several aspects such as the availability of soil nutrients, the rhizosphere's water status and vegetation type. The root exudates produced by plants are one of the leading drivers of rhizobacterial dynamic modulation (Siyar et al. 2019). Citric acids produced by cucumber roots have been effective in the root colonisation of the host plants with plant growth-promoting rhizobacteria (Zhang et al. 2014).

The role of secondary metabolites released from the host roots in the effective interaction between plant growth-promoting rhizobacteria and plants was described by Huang et al. (2014). Ahemad and Kibret (2014) suggested that amino acids, organic acids, carbohydrates, vitamins, enzymes and various inorganic salts are found in the root zone, which can drive plant growth-promoting rhizobacteria to the root surfaces or the rhizosphere in a particular zone. Rasmann and Turlings

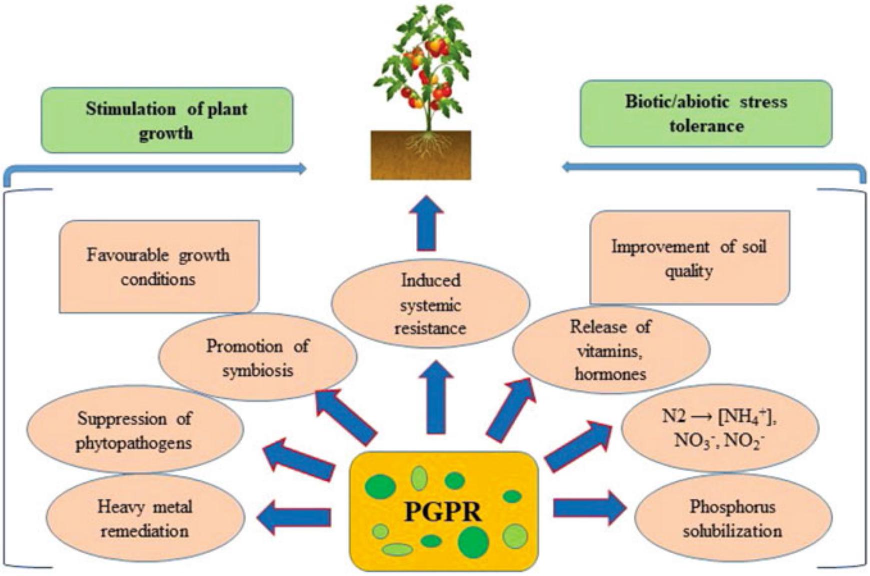

Fig. 25.1 An illustration of the mechanism of action of PGPR in plant growth improvement

(2016) indicated that roots produced organic compounds that serve as 'attracting' signals for plant growth-promoting rhizobacteria accommodation in the root region. Schulz-Bohm et al. (2018) indicated that various volatile organic compounds produced by the plant roots play a significant part in attracting plant growth-promoting rhizobacteria and the symbiotic association formation. Figure 25.1 provides an example of the mode of action of plant growth-promoting rhizobacteria in promoting plant growth. The stimulation of plant growth by plant growth-promoting rhizobacteria occurs primarily through two mechanisms that is, primary and indirect mechanisms. Direct growth promotion is assisted by plant growth-promoting rhizobacteria when they produce several types of organic molecules which range from enzymes, amino acids and carbohydrates to inorganic substances (Ahemad and Kibret 2014). Certain plant growth-promoting rhizobacteria species can directly boost growth and development of plants by producing hormones and growth-regulating substances such as abscisic acid, auxins, gibberellic acid and cytokinins, (Timmusk et al. 2017). Several free-living species similar to those associated with legumenous plants can fix nitrogen and thus help plants meet their nitrogen requirement (Pham et al. 2017; Siyar et al. 2019). Chauhan et al. (2017) presented evidence that *Aneurinibacillus aneurinilyticus* strains were able to efficiently convert phosphate to soluble forms and nitrogen to simple forms. The *Pseudomonas*, *Erwinia*, *Flavobacterium*, *Aerobacter* and *Bacillus* species have also been reported for phosphate solubilisation and growth improvement of various plants (Hayat et al. 2010). The active role played by plant growth-promoting rhizobacteria in reducing salinity stress (Shrivastava and Kumar 2015; Kumar et al. 2018; Siyar et al. 2019), drought tolerance (Jatan et al. 2019), remediation of heavy metals (Sobariu et al. 2017; Saif and Khan 2017) and disease resistance and disease intensity reduction are indirect mechanisms involved in plant-growth enhancement (Verma et al. 2018). Majeed et al. (2018) suggested that there are several plant growth-promoting rhizobacterial strains that induce systemic plant resistance to various pathogens and suppress pathogenic agents directly through antagonism, competition and antipathogenic compounds.

25.5 Effects of Plant Growth Promoting Rhizobacteria

The plant growth-promoting rhizobacteria influence plants, both indirectly and directly. These rhizobacteria directly supply substances synthesised by bacteria or promote the absorption of certain plant nutrients from the atmosphere to the plant. The plant growth-promoting rhizobacteria counteract the negative effects of one or more phytopathogenic microbes, resulting in indirect plant-growth promotion (Kokalis et al. 2002; Ortega et al. 2017).

25.5.1 Biological Nitrogen Fixation

The nitrogen-fixing microbes can live in symbiosis or freely with some plants, specifically with legumes where they produce root nodules. These microorganisms take nitrogen from the air and provide it to plants in the form of assimilable compounds, collecting carbohydrates from the roots in the process (Ortega et al. 2017). Some examples of vegetable inoculation with nitrogen-fixing PGPR are *Rhizobium* sp. TN42, *Azotobacter chroococcum* in potato (Naqqash et al. 2016), Azotobacter + PSB in radish (Ziaf et al. 2016), Azotobacter, Azospirillum and VAM in cabbage (Sharma et al. 2013).

25.5.2 Solubilisation of Phosphorus

Phosphorus is one of the significant macronutrients in plants, along with nitrogen and potassium. Although agricultural soil normally has adequate amounts of phosphorus due to fertiliser inputs, most of it is generally not accessible to plants in insoluble form. However, there are several microbes which are able to convert insoluble phosphorus to orthophosphates, a soluble form (Chen et al. 2006; Selvakumar et al. 2009). Many microbes are identified in the rhizosphere of horticultural crops with phosphate solubilisation effects such as *Bacillus megaterium* TV-91C, *Pantoea agglomerans* RK-92 and *B. subtilis* TV-17C in cabbage (Turan et al. 2014), *Agrobacterium rubi* A16, *Burkholderia gladii* BA7, *P. putida* BA8, *B. subtilis* OSU142, *B. megaterium* M3 in mint (Kaymak et al. 2008), *Azospirillum, Pseudomonas fluorescens*, and *Bacillus subtilis* in bitter gourd (Kumar et al. 2012).

25.5.3 Production of Stimulants of Plant Growth

Phytohormones such as cytokinins, gibberellins, auxins and ethylene are released by plant growth-promoting rhizobacteria, which influence a variety of activities like stem and root growth, flowering and fruit production (Ahmad et al. 2005; Ortega et al. 2017).

25.5.4 Antagonistic Activity and Biocontrol Agents

The bacteria that reduce plant disease incidence are regarded as biocontrol agents (Beattie 2007), whereas antagonists are the microbes that have antagonistic behaviour against plant pathogens (Ortega et al. 2017). Among these activities, the following actions can be illustrated.

(a) **Hydrolytic enzyme synthesis:** The different hydrolytic enzymes, for example, proteases, glucanases, chitinases and lipases, are synthesised that can lyse pathogenic fungal cells (Maksimov et al. 2011).
(b) **Siderophores production:** In iron (Fe)-limiting situations, plant growth-promoting rhizobacteria can create siderophores, which are iron-chelating chemicals that trap the available iron and deliver it to plants, encouraging their growth (Whipps 2001). It also has an antagonistic influence by stopping iron from being taken from the soil by other harmful bacteria and fungi. Four kinds of bacterial siderophores are classified as hydroxamates, carboxylates, phenol catecholates and pyoverdines (Crowley 2006).
(c) **Antibiotics production:** There are six groups of antibiotic compounds produced by plant growth-promoting rhizobacteria that are associated with root disease control, according to Haas and Défago (2005), which include phloroglucinols, phenazines, pyrrolnitrin, pyoluteorin, hydrogen cyanide and cyclic lipopeptides. In addition to other antibiotics, the pathogenic bacteria and fungi are active against colistin polymyxin and circulin (Ortega et al. 2017).

25.5.5 Induced Systemic Resistance and Systemic Acquired Resistance

Induced systemic resistance and acquired systemic resistance are two distinct events in plants, yet both trigger an immunological response to pathogenic agents. Induced systemic resistance consists of plant growth-promoting rhizobacteria or non-pathogenic rhizobacteria-induced self-plant resistance (Pieterse et al. 2003). Systemic acquired resistance is resistance that develops as a result of exposure to a pathogen. Induced systemic resistance and systemic acquired resistance act via different metabolic pathways. Although systemic acquired resistance induction is done via salicylic acid, jasmonic acid is required for induced systemic resistance (Pieterse et al. 2003). Induced systemic resistance-mediated protection is considerably lower than that provided by systemic acquired resistance (Van Loon 2000). Nevertheless, the two forms of protection occur with a greater impact at the same time than each separately (Ortega et al. 2017).

25.5.6 Industrial Applications of Plant Growth-Promoting Rhizobacteria

As can be inferred from the above, there are various applications of plant growth-promoting rhizobacteria and typically result in an option that is more environment friendly than pesticides and chemical fertilisers (Vessey 2003; Adesemoye et al. 2009). Optimal plant growth-promoting rhizobacteria for commercialisation must have the ability to contend with other microbes, expedite plant growth, have a large spectrum of activity, and be resistant to UV radiation, heat and oxidising chemicals (Nakkeeran et al. 2005). Despite several decades of commercial application of the *Enterobacter*, *Bacillus*, *Azotobacter*, *Klebsiella*, *Variovorax*, *Serratia* and *Azosprillum* species and with promising new laboratory studies, their effects on crops are not fully satisfactory (Ortega et al. 2017). For example, the use of plant growth-promoting rhizobacteria as fertiliser includes losses as a result of ecological conditions and runoff, during aerial application. There are, however, numerous ways to help the development of plant growth-promoting rhizobacteria. They are also applied to plant seeds (Bloemberg and Lugtenberg 2001) and, by taking advantage of plant exudates, plant growth-promoting rhizobacteria should be capable of settling in the rhizosphere once sown. Nano encapsulation-based technology, on the other hand, can be used as a tool for the safety of plant growth-promoting rhizobacteria and allow the more regulated release of plant growth-promoting rhizobacteria (Vejan et al. 2016). Experiments with genetic modifications can also enhance the establishment and functionality of the plant growth-promoting rhizobacteria (Ortega et al. 2017). Despite the large number of investigations related to mechanisms adopted by plant growth-promoting rhizobacteria and the mode of their activity, the complexity of interactions between plant growth-promoting rhizobacteria and plants makes it important to broaden the knowledge of this subject. Genetic and molecular research (Bloemberg and Lugtenberg 2001) could make it possible for these interactions in the rhizosphere to understood these better and help create new commercial products. Finally, due to recent developments in bioinformatics, DNA amplification refinement and computational progress, these studies can be reinforced by metagenomic advance (Leveau 2007). This will promote the identification of bacterial species in experimental crops and the tracking of population time periods during culture cycles (Bashan and de-Bashan 2002).

25.6 Rhizosphere Engineering

According to Hiltner (1904), the rhizosphere has been described as the soil compartment that is influenced by plant growth. This effect results from the release of organic materials by the plant, a process called rhizodeposition, consisting mainly of metabolites of plants (exudates) and plant debris (Hartmann et al. 2009). Jones et al. (2009) stated that this carbon loss is a large part of the photosynthetically fixed carbon allocated to the underground root system (from 20 percent to 40 percent). As a result, while most of the bare soils are considered oligotrophic environments, rhizosphere soils are defined as mesotrophic, promoting the growth of bacteria, archaea, viruses and fungal populations (Philippot et al. 2013). These microbes have various effects on the plant and on the overall functioning of the rhizosphere. They are the parts of the carbon cycle that help in the growth and development of plants and also provide resistance against pathogens. Plant rhizodeposition may change qualitatively and quantitatively due to microbial activity affecting microbial components, which is known as the characteristic feedback loop of the rhizosphere that keeps the rhizosphere in a dynamic equilibrium. Such a complex relationship indicates that the rhizosphere can be engineered to promote plant growth and health, or to minimise the effects of different biotic or abiotic stresses, a feature of significant interest in the current global climate change scenario and the need for more sustainable agricultural practices. Basically, all three components of the rhizosphere can be manipulated. The soil can be altered to change its physicochemical properties or improve its overall quality, the plant can be engineered to select or introduce a novel trait of interest, and the microbial populations can be selected to promote plant growth and health. For the development of sustainable agriculture, rhizosphere engineering can improve plant health and change the activity of root-associated bacteria. *Rhizobia, Pseudomonas, Bacillus, Burkholderia, Azospirillum, Klebsiella* and *Gluconacetobacter* are among the phytomicrobiomes used in diverse horticultural applications (Rahi 2017). It is important to identify the various root exudate molecules and their interactions with the rhizosphere microflora in order to use the capacity of the rhizosphere for plant growth and the associated climate. Knowing the interactions of the rhizosphere is important for the production of sustainable agroecosystems (Bhatt et al. 2020). Manipulation of the plant and its associated microbes affects the rhizosphere by releasing root exudation molecules that favourably influence microbial signalling compounds. Exudates from the roots differ depending on the genotype and species of the plant. Phytohormones, extracellular enzymes, organic acids, antibiotics, volatile signals and quorum sensing molecules are among the signalling substances secreted by microorganisms (Li et al. 2019). The alteration of plant and rhizosphere microflora for the enrichment of the rhizosphere zone for sustainable agriculture has been investigated in several studies (Fig. 25.2). Plant scientists have created genetically altered plants to address a variety of biotic and abiotic stress problems in the soil. Engineered plants change the pH of the rhizosphere and the secretion of root exudation. This change in the rhizosphere promotes a positive shift in microbial behaviour. Microbial engineering, in addition to plant engineering, is critical in agricultural research, particularly in the

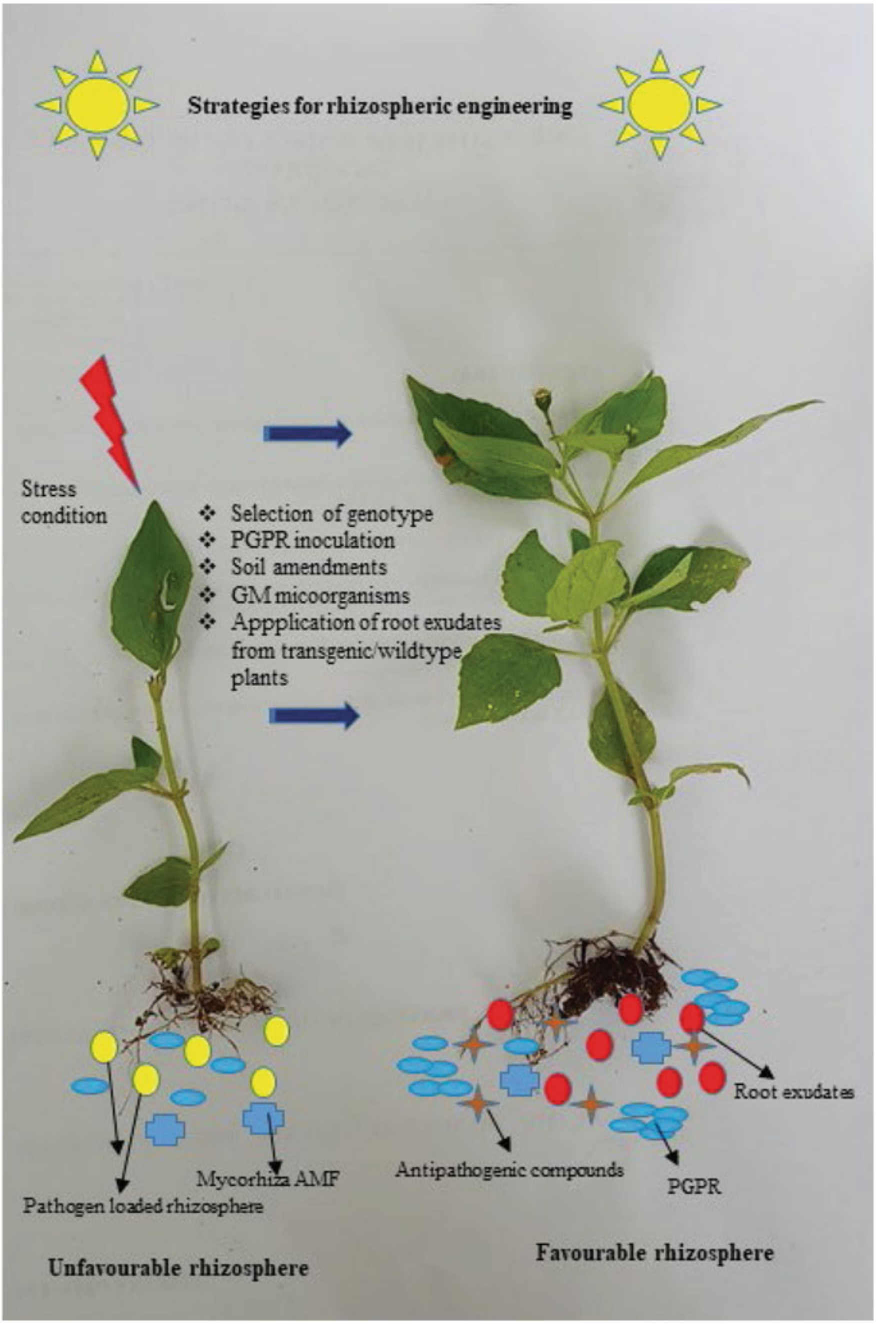

Fig. 25.2 Rhizosphere engineering for improving plant growth-promoting activities and balancing the soil environment

areas of PGPR, biological nitrogen fixation, phosphate and iron solubilisation, phytohormone regulation and biocontrol activities (Jochum et al. 2019). Because of the rhizosphere's numerous interconnected biomes, a shift in these interactions has the potential to alter plant growth and health, and soil qualities. Rhizosphere engineering is promising for soil enhancement, crop quality and production. The engineering of rhizospheres has been supported by a number of research studies. Resistance to major diseases including *Pseudomonas syringae* and the *Xanthomonas* genus has been produced using traditional breeding methods. PGPR microbe engineering involves rescuing chromosomes from resistant to susceptible wheat lines, such as replacing the susceptible wheat line S-615 chromosome for Apex chromosome 5B, resulting in SA5B chromosome substituent lines that are as resistant to common rot as Apex (Zhang et al. 2018; Guichard et al. 2019; Chu et al. 2020). Plant density plays a crucial role in phytomicrobiome existence. Traditional breeding considered plant density as an important factor in crop improvement. The genetic capacity for yield and other traits are completely expressed when plants are grown at extremely low plant densities, but it is greatly inhibited at high plant densities (Fasoula 2013).

The intimacy of this contact between the plants and their environment is critical for the acquisition of water and nutrients, as well as for beneficial interactions with soil-borne microbes. This same intimacy, however, increases plants' susceptibility to a variety of biotic and abiotic stresses. Plants have developed a number of mechanisms to adjust the rhizosphere in order to reduce the effects of various environmental stresses, and an understanding of the processes involved can reveal ways in which the rhizosphere can be exploited to improve plant health and productivity. Rhizosphere engineering will eventually minimise our reliance on agrochemicals by replacing their role with helpful bacteria, biodegradable biostimulants or transgenic plants. Some of these materials and procedures are still in the development stage, while others are being tried in the field. Engineered horticulture plants for rhizosphere enrichment are shown in Table 25.3.

25.7 Benefits of Rhizosphere Engineering

Various benefits of rhizosphere engineering in horticultural crops have been illustrated in Fig. 25.3 and discussed below.

25.7.1 Drought Resistance

In the twenty-first century, a major problem for agriculture is making the most productive use of water supplies possible. The broad availability of input services such as fertiliser and water has been the basis of modern agricultural production, but it is now widely acknowledged that water shortages are one of the main constraints in meeting the inevitably rising demand for food in the world as the world's population continues to grow. Therefore, increasing the resistance of plant drought

Table 25.3 Rhizosphere engineering: engineered horticultural plants for rhizosphere enrichment

Engineered Plant/ microbes	Gene	Host	Effect	Reference
Papaya	Papaya ring spot coat protein gene	Papaya	Virus resistant plants	Azad et al. (2014)
Cucumber, Canola	*Pseudomonas fluorescens* (CHA 0) transformed with ACC deaminase gene acdS from *P. putida* UW4	–	Improved root architect and plant protection	Wang et al. (2000)
Potato	Bacterial lactonase gene Aii A	*Bacillus* sp.	Protect from plant pathogen Pectobacterium	Dong et al. (2000)
Lotus corniulatus	Opines biosynthesis gene	Agrobacterium tumefaciens	Phytoremediation	Savka et al. (2002)
Citrus sweet orange	Pattern recognition receptor FLS 2	Tobacco (*Nicotiana benthamiana*)	Canker resistance and defence	Hao et al. (2016)
Radish	Heterologous gene encoding siderophore responsible for iron uptake	*Pseudomonas fluorescens*	Improved the competitiveness in soil	Raaijmakers et al. (1995)
Yellow lupin	pTOM toluene-degradation plasmid	*Burkholderia cepacia* G4	Phytoremediation	Barac et al. (2004)
Tomato	Cf-4 (Fungal gene)	Wild tomato	Resistance to the fungal tomato pathogen *Cladosporium fulvum*	Oliver et al. (2000)

and improving the capacity of agricultural crops to extract water from the soil are the main research objectives. To increase water efficiency in crop production, many advocate the use of genetic modification. Genetically modified crops, however, may have unforeseen evolutionary repercussions for ecosystems. Another potential technique used by plants to maximise water absorption is to change the rhizosphere, the atmosphere in which the roots grow and interact with them (York et al. 2016). The presence of mucilage, a polymeric gel that exudes from most plant roots, is of particular interest. Recent studies have drawn attention to the role of mucilage in forming hydraulic properties in the rhizosphere and controlling the absorption of root water. The mucilage keeps the rhizosphere moist and conductive during drying, but it becomes hydrophobic upon drying, restricting the absorption of root water. Ahmed et al. (2018) used the concept of rhizoligands, defined as additives that (i) rewet the rhizosphere and (ii) decrease mucilage swelling, thus reducing the conductivity of the rhizosphere. They stated that the interaction between rhizoligands and the mucilage exuded by roots caused a decrease in transpiration in *Lupinus albus*. Rolli et al. (2015) reported that *Acinetobacter* sp. S2 and *Bacillus*

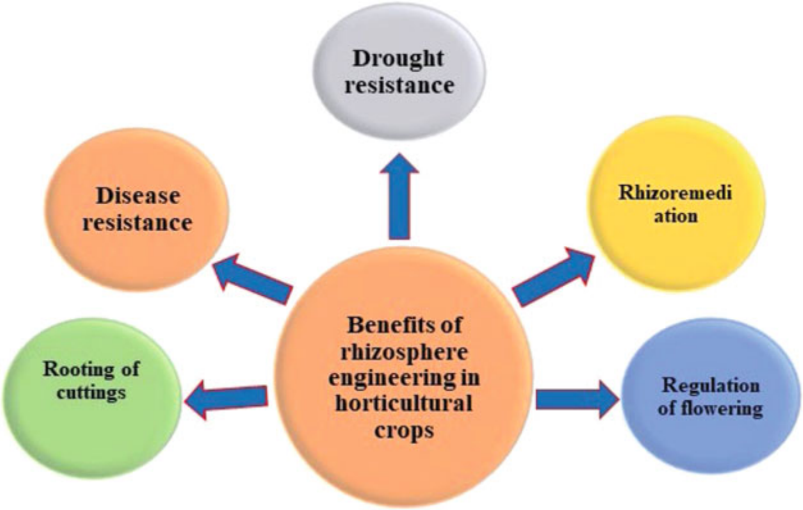

Fig. 25.3 Benefits of rhizosphere engineering in horticultural crops

sp. S4, *Sphingobacterium* sp. S6, *Enterobacter* sp. S7 and *Delftia* sp. S8 enhanced drought tolerance in *Capsicum annuum*, as well as increased fresh root, aerial biomass and photosynthesis. Nordstedt et al. (2020) identified two Pseudomonads, *P. poae* 29G9 and *P. fluorescens* 90F12–2, that enhance the quality of three ornamental plants, that is, *Petunia* × *hybrida*, *Impatiens walleriana*, and *Viola* × *wittrockiana*, under drought conditions.

25.7.2 Disease Resistance

In general, bacterial and fungal pathogens are a significant threat to sustainability, quality and yield of horticultural crops. Therefore, growers follow various practices such as proper sanitation of the planting fields, crop rotation, use of disease-resistant cultivars and indiscriminate use of chemicals to minimise the yield losses caused by phytopathogens and therefore to maximise vegetable output. Despite following too many tactics, including the excessive use of chemicals, significant success in combating plant diseases has not been achieved in horticultural production. Therefore, to boost production and to increase the yield by minimising the losses due to pathogen attacks, in recent times, emphasis has been shifted towards the use of cheap, eco-friendly and viable alternatives such as PGPR in the management of plant diseases to minimise yield losses therefrom. This has significantly increased the growth, yield and quality of many vegetables due to the application of PGPR. Shanthiyaa et al. (2013) reported that late blight of potato can be effectively controlled by applying PGPR, that is, *Chaetomium globosum*; *Burkholderia cepacia*. *Pseudomonas* spp. isolated from pea, wheat, cotton, tomato, sugarbeet and tobacco reduced disease severity and abundance of *Ralstonia solanacearum* in tomato plants (Hu et al. 2016). Jayapala et al. (2019) stated that rhizobacteria *Bacillus* spp. induce resistance against anthracnose disease in chilli by activating host defence response.

25.7.3 Rhizoremediation

An emerging field of interest is the use of plants and microbes for the rehabilitation of heavy-metal-contaminated habitats because they provide an ecologically sustainable and healthy approach for restoration and remediation. Wu et al. (2006) showed that the expression of a metal-binding peptide (EC20) in *Pseudomonas putida* 06909, a rhizobacterium, not only strengthened cadmium binding, but also alleviated cadmium cell toxicity. More significantly, inoculation of the sunflower roots with the engineered rhizobacterium resulted in a substantial decrease in the phytotoxicity of cadmium and a 40 percent increase in the accumulation of cadmium in the plant roots. The use of EC20-expressing *P. putida* with organic-degrading capabilities could be a promising strategy for remediating mixed organic-metal-contaminated sites due to the significantly improved growth characteristics of both the rhizobacterium and the plant. Wang et al. (2020) screened bacterial population for heavy metal resistance Its effect on reducing Cd2+ and Pb2+ concentrations in water spinach (*Ipomoea aquatic* Forsk.) and reported three heavy metal-immobilising bacteria, *Enterobacter bugandensis* CQ-7, *Bacillus thuringensis* CQ-33 and *Klebsiella michiganensis* CQ-169 produced siderophores and IAA and were highly resistant to Cd2+ and Pb2+. The results showed that heavy metal-immobilising bacteria played significant role in vegetable growth and accumulation of metals. The findings also highlighted that the efficacy of heavy metal-immobilising bacteria-vegetable systems must be checked and experimental designs should be developed in managed vegetation, taking into account the precise matching of vegetables and bacteria.

25.7.4 Regulation of Flowering

It was found that microbial communities in the rhizosphere could modulate the timing of *Arabidopsis thaliana* flowering. By converting tryptophan to the phytohormone indole acetic acid (IAA), rhizosphere microorganisms that increased and prolonged N bioavailability via nitrification delayed flowering, thereby downregulating genes that cause flowering, and stimulating further plant development (Lu et al. 2018). Manipulation of flowering time through microbial community is a very good approach which will help the horticulturist produce crops during the off-season.

25.7.5 Rooting of Cuttings

Most of the fruits and floriculture crops are being propagated by vegetative means. PGPR play a very important role in the rooting of plants. To obtain organic nursery material, the use of PGPR for the multiplication of nursery material may be necessary because the use of all formulations of synthetic plant growth regulators such as indole-3-butyric acid (IBA) are banned worldwide. In addition, the success of root promotion depends on the strain and genotypic response of the plant species used. Erturk et al. (2010) studied the effect of PGPR (*Bacillus* RC23, *Paenibacillus polymyxa* RC05, *Bacillus subtilis* OSU142, *Bacillus* RC03, *Comamonas acidovorans* RC41, *Bacillus megaterium* RC01 and *Bacillus simplex* RC19) on rooting of cuttings of Kiwifruit and revealed that the highest rooting ratios were obtained when the cuttings were treated with *Bacillus* spp. Sezen et al. (2014) reported that inoculations with PGPR stimulate adventitious root formation on semi-hardwood stem cuttings of *Ficus benjamina*. Researchers reported that *Bacillus subtilus* (BA-142) may possess great potential for promoting adventitious root formation in *Ficus benjamina*. The abovementioned finding suggests that PGPR can be used in the organic production of nursery material.

25.8 Conclusion

Numerous microbial inoculants have been established due to the high potential of microorganisms to enhance plant development, stress tolerance and health, but many of them show poor performance in the field. Several approaches can contribute to improved field performance, such as the design of the able microbial consortia, the selection of agricultural management practices that favour beneficial microbiota or a new generation of approaches to plant breeding. Various studies have shown that the rhizosphere can be engineered by selecting the right crop species and varieties, by adding microorganisms or soil alterations, and by manipulating plant and microbial processes genetically. The development of molecular technology now allows for the direct alteration of genes that influence rhizosphere functions. Biotechnology would ensure more progress in the future. Our capacity to alter the rhizosphere efficiently and predictably remains a problem, despite encouraging improvements in a variety of areas. It is important for the scientists to continue their work so that the public can be benefitted from secure, sustainable and environment-friendly agricultural practices.

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Conflict of Interest Authors declare no conflict of interest.

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