Manoj Kumar Solanki
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# Phytobiomes: Current Insights and Future Vistas

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# Preface

This book addresses the updates about the plant-associated microbiomes and their contemporary uses. To satisfy the food demands of the global population, advanced technology-based research is needed that can extract the information from the plant metabolism and microbial gene pools up to the complexity. Modern biotechnological tools can unlock the limitations of agricultural practices. However, the application of these tools is not well-equipped. Eco-friendly agriculture using microbial inoculants had positive influences on soil/plant health. Exploring the plant-associated microbial niches, especially endophytes, epiphytes, and soil microbes and how they are benefitting each other, can open new insights to develop sustainable agriculture practices by using consortia of microbes as plant helpers that recover the imbalanced agriculture systems and manage pathogenic diseases.

This book inculcates the gap between soil and plant helper microbiomes and their importance in the intensification of sustainable agriculture. New insights of phytobiome are explored in various chapters on a variety of interrelated aspects of the fascinating areas like plant microbial interaction, integrated pest management, soil fertility intensification, sustainable crop production, and disease management. This book is also entitled to yield a plethora of information about how beneficial plant microbiomes are currently being utilized for smart farming practices. To resolve the global food problem without harming the soil and environment health, this book covers valuable information regarding the significance of microbes in the amelioration of plant and soil health. Application of advanced molecular tools in plant disease diagnosis and pathogen isolation has also been discussed in order to advance the crops and human health. Some chapters have also been written to present the latest information related to phytobiomes and their range as far as their utility is concerned. Information on plant health promoter, endophytes as microbial elicitors, fly ash and plant health, zinc solubilizers, and their role in ecosystem engineering towards sustainable agriculture have also been presented.

This book is intended for everyone who is directly or indirectly involved in agriculture, bioinformatics, and all disciplines related to microbial biotechnology. These include academicians, scientists, and researchers at universities, institutes, industries, and government organizations who want to understand microbial linkages in a shorter time. This book also contains essential information that will help the nonspecialist readers to understand progressive research. We are confident that the current edition will be a milestone as chapters having updated information are

anticipated to be published in *Phytobiomes: Current Insights and Future Vistas*. The views conveyed by the expert/authors in their respective chapters are based on their vast experience in the plant-microbe interaction and associated research areas. We thank all the contributors to this book, and we are obliged for their valuable contributions.

Rishon LeZion, Israel — Manoj Kumar Solanki
Karnal, Haryana, India — Prem Lal Kashyap
Hazaribag, Jharkhand, India — Baby Kumari

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# Phytobiomes: Role in Nutrient Stewardship and Soil Health

1

Madhumonti Saha, Abhijit Sarkar, Trisha Roy, Siddhartha Shankar Biswas, and Asit Mandal

**Abstract**

The enormous potential of the phytobiome for better plant growth and productivity is an essential tool for sustainable agronomic practices for eco-friendly cultivation processes. However, microbes in relation to plants, environment, omics, and their interactions are still to be clearly explored. Current predictive formulae like niche origin, ecological traits, evolution, genetics or heterosis, and resource trades are not mutually exclusive. This chapter mainly emphasizes on phytobiome related to soil fertility, nutrient cycling, plant growth, and soil health. Plant-associated phytobiome such as rhizospheric and phyllospheric played a significant role in the enhancement of plant growth and yield. These organisms form multifarious networks that are established and regulated through nutrient cycling, competition, antagonism, and chemical communication mediated by a diverse array of signaling molecules. The integration of knowledge of signaling mechanisms with that of phytobiome members and their networks will lead to a new understanding of the fate and significance of these signals at the ecosystem level. Such an understanding could lead to new biological, chemical, and breeding strategies to improve crop health and productivity. Soil organic matter (SOM) is a heterogeneous mixture of materials that range in the stage of decomposition from fresh plant residues to highly decomposed material known as humus. SOM is made of organic compounds that are highly enriched in carbon. Though half of the global population depends on fertilizer N, atmospheric N fixation by rhizospheric microbes is essential for plant productivity in low N soils. Many plants form symbiotic associations between their roots and specialized fungi in the soil

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known as mycorrhizae; the roots provide the fungi energy in the form of carbon, while the fungi provide the plant with often-limiting nutrients such as phosphorus. Besides, some soil microbiomes play a crucial role in the solubilizing stable form of potassium and micronutrients by releasing some metabolites which act as chelating agents. We explain recent information and cracks in these areas using phytobiomes.

**Keywords**
Phytobiome · Soil fertility · Nutrient cycling · Soil organic matter · Soil health

## 1.1 Introduction

Developing countries like India need to produce a double quantity of foods to feed the population of the nation as well as the global crowds, whereas population growth touches 1.1% or 83 million annually. Feeding is not only mean full stomachs, but it mainly focused on nutritional security. The overall impact of rising demand for food production will depend on improved productivity via high-yielding varieties and sustainable use of bioenergy (FAO 2017), whereas these higher yields of agricultural crops are vigorously influenced by regular nutrient accessibility and higher soil quality for promoting plant growth. Hence, there is an excellent chance of input-output imbalance, meticulous use of chemicals, organic matter depletion, removal of nutrients, and massive exhaustion of soil system through the anthropogenic activity for sustainable food production (Godfray et al. 2010). A sustainable food production system, which delivers both adequate nutrition and appropriate energy to people in the society, also improves the natural environment intergenerationally. To maximize the sustainability of production, we require a good strategic plan, which would help to acquire knowledge regarding components that may establish and sustain a healthy, productive agroecosystem. Integration and incorporation of this information into the present crop production system may further increase the total coverage of existing farmlands (mostly rehabilitated from degraded and marginal lands). Hence, to achieve the food and nutritional security with limited increased lands, we should first concentrate the interactions among the components of phytobiomes.

The word "phytobiome" comprises *phyto* (plants) and *biome* (ecological area), which are very specific for plants and their surroundings. It includes plant interaction biology with sounding environments and living and nonliving objects (Leach et al. 2017). Living entities include microorganisms, macroorganisms, and other plants and animals, and nonliving entities include soil, air, and climate. Simply, we can explain phytobiome as a conjoint venture of the rhizosphere, phyllosphere, biosphere, spermosphere, hyphasphere/mycorrhizosphere, and detritusphere (Nelson 2004; Frey-Klett et al. 2007; Vorholt 2012; Hacquard et al. 2015; Leach et al. 2017). Thus it is easy to say that phytobiome includes all entities that have a direct and indirect influence on plants and vice versa (Beans 2017). Phytobiome extends

beyond the plant microbiome and unites facets including crop improvement and production, weather data modeling and forecasting, pest pollinators and pathogens, and data generation along with dissemination (Fig. 1.1). Moreover, phytobiome considers animals, pests, physical environment, management, and other pillars of sustainable agroecosystem that boost our scientific understandings and analytical aspects about plant microbiome also leveraging the computational power of "omics" (Young and Kinkel 2017). Spanning from fundamental to applied research aspects, researcher associated with phytobiome cover the areas of biotechnology, genetics-breeding, seed technology, agronomy, soil fertility and fertilizers, microbiology, plant pathology, nematology, entomology, meteorology, crop physiology, economics, statistics, extension, and more branches of agriculture (Fig. 1.1, Young and

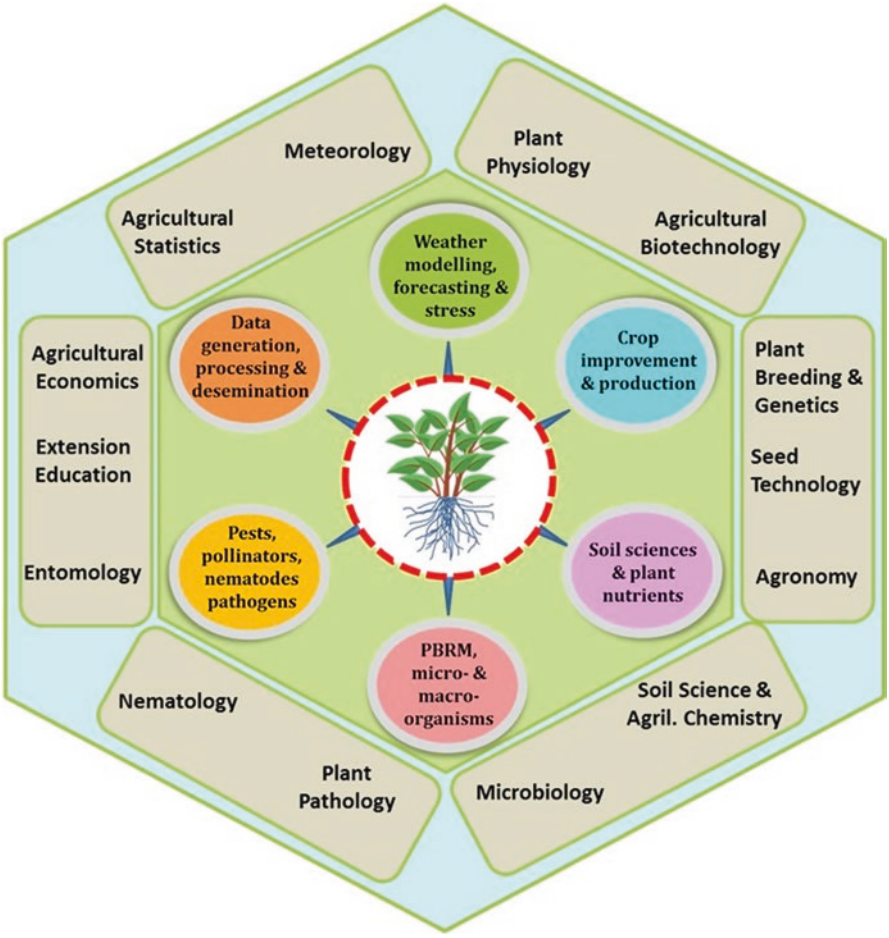


**Fig. 1.1** Phytobiome components and their interrelationship with different branches of agriculture. (Conceptualized and modified from Phytobiomes: A Roadmap for Research and Translation 2016; Young and Kinkel 2017)

Kinkel 2017). However, composition, characteristics, and functions of healthy phytobiome, principally soil-plant-microbe interaction, are yet to be understood entirely.

## 1.2 Plant-Soil-Microbe Interactions in a Typical Phytobiome

In a typical phytobiome, phyllosphere gets more affected from the discontinuity of weather abnormalities (diurnal cycle), leaf surface, plant metabolism, environmental conditions (rain, wind, direct sunlight, and UV radiation), and nutrient availability (oligotrophic) than the rhizosphere (Leveau and Lindow 2001; Miller et al. 2001; Remus-Emsermann et al. 2012). Phyllosphere is a partial environment as compared to rhizosphere (Vorholt 2012). Though phyllosphere is also an essential aspect in the phytobiome study, nutrient cycling and soil health are controlled by the rhizosphere. Accordingly, we would like to concentrate on rhizosphere and rhizospheric engineering associated with plant nutrition and soil health. Discussion on soil-plant-microbe interaction in the rhizosphere can provide information regarding the influence of phytobiome on soil health, soil fertility, nutrient dynamics, as well as crop productivity. This rhizosphere is mainly crammed with plant roots having a significant impact on microbial composition and diversity as well as their activities (Andersen and Winding 2004; de Vries and Shade 2013). These organisms constituted with macrofauna like earthworms, ant, and termite; mesofauna like collembolan and acari; microfauna like nematodes and protists; macroflora like plant roots; and microflora like bacteria, fungi, actinomycetes, and algae (Coyne 1999; Mendes et al. 2013; Sarkar et al. 2017a). The further rhizosphere is differentiated into the endorhizosphere (mucoid layer coated root) and ectorhizosphere (rhizosphere soil).

The community of soil microbes is so diverse than any other group of organisms that we are still able to explore very little diversity of genetic resources. Starting from decomposition, mineralization, or immobilization and subsequent humification in soil system up to plant growth and the functions of diversified microbial communities are affected mainly by management practices (Kennedy and Stubbs 2006). Nevertheless, soil respiration, microbial population, biomass, and enzymatic activities are significantly influenced by soil properties like pH, redox potential (Eh), clay mineralogy, soil organic matter content, and other nutrient levels (Sessitsch et al. 2001). In the humid subtropical mountainous ecosystems of India, urease activity was higher in the grassland ecosystem, whereas microbial population, dehydrogenase activity, and nutrient status were higher at Sacred Orchard (Arunachalam et al. 1999). While focused on soil structure, literature demonstrated that microbial biomass was the utmost concerted in silt and clay-sized soil particles, especially in silt and clay particle-associated micropores (5–30 mm) (Hassink et al. 1993a; van Gestel et al. 1996; Kandeler et al. 2000). Additionally, invertase, urease, and alkaline phosphatase activities were reported to be highest in the silty and clayey type of soil. Xylanase activity, which is considered to be the indicator of fungal activity (Kandeler et al. 2000), was found to be higher in sand particles (Stemmer et al. 1998a, b; Kandeler et al. 1999; Kirchmann and Gerzabek 1999).

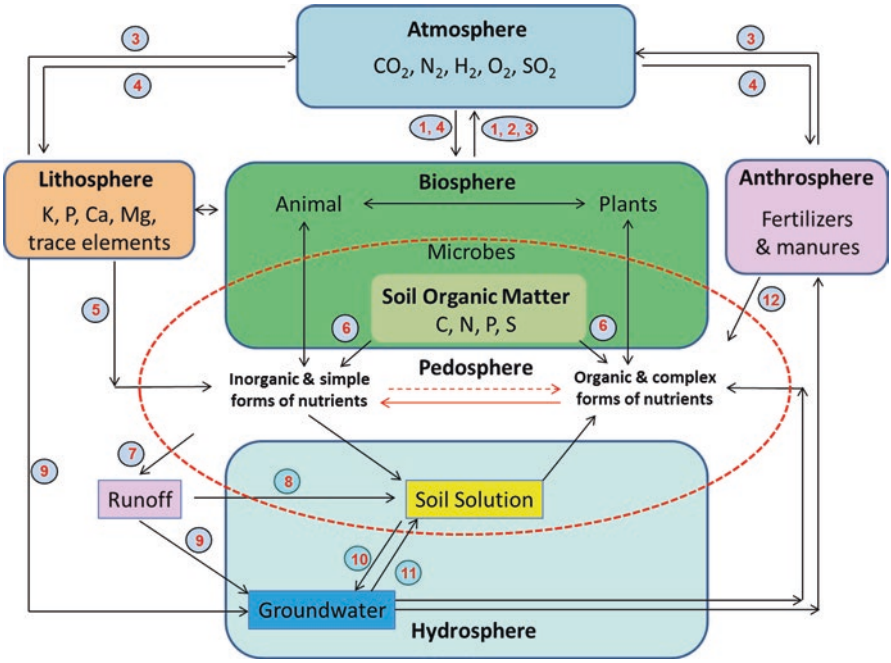


**Fig. 1.2** Nutrient cycling and interconnectivity of the atmosphere, lithosphere, biosphere, pedosphere, hydrosphere, and anthrosphere in a typical phytobiome
[1]photosynthesis; [2]respiration; [3]gaseous exchange; [4]deposition; [5]weathering; [6]decomposition; [7]erosion; [8]infiltration; [9]percolation; [1]leaching; [11]capillary rise; [12]manual application; red dashed arrow, immobilization; red solid arrow, mineralization

Though the population of fungi is much lower in grassland soil and arable fields, but its crucial role in the initial fragmentation of soil organic matter could not be ignored (Brussaard et al. 1990; Hassink et al. 1993b). Nutrient cycling and interconnectivity of the atmosphere, lithosphere, biosphere, pedosphere, hydrosphere, and anthrosphere in a typical phytobiome are represented in Fig. 1.2.

## 1.3 Beneficial Rhizospheric Microbes and Phytobiome

Rhizospheric microbes have an immense potential in sustainable, cutting-edge crop production. Furthermore, beneficial rhizospheric microbes (BRMs) are obligatory in biogeochemical nutrient cycles that drive soil fertility and crop productivity for the span of decades (Sarkar et al. 2017a). In general, these BRMs enhance the soil and plant productivity by means of (a) secreting hormones and other regulatory growth chemicals, (b) acquisition and bioavailable felicitation of essential nutrient elements, (c) inhibition of pathogens through biocontrol agent production, and (d) abiotic stress tolerance (Yang et al. 2009).

A better understanding of environmental stress and its bio-modulation has also been possible with PBRMs (Schrey and Tarkka 2008; Ryan et al. 2009). Symbiotic association of rice and arbuscular mycorrhizae shrinks the drought stress effects by producing an antioxidative response and photosynthetic efficiency (Ruiz-Sanchez et al. 2010). Even though PBRMs and other rhizospheric microbes are insignificant in size, their population and activity are so significant that they are also considered as "dinner in the dark: illuminating drivers of soil organic matter decomposition" (van der Waals and de Boer 2017).

Rhizospheric and non-rhizospheric microbes interact with each other either synergistically or antagonistically and are possible to change microbial community structure through differential soil, plant growth stages, feeding behavior on other taxa, acidity, reducing prey abundance, etc. (de Vries and Shade 2013; Leach et al. 2017). Preferential feeding like predation of gram-negative bacteria by protozoa (Andersen and Winding 2004) and predation over harmful bacteria by nematode may change the bacterial community. Interestingly, some *Pseudomonas* sp. could be able to prevent protozoan predation by producing antibiotics. The microbial collaborations and their network are recognized and synchronized but sometimes passive, through the fabrication and sensitivity of somatic and biochemical signals (Leach et al. 2017).

## 1.4 Phytobiome and Nutrient Stewardship in Soil

Though plants exclusively utilize inorganic forms of nutrients, soil organic matter (SOM) plays the "key to soil fertility" and nutrient dynamics in every soil systems, because SOM is the source for almost every essential plant nutrients. The content of SOM is considered as the indicator soil quality, microbial activity, and buffering capacity of the soil. Correspondingly, SOM could also be regarded as a critical element of phytobiome. Along with SOM dynamics, soil structure and nutrient cycling in the soil are greatly influenced by microbial processes, which are frequently affected by management (Kennedy and Stubbs 2006).

### 1.4.1 C-Cycle and Phytobiome

Natural and agricultural-based soils are the significant source and sink for the dynamic flow of atmospheric $CO_2$ as a result of the soil microbe-derived respiratory flux of $CO_2$. Hence, concentrations of atmospheric $CO_2$ are more sensitive to minute changes in the soil carbon cycle. Higher plants utilized atmospheric $CO_2$ for photosynthesis, whereas microbes play the key role to maintain the source-sink relationship of SOC by altering the mechanisms involved in plant symbionts, detritivores, and phytopathogens. Moreover, preserve soil C by their death cells and plant roots (Amato and Ladd 1992; Lal 2004; King 2011). It has been reported that land use and management system influence soil ecosystems with the proliferation of microbial diversity which favor sequestration of C in soil (Singh et al. 2010). So, here we will discuss

microorganisms responsible in the global carbon cycle as well as climate change. This detail is required to maintain practical management of the soil-plant continuum to nourish the microbes for stimulating soil carbon storage in agricultural soil.

#### 1.4.1.1 Endurance of Soil Organic Matter and Its Microbial Decomposition

The input of organic matter into the soil system is followed by two ways: (a) aboveground plant litter, crop residue, and organic manure including soluble organic carbon which penetrate into the soil with irrigation water and rainfall and (b) belowground roots and its rhizodeposition. Rhizodepositions often consist of simple organic compounds like carbohydrates, amino acids, organic acids, alcohols, etc. Perhaps with time rhizodeposition becomes humidified, which contains complex compounds like cellulose, hemicellulose, lignin, etc. (Wallenstein and Weintraub 2008). Mycorrhizal fungi play a vigorous role in the denaturation of the humified polymer. This mycorrhizal symbiosis seems to be found in approx. 85% of all plant communities usually in herbaceous crop (Smith and Read 2008). An experimental study shows that fungal partners are satisfied with the shifting of 20% (Nakano-Hylander and Olsson 2007) or even 30% (Drigo et al. 2010) of total assimilated carbon by plants having intense effects on rhizodeposition. A fraction of the plant carbon that is moved to the mycelia is rapidly respired back to the atmosphere, which results in a crack in the soil carbon cycle.

A large portion of biomass is decomposed by heterotrophic microbial respiration causing loss of soil C. In addition, a small amount of the original C is reserved in the soil through the formation of stable organic carbon (Reynaldo et al. 2012) that results in increased SOC stocks through C sequestration over time. The main microbial modulators for soil C storage are fungi and bacteria. The fungal/bacterial ratio is closely related with C sequestration potential in soils with more massive fungal abundance being correlated with higher C stock. Greater storage of C in fungal-dominated soils can be endorsed to improved C use efficiency, more extended protection of C in living biomass, and recalcitrant portion resulting in longer resident time of C. Nevertheless, these observations are only relative, and it is still questioned whether fungal communities support soil C storage or whether soil with higher organic C favors soil fungi. Moreover, it can also be debated that fungi can negatively affect C storage due to their higher efficacy to decay recalcitrant litter (Cheng et al. 2012).

#### 1.4.1.2 Microbial Functions in Soil Carbon Cycle Subsequent Climate Change

Responses of climate change through carbon cycle are tangled because of microbial aids, their individual effects, and collaborations with other factors (Bardgett et al. 2008; Singh et al. 2010). A simple example of response to global warming is microbial activity, organic carbon decomposition, and $CO_2$ release, which may be hastened in response to an increase in temperature (Davidson and Janssens 2006). This is further established by field observations that confirmed the positive correlation between higher respiration rates and elevated temperature regimes. Besides, a sign

of secondary positive feedback to raise $CO_2$ is a result of the photosynthetic production of carbon fertilization, whereas increased atmospheric $CO_2$ accelerates photosynthesis (Bond-Lamberty and Thomson 2010) and the release of root exudates; consequently more labile carbon will be available for microbial decomposition and respiration. Readily available exudates after increased root deposition may "prime" the turnover of a passive pool of SOM that otherwise would not be subject to decomposition (Koyama et al. 2018). This difficulty is intensified by the diversity of soil ecosystems across the globe, which varies in their function due to their differences in forming factors including climate, organisms, relief, parent material, and time. Main interests on microbial activity in carbon cycle have been raised in peaty and frozen soils, where climatic conditions are not predicted for the addition or conservation of organic material, which may not be in favor of future climate, eventually in the release of sufficient quantities of $CO_2$ to the atmosphere (Srivastava et al. 2017). Further research in this area is very crucial if we can calculate the impacts and feedbacks between climate change and microbial function.

#### 1.4.1.3 Microbial Growth Dynamics and Energy Balance

Chemotrophic soil microbes fix soil C and N by synthesizing new biomass (Sokatch 1969; Dawson 1974). The energy balance and consequent thermodynamics equations are listed subsequently (adapted from Chen et al. 2003):

$$\varepsilon X \Delta Gr = -\Delta Gs \tag{1.1}$$

where $\Delta Gr$ is the energy released during respiration, $\Delta Gs$ is the energy carrier (e.g., ATP) required to synthesized biomass, $\varepsilon$ is the coefficient of energy transfer from ATP, and $X$ is the balanced ratio of $\Delta Gr$ (ATP used) and $\Delta Gs$ (microorganisms involved).

Stoichiometric yield coefficient ($Y$, biomass formed the unit amount of C or inorganic ingredients consumed (Dawson 1974; Chen et al. 2003)) is calculated as follows:

$$Y = \frac{\alpha}{\beta\left(1+X\right)} \tag{1.2}$$

where $\alpha$ is the biomass (mg) formed from unit kcal energy consumption and $\beta$ is the mass of organic and inorganic substances (mg) used to produce unit kcal energy:

$$\gamma \max = Yk \tag{1.3}$$

$$k = \frac{Y}{\gamma \max} \tag{1.4}$$

where $\gamma$max is the maximum specific microbial growth rate ($h^{-1}$ $mg^{-1}$ biomass) and $k$ is the maximum specific substance utilization rate (mg $mg^{-1}$ biomass $h^{-1}$) when ignoring microbial decay or maintenance. The rate of respiration, i.e., energy-yielding reactions, is constant and varying between 0.5 and 2.0 $mg^{-1}$ biomass $h^{-1}$ in many heterotrophs, autotrophs, aerobes, and anaerobes.

From this declaration, the maximum specific substance utilization rate ($k$) and the maximum specific microbial growth rate ($\gamma$max) are expressed as Eqs. 1.5 and 1.6, respectively:

$$k = \frac{\beta\left(0.5 \sim 2.0\right)\left(1+X\right)}{X} \tag{1.5}$$

$$\gamma \max = \frac{\left(0.5 \sim 2.0\right)\alpha}{X} \tag{1.6}$$

From Eq. 1.6, it is clear that both energy sources and microorganisms determine the maximum specific microbial growth rate ($\gamma$max). Thus, microbial growth in soil is dependent upon the types and diversity of microbes, and SOM content is described by Monod's equation (Sokatch 1969; Dawson 1974; Chen et al. 2003):

$$\gamma = \gamma \max \frac{S}{\left(Ks + S\right)} \tag{1.7}$$

where $S$ represents limiting nutrient (mg $g^{-1}$), γ represents the specific growth rate ($h^{-1}$ $mg^{-1}$ biomass), and $Ks$ is the half-velocity constant (Michaelis-Menten constant). $Ks$ reproduces the affinity of the microorganisms for the growth-limiting nutrient and governs how quick the specific growth rate can reach the maximum rate and for efficient microbial work ($Ks$ should be kept as small as possible). The Contois equation designates heterotrophic microbial growth; Eqs. 1.8 and 1.9 represent the dynamics of SOM depletion and biomass formation:

$$\frac{dA}{dt} = \frac{\gamma \max SA}{Ks + S} - kdA \tag{1.8}$$

$$\frac{dS}{dt} = -\frac{1}{Y}\left[\frac{\gamma \max SA}{Ks + S} - kdA\right] \tag{1.9}$$

where $A$ is the biomass (mg $g^{-1}$) and $k_d$ is the specific microbial decay rate ($h^{-1}$ $mg^{-1}$ biomass). Complete understanding of the terrestrial microbial association and particular processes that govern the degree and fate of C dynamics will increase the prospect of successful management of the terrestrial ecosystem for increasing the stable C reservoir.

### 1.4.2 N Cycle and Phytobiome

Nitrogen (N) is an essential nutrient required by plants for their growth and metabolism. The rate of N inputs in the agricultural system has doubled during the last century, potentially affecting both terrestrial and aquatic ecosystems. Even after excess application, it is often misplaced through volatilization, denitrification, or leaching and causes to limit its availability in most of the cultivated soils.

### 1.4.2.1 Nitrogen Fixation and Transformations

Although N abundantly exists in the atmosphere, the diatomic ($N_2$) molecule is a comparative inert. Thus, N fixation by reducing the inert $N_2$ molecule into $NH_3$ is a complex process with the expense of an enormous amount of energy (Postgate 1982). Soil N stock and its variation depend on N transformation based on microbial dynamics and environmental conditions. The N transformation is mainly controlled by soil and residue C/N ratio (Chen et al. 2003). Other than C/N ratio, soil texture, pH, fertilizer quantity, crop rotation, soil and air temperature, soil moisture, and rainfall are some of the controlling factors of N transformation and stock variations (Nave et al. 2009; Schipper and Sparling 2011). The diazotrophic microbes, which belong to the prokaryotic groups, fix atmospheric $N_2$ with their normal metabolic process (Galloway et al. 2008). Some of these microbes are free-living in the soil like *Cyanobacteria*, *Proteobacteria*, *Archaea*, and *Firmicutes* (Reed et al. 2011). Other organisms, including *Azotobacter* and *Azoarcus* genera, are also present at equivalent densities in the rhizosphere and non-rhizosphere soil. Bacterial genera like *Herbaspirillum* and *Azospirillum* colonize only in the higher plants' rhizosphere (Mrkovacki and Milic 2001). On the other hand, some symbionts like *Rhizobia* are capable of infecting the root and form root nodules. Interestingly, this symbiosis has often limited to legumes. Thus, N fixation related to *Rhizobium* sp. has become a more exciting issue for researchers to unveil these complex environmental phenomena. A higher impact on primary productivity and economic feasibility is also one of the pillars for a sustainable agricultural ecosystem.

Bacterial N fixation is a complex microbial process, where enzyme complex called *nitrogenase* (*dinitrogenase reductase* and *dinitrogenase* metal cofactor) takes part in the electron transport chain: the former enzyme serves as an electron donor, and the latter (substrate reduction component) accepts the energy of the electron to convert $N_2$ to $NH_3$. To generate one mole of $NH_3$, 16 moles of adenosine triphosphate (ATP) is required, which are obtained from the oxidation of organic molecules. The N cycle and its processes are dynamic and simultaneously proceed to maintain equilibrium to nature. A diverse pool of nitrogenous compounds including organics (proteins, urea, amines, etc.) and inorganics ($NH_4^+$ and $NO_3^-$) in soil along with gaseous forms like NO and $NH_3$ in troposphere determines N dynamics and bioavailability to the plants. Other than this, nitrification that is controlled by nitrifying bacteria includes nitrifiers (oxidizing ammonia to nitrite) and nitratifiers (oxidizing nitrite to nitrate). There are five common species of nitrifiers, *Nitrosomonas europaea*, *Nitrospira briensis*, *Nitrosococcus nitrous*, *Nitrosococcus oceani*, and *Nitrosolobus multiformis*, and three nitrifiers, *Nitrobacter winogradskyi*, *Nitrospina gracilis*, and *Nitrococcus mobilis*. Denitrifying microorganisms include *Thiobacillus denitrificans* and *Micrococcus denitrificans* and some species of *Serratia*, *Pseudomonas*, and *Achromobacter* (Delgado and Follett 2002).

### 1.4.2.2 N in Soil and Its Mineralization

Being the most extensively limiting plant nutrient in agriculture, fertilizer N is the main depending material for half of the global population for their daily

hand-to-mouth activities. Total N content varies from 0.02% to 0.44% across the globe, the tropical country like India lacks SOM, causing consequently low soil N in surface soil layers. In India the total N content of surface soil ranges from 0.02% to 0.13% (Motsara 2002). Whether in the forest cover or the agricultural fields, crop-covered soils result in 10–20 times higher soil N content than the open fallow lands. Excessive cultivation leads to disintegration and decomposition of SOM, which leads to a decrease in N content in soils. Studies have shown that up to 3.5% of organic N in soil is mineralized annually. Even this rate of mineralization can take care of the normal growth of plants, except under sandy soils with inferior organic N; also crop demand of N is more than the mineralization rate (Chen et al. 2003). In a sandy loam soil, a 50% combination of sewage sludge and fertilizer could be one of the possible and best alternatives to curb fertilizer N and enhance N use efficiency (Biswas et al. 2017). As of advances in technology, the N stable isotope ($^{15}N$) technique has been widely used as a measuring tool in ecological studies of N cycling. This because of $^{15}N$ natural abundance values (δ15N) of soil samples is the net result of many biogeochemical processes that can cause $^{15}N$ refinement (Frank et al. 2000; Ghosh et al. 2018).

### 1.4.3 Phosphorus Nutrition and Phytobiome

Phosphorus (P) is one of the major nutrients essential to sustain all forms of life and is indispensable for the functioning of virtually every living cell on this planet. It is essential for energy metabolism and the principal component of adenosine diphosphate (ADP) and adenosine triphosphate (ATP). It is an important constituent of the genetic components deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), and plays an important role in almost all metabolic processes in plants. An adequate amount of P is essential for root development, growth, and maturity of all the crop plants. It is second only to nitrogen (N) in terms of its limiting nature to plant growth, in almost all arable soils across the globe.

#### 1.4.3.1 Phytobiomes and P Nutrition in Plants

The complex chemistry of P and its widespread deficiency in the world soils have driven the plant community to develop and adopt several strategies to improve the acquisition of P from marginally P-deficient soils. Changes in the root configuration, morphology, and distribution (de Souza et al. 2016), production of different organic compounds by the roots which helps in dissolution of inorganic P compounds in soil, and alliance of the plant roots with beneficial microorganisms in the rhizosphere (Zhou et al. 1992; Hasan 1996; Sharma et al. 2007) are some of the unique strategies adopted by the plants to strive over P-deficient situations. It is estimated that the amount of P fixed in world soils would be able to sustain crop production for the next 100 years if properly mined using available techniques (Goldstein et al. 1993).

### 1.4.3.2 Microbiome and P Nutrition in Plants

The phosphate-solubilizing microorganisms (PSMs) are essential for P nutrition of plants, and in the soil, the population of phosphate-solubilizing bacteria is higher by 2–50-fold compared to fungi. However, the fungal isolates in both solid and liquid culture media exhibited higher P-solubilization capacity compared to bacteria (Gaur et al. 1973; Banik and Dey 1982; Kucey 1983; Harrison 1987; Kucey et al. 1989). Of the various P compounds present in the soil, the Ca-P is more readily solubilized by the PSMs, while very few are reported to solubilize the Fe and Al-P which are the major compounds in acid soils like alfisols (Fig. 1.3). The PSMs are very effective in insolubilization of rock phosphates (RP) which are generally Ca-apatites and increase the P availability to crops when applied along with rock phosphates in soil. Currently, different species of bacteria such as *Azotobacter chroococcum*, *Bacillus subtilis*, *Bacillus cereus*, *Bacillus megaterium*, *Arthrobacter ilicis*, *Escherichia coli*, *Pantoea agglomerans*, *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Microbacterium laevaniformans*, and *Micrococcus luteus* have been identified as effective P solubilizers (Kumar et al. 2014).

The microbial association thus plays a significant role in P nutrition particularly in the low P soils, and often the level of soil P determines the nature of microbes that get associated with plant roots (Gomes et al. 2018). Different maize genotypes with variable P acquisition capacity had a different association with microorganisms, and the P levels in the soil primarily guided this. The slow-growing microorganisms were more abundant in soil with low P levels, while the fast-growing

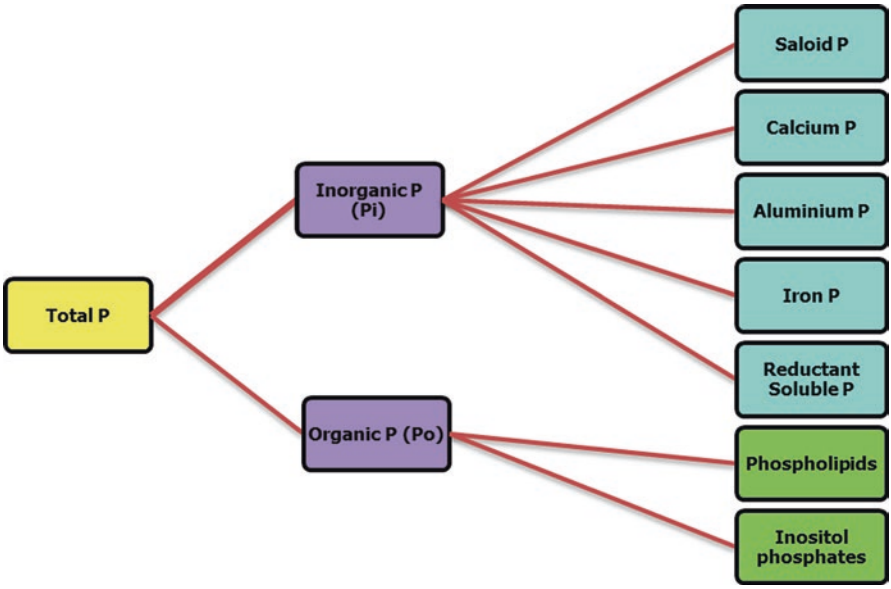


**Fig. 1.3** Different fractions of soil P based on sources and chemical properties

microorganisms were predominant in the soils with high P levels (Gomes et al. 2018). In general, several bacterial species like the *Pseudomonas*, *Enterobacter*, *Azotobacter*, *Burkholderia*, *Rhizobium*, etc. are associated with P-solubilization and promote plant growth (Oliveira et al. 2009; Glick 2015; de Souza et al. 2016; Alori et al. 2017). However, the population of phosphate-solubilizing organisms was not affected by the level of P in soils (Browne et al. 2009; Gomes et al. 2018; Robbins et al. 2018).

Besides the P-solubilizing bacteria, the arbuscular mycorrhizal fungi (AMF) play an essential role in the P nutrition of plants. The hypha of the AMF acts as an extension to the plant roots and helps in exploring more soil volume, increasing the P availability to plants, and acting as an unhindered pathway for translocation of P (Schweiger and Jakobsen 1999). However, the soil P level is again critical, which regulates the abundance of AMF association with plant roots. Low soil P level increases the AMF association with plant roots, while P fertilization harms the AMF establishment. The AMF colonization in maize reduced from 29.2% to 13.7% when the soil P concentration was raised from 15 ppm to 70 ppm (Gosling et al. 2013). The plant species is an essential factor determining the association of AMF, and it is higher for crops like maize, cotton, pigeon pea, sunflower, mung bean, and sorghum while lower for oats, wheat, barley, etc. (Seymour 2009).

Sometimes even the non-P-solubilizing microbes play an essential role in P nutrition. These organisms take up sparingly soluble P compounds from the soil with the help of high-affinity P transporters, which becomes available to plants upon the death of the microbes (Gyaneshwar et al. 2002). The extremely insoluble P compounds are also cycled and become available for plant nutrition. *Escherichia coli*, a non-P solubilizer helps in the P nutrition of plants (Wanner 1996). Under P-limiting situations, the P transporters in *Rhizobium* are activated, resulting in higher accumulation of P and enhanced alkaline phosphatase activity (Al-Niemi et al. 1997).

#### 1.4.3.3 Rhizosphere Engineering and P Nutrition

Root-induced changes in the rhizosphere also help in P acquisition and improve the P availability to plants (Hinsinger 2001). The plant root secretes a large number of substances that alter either the soil pH or chelating substances which are capable of releasing P from sparingly soluble P compounds. The secretion of organic acid molecules like citrate, oxalate, etc. also helps in P release through ligand exchange and increases P availability to plants (Shen et al. 2011). The plant roots also secrete enzymes like phosphatase or phytase, which can mobilize the organic P from the soil and make it phyto-available (Neumann and Romheld 2002; Zhang et al. 2010). Some of the new hypotheses are postulated based on the PSM-inspired chemistry by deriving polymer-coated P fertilizers to enhance PUE (Sarkar et al. 2017b; Sarkar et al. 2018). Also, nano-formulation of natural resources like phosphate rock increases the specific surface area that helped in better microbial activity and further improved PUE with or without organic acids (Roy et al. 2015, 2018a, b).

### 1.4.4 K-Bioavailability and Phytobiome

For achieving sustainable crop productivity, nutrients are the necessary inputs, and potassium is one of them. The flaws between depletion and the use of K fertilizers in agriculture are flaring day by day. So, it is relative to know the potassium dynamics in soil corresponding with potassium needed for the crops which offer balanced nutrition and retain its status in soils. Addressing this nutrient inequity and deficiencies, increased manufacture of potassium fertilizer is necessary for India and other developing countries. In these situations, it is required to prosper natural mineral sources of K to replace costly fertilizers. Although these minerals are not readily soluble, the combination of potassium minerals with a potassium-solubilizing microorganism (KSM) would be a better and available knowledge to increase bioavailability of K from K-bearing minerals, which could help in maintaining the ecological balance and sustaining agricultural production and environmental quality. Now, the question is how these soil organisms solubilize K. The processes involved in K solubilization are listed and described in the following (Fig. 1.4).

#### 1.4.4.1 K-Solubilizing Microbial Species

Soil presents a variety of microbiomes including bacteria and fungi which boost the solubility of K minerals through the production of organic synthetics including chelating agents, exudates, extracellular enzymes, metabolic by-products, and both simple and compound organic acids (Meena et al. 2014; Saha et al. 2016a, b). These enzymes and organic metabolites triggered the biotic weathering of K-bearing rocks

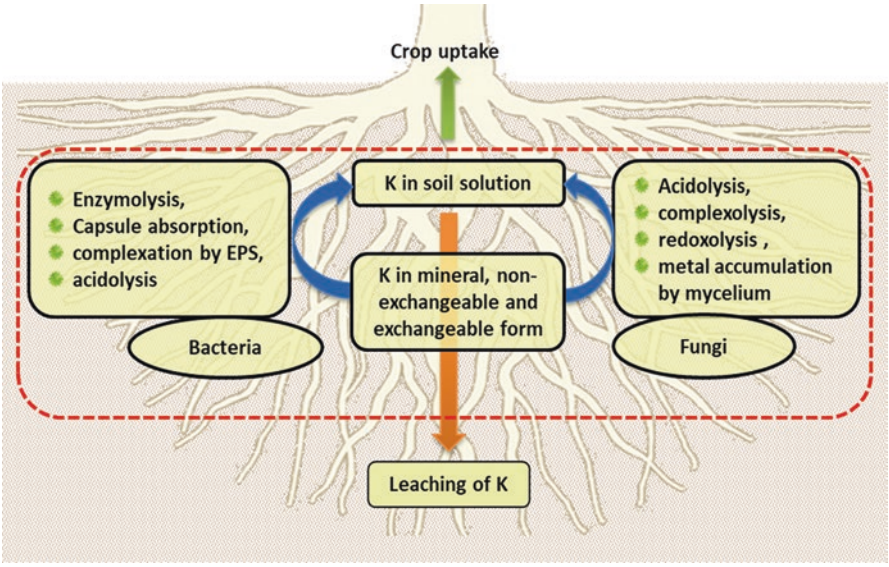


**Fig. 1.4** Mechanisms of K solubilization in a typical phytobiome. (Conceptualized and modified from Meena et al. 2014; Saha et al. 2016a, b)

and minerals. Especially rhizobacteria produce 2-keto-L-gluconic acid which binds Ca and acts as a strong weathering factor for underlying rocks. Otherwise, soil microorganisms respire and produce $CO_2$ that reacts with water and forms carbonic acid. This carbonic acid contributes to chemical weathering by dissolving $CaCO_3$ and K minerals through dissolution reactions.

Negatively charged soil particles adsorb maximum cationic nutrients like K and retard bioavailability. However, acidification of soil by the soil microbiomes assists in ion exchange processes. In this way, K is released into the soil solution and becomes readily available to the plants. Similar charge species are also involved in ion exchange when their presence is higher in the soil solution. For example, when $Ca^{2+}$ is present in excess in the soil solution, it can exchange two $K^+$ ions from the soil adsorption sites and contributes to desorption and K solubilization. These ions also transfer K, which is trapped in the interlayer spaces of the minerals to some extent. Certain bacteria produce mucilage like extracellular polysaccharides (ESP) which form a wrapper around the bacterial cell, attack the clay minerals chelating with silicon, and consequently release K from that structure.

Fungi are also considered as a biological weathering referee of rocks, minerals, and building blocks. Ectomycorrhizal hearting networks and the arbuscular structure of non-ectomycorrhizal trees, embedded in biofilms, conduct nutrients to the host. Here, biofilms assist in stimulating the weathering of minerals and thereby increasing uptake of nutrients to the plant. Some rock-decaying fungi can ooze out organic ions having low molecular weight, which forms a microscopic tunnel in the vicinity of exudates at hyphal tips within the minerals which enhances the weathering rates in that soil.

This is supported by numerous studies, whereas a wide variety of bacteria like *B. mucilaginous*, *B. edaphicus*, *B. circulans*, *Burkholderia*, *Acidithiobacillus ferrooxidans*, *Paenibacillus* spp., and *Pseudomonas* spp. have been demonstrated to solubilize K from K-containing minerals in the soils (Liu et al. 2012; Meena et al. 2014). Zhang and Kong (2014) isolated and identified that strains of *Pantoea agglomerans*, *Agrobacterium tumefaciens*, *Microbacterium foliorum*, *Myroides odoratimimus*, *Burkholderia cepacia*, *Enterobacter aerogenes*, *E. cloacae*, and *E. asburiae* remain effective in K solubilization in both solid and liquid media. Several other studies also validated the significant role of plant growth-promoting rhizobacteria (PGPR) in K solubilization and its mobilization in the plant root systems (Kumar and Singh 2001; Kukreja et al. 2004; El-Fattah et al. 2013).

### 1.4.5 S Cycle and Phytobiome

Sulfur (S) containing amino acids (cysteine, cystine, and methionine) is present in almost all the proteins, which makes it indispensable to more or less all living organisms. These amino acids may be present as free or in combined states. S cycle includes the transformation of S from organic to inorganic or inorganic to organic form and oxidized state to reduced state or reduced to oxidized state. These processes are mediated by various microorganisms, especially bacteria (Schoenau and Malhi 2008).

#### 1.4.5.1 Mineralization and Immobilization of Sulfur

Sulfur mineralization is the conversion of plant-unavailable organically bound forms of sulfur (organic sulfates and carbon-bonded sulfur) to the plant-available inorganic forms of S (sulfate, $SO_4^{2-}$) in soil. Whereas immobilization is exactly the opposite process, where inorganic S is assimilated by living organisms (microbes) and converted to the organic forms, that plant cannot absorb. In unfertilized soil S mineralization is the dominant source of S, in S pool for plants throughout the year (Bettany et al. 1979). The main factor which decides whether an organic S source will be mineralized or immobilized is the C/S ratio. When the C/S ratio is less than 200:1, net mineralization occurs, and organic S contributes to plant-available pool of S; in case the C/S ratio of the organic matter exceeds 400:1, net S immobilization occurs, and plant-available S is converted to organic S (Schoenau and Davis 2006). Organic S consists of (1) organic sulfate (S and C are not directly bonded like thioglucosides, sulfate esters, and sulfamates; here S is bonded to C via oxygen or nitrogen) and (2) carbon-bonded sulfur (here S is directly bonded to C like sulfonic acids, sulfur-containing amino acids in proteins). Organic sulfates cover 30–70% of the total soil organic S. S-containing amino acids and organic sulfates are the labile forms of organic S; they mineralize very easily by extracellular enzyme arylsulfatases (Tabatabai and Bremner 1970). Through organic mineralization, S is converted to sulfate ($SO_4^{-2}$) and thiosulfate ($S_2O_3^{-2}$) which are absorbable by plants. Humus soil has a C/S ratio nearly 100:1. Thus decomposition of it results in net mineralization (Roberts et al. 1989).

#### 1.4.5.2 Microbial Oxidation and Reduction of S

Soils where reduced forms of S (such as sulfides, elemental sulfur) are present, in those places microbial oxidation of reduced inorganic S is an important process. Through oxidation, reduced (–2, 0) forms of S ($S^{2-}$, $S^0$) are converted to higher oxidation states (+6) like sulfate ($SO_4^{2-}$). Both autotrophic and heterotrophic microorganisms perform microbial oxidation, including species of *Thiobacillus* (autotroph); heterotrophic bacteria like *Bacillus*, *Arthrobacter*, and *Pseudomonas*; and some fungi (Lawrence and Germida 1988). In some cases, heterotrophic sulfur oxidizers may dominate, especially in the rhizosphere (Grayston and Germida 1990). S oxidation is an acidifying process. It can be depicted by the following equation where elemental S, a reduced form of S, is oxidized to sulfuric acid:

$$2S^0 + 3O_2 + 2H_2O \rightarrow 2H_2SO_4$$

As a result of flooding, reduced aeration and high oxygen consumption produce reducing conditions, where redox potential decreases and drives the microbial conversion of sulfates to sulfide after other electron acceptors including oxygen and nitrate are depleted. This is termed as dissimilatory sulfate reduction, which is performed by the species of *Desulfovibrio* and *Desulfotomaculum* bacteria. Hydrogen sulfide gas is retained in the soil by reaction with iron to form an iron sulfide mineral, depicted in the following equation:

$$SO_4 \rightarrow H_2S(g) + Fe \rightarrow FeS(\text{mineral})$$

These sulfides may be oxidized back to sulfates if the soil becomes aerobic again (Schoenau and Malhi 2008).

### 1.4.6 Trace Metals and Phytobiome

The huge quantity of effluents generated from households and industries is continuously contaminating the land, river, pond, as well as groundwater aquifers (Saha et al. 2017a). Subsequent contamination of the human food chain from a significant amount of metals and pollutants is accumulated in soil following crop accumulation. As metals like iron (F)e, manganese (Mn), zinc (Zn), copper (Cu), and nickel (Ni) are essential for plant growth (Hapke 1991; Lenka et al. 2016; Saha et al. 2017b), apart their required quantity is significantly lesser than the N, P, and K. Thus, these metals along with other beneficial and harmful cationic metals are often defined as trace metals, whereas the term "heavy metal" is a cumulative term that includes both metals and metalloids having an atomic number of more than 20 (Ca) or atomic density more than 5 g $cc^{-1}$ which also cause toxicity at very trace concentration. Moreover, trace metals like chromium (Cr), cadmium (Cd), cobalt (Co), selenium (Se), mercury (Hg), lead (Pb), and arsenic (As) are responsible for different types of malfunction or even death due to toxicity in plants as well as animals and adversely affect the soil system. However, the levels of toxicity are determined by the combination of different factors like soil pH, conductivity, and other ionic activities in soil solution and particular metal species (type) and concentration present in ecosystems (Tchounwou et al. 2012; Saha et al. 2017c). Metal toxicity induced poor plant growth, retarded carbohydrate and protein metabolism, obstructed growth hormone formations and enzyme activities, and simultaneously insisted reduced biological diversity in the rhizosphere and non-rhizospheric soils (Singh et al. 2011).

#### 1.4.6.1 Conducive Soil Condition for Trace Metal Toxicity

Certain conditions induce trace metal toxicity for crops and humans. These trace element toxicities occur in the following (Bucher and Schenk 2000; Broadley et al. 2007; Aref 2011):

(a) Superfluous application of untreated sewage sludge, municipal waste, tannery and distillery effluents, coal combustion ashes, etc.
(b) Light-textured soil with low pH and having impeded subsurface soil layer with very low hydraulic conductivity
(c) Mineral soil having deficient soil organic matter
(d) Intensive raw minerals (e.g., waste mica, rock phosphate, pyrite, etc.) and fertilizer application
(e) Irrigation with polluted groundwater
(f) Type and concentration of trace metals in the environment

Heavy-trace metals are distributed over the solid phase, liquid phase, and also the gaseous phase, and their partitioning influenced and depends on complexation with

ligands, ion exchange, adsorption-dissolution-precipitation reaction, and oxidation-reduction reaction (Vlek et al. 1974; Singh and Saha 1997; Rattan et al. 2008). These interacting properties are the controlling factors of trace metal solubility, mobility, and bioavailability in soil and water systems. Other than mechanical and chemical processes, microbial inoculation has the potentiality to reduce the metal toxicity by either decomposition or immobilizing the metals from the soil (Abou-Shanab et al. 2003; Seshadri et al. 2015).

#### 1.4.6.2 Trace Metals' Crop Response and Phytoremediation

Negative interaction of cationic trace metals induces other trace metal deficiencies in plant tissues. Khan and Khan (2010) reported that Fe and Zn uptake was reduced from the excess application of Ni and developed chlorosis symptoms on leaves. Other reports also indicated that Cd toxicity induces poor Cr uptake (Dotaniya et al. 2017); and Zn toxicity induces Fe and Mn deficiency in plant tissues (Sivasankar et al. 2012). Correspondingly, specific metal toxicity also affects the dynamics of nutrient mineralization and nutrient release kinetics and results in poor plant growth; plants appeared brushy (Singh et al. 2016). Trace metals' (heavy metal) toxicity also retard soil enzymatic activity by replacing the native essential metals from binding sites (Bruins et al. 2000). For example, excess concentrations of $Cd^{2+}$, $Ag^{2+}$, and $Hg^{2+}$ in the soil-solution exchange phase are likely to bind with SH groups of some sensitive enzymes, which retard the activity of the enzyme (Nies 1999). Thus, increasing the use of wastewater, municipal wastes, industrial effluents, and coal combustion ashes triggers the toxic trace metal concentration in soil, which further accelerates the metal accumulation in ecological habitats (Rajkumar et al. 2012). Apart from this, higher Cr concentration was reported in plant roots compared to shoot, because metal excluder plants can store an excessive amount of Cr in root cell vacuole (Oliveira 2012; Nematshahi et al. 2012). Contrastingly, untreated sewage sludge application enhances crop yield, soil nutrient content, organic C content, and soil biological activities (Roy et al. 2019). Integrated approach (treated organic waste and inorganic fertilizers) is the best-recommended method for sustainable soil management and crop production. Neither the excess of essential nutrients nor other trace elements are beneficial for the plant-soil-microbe system, because specific element toxicity damages the DNA structure of soil enzymes and disturbs the normal functionality in the environment (Bruins et al. 2000).

#### 1.4.6.3 Siderophore and Phytosiderophore Engineering and Trace Metal Chemistry

Due to certain unwanted and challenging environmental conditions, the release rate of plant root exudates enhances the rhizosphere. These exudates help both ways by accelerating essential nutrient elements and inhibiting toxic elements (Marschner 1995). During Fe and Zn deficiency, graminaceous plants like barley, wheat, etc. exude phytosiderophores. While these exudates are produced from soil microbes, other higher plants are termed as siderophores. Siderophore and phytosiderophore are hexadentate non-proteinous amino acids, which act as a ligand and coordinate with $Fe^{3+}$, $Zn^{2+}$, or $Mn^{2+}$ by the carboxylic and amino groups (Romheld 1991). In the

rhizosphere, metal chelation is not specific for $Fe^{3+}$. Subsequently, phytosiderophores can transport a wide range of metals like Zn, Cu, Mn, Cd, and Ni (Awad and Romheld 2000). The diurnal variation of phytosiderophore release is also specific with plants and parts of plant roots. Generally, maximum phytosiderophore release and microbial activity are observed near the apical root zones due to localized microbial degradation and distribution of phytosiderophore (Marschner et al. 1986; Romheld 1991). The rate of phytosiderophore (PS) release differs among interspecies, plant to plant, and depends on daytime, sunshine hours, light intensity, and micronutrient status (Cakmak et al. 1994). However, this correlation with phytosiderophore quantity is not always consistent. Siderophores and phytosiderophores associated with metal chemistry and metal bioavailability in the soil are listed in Table 1.1. In contrast with Zn-PS and Mn-PS, the preferential uptake of Fe-PS is more prominent, which is mainly regulated by soil Fe nutritional status. The affinity of mugineic acids (MA) for heavy metal cations decreases in the order of $Cu^{2+} > Fe^{3+} > > Zn^{2+} > > Mn^{2+}$ (Dotaniya et al. 2014). Fe-PS and Zn-PS complex uptake rate corresponds to ~100 and ten times higher than that of free Fe and Zn. Thus, PS production and subsequent uptake are regulated by the transporters of the YS1/YSL protein family, which is induced by Fe deficiency (Meda et al. 2007). Due to increased nutrient availability, this process is also considered as a lifesaving mechanism for plants.

## 1.5 Phytobiome: An Early Indicator of Soil Health

Typically, soil health is defined as "a state of dynamic equilibrium between flora and fauna and their surrounding soil environment in which all the metabolic activities of the former proceed optimally without any hindrance, stress or impedance from the letter" (Goswami and Rattan 1992). Simultaneously the soil quality, which is analogous to soil health, is often used in the scientific literature, as scientist prefers soil quality. Soil quality is defined as "the capacity of soil to function within the ecosystem and land-use boundaries, to sustain biological productivity, maintain environmental quality, and sustain plant, animal, and human health" (Doran and Parkin 1994). On the other hand, soil health is generally dealt with by the producers (e.g., farmers). There are few conceptual differences between soil quality and soil health, where the former considers soil usefulness for a particular purpose for a long time scale and the latter considers the state of soil for a particular time (Larson and Pierce 1991).

Healthy soils are very crucial for the reliability of global ecosystems to stay integral or to recuperate from biotic and abiotic stresses as well as anthropogenic exploitation with agriculture (Ellert et al. 1997). In soil, phytobiome has the capacity to provide an integrated standard tool for soil health and quality, which cannot be obtained from physical or chemical analyses of soil. Microbiomes quickly acclimatize to environmental conditions as they have close relations with their surroundings due to their higher surface-to-volume ratio and respond rapidly to changes. Hence, the potentiality of these biomes can be adapted as an excellent indicator of soil health assessment. In some cases, alterations in microbial populations and activity

**Table 1.1** Siderophores and phytosiderophores associated with metal chemistry and metal bioavailability in soil

| Plants and microorganisms | | Siderophore and phytosiderophore production |
|---|---|---|
| Plants | *Graminaceous* family | Phytosiderophores: mugineic acid (MA), deoxymugineic acid (DMA), epoxy-mugineic acid (EMA), hydroxy-mugineic acid (HMA) |
| Microorganisms | | Siderophores: |
| Bacteria | *Bacillus* | Schizokinen, bacillibactin, ferrioxamines |
| | *Nocardia*, *Arthrobacter* | Ferrioxamines |
| | *Escherichia coli* | Enterobactin |
| | *Staphylococcus* | Staphyloferrin |
| | *Erwinia chrysanthemi* | Chrysobactin, achromobactin |
| | *Mycobacterium tuberculosis* | Mycobactin |
| | *Pseudomonas* sp. | Pyoverdines |
| | *Bordetella* sp. | Alcaligin |
| | *Azotobacter*, *agrobacterium* | Catecholate |
| Arbuscular mycorrhizae | *Cenococcum geophilum*, *Wilcoxinarehmii* | Ferricrocin |
| | *Glomus etunicatum*, *G. mosseae*, *unidentified Glomus* sp. | Glomuferrin |
| Fungi | *Rhizopus* | Rhizopherin |
| | *Ustilagosphaerogena* | Desferriferrichrome |
| | *Fusarium roseum* | Fusarinines, malonichrome, triacetylfusarinines |
| | *Neurospora crassa* | Ferricrocin |
| | *Aspergillus fumigants*, *Penicillium bilaiae* | Pistillarin |
| | *Aspergillus ochraceus* | Ferrichrome |

Adopted and modified from Matzanke et al. (1988), Hider and Kong (2010), Dotaniya et al. (2014), Winkelmann (2017), Sarkar et al. (2017a, b), Hussein and Joo (2017)

before detecting changes in soil rather than physical and chemical properties thus provide an early signal of soil improvement or an early indication of soil degradation (Pankhurst et al. 1995). The turnover rate of microbial biomass is much faster (1–5 years) than the total soil organic matter turnover (Carter et al. 1999). Phytobiomic indicators of soil health involve various sets of microbial dimensions due to the multifunctional characteristics of microbial groups in the terrestrial ecosystem. Some indicators of soil health are as follows:

1. *Biodiversity*: genetic diversity, functional diversity, structural diversity
2. *Carbon cycling*: soil respiration, organic matter decomposition, soil enzymes, methane oxidation
3. *Nitrogen cycling*: N-mineralization, nitrification, denitrification, N-fixation
4. *Soil biomass*: microbial biomass, protozoan biomass

5. *Microbial activity*: bacterial DNA synthesis, bacterial protein synthesis, RNA measurements, bacteriophages
6. *Bioavailability*: biosensor bacteria, plasmid-containing bacteria, antibiotic-resistant bacteria, catabolic genes

In addition to the influence on nutrient cycling, microbiomes also affect the physical properties of soil by producing extracellular polysaccharides and other cellular trashes that help in maintaining soil structure. As these biochemicals function as chelating agents, they stabilize soil aggregates and affect water retention capacity, crusting, erodibility, infiltration rate, as well as susceptibility to compaction.

## 1.6 Future Outlook

Attempts are to be made to enhance the food, feed, and fiber production worldwide by exploring each and individual component of phytobiome and in between interactions. Due to increased food demand and high-intensity agriculture, integration and optimization of phytobiome-based pieces of knowledge, resources, and site- and condition-specific solutions become one of the most treasured materials for sustainable agriculture and soil health. Further, an advanced association of scientific society to explore this concept is a much important future aspect of phytobiome studies.

## 1.7 Conclusions

This chapter is an effort to enlighten the idea of phytobiome and its role in nutrient regulation, maintaining soil quality. The modern principles of different utilizations of this phytobiome have been characterized to excerpt a high prospect of their functionality and applicability for sustainable agriculture. So, these phytobiomes are evolved as plant growth-promoting microbiomes, which are important for keeping good soil health, better soil conditions, and persistent agricultural productivity. These biomes do not become alive independently but make interconnected coordination with the environment. All are engaged in the nutrient cycle of the terrestrial ecosystem by weathering and solubilizing complex and stable nutrient sources. The contest of this section is to develop new approaches for addressing the nutrient use efficiency as well as directed plant parts (within metabolomes of rhizospheric groups) to assist a significant change in the level of perception. The ultimate aid from this section will be the information about modification of the soil-plant system to support microbial chemistry or their effects on soil physico-chemical properties that are most important in promoting nutrient acquisition in plants across the diversified global agricultural condition. These provide an effective substitute for advanced crop production.

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# Role of a Quorum Sensing Signal Acyl-Homoserine Lactone in a Phytobiome

2

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**Abstract**

A phytobiome is influenced by its many members, which includes plants, soils, microbes, animals (insects), and the constantly fluctuating environment. Plant-microbe interactions represent one of the most impactful relationships inside a phytobiome and may have a beneficial, harmful, or neutral effect on one another. This chapter provides a comprehensive analysis of the complex network of signal exchange between microbes and plant in a phytobiome, via the quorum-sensing circuit with a special focus on N-acyl homoserine lactones (AHLs) signaling. Incorporating the current understanding of this plant-microbe dynamic by tracing their signals is one of the major tools to customize a sustainable phytobiome. There are still many gaps to cover such as understanding a system-level communication of the phytobiome and the molecular nitty-gritty of signal transport within plants and molecular pathways coordinating plant physiological changes. Future advances would depend on the collaborative effort of interdisciplinary scientist groups backed by advance "omic" techniques to link all the biotic and abiotic components and understand the synchronized dynamic of a phytobiome.

**Keywords**

Phytobiome · Quorum sensing · N-Acyl homoserine lactones · Signaling · Microorganism

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## 2.1 Introduction

Given the ever-increasing food demand and energy crisis in the world today, increasing biomass yields for greater food supply and low-cost, low-maintenance energy crop production without the use of harmful synthetic agrochemicals is the need of the hour. In recent years, there has been a paradigm shift in the understanding of the nature of plant-associated community, from the perspective that a plant is strongly interconnected with different biotic factors such as plants, animals, and microorganisms and abiotic factors like soil, light, and water and that sustainable improvement in agro-productivity and agro-quality would require a system-level approach, taking into account all the optimum biotic and abiotic set of influences on a plant (Leach et al. 2017). Phytobiome is defined as the totality of a plant, its physical environment, and the entire population of microorganism in, on, and around the plant. The composition of biotic community associated with the plant depends on the host genotype, local niche, or the plant compartment (spermosphere, endosphere, rhizosphere, phyllosphere) and environment such as soil type (Hacquard et al. 2015; Leach et al. 2017). For example, phyllosphere, the aerial plant part, experiences a highly fluctuating environment and nutrient availability and hosts a more distinct microbiome as compared to a more environmentally stable rhizosphere (Remus-Emsermann et al. 2012).

Chemical communication plays a major role in the growth, development, and evolution of plants (Witzany 2006). For example, plants release volatile compounds to invite insects to help them spread their pollen for reproduction (Baldwin 2010). A good understanding of the integrity and the basic administration in a phytobiome would help to design strategies toward sustainable agriculture and environmental preservation. This would require a sound understanding of the communication among the various members of a phytobiome. However, since signals can be quite often co-opted, modified, or even destroyed by another member of the community, there is the need for a system-level analysis of communication within the phytobiome (Leach et al. 2017).

Microbes represent the largest biotic component of a phytobiome, which also exist in the closest proximity, that is, on, around, and inside of a plant. Plants and microbes have co-evolved over time, interacting both in symbiosis and pathogenesis, and so has the communication signaling network between the two kingdoms (De-la-Peña and Loyola-Vargas 2014). The microbial world communicates via signaling molecules based on the population size-driven phenomenon of quorum sensing (QS) (Fuqua and Winans 1994). Bacterial QS machinery consists of an autoinducer, its cognate receptor protein, and a synthase gene for signal synthesis. QS-controlled bacterial phenotypes include bioluminescence, biofilm formation, motility, conjugation, sporulation, production of antibiotics, and the expression of virulence factors (Williams 2007). Disruption of the QS system or quorum quenching (QQ) takes place by (1) signal degradation by enzymes and physical agents, (2) inhibition of signal synthesis, and (3) receptor blocking (Uroz et al. 2009). Given this myriad of roles of QS in both pathogenic and symbiotic interaction, QS and QQ find multiple applications in agriculture and medicine (Grandclément et al. 2015).

Common bacterial QS signal molecules, such as *N*-acyl homoserine lactones (AHLs), diffusible signal factors (DSF), and signaling peptides, are among the best-studied communication signals in the phytobiome (Leach et al. 2017). AHLs produced by Gram-negative bacteria are the most reported autoinducer signal molecules. AHLs can be defined by the length of their acyl chain, with short-chain ($C_4$-$C_6$), medium-chain ($C_6$-$C_{10}$), and long-chain ($C_{10}$-$C_{16}$) AHLs. AHLs are produced by both pathogenic bacteria and plant growth-promoting rhizobacteria (PGPR). Table 2.1

**Table 2.1** Different QS signaling molecules and their effect on plants along with the probable mechanism of action

| QS molecule | Producers | Probable mechanism of action | Effect on plant | References |
|---|---|---|---|---|
| *N*-Acyl homoserine lactones (AHLs) | Gram-negative bacteria | LuxR receptor/G--protein signaling Induction of salicylic acid and oxylipin-dependent pathways | Seed germination and plant development ($C_6$HSL) Root elongation ($3OC_6$HSL, $3OC_8$HSL) Primary root growth, lateral root formation, and root hair development | Moshynets et al. (2019) Jin et al. (2012) Ortíz-Castro et al. (2008) |
| | | | Increased defense and systemic resistance against fungal pathogens "Priming" for induced resistance and rapid and robust response to abiotic stresses ($C_{12}$HSL, $OC_{14}$HSL) | Schuhegger et al. (2006) Schenk et al. (2014) and Schenk and Schikora (2015) |
| Aryl HSLs | *Rhodopseudomonas palustris*, *Bradyrhizobium* | RpaR, BraR | Not known | Schaefer et al. (2008) and Ahlgren et al. (2011) |
| Diffusible signal factor (DSF) | *Stenotrophomonas maltophilia*, *Xanthomonas*, *Burkholderia cenocepacia*, *Pseudomonas aeruginosa* | RpcF/RpfG two-component system | Promotes seed germination and plant growth, enhanced biocontrol, elicit innate immunity in plants | Alavi et al. (2013) and Venturi and Keel (2016) |
| Cyclodipeptides/ diketopiperazines | *Pseudomonas aeruginosa* | Auxin signal mimics | Promote plant growth, lateral root development | Ortiz-Castro et al. (2011) |
| Autoinducing peptides (AIPs) | Gram-positive bacteria | Not known | | Monnet et al. (2016) |

provides a list of QS signal molecules reported for their effect on plants and possible mechanism of action. Many reports have shown that plants can detect and respond to AHLs, and the responses differ based on the acyl chain length and substitution of the AHL molecule and the plant species-AHL combination (Mathesius et al. 2003; Schuhegger et al. 2006; Schikora et al. 2011; Schenk et al. 2014). This chapter summarizes the current knowledge of (a) how AHL signaling in a phytobiome impacts plants, b) how plants manipulate AHL signals to their advantage, c) the basic dynamic of the plant-microbe signal exchange, and finally d) how this knowledge can be incorporated in the bigger scheme of creating a healthier phytobiome.

## 2.2 Plant Senses and Responds to AHL

The first report on the impact of bacterial AHLs on plant physiology was a differential proteome analysis of *M. truncatula* roots, studying their response to the exposure of $3OC_{12}$-HSL and $3OC_{16:1}$-HSL, in nanomolar (nM) concentrations (Mathesius et al. 2003). This work revealed complex functional responses to AHL application in the plant, with significant changes in the expression of over 150 proteins. The proteins detected were associated with defense, stress responses, energetic and metabolic activities, transcriptional regulation, protein processing, cytoskeletal activities, and plant hormone responses. In another proteomic study done on the responses of *A. thaliana* seedlings to $3OC_8$-HSL treatment (Miao et al. 2012), 53 proteins showed alteration in expression pattern, and 34 of these proteins were recognized to be associated with energy and carbohydrate metabolism, protein synthesis, plant defense, signal transduction, cytoskeleton remodeling, etc. The chloroplast was observed to be the most sensitive intracellular organelle to $3OC_8$-HSL treatment. Such findings strongly suggest that plant displayed extensive functional responses to bacterial AHLs that might be important for inter-kingdom interactions. In a recent proteomic study on the effect of exogenous AHLs on *Arabidopsis thaliana* seedlings under salinity stress, AHL application was found to impart salt tolerance and growth, and it might involve 97 proteins associated with defense/stress/detoxification, photosynthesis, protein metabolism, signal transduction, transcription, cell wall biogenesis, energy metabolisms, etc. (Ding et al. 2016).

## 2.3 AHL Impact on Plant Root Growth and Architecture

Mathesius and coworkers (2003) reported the activation of auxin-inducible GH3 promoter upon AHL application, from their proteomic analysis of *M. truncatula* roots post AHL treatment. In a later study, von Rad et al. (2008) also reported $C_6$-HSL-induced changes in the expression of several plant hormone-related genes, resulting in a higher expression of auxin and lower cytokinin concentrations. Moreover, they observed a significant increase in primary root growth in the presence of short-chain AHLs ($C_4$-$C_6$HSL) at 1 nM and a concentration above 10 micromolar (μM), respectively, in *A. thaliana*, whereas the long-chain $C_{10}$-HSL remained

neutral. The authors attributed this different plant response to the different acyl chain length of AHLs to the difference in their hydrophobicity. Long-chain AHLs with higher hydrophobicity are not transported from root to the shoot, and their accumulation in the root makes them toxic, thereby inhibiting the growth of root tissue. Since auxin induces root growth, the change in auxin/cytokinin ratio upon AHL treatment might be a potential mechanism behind short-chain AHL-induced root growth (von Rad et al. 2008).

Modification of overall root architecture of *A. thaliana* on exposure to μM concentration of long-chain AHLs, especially $C_{10}$-HSL, has been observed as a result of inhibition of primary root growth and promotion of lateral root and root hair growth (Ortíz-Castro et al. 2008). This change in root morphology was associated with alterations in cell division and differentiation in the primary root meristem. Although auxin-treated *Arabidopsis* seedlings show similar changes in root morphology, $C_{10}$-HSL action was independent of the auxin-regulated process. Enhanced lateral root and root hair growth could increase the absorptive capacity of the plant and also provide a larger surface area for bacterial colonization. These findings suggest the advantage of long-chain AHL-producing rhizobacteria to form a symbiotic association with the plant host.

Short-chain $C_6$-HSL has been shown (Schenk et al. 2012) to increase the shoot biomass and cause primary root elongation in *Arabidopsis*, with eventual decrease and loss of activity with an increase in acyl chain length (Fig. 2.1). Liu et al. (2012) demonstrated promotion of primary root growth in *Arabidopsis* by AHLs with modification at the C3 position of their branched chain, $3OC_6$-HSL and $3OC_8$-HSL treatment, in a concentration-dependent manner similar to $C_3$ unsubstituted $C_6$-HSL. On the other hand, long-chain AHLs, $C_{12}$-HSL, and $C_{14}$-HSL, promoted lateral root

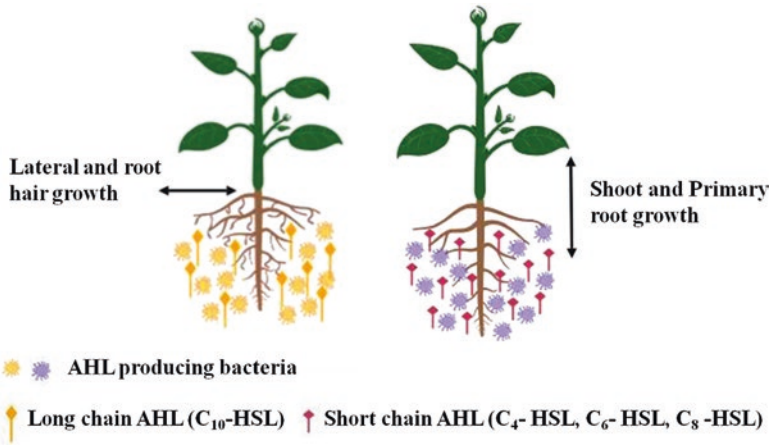


**Fig. 2.1** AHL influences plant root growth and morphology in *Arabidopsis*. Changes varied based on AHL acyl chain length. Short-chain AHLs ($C_6$-HSL, $C_8$-HSL) causes the growth of primary root, whereas long-chain AHL ($C_{10}$-HSL) causes the growth of lateral root and root hair (von Rad et al. 2008; Bai et al. 2012; Liu et al. 2012)

growth and inhibited root elongation, suggesting separate signaling pathways for the long- and short-chain AHLs (Liu et al. 2012).

A different model plant, mung bean, was studied by Bai et al. (2012) for AHL treatment response. They found enhanced auxin-mediated adventitious root formation post AHL treatment. AHLs with C3 modification, particularly 3O$C_{10}$-HSL, showed higher activity than their unmodified analogs such as $C_8$-HSL, $C_{10}$-HSL, and $C_{12}$-HSL. This preference for specific AHL structures may be the result of spatial specificity, required by some plant proteins (Bai et al. 2012). In contrast to these reports, there was no significant effect on the growth of yam and barley plants when exposed to $C_6$-HSL, $C_8$-HSL, and $C_{10}$-HSL (Götz-Rösch et al. 2015). These findings strongly suggest that different plants respond differently to AHLs, which may be a result of variations in AHL signaling cascades (Götz-Rösch et al. 2015). Figure created using BioRender.

## 2.4 AHL as Plant Strengthener

Plants possess a wide range of defenses that get activated in response to various stress, pathogens, and parasites (Gimenez et al. 2018). Induced resistance in plants is an important defense mechanism where defenses are preconditioned by prior infection or treatment resulting in resistance (or tolerance) against future infection (Vallad and Goodman 2004). The prior attack acts as a warning signal, preparing the plant for a stronger defense. The sensibilization mechanism, before an attack, called the priming phase, can be induced by a diverse range of low-molecular-weight metabolites and natural compounds, including AHLs (Mauch-Mani et al. 2017).

Tomato plants get primed via SA- and ethylene-dependent defense genes, upon exposure to AHL-producing rhizobacteria, *Serratia liquefaciens* MG1, and *Pseudomonas putida* IsoF, against the fungal pathogen *Alternaria alternata* (Schuhegger et al. 2006). A similar AHL-mediated antifungal activity that involved on SA- and ethylene-related defense reactions was reported in cucumber (*Cucumis sativus*) by *Serratia plymuthica* HRO-C48 against the damping-off disease caused by *Pythium aphanidermatum*, as well as in bean and tomato plants against the gray mold fungus *Botrytis cinerea* infection (Pang et al. 2009).

Among long-chain AHLs, 3-oxo-$C_{14}$-HSL conferred systemic resistance in monocotyledonous (barley), and dicotyledonous plant (*Arabidopsis*) plants toward biotrophic and hemibiotrophic fungus, but not against necrotrophic fungal pathogens (Schikora et al. 2011). This resistance is associated with an increased expression of mitogen-activated protein kinases, AtMPK3, and AtMPK6, which consequently increases the expression of defense-related transcription factors, WRKY22 and WRKR29, and the Pathogenesis-Related1 gene (PR1). However, 3-oxo-$C_{14}$-HSL did not show any impact on root and shoot growth as reported for long-chain AHLs (von Rad et al. 2008). A similar effect of 3-oxo-$C_{14}$-HSL imparting systemic resistance was reported by Zarkani et al. (2013) in *A. thaliana* when inoculated with *Sinorhizobium meliloti* strains producing oxo-$C_{14}$-HSL. They observed that neither 3-oxo-$C_{14}$-HSL-negative *S. meliloti* nor

3-oxo-$C_8$-HSL-positive *Rhizobium etli* produced a similar effect. These findings revealed that the resistance induced by AHLs does not necessarily require a host-symbiont relationship to start with, as *A. thaliana* is not a symbiotic or nodule-forming plant.

Schenk et al. (2014) reported the priming effect of AHL on exposure to oxo-$C_{14}$-HSL leading to increased deposition of lignin, phenolic compounds, and callose on the cell wall, reinforcing the cell wall to increase resistance to pathogens. A second possible mechanism of resistance by oxo-$C_{14}$-HSL could be the enhanced stomatal closure observed due to AHL-induced accumulation of cis-12-oxo-phytodienoic acid (OPDA) and salicylic acid (SA) in plant leaves. Stomatal closure is an important part of innate immunity. Another interesting mechanism of resistance by AHL-induced priming is the elevated level of hypersensitive response (HR) after infection. Barley primed with *S. meliloti* expR+ AHL showed accumulation of reactive oxygen species (ROS) and increased expression of Peroxidase7 as an HR, following infection with *Blumeria graminis* (Hernández Reyes et al. 2014). Given these various mechanisms of resistance induced by AHL priming, AHL-induced resistance seems to differ from the systemic acquired and the induced systemic resistances, providing new insight into inter-kingdom communication (Schenk et al. 2014). Moshynets et al. (2019) used $C_6$-HSL as a seed primer for winter wheat (*Triticum aestivum* L.), which was found to improve germination levels significantly, biomass at filtering stage, crop structure, and productivity at maturity of the crop. Thus, AHLs could find application in improving growth and productivity of cereal crops.

## 2.5 Effect of AHL on Symbiosis and Nitrogen Cycle

Many strains of nodulating rhizobia such as *Rhizobium leguminosarum* bv. viciae, *R. leguminosarum*, *S. meliloti* Rm41, and *A. tumefaciens* are associated with quorum sensing gene regulation systems (Gonzalez and Marketon 2003). The process of legume-host symbiosis is a complex interplay of communication between various signal molecules that includes flavonoids from root exudates that attract the rhizobia, exopolysaccharides for bacterial attachment, Nod factors for initiation of root cell division to form the infection threads, and AHLs. Once bacteria reach the host, a further process of symbiosis depends on reaching a threshold cell density, coordinated by the QS regulatory system (Gonzalez and Marketon 2003; Sanchez-Contreras et al. 2007; Downie 2010).

A few QS regulatory systems directly linked to symbiosis are discussed here. Association of root nodule formation with an unidentified AHL was initially reported in *Rhizobium leguminosarum* by Gray et al. (1996). Rosemeyer et al. (1998) identified *luxI*- and *luxR*-homologous genes, *raiI*, and *raiR* in *Rhizobium etli* CNPAF512 and found *raiI* to have a restrictive effect on the number of nodules in the host plant *Phaseolus vulgaris* without affecting the nitrogen-fixing capacity per nodule. On the other hand, the second quorum sensing locus, *cinI*, and *cinR* in *Rhizobium etli* CNPAF512 was essential for nodule bacteroid differentiation and involved in symbiotic nitrogen fixation (Daniels et al. 2002). However, the same

genes showed varying implications in different *Rhizobium* strains. Mutation of *cinR and cinI* in *R. leguminosarum* had little or no effect on the growth of the bacterium and in the symbiosis with peas (Edwards et al. 2009).

QS-deficient mutants of *Mesorhizobium huakuii* showed an inability to form root nodules (Gao et al. 2006). *MrtI-MrtR* pair was identified as *LuxI-LuxR* homologs in *M. tianshanense* and played an indispensable role in root hair adherence and nodulation on the host plant *Glycyrrhiza uralensis*; QS-deficient mutants showed the absence of nodulation (Zheng et al. 2006; Cao et al. 2009). Increase in several nodules was observed in *M. truncatula* after treatment with 1 μM 3-oxo-$C_{14}$-HSL with no effect on lateral root number (Veliz-Vallejos et al. 2014). Study on ExpR/Sin QS system in *Sinorhizobium meliloti* by Gurich and González (2009) revealed that QS stays active during all the growth phases till symbiosis is established, after which the bacteroids' metabolic functions focus on nitrogen fixation.

Besides nitrogen fixation, nitrogen mineralization is also responsible for providing plants the utilizable form of nitrogen, where microbes enzymatically break down the organic nitrogen sources such as chitin, to the simpler inorganic form of nitrogen such as ammonia. DeAngelis et al. (2008) studied the link between QS and extracellular enzyme activity of 533 bacterial isolates, dominated by α-proteobacterial isolates such as *Agrobacterium rhizogenes*, *Inquilinus ginsengisoli*, and *Burkholderia* sp. in the rhizosphere of *Avena* and observed compromised chitinase or protease activity in all but one isolates upon disruption of QS. This strongly suggests that QS plays an important role in the regulation of exoenzyme production by bacteria in soil, and thus targeting QS for disease control should also take into account the resultant risk of disturbing soil nitrogen cycle.

## 2.6 AHL-Induced Plant Diseases

Phytopathogenic bacteria employ QS regulatory system to control virulence and may carry one or more QS regulons. The most well-characterized bacterial signal molecules involved in plant pathogenesis include AHLs, DSF, and 3-hydroxy palmitic acid methyl ester or methyl 3-hydroxypalmitate (Sibanda et al. 2016).

The LuxR homologs *aviR*, *avsR*, *and avsI* in *Agrobacterium vitis* are required for causing necrosis in grapes and hypersensitive-like response on tobacco (Zheng et al. 2003; Hao et al. 2005; Hao and Burr 2006). Ferluga et al. (2007) reported a LuxR family-type regulator, OryR, in *Xanthomonas oryzae* pv. *oryzae* (Xoo), essential for virulence in rice, and it required macerated rice for activity. The plant pathogen, *Pantoea ananatis* SK-1 depends on the quorum sensing system for the biosynthesis of extracellular polymeric substances (EPS), biofilm formation and causes center rot disease in onion (Morohoshi et al. 2007).

Liu et al. (2008) demonstrated a greater role for QS in pathogenesis as a "master regulator," controlling other regulatory systems that further coordinate to control virulence genes. The study monitored the progression of soft rot in potato caused by *P. atrosepticum* using virulence factors and plant cell wall-degrading enzymes (PCWDEs) under the control of the QS system. Differential transcription of up to

26% of the *P. atrosepticum* genome was observed in a QS mutant. They also identified many novel components of the QS regulon namely type I, II secretion systems associated with secretion of PCWDE; type III secretion system and coronafacoylamide conjugates for manipulation of plant defenses; T6SS (Type VI secretion system) and VirS, a novel potential regulator essential for complete virulence.

It is interesting how some plant pathogens exploit changes in plant chemicals to mount an optimal attack. This is a common phenomenon among plant pathogens carrying the "orphan or the solo" LuxR homologs, which do not possess a cognate LuxI homolog (Subramoni and Venturi 2009). LuxR solos occur in both AHL-producing and AHL-nonproducing bacteria, allowing them to respond to signals from other bacteria or eukaryotes. These LuxR solos may act as important interspecies and inter-kingdom signals (Ferluga and Venturi 2009; Subramoni and Venturi 2009). For example, OryR protein of *Xanthomonas oryzae* pv. *oryzae*, solubilized in the presence of rice extract, however became insoluble in the presence different AHLs. This indicates the presence of a molecule in rice extract that might interact with and stabilize OryR (Ferluga et al. 2007) and that this molecule could be closely related to AHLs, as OryR possesses an AHL-binding motif.

Similarly, XccR, the LuxI homolog in *Xanthomonas campestris* pv. *campestris*, causes infection in cabbage. XccR associates with an unknown plant factor and binds to a lux-box present in the promoter of the proline aminopeptidase (*pip*) virulence gene (Zhang et al. 2012). It is probable that OryR, XccR solos, and their plant partner participate in inter-kingdom signaling, and identification of the plant compounds would set novel insights into inter-kingdom signaling (Subramoni and Venturi 2009).

## 2.7 AHL Uptake in Plant and Possible Signaling Pathways for Plant Response to AHL

There are a few reports addressing this basic question of whether AHLs coordinate with other signals upon perception by plant or are taken up by plants by some physical mechanism (Schikora et al. 2016). Götz and coworkers (2007) tested the uptake of radiolabeled $C_6$-HSL, $C_8$-HSL, and $C_{10}$-HSL in barley (*Hordeum vulgare* L.) and yam (*Pachyrhizus erosus* L.). Ultra-performance liquid chromatography (UPLC) and Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) analysis revealed short AHLs, only $C_6$-HSL, and $C_8$-HSL, transported from roots into barley shoots and $C_6$-HSL in case of yam shoots. *A. thaliana* also transported $C_6$-HSL from root to leaves in a time-dependent manner, while $C_{10}$-HSL remained accumulated in root region (von Rad et al. 2008). Similarly, it was observed that the long-chain AHLs, oxo-$C_{14}$-HSL, were not taken up within the *Arabidopsis* plant.

Moreover, it showed no effect on root growth, whereas short-chain AHL $C_6$-HSL got systemically translocated from root to shoot and affected growth-promoting effect (Schikora et al. 2011). A possible reason behind the difference in uptake of AHLs might be the reduced motility with an increase in acyl chain length or higher hydrophobicity of longer AHLs (von Rad et al. 2008; Schikora et al. 2011).

However, Sieper et al. (2014) reported systemic transport of $C_8$-HSL and $C_{10}$-HSL into the barley shoots with a molecular analysis of the process backed by autoradiography analysis, sensor strain assays, and monoclonal antibodies. They suggested a probable active or semi-active process of translocation of AHLs with the involvement of ATP-binding cassette (ABC) transporters based on the inhibition of AHL uptake in the presence of orthovanadate, an inhibitor of ABC transporters. All these reports point toward different signaling pathways and receptors for different AHLs. Götz-Rösch et al. (2015) observed a distinct pattern of selected detoxification enzymes activity in barley and yam plants, upon $C_6$-, $C_8$-, and $C_{10}$-HSL treatment. The activities of glutathione S-transferase and ROS scavenging enzymes were mostly tissue specific. $C_6$-HSL was readily transported into all barley plant parts without degradation with the most prominent influence on the leaf-located enzymes.

On the other hand, the yam plant showed no such change in detoxification enzyme activity, which might be a result of the absence of AHL-transport machinery toward the shoot in yam bean. In both the plants, $C_{10}$-HSL was almost broken down completely before it entered the shoot in its initial form. Interestingly, the metabolization was faster in yam plant than in barley, which may be related to the fact that symbiotic yam beans are naturally exposed to AHL (Götz-Rösch et al. 2015).

Other studies have aimed at deciphering the molecular mechanisms behind the plant-AHL interactions, such as AHL perception mechanism in plant and signal transduction pathways involved (Jin et al. 2012; Song et al. 2011; Liu et al. 2012; Zhao et al. 2016). G-protein signaling is considered one of the most conserved signaling pathways in plants and is associated with the transduction of many extracellular signals. It is involved in many physiological functions in plants like stomatal opening (Urano and Jones 2013), phytochrome-dependent cell death (Zhang et al. 2012), stress signaling (Tuteja and Sopory 2008), response to plant hormones, and agronomical traits like nitrogen fixation, seed size, and number (Pandey et al. 2019). In typical G-protein signaling, the G-protein complex comprised of Gα, Gβ, and Gγ subunits remains inactive with GDP bound to the Gα subunit. In animals, the G-proteins get activated via a membrane protein called G-protein-coupled receptor (GPCR) upon binding with a ligand, leading to the exchange of GTP with GDP and the dissociation of GTP-Gα from the Gβγ subunit. The free Gα and Gβγ subunits further initiate different intracellular signaling pathways (Tuteja 2009). However, in plants, the G-protein signaling is reported to be independent of GPCR, and the plant G-proteins release GDP spontaneously and thus are self-activating (Urano and Jones 2013). Although some authors think this hypothesis is supported by genetic and physiological findings, it is still not clear whether the hypothesis applies to all G-proteins in all plant species (Pandey et al. 2019). Song et al. (2011) hypothesized that AHLs in plants is received by GPCRs, which activate the G-protein α subunit and $Ca^{2+}$ channels to allow $Ca^{2+}$ influx. $C_4$-HSL treatment on *A. thaliana* roots was shown to cause a transient rise in cytosolic $Ca^{2+}$ as a result of influx from the extracellular matrix. The authors suggest $Ca^{2+}$ signaling might help plant cells to sense QS signals. However, considering the possibility of the absence of GPCR, AHL might also interact directly with intracellular receptors without the help of transmembrane proteins (Jin et al. 2012; Song et al. 2011).

Many G-protein-coupled receptors (GPCRs) have been reported to mediate AHL-induced root elongation in *A. thaliana.* Expression of GPCRs, Cand2, Cand7

(Jin et al. 2012), GCR1, and GCR2 (Bian et al. 2011) increased in *A. thaliana* root in response to 1 μM $3OC_6$-HSL and 10 μM $3OC_8$-HSL treatment. GPA1 was also added to this list of GPCRs affecting $3OC_6$-HSL and $3OC_8$-HSL action on root growth in *A. thaliana* in a concentration-dependent manner (Liu et al. 2012). Involvement of $H_2O_2$ and NO-dependent cGMP signaling pathways behind this AHL-induced auxin-dependent lateral root formation has also been proposed (Bai et al. 2012). A recent report on $3OC_6$-HSL enhancing root elongation involves the transcriptional factor AtMYB44 in *A. thaliana* (Zhao et al. 2016).

## 2.8 Plant Interference with QS Signals

Not only do plants respond to AHL and QS systems in various ways, from reaping symbiotic benefits to enhancing disease resistance, they also evolve strategies to interfere with the microbial signaling systems for their advantage, by producing signal mimics, certain plant metabolites, and signal-degrading enzymes (Fig. 2.2).

### 2.8.1 Plant Interferes with QS via AHL Mimics

AHL mimic compounds are reported to be produced by many plants to create confusion in microbial communication (Bauer and Mathesius 2004). Halogenated furanones from *Delisea pulchra*, a marine red alga, were reported to have strong

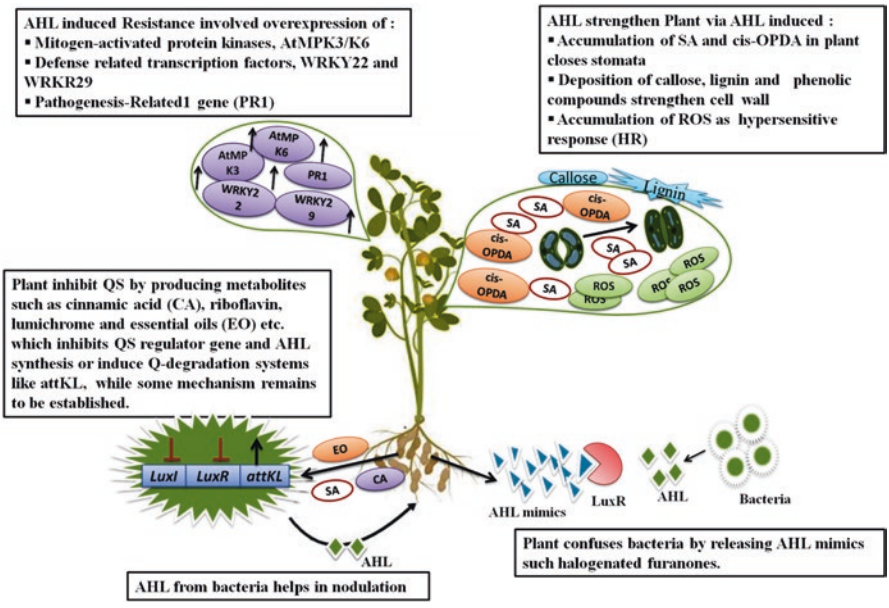


**Fig. 2.2** Schematic presentation of how plants resist and manipulate QS signaling to their advantage. Figure created using BioRender

inhibitory effects against AHL-controlled phenotypes in *Serratia liquefaciens*, *Escherichia coli*, and *Proteus mirabilis* models (Givskov et al. 1996; Gram et al. 1996). Later, the structural similarity of furanones to AHLs was confirmed alongside their possible mode of action of displacing AHLs competitively from the AHL LuxR receptor (Manefield et al. 1999). Further clarity into the mechanism of furanones came from the finding that halogenated furanones destabilized LuxR protein, accelerating its degradation (Manefield et al. 2002).

The leaves and stem of rice plants produce AHL mimics, which could activate three different bacterial AHL biosensors and were very sensitive to the AiiA lactonase. Given the highly specific nature of lactonases, these rice compounds may be another AHL or a compound of close structure (Degrassi et al. 2007). Teplitski et al. (2000) reported the presence of AHL mimics in pea seedling exudates using various AHL reporter strains, *C. violaceum* CV026, *P. aureofaciens* 30-84I, and *S. liquefaciens* MG44. Activity analysis of different HPLC fractions revealed the presence of both inhibitory and stimulatory substances in pea plant. AHL mimic activity was also shown by seedlings of other plant species, rice, soybean, tomato, crown vetch, and *Medicago truncatula*, suggesting AHL mimic synthesis is a common response among higher plants (Helman and Chernin 2015). Gao et al. (2003) reported around 15 to 20 separable unidentified QS mimic compounds in *M. truncatula* young seedling, capable of specifically stimulating or inhibiting responses in QS reporter bacteria, *Vibrio harveyi* BB170, *Pseudomonas putida* pAS-C8, *C. violaceum* CV026, and *E. coli* strains JM109, p(SB401), p(SB536), and p(SB1075). However, the precise chemical structure of these QS mimics in pea, rice, and *M. truncatula* is not yet known, except for the possibility of a structure close to that of AHLs. It is also suggested that such mimic compounds might be degradation products of bacterial AHLs as the result of the action of plant AHL-degrading enzymes such as lactonases (Mathesius and Watt 2010). Pérez-Montaño et al. (2013) reported AHL mimic molecules produced by *Oryza sativa* (rice) and *P. vulgaris* (bean) plants that specifically interfere with the QS-regulated biofilm formation of two plant-associated bacteria, *Sinorhizobium fredii* SMH12 and *Pantoea ananatis* AMG501. These mimic compounds are considered non-AHL-type mimics as they lack a lactone ring typical of AHLs. Seed and root exudates of *Arachis hypogaea* L. (peanut) contain unidentified long-chain AHL-like mimics and short inhibitory chain AHL-like compounds, which are tools for manipulating the bacterial behavior in the rhizosphere (Nievas et al. 2017).

### 2.8.2 Plant Metabolites and Enzymatic Interference of QS

Many plant secondary metabolites interfere directly and indirectly with bacterial QS. Keshavan et al. (2005) coined the term quorum sensing-interfering (QSI) compounds for the second group of plant signals that do not resemble AHLs. They observed inhibition of violacein production in *C. violaceum* CV026 by Alfalfa seed and seedling exudates without affecting cell growth, indicating interference of QS. Structural characterization of a molecule purified from the exudates revealed it

as L-canavanine, an arginine analog. The Alfalfa seed exudates also inhibited the synthesis of exopolysaccharide EPS II, QS-regulated phenotype, required for establishing symbiosis in *S. meliloti*, the natural symbiont of Alfalfa. A similar effect was demonstrated by synthetic L-canavanine, which did not affect protein synthesis or synthesis of AHL in *S. meliloti.* A possible mechanism of action may be the incorporation of L-canavanine in place of L-arginine in the QS regulator protein, thus impairing protein folding. Alternatively, some bacteria such as *Streptococcus faecalis* can degrade L-canavanine to homoserine (Kalyankar et al. 1958), which if converted to AHL mimic scan also contribute to the QS inhibition property of L-canavanine.

Salicylic acid (SA) produced by plants downregulates the virulence genes and activates the quorum degradation system, *attKLM* operon in *Agrobacterium*, which includes the *att*M gene encoding a lactonase (Yuan et al. 2007). Thus, SA might act as a powerful plant defense signal acting against *Agrobacterium* and other QS bacteria in the phytobiome. A similar interference with QS mechanism was observed when plant phenolic acids, cinnamic acid (CA), and SA inhibited expression of QS regulator gene; QS regulated virulence genes and reduced AHL level in *Pectobacterium aroidearum* and *P. carotovorum* (Joshi et al. 2016). Cinnamaldehyde is a naturally occurring compound in the bark of cinnamon trees and widely used in food industries. Niu et al. (2006) reported inhibition of 3-hydroxy-$C_4$-HSL- and $3OC_6$-HSL-mediated QS by very low concentrations of synthetic cinnamaldehyde. p-Coumaric acid, a natural phenolic compound produced by plants, showed both stimulatory and inhibitory effects on QS in a concentration- and strain-dependent manner, whereas garlic extract inhibited QS receptors LuxR, AhyR, and TraR, which become toxic at higher concentrations (Bodini et al. 2009). It is interesting that coumaric and cinnamic acids have also been implicated in the synthesis of aryl HSL signals in certain nodulating rhizobacteria (Schaefer et al. 2008; Ahlgren et al. 2011).

The vitamin riboflavin and its derivative, lumichrome, are synthesized by bacteria such as *B. subtilis*, *E. coli*, *Photobacterium phosphoreum*, and *Actinobacillus pleuropneumoniae* (Vitreschak et al. 2002), a majority of plants (Roje 2007), and algae like *Chlamydomonas* (Palacios et al. 2014). Riboflavin and lumichrome purified from the green alga *Chlamydomonas reinhardtii* can bind to and activate the AHL receptor LasR and thus interfere with bacterial QS (Rajamani et al. 2008). Change in response to riboflavin and lumichrome after mutation of LasR residues required for AHL binding indicated a similar binding site for all the three signals. These molecules may have a larger and complex role, given the fact that they are produced by plants, bacteria, and algae (Mathesius and Watt 2010).

Medicinal plants also contribute a vast repertoire of secondary metabolites with anti-QS effects. Curcumin, the phenolic bioactive compound of *Curcuma longa* (turmeric), is a popular example. It can attenuate *Pseudomonas aeruginosa* (PAO1) virulence by interfering with AHL production, biofilm formation, and inhibiting elastase and protease activity (Rudrappa and Bais 2008). It is associated with reduction in biofilm formation by uropathogens, *E. coli*, *Proteus mirabilis*, *P. aeruginosa*, and *Serratia marcescens* that cause persistent urinary tract infection via QS-dependent virulence mechanisms (Packiavathy et al. 2014). Some eminent

examples of medicinal plants with anti QS activity include *Terminalia chebula*, *Ocimum sanctum*, *Amphipterygium adstringens*, and *Centella asiatica* (Bouyahya et al. 2017). The primary bioactive compounds present in these medicinal plants are polyphenols, terpenoids, flavonoids, quinines, tannins, anthocyanins, polyamines, cytokinins, and polysaccharides (Bouyahya et al. 2017).

Many plant volatiles such as essential oils (EO) and their components compounds also act as a signal and affect bacterial QS (Ahmad et al. 2015). *Eucalyptus globulus* and *E. radiate* essential oils contain eucalyptol and limonene as the main bioactive component, respectively, which inhibited violacein pigment of the reporter strain *C. violaceum* CV026 without changing cell growth parameters, indicating anti-quorum sensing activity (Luís et al. 2016). A similar effect was seen in EOs from different species of *Piper* (Olivero et al. 2011). Khan et al. (2009) reported the anti-QS property of an unknown component of clove oil, whereas eugenol, the main constituent of clove oil, could not inhibit QS activity, indicating the anti-QS property of other minor components of the oil such as α-caryophyllene and β-caryophyllene. Citrus reticulate EOs also showed inhibition of biofilm formation and AHL production (Luciardi et al. 2016). *Thymus vulgare* EO inhibited the expression of the AHL-related flagella gene *flg*A required for initial bacterial attachment for biofilm formation by the foodborne pathogen *P. fluorescens* KM121 strain (Myszka et al. 2016).

The significance of the AHL-based signaling systems is reflected in the abundance of quorum quenching activities in plants and animals, which have the role of destroying the signaling activity of bacterial pathogens (Schikora et al. 2016). Plants can interfere with bacterial QS by producing enzymes as well. Delalande et al. (2005) reported rapid disappearance of AHLs from the root region of germinating *Lotus* plants, and a possible enzymatic mechanism is suspected. AHLs are reported to alter *Arabidopsis* postembryonic root development (Ortíz-Castro et al. 2008). Fatty acid amide hydrolase expressed by *Arabidopsis* could degrade AHLs, as evident from the increased resistance to AHLs in overexpressing mutants, and AHLs induced susceptibility to developmental changes in fatty acid amide hydrolase mutant (Ortíz-Castro et al. 2008).

## 2.9 Role of Endophyte in a Phytobiome

Endophytes are microbes that symbiotically live whole or at least a part of their life cycle within a living plant without showing any signs of infection. However, commonly known endophytes are mostly considered commensals and do not affect host plant functioning, while less common are either mutualistic with beneficial effects or antagonistic as latent pathogens (Hardoim et al. 2015). The beneficial effects of an endophyte include the promotion of plant growth, health, and contribution to host defense response against a pathogen and abiotic stresses (Khare et al. 2018). One of the most notable properties of endophytes is their ability to either synthesize or induce a host plant to synthesize metabolites that promote plant growth and help them adapt better to the environment (Varma et al. 2017). Thus, they promise eco-friendly sources of useful bioactive compounds such as antibiotics and anticancer

drugs (Hardoim et al. 2015). Moreover, endophytic make up of edible plants becomes a concern as it is known that some plant-friendly endophytes could be potential human pathogens (van Overbeek et al. 2014).

Although it is accepted that the interaction of endophyte and its host plant may be multidimensional (Khare et al. 2018), many fundamental questions remain to be answered. How do plants recruit endophytes (Kandel et al. 2017)? How does the endophyte survive the first line of defense of the host plant? How do they influence host gene expression and how different members of the endophyte community interact and influence one another?

There are a few reports on QS activity among endophytes and about its influence on the host plant. Hudson et al. (2010) isolated and identified several culturable endophytic strains from stem tissue of sugarcane and from xylem fluids of grapevine. Five of six sugarcanes and fourteen of fifteen grapes with identified endophytes showed a significant response in at least one AHL-dependent biosensor. AHL lactonase activity has been reported in endophytic population isolated from potato tuber peel (Ha et al. 2018) and *Pterocarpus santalinus* (Rajesh and Rai 2014). Two AHL-based quorum sensing systems, SplIR and SpsIR, have been reported from endophytic *Serratia* sp. strain G3 (Liu et al. 2011). While similar to the free-living strain of *Serratia* HRO-C48, antifungal activity, indole-3-acetic acid and exoenzyme production in the G3 strain are regulated by QS, however, QS control of swimming motility and biofilm formation was a strain-specific phenotype, reflecting a lifestyle driven by the evolution of the QS systems in free-living and endophytic strains. Jiang et al. (2014) reported production of $3OC_8$-HSL by *Pantoea agglomerans* YS19, an endophytic diazotrophic bacterium isolated from rice, and revealed the importance of $3OC_8$-HSL in promoting bacterial growth and also in the formation of a multicellular aggregate structure called symplasmata, which is a characteristic of the bacterium.

*Pseudomonas* sp. strain GM79, an endophyte isolated from cottonwood (*Populus deltoides*), possesses an orphan OryR homolog PipR, which was shown to require an unknown signal derived from the cottonwood leaf macerates instead of an AHL (Schaefer et al. 2016). The signal was later identified as a chemical derived from ethanolamine activity (Coutinho et al. 2018). Similar roles of plant signals for activation of different solo/orphan Lux R homologs have been reported in many other free-living bacteria (Ferluga et al. 2007; Ferluga and Venturi 2009; Subramoni and Venturi 2009). Thus, the current understanding of endophytes signifies their immense potential and advantage in improving the quality of a phytobiome. At the same time, the need for a deeper understanding of the physiology of endophytes is also obvious, given the limited number of reports and a nonsystemic approach of study used so far (Hardoim et al. 2015).

## 2.10 Conclusion and Forthcoming Prospects

Managing the phytobiome may offer an important biotechnological area for improving agricultural yield and quality with a minimum participation of harmful agrochemicals. Phytobiome studies may provide more precise insights into the

mechanisms and consequences of disease (and resistance) and identify microbial indicators of disease (and resistance) progress. In the field, it can be assumed that communication signals are exposed to many interfering and mimic signals from the neighbors, and hence a system-level understanding of phytobiome is the appropriate approach for successful application of this understanding.

One of the pivotal points of control to bring desired traits in a phytobiome is the manipulation of the interspecies and intraspecies communication between plants and microbes, either by introducing selected beneficial microbes as microbial biofertilizers and biopesticides or by genetic engineering of plants and microbes. A known plasmid pME6863 carrying a lactonase coding gene *aiiA* from *Bacillus* sp. A24 to *Pseudomonas fluorescens* P3 exhibited the ability to degrade AHLs and significantly reduced soft potato rot caused by *Erwinia carotovora* and crown gall of tomato caused by *Agrobacterium tumefaciens* to a similar level as *Bacillus* sp. A24. Tobacco and potato plants transformed with AHL lactonase from *Bacillus* sp. showed enhanced resistance to *E. carotovora* infection.

The use of advanced "omic" technologies, such as metabolomics and proteomics, allows a more reliable analysis of phytobiome interactions in unprecedented detail and provides insights into the resistance mechanisms that consider both simultaneous attacks of various pathogens and the interplay with beneficial microbes. In this context, the metagenomic study of unculturable microorganisms would also provide an indispensable solution to this whole complex equation in a phytobiome. Moreover, a multidisciplinary analysis should be incorporated by collaborative discussions among different disciplines such as biologists, ecologists, plant pathologists and agricultural entomologists. It should be soon possible to provide a systemic picture of how and to what extent plants can shape their own detrimental or beneficial community.

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# Plant Microbiomes: Understanding the Aboveground Benefits

3

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**Abstract**

Soil and plant root are known as the microbial reservoir, and these microbes are found broadly in the plant rhizosphere and tissues. Phytobiome generally exists as epiphytic, endophytic, and rhizospheric that undertakes a critical role in plant development. These microbiomes may shape networks, to stabilize the function among different kinds of plant-associated factors to propagate or transmit in a different part of the plant. Microbial networks linked with plant health give crucial beneficial insights to look upon. The present section covers the features of such microbial networks that build the phytobiome. The chapter highlights their ability to better uptake nutrients or plant growth regulators in a stressed environment and further extends an evolution of studies depicting the supporting components that shape the phylogenetic and plant-related networks. The chapter advocates the possibility to understand the techniques by which plants select and connect with their microbiomes and affect plant improvement and well-being, thereby laying the foundation of novel microbiome-driven systems to the advancement of sustainable agriculture. The microbiome is unpredictably

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engaged with plant well-being providing extra qualities to the plant. To understand the guideline of plant characteristic articulation, henceforth plant execution, and how this impacts the biological systemic network, it is required to get well versed with phytobiome and its usefulness. In the present section, the significance of the phytobiome to plant genomics is tended to describe the phytobiome in assembly to the environment of the outline with attention on natural surroundings happening subterranean at the plant-soil between face, where the center is around the job of exudates as currency in this framework.

**Keywords**
Microbial communities · Rhizosphere · Endophytes · Phyllosphere

## 3.1 Introduction

Nature allows the coexistence of healthy and asymptomatic plants with diverse microbes such as archaea, bacteria, fungi, and protists where a complex microbial consortium is formed to impact plant growth and productivity (Vorholt 2012; Kumari et al. 2019; Solanki et al. 2019). Phytobiome has either neutral or helpful roles in the plants' fitness (Mendes et al. 2013). The useful impacts on plants include disease suppression (Ritpitakphong et al. 2016; Solanki et al. 2012), plant immunization (Van der Ent et al. 2009), induction of systemic resistance (Zamioudis et al. 2015), increased nutrient acquisition (Van der Heijden et al. 2016), increased tolerance to abiotic stresses (Rolli et al. 2015; Wang et al. 2018), adaptation to environmental variations (Haney et al. 2015), or enhancement of the mycorrhizal colonization (Garbaye 1994). Microorganisms can also target agricultural productivity by providing nutrient availability/acquisition (Kavamura et al. 2013). Lack of precise methodologies has led to limited access to nonculturable microbial groups, and thus, most of the work relies on single microbial groups associated with plants (Andreote et al. 2009). Mycorrhizal association with a plant (Chagnon et al. 2013) and microbial diazotrophs (Raymond et al. 2004) are the few examples that need to be explored in-depth. Nevertheless, an inclusive map of this system laid stress on the interactions happening between diverse groups of microbes, permitting the term "microbiome." Joshua Lederberg coined the term "microbiome" for the first time and described it to be the "ecological community of commensal microorganisms, symbionts or pathogens that occupy a space in our body" (Lederberg and McCray 2001). New terminology for "microbiome" was suggested by Boon et al. (2014) that relates to host-associated genes in a defined surrounding, thereby bypassing the abundance of the microbial community of low significance. Plant-associated microbial groups work in multidimensional ways as host plant delivers unique metabolic adeptness to attract beneficial microbial niches that can have a positive (mutualistic), neutral (communalistic), or deleterious (pathogenic)

effect on plant health (Thrall et al. 2007). Microbes are the main component of plant functional traits such as soil formation, organic matter decomposition, nutrient mobilization, and improvement in plant productivity (deBello et al. 2010). Rhizospheric prokaryotes are known as plant helpers due to their beneficial activities such as nitrogen ($N_2$) fixation (Martinez-Romero 2006), solubilization of insoluble minerals, and stimulation of phytohormones (Hardoim et al. 2008). Genome duplication (polyploidization) is defined as macroevolutionary events of host that can change microbiome structure. The phytobiome exerts influences on plant trait expression through upstream and downstream regulation of nutritional uptake, thus supervising plant's performance. To unlock the subtleties inside the ecosystem, and the regulation of plant trait expression, impacts of the microbiome are needed to be observed. Bernedsen et al. (2012) reported that plant microbiome interface aligned as "microbe-soil-microbe-plant-microbe interface" rather than the "soil-microbe-plant interface." Plant genome is itself a complex system, and microbial interaction is coined as the plant's "second genome" because it extends the plants' genetic compendium extensively.

This chapter also contains a detailed description of beneficial phytobiome interactions. Three microbial groups (bacteria, fungi, and protists) that abundantly originate on plant tissues are deliberated, and diverse mechanisms used to cooperate and compete in planta are defined. Nevertheless, the activity of microbiomes is a new systematic approach that is required to understand the multidimensional actions of microbial communities (Bashiardes et al. 2018). To some degree, microbiome applications would include an emphasis on enlightening basic components that can improve crop production such as management of plant nutrients, soil health, and environmental safety (Syed Ab Rahman et al. 2018).

## 3.2 Soil Microbiome Characterization

Phytobiomes are discrete that comprise unfavorable pathogens, potential endophytes, and helpful symbionts (Rosenblueth and Martínez-Romero 2006; Wang et al. 2017; Malviya et al. 2019). Be that as it may, traditionally, the microbial assorted variety was assessed by segregating and refined on various supplement media and development conditions. Microbial metabolism fulfills the nutritional and regulatory prerequisites of plants (Lugtenberg and Kamilova 2009). These healthful necessities, for the most part, incorporate nitrogen, phosphorous, and iron. Moreover, these elements also control plant growth by stimulating the production of plant growth regulators. Screening of the most suitable bacteria would require culture-based methods (Taulé et al. 2012). On a routine basis, the procedures normally contain an agar plate assay or a broth medium to multiply the microbes. These assays also help in locating genetic components of microbes. However, these protocols failed to explore the microbial diversity of nonculturable microbiota.

For investigating the entire microbiome, the very first effort is initiated with sequencing of a conserved gene region such as the 16S rRNA gene that is widely

applied for microbial identification (Mullis et al. 1987). To contemplate and comprehend the microbiome in a brief span, thorough upgrades have been accomplished by this technique, thereby yielding metagenomics. These techniques incorporate beginning with the entire metagenome examining, trailed by refinement, partition, and sequencing and lastly information investigation and elucidation. Particularly, the sequencing innovation is experiencing fast improvement, as it gives wide and top to bottom perspectives on metagenomics, and now it is extensively named as high-throughput sequencing (HTS) or cutting-edge sequencing technology. HTS methods incorporate the utilization of the AB SOLiD System (Life Technologies), the HiSeq 2000 (Illumina), and the 454 Genome Sequencer (Roche Diagnostics) (Yergeau et al. 2014). Besides, other propelled methods, for example, DNA/RNA-SIP and DNA arrays (PhyloChip and practical quality exhibits), likewise have prospective highlights in the examination of microbiomes, mostly their useful parts (Uhlik et al. 2013). At present, there is a change from metagenomics to metatranscriptomics, as the latter helps in understanding the numerous microbial functions and structure (Turner et al. 2013).

In the metatranscriptomics approach, complementary DNA analysis aligned with quantitative reverse transcription-PCR and RNA-SIP explored the microbial functionality associated with the soil and rhizosphere (Uhlik et al. 2013). RNA-SIP significantly is used to crack the complexity in interactions especially between root-derived carbon and microbiome so as to provide sequence as first and second utilizers of carbon within the microbiome. This method is dissimilar to DNA-SIP because it provides higher amounts of labeling and does not rely on cell multiplication. Challenges coming with these cutting-edge innovations include choosing either mRNA or rRNA alone and accomplishing more extensive inclusion of environmental RNA pool that gives naturally vital information through the sequencing. Peiffer et al. (2013) demonstrated noteworthy community contrasts among 27 maize innate lines (a genetic variant of a single species) with a normal enhanced population in the maize rhizosphere. Metaproteomics, on the other hand, has a different approach as it focuses on the dynamic function of the phytobiome and extracts samples of metaproteome and performs peptide fingerprinting by mass spectrometry (Kolmeder and de Vos 2014; Lakshmanan et al. 2014). Using metagenomic and metaproteomic (existing and future) information is an essential process-driven methodology and should be supplemented by different strategies to decide the diversity and functional relatedness of the rhizospheric microbiome (Keiblinger et al. 2012).

Molecular methods (molecular fingerprinting) and plate count anomaly (culture-dependent methods) demonstrate the entire bacteria community structure (Amann et al. 1995). Therefore, both approaches are utilized, for thoughtful knowledge of separate classification and communication with host plants. Dini-Andreote and van Elsas (2013) have, however, stressed the present need for a change in outlook from HTS (or comprehensive endeavors) to investigations of basic studies.

## 3.3 Structural and Compositional Factors in Plant-Associated Microbial Network

### 3.3.1 Plant-Associated Bacterial and Archaeal Microbiomes

Plant-associated bacterial population detected on plants does not look arbitrary; relatively numerous components participate in controlling the structure of microbiomes such as soil type (Lundberg et al. 2012), plant compartment (Leff et al. 2015), host genotype/species (Tkacz et al. 2015), plant invulnerable framework (Horton et al. 2014), plant attribute variety/developmental stage (Donn et al. 2015), and residence time/season (Shi et al. 2015). Hacquard et al. (2015) described that *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes* are the major bacterial phyla that exist in both substrata such as above- and belowground plant tissues, and they influence the plant metabolism. Broad cover among root- and leaf-related network individuals has been portrayed at OTU (operational taxonomic unit) level determination in different plants such as *Arabidopsis thaliana*, wild mustard, grapevine, and agave (Wagner et al. 2016), and microbiota reconstitution experiments with germ-free *A. thaliana* approved that root- and leaf-related bacterial networks have reciprocal relocation. Regardless of the conspicuous elementary uniformities saw between *A. thaliana* leaf- and root-related bacterial networks, it is observed that related microbiota individuals are particular and adjusted to their separate related plant organs (Bai et al. 2015). Among all phytobiomes, the nonpathogenic segregation of archaea has been depicted. The plant endophytic archaeal taxa of the phyla *Thaumarchaeota*, *Crenarchaeota*, and *Euryarchaeota* have plant-associated functional significance (Müller et al. 2015).

### 3.3.2 The Fungal Microbiota of Plants

*Ascomycota* and *Basidiomycota* are two noteworthy phyla that colonize both above- and belowground plant tissues (Hardoim et al. 2015). In roots, even though arbuscular (*Glomeromycota* phylum) and ectomycorrhizal growths have been for the most part contemplated, ongoing network profiling information demonstrates that other endophytic organisms too make up for root microbiota (Toju et al. 2013). The structure of fungal communities on plants relies upon different kinds of soil, plant parts, plant genotypes, or seasons (Coince et al. 2014) and is subjected to stochastic variations (Wang et al. 2013) and reacts distinctively to ecological elements (Thomson et al. 2015). Thus, mostly dispersal restriction and atmosphere clarify the worldwide biogeographic conveyance of growths and have been recommended to compel contagious dispersal, supporting high endemism in parasitic populaces (Talbot et al. 2014). Steady with that, the synchronous examination of both contagious and bacterial networks related to plants recommended a more prominent significance of biogeography for organizing parasitic networks contrasted with bacterial networks (Hacquard. 2016). Regardless of using molecular markers such as 16S rRNA and ITS, their loci need to be elucidated (Peay et al. 2016).

Recently, Yunshi et al. (2018) quantified the prokaryotic and fungal groups within the phyllosphere and rhizosphere of six spruce (*Picea* spp.) tree species through illumine amplicon sequencing. In brief, this microbial quantification experiment is performed in a common garden, and linkages among phenotypic characters of their plant hosts and bacterial/archaeal and fungal community are analyzed. Correlation results among plant microbiome and different phenotypic characters of host plants (such as leaf morphology, water content, water storage ability, dry biomass, nitrogen, etc.) which suggests that plant genotype played a significant role to shape its microbiota by improving plant phenotypes.

### 3.3.3 Plant-Associated Protists: The Outcasted Fraction of the Plant Microbiota

Protists are a vital constituent of the soil microbiome, and method progresses now extended to our thoughts of the real taxonomic and efficient diversity of soil protists. The Stramenopiles-Alveolata-Rhizaria (SAR) group is known as a large group of plant-associated protists (Ruggiero et al. 2015) and especially those having a place with the *Oomycota* (Stramenopiles) and *Cercozoa* (Rhizaria) lineage. Inside *Oomycota*, a couple of individuals having a place with the genera *Peronospora*, *Phytophthora*, *Pythium* (and other wool buildup genera), or *Albugo* frequently exist in the plant roots or leaves (Agler et al. 2016). Root colonization by oomycetes (i.e., *Pythium oligandrum*) provides positive benefits to the host (Van Buyten and Hofte 2013). Even though plant tissue-associated oomycete network profiling stays scanty, an exceptionally low decent variety is demonstrated with individuals from the *Pythiaceae* family being the most spoken about to be present on plant tissues (Sapp et al. 2018). Inside *Cercozoa*, one of the prevailing protistan bunches in biological systems, network profiling information uncovered a surprisingly high diversity in plant roots and leaves (Ploch et al. 2016), also giving a piece of strong evidence that indicates the plant stress tolerance and metabolic behavioral changes governed by special community structure. Thus, *Oomycota* and *Cercozoa* individuals are significantly important for holobiont wellness. Recent reports concluded that plant microbe linkages are outlined well under the evolutionary measure and it helps to unlock the complex interactions of plant and microbes in more depth, and plant-microbe or plant bacterial interaction is new as compared to the bacterial and other kingdom interactions (Lücking et al. 2009; Hassani et al. 2018).

## 3.4 Microbial Currency: Exudates

Plants and microbes release certain chemicals called exudates, which help them to communicate with each other and to accelerate the disease tolerance against biotic and abiotic factors, stabilize the plant and microbial growth during nutrient scarcity, and remediate the toxic elements. Microbes utilized exudates as a food source, particularly carbon and other acids. This section discussed the two-way interaction of

plant and microbial exudate that is influenced by plant and microbial metabolism. Huang et al. (2014) reported the significance of plant root exudates to regulate the microbial structure in the plant rhizosphere that is influenced by plant variety, growth stages, disease-suppressive soils, root exudate composition, and plant hormone signaling. Plant-microbe interaction is a complex system that is mediated by numerous compounds, and these compounds are released under specific conditions. These compounds play an indispensable role to shape the microbial community and unified the microbes and their functions up to species level. For example, legumes and rhizobia symbiosis is signaled by flavonoids, plant mycorrhizal association is stimulated by strigolactones, malic acid regulates the quorum sensing (QS) of plant microbial helpers and major chemoattractants of microbes such as sugars and amino acids attract the beneficial microbial niches toward the plant roots to protect the plant against the multiple stresses. However, various protein molecules are released from the root in the rhizosphere that are less explored to understand their mechanism in plant fitness. Besides, root exudates played intermediate role in several other interactions such as plant attract the nematodes, and these nematodes are the vectors of rhizobia that enhanced the nodulation of root to fix the nitrogen, plant nodulation efficiency enhanced by the interaction of rhizobia with PGPR and arbuscular mycorrhiza (Huang et al. 2014). A multitude of rhizospheric interactions is mediated by root exudates, which are depicted in Fig. 3.1.

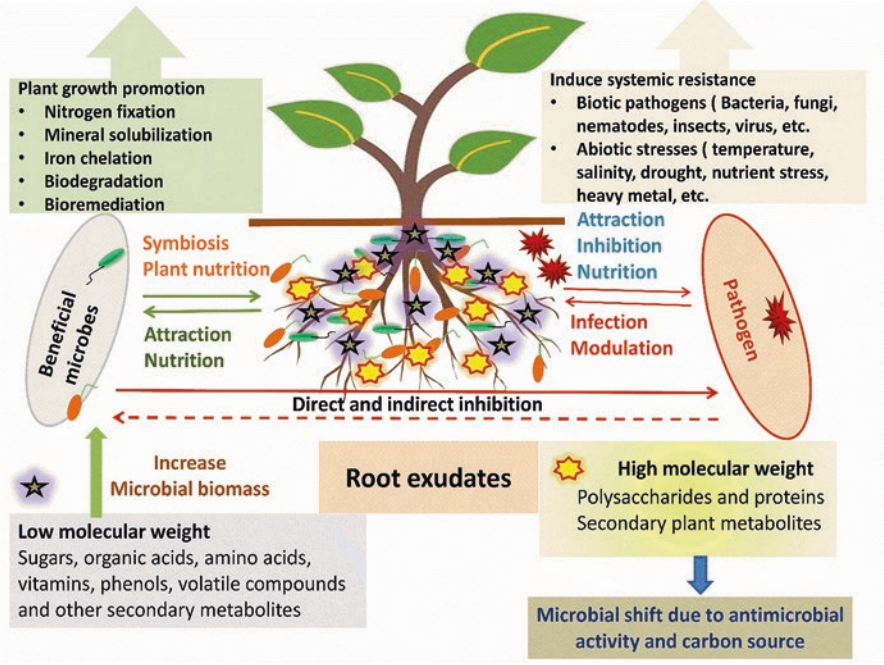


**Fig. 3.1** The complex plant microbe system of rhizosphere that is mediated by plant root exudates. Root exudates improve plant health status directly and indirectly as well

### 3.4.1 Plant Uptake and Release

Plant exudate components and assembly are specific to plant species and incorporate high-molecular-weight particles (e.g., sugar molecules, proteins, and fatty acid) and low-molecular-weight signaling molecules (e.g., natural compounds, metabolites, and amino acids) (Badri and Vivanco 2009). Jaeger et al. (1999) reported that plant root exudate contains sugar molecules and amino acids that help bacteria and other microbes to attract toward the plant root. Exudates assist numerous jobs such as stimulate the antagonism, allelopathic particles, and pathogen/herbivore safeguards. A large number of these exudates likewise fill in as a vitality hotspot for the microbiome; prokaryotes can use plant exudates as nutrient sources. For instance, grass *Sorghum halepense* excretes the exudate sorgoleone from root hairs having allelopathic properties (Kagan et al. 2003) which can be used as microbial nutrients (Gimsing et al. 2009). The different elements of plant exudate repeat the significance it fills in as numerous monetary forms of the phytobiome.

Roots participate in taking up the nutrients and signaling molecules from the rhizosphere while at the same time saving these supplements and concoction signaling molecules into this equivalent space required for evoking defense reactions. Terpenoids, flavonoids, and isoflavonoids contain a large number of the plant's antimicrobial barriers. Isoprenoids being the most diverse primary metabolite is required to control cellular processes such as photosynthesis (as phytopigments) and seed growth stimulation (as gibberellic and abscisic acids), and allelopathic molecules also protect the plants from the pathogens (Hardoim et al. 2008).

### 3.4.2 Microbial Uptake/Release

Nitrogen fixation requires a constant need for rhizobium-legume symbiosis inside the biosphere. The ability of this methodology to enhance agricultural yield has produced attention in knowledge to manipulate this process for better use. A number of the study approaches were procured inside the examiner of rhizobia, and the precise knowledge collected from these numerous tools is used to focus on the genome- and systems-level procedures (diCenzo et al. 2019). Exudates are essential methods for correspondence within the environment for the microbial network. The uptake of exudates such as sugars, organic, and amino acids has been a noteworthy focal point of many years of research in a microbial environment utilizing different estimations of respiration or carbon substrate use measures, for example, those utilizing ECO MicroPlates™ (Biolog®). Microbial community behaves according to expanded or diminished centralizations of promptly accessible supplements that require insignificant vitality to absorb. Moreno et al. (2009) reported that a PGPB *Pseudomonas putida* KT2440 utilized amino acids and sugar that is concluded by the identification of proteins that regulate the amino acid and sugar uptake. The microbiome of rhizosphere has a perplexing task in nutrient cycling and includes a horde of nutrient transformations in soils. Microorganisms act as a catalyst for chemical changes in the soil during biogeochemical cycling. These changes plant

supplement (N and P) uptake, soluble metal ($Ca^{2+}$, $Mg^{2+}$, $K^{+}$, and $Na^{+}$) uptake, and micronutrient uptake ($Zn^{2+}$, $Fe^{2/3+}$, $Cu^{+}$, and $Mn^{2+}$) (Stevenson and Cole. 1999).

Nitrogen-fixing prokaryotes played a vital role to fix nitrogen gas ($N_2$) into ammonia (NH3), and this method helps the plants. Howard and Rees (1996) reported that physiological and genetic drivers $N_2$-fixing prokaryotes have a highly conserved protein complex that is nitrogenase, and it is used to assess the abundance of $N_2$-fixers in diverse ecological zones (Zehr et al. 2003). Specific bacterial metabolites worked as important plant hormones, for example, indole-3-acidic corrosive (IAA) engaged with managing plant hormone flagging (e.g., 1-aminocyclopropane-1-carboxylate (ACC) deaminase). These hormone flagging particles can advance plant development.

When wheat is inoculated with rhizosphere bacteria expressing ACC deaminase activity, expanded root improvement, and consequently expanded nutrient uptake, has been recorded (Shaharoona et al. 2008; Honma and Shimomura. 1978). ACC deaminase-producing bacteria manage the ethylene production in the plant, in this manner limiting effects of different ecological anxieties, which typically trigger expanded ethylene generation (Hardoim et al. 2008). Microbial exudates involve a significant part of antimicrobial and antifungal compounds. Several prokaryotic and eukaryotic microorganisms are able to secrete or discharge antimicrobial substances, but among all very few are cultivable (Piel 2011); due to this to understand the complex functions of these substances, metatranscriptomics and metabolomics tools need to be applied. It is found that 35% of *Escherichia coli* strains produce the antimicrobial compound known as calcium. The discoveries bolster the theory that antimicrobial cooperations inside microbial networks serve to look after diversity; this thought was created utilizing recreation models (Czaran et al. 2002).

Notwithstanding oozing antimicrobials that encourage plant resistance, microorganisms additionally release low-molecular-weight compounds to the plants, and plant sensors identify the microbes as a pathogen or beneficial and then trigger the reaction (Boller and He 2009). In this way, microbial metabolites can act straightforwardly on different microorganisms inside the microbiome in suppressive components or can act specifically on the plant to revitalize secure reactions, regularly activating plant exudation. As modern biotechnological tools improve our knowledge to measure these microbial metabolites in the soil matrix, multiple functions of the same microbiological substances are discovered.

## 3.5 Ecological Considerations for Utilizing Plant's Benefits in the Farmer's Field

An effective microbial inoculant needs to attack the pathogens and survive in varying abiotic conditions, also to set up good cooperation with the host that incorporates molecular passivity with the plant resistant framework. All through the developing season, microbial network experiences progression in both over the ground and subterranean (Edwards et al. 2015; (Copeland et al. 2015) portions of the plant. Along these lines, regardless of whether PGP inoculants colonize the plant at first,

their constancy after some time isn't ensured. Estimating the determination of bacterial inoculants in the soil presents technical difficulties, as the inoculant should be distinguished from a complex network. Strategies such as culture-based count utilizing re-separation of antibiotic-resistant inoculants or culture-autonomous estimation of relative bounty of the inoculant's 16S rRNA quality in the soil, by means of DGGE (Schreiter et al. 2014), amplicon sequencing (Haney et al. 2015), or metagenomic sequencing (Krober et al. 2014), are used to determine the persistence of microbes.

Ecological components impacting root exudate organization and amount include raised dimensions of $CO_2$, dry season, and nutrient deprivation (especially nitrogen and phosphorus). Increased carbon allocated in roots is observed in $CO_2$-fertilization experiments, resulting in shifts in exudate composition and concentration that differ with plant species (Cheng and Gershenson 2007). These species-explicit effects can result in increased yield, in no net profitability increment, or can be unfavorable to plant development and generation. For example, positive biomass reactions in rye and clover to $CO_2$ preparation were observed, while maize demonstrated no net biomass advantage (Phillips et al. 2006). Be that as it may, maize showed expanded exudation of a few amino acids under $CO_2$ treatment. These discoveries are not amazing thinking about that the $C_4$ photosynthetic pathway encourages development under elevated amounts of $CO_2$; in any case, the effects of expanded arrival of amino acids into the rhizosphere by the $C_4$ grass (maize) may assume a job in a large number of criticisms between different plants and organisms (Klironomos 2002).

### 3.5.1 Impact on Plant Functions

Plants participate in nutrient exchange and exudate correspondence depending on the molecule and energy required for the plant (alone or through help from the microbiome) to acquire or release exudate currency. An active transport system using ATP-restricting tape transporter participates in root exudation creation and fixation (Badri et al. 2009). Low-molecular-weight particles such as amino acids can be discharged through membrane diffusion or through protein channels (Badri and Vivanco 2009).

Plants utilize those microbes which can communicate with increased levels of N-acyl-L-homoserine lactones. AHL-degrading enzymes in the presence of a pathogen subsequently suppress gene expression of pathogens (Reading and Sperandio 2006). Plants in the same manner also help in AHL degradation inside the microbiome (Teplitski et al. 2000). Fluorescent pseudomonads which are fundamental to the rhizosphere of the different clusters of the plant are used to deliver the antimicrobials 2,4-diacetyl phloroglucinol (2,4-DAPG) and phenazine (Phz) derivatives (Mavrodi et al. 2011). These antimicrobials are of wide range and act against a number of plant pathogens that are contagious leading to their suppression (Raaijmakers et al. 2009). 2,4-DAPG and Phz derivatives are evident in the rhizosphere and are associated with the suppression of disease in wheat called as

take-all in wheat. These plant hormones administrate the plant performance and its marking abilities to adjust to exudate profiles and enhance the plant's immunity (Doornbos et al. 2012).

Most of the microorganisms are capable of producing and controlling the majority of plant hormones (Friesen et al. 2011), thereby modifying plant physiological pathways. In plant rhizospheres, 80% of bacterial taxa accounts for IAA production (Loper and Schroth 1986). In plants, root development is accelerated with a low concentration of IAA (Glick 1999), while its high concentration represses plant's development, hence making plants prone to pathogen's attack (Sarwar and Kremer 1995). An example of this is seen in *Sorghum halepense*; microbes of the invasive grass secrete high concentrations of IAA in contrast to other prokaryotes that secrete lower levels of IAA (Rout et al. 2013). Environmental stress and plant phenology drive the changes in plants that need to increase or decrease the hormones. PGPB are competent cells having multiple genes required for plant-microbial association (Hardoim et al. 2008). Yield expansion, organic methodologies, intercropping, and other cultural practices are utilized for possible farming production. New strategies are formulated to modulate the plant microbiome in an ideal course (Fig. 3.1). Distinctive microbiota is induced by diverse agro-management in viticulture (natural, biodynamic, or biodynamic with green compost) (Longa et al. 2017). It is observed that in an integrated management system, soil has diminished bacterial species richness as compared to organic management, even though microbial composition was similar to organically and biodynamically managed soils (Hendgen et al. 2018).

### 3.5.2 Impact on Bacterial Functions

Microbiome present in the rhizosphere possesses varied phenotypic expressions due to root exudates. Inhabitant microflora of plants perform different functions such as chemotaxis, stress tolerance (Amador et al. 2010), polychlorinated biphenyl degradation (Toussaint et al. 2012), modulation of genes involved in competence and sporulation (Mader et al. 2002), and biofilm formation on plant roots (Rudrappa et al. 2008). Plant exudates such as terpenoids, flavonoids, and isoflavonoids protect and control the internal structure of plants and outward surface of roots from microbial inhabitant (Hardoim et al. 2008). Microbiome symbionts can be epiphytes or endophytes, which survive for a short growth period and may encompass not only the pathogens but plant growth-promoting microbes using a mechanism of hormone signaling. Organic farming impacts the community composition on soil and roots of winter wheat (Hartman et al. 2018). The structure of bacterial communities is taken care of by tillage. Root bacteria respond to management types, whereas fungal communities respond to both. Different agricultural practices are parameters in affecting the microbial structure with differences in soil, roots, bacteria, and fungi and hence bringing around 10% of the variation in microbial communities.

## 3.6 Formation of Biofilm

Microbial communities act as a unit and secrete polymeric substances to produce a network known as biofilms (Stoodley et al. 2002). Microbes, when present in a biofilm as consortia, are highly protected from their competitor, antimicrobial agents, enzyme degradation, and acquisition of new genes through horizontal gene transfer (Van Acker et al. 2014; Nadell et al. 2009; Zhang et al. 2014). *Enterobacter* spp., a root-occupying bacterial endophyte, when forming a biofilm, inhibits the entry of root-colonizing pathogen *Fusarium graminearum* (Mousa et al. 2016). Bacteria commonly produce biofilms on fungi, but it is rarely seen on the hyphae of ascomycete fungi. An example of this is seen in *Pseudomonas fluorescens* BBc6 which formulates biofilm on the hyphal region of the ectomycorrhiza *Laccaría bicolor* specifically at its root tip, thereby establishing ectomycorrhizal beneficial symbiosis and promoting bacterial biofilm on fungal host surfaces (Guennoc et al. 2017).

## 3.7 Molecular Communications

The mechanism of quorum sensing is used by microbes to sense their counterparts. Gram-negative microorganism secretes signaling molecule N-acyl-l-homoserine lactone (AHL) to screen out their populace densities (Eberl 1999). Regulation and secretion of signaling molecules are evident in *Saccharomyces cerevisiae* and *Candida albicans* (human fungal pathogens) that secrete farnesol to control filamentation (Oh et al. 2001), constrain biofilm formation, and activate oxidative stress responses or drug efflux (Sharma and Prasad 2011). Quorum sensing mechanisms are not defined thoroughly for plant-associated fungi. Signaling compounds such as volatile organic compounds (VOCs), oxalic acid, trehalose, glucose, or thiamine accelerate fungal bacterial associations (Schmidt et al. 2016).

## 3.8 Ecology of the Microbiome

The plant microbiome is localized in three different regions, namely, rhizosphere, endosphere, and phyllosphere (Hirsch and Mauchline 2012). Rhizosphere presents a microbial community in requirement with plant metabolism; endosphere presents those microorganisms which interact with host closely and inhabit inner part of plant tissues asymptomatically (Hardoim et al. 2008); phyllosphere, on the contrary, is composed of those microbes which inhabit plant surfaces (Lambais et al. 2006). Irrespective of different plant habitats, specific microbes are present in all, also known as "keystone" species which interact with other microbes within the networks and affect the microbial structure (Bakker et al. (2014).

Rhizosphere and endosphere account for root microbiome that was key for the advancement of land plants and underlay crucial ecosystem processes. It is reported that nearly 30 angiosperm species affect root bacterial diversity and composition (Fitzpatrick et al. 2018). A competitive interaction gets affected when there is a

similarity in root microbiomes between hosts among plant species. Climatic parameters such as drought affect the root microbiome composition, by elevating the *Actinobacteria* population. In the endosphere, *Streptomyces* are associated with host drought tolerance influencing drought response crosswise over host plant species bringing host-specific changes.

### 3.8.1 Rhizosphere and Rhizoplane

Plant health is influenced by the rhizosphere using next-generation and third-generation technologies (Hiltner 1904). Rhizospheric soil shows a significant difference in contrast to bulk soil due to abiotic and biotic stresses impacted by the atmosphere. Properties such as higher water holding capacity, expanded nutrient availability, and diverse microbial biomass mark its importance than bulk soil (Schade and Hobbie 2005). Spatiotemporal movements are observed in the rhizosphere microbiome (Kaplan et al. 2013); however, it is still to affirm how much abiotic stresses impact the microbiomes. Protection from a wide range of pathogens both aboveground and belowground is provided by microbiomes. For example, induction of systemic resistance (ISR) is initiated where jasmonic acid-inducible genes are secreted in leaves (Pineda et al. 2010).

### 3.8.2 Epiphytes and Endophytes

The epiphyte and endophyte microbial communities in root involve the acknowledgment and selection of those microbiomes that establish a homeostatic association with the plant. Technologies such as metabolomics and metatranscriptomics are used to observe microbial members that colonize in adherent (epiphytic) or internal (endophytic) parts of plants. Microbes colonize the outside root surfaces. For instance, secondary metabolite root exudates were released due to an ISR response in maize that appoints PGPB *P. putida*, based on chemotaxis inclinations (Neal et al. 2012). Plant chemical exudate is secreted, and valuable PGPB is selected in a plant-mediated reaction as observed in tomato, where natural acids are the major chemotactic operator (De Weert et al. 2002), while in rice, amino acids serve the purpose (Bacilio-Jimenez et al. 2003). Root microbes that laid distinctive qualities such as that code for the sort IV pilus and twitching motility (Bohm et al. 2007), isoflavonoid efflux siphon (Palumbo et al. 1998), and DNA improvements influence colony aggregation (Dekkers et al. 1998).

Endophytes are inhabitant to both wild and domesticated crops including intrusive species (Compant et al. 2008; Rout and Chrzanowski 2009). Biotechnology and agriculture ensure to utilize plant developing qualities of microbiomes as seen in phosphate-solubilizing *Bacillus* strains, where apart from secreting protein ACC deaminase, these also show plant development advancements (Baig et al. 2012). Thus, a microbiome serves a double attribute working together with mycorrhizal parasites to improve plant development advancement (Zaidi and Khan 2005).

Plant phenology correlates with endophyte microbiome composition shifts (van Overbeek and van Elsas 2008) which further depends upon colonization and similarity (Hardoim et al. 2008). This collaboration inclines more toward mutualism than parasitism. Most of known plant endophytes and epiphytes are horizontally transmitted (Friesen et al. 2011). This empowers host-to-host exchange of endosymbionts without the association of plant sexual reproduction. Endophytes also show vertical transmission depending upon host wellness and present more host benefits than horizontal transmission (Clay and Schardl 2002; Sachs et al. 2004). But the environmental hypothesis proposes that the presence of an accessible host allows for horizontally transmitted life forms. It is observed that horizontally transmitted endophytes were positively related to plant thickness reliance, while vertically transmitted endophytes did not demonstrate this pattern (Rudgers et al. 2009).

### 3.8.3 Phyllosphere Region

Phyllosphere is regarded as a third segment of the plant microbiome that colonizes the outside region of the external area of plant tissues specifically when describing the leaf surface (Vorholt 2012). The microbiomes in the phyllosphere perform nitrogen fixation, securing plants against attacking pathogens and biosynthesizing phytohormones (Kishore et al. 2005). These can be beneficial in carbon sequestration (Bulgarelli et al. 2013), and they can also participate in sustainable agricultural practices. Fungi (filamentous and yeasts), bacteria, and algae make phyllosphere network, and at lower frequencies, protozoa and nematodes are seen (Lindow and Brandl 2003). The bacterial population is the most abundant group of microorganisms present in the phyllosphere at numbers ranging from $10^5$ to $10^7$ cells for each $cm^2$ (Andrews and Harris 2000). These microbes can thrive in harsh environmental conditions such as limited availability of nutrients and variable conditions of humidity, UV radiation, pH, and temperature (Andrews and Harris 2000). The phyllosphere community is created with the help of various hotspots as air, soil, and water (Bulgarelli et al. 2013). Agricultural plants also show specificity to phyllosphere microbiomes as seen in beans, cucumber, grasses, lettuce, and maize (Rastogi et al. 2012). Plant genotype plays a significant effect on the composition of phyllosphere microbiomes (Bokulich et al. 2014). The microbial population shows intraspecific variations in its composition which are due to nutritional heterogeneity observed in regions on the leaf surface where heterogeneous carbon sources such as glucose, fructose, and sucrose are utilized near the stomata and surface appendages (Vorholt 2012). At times, this heterogeneity is observed when microbial cells aggregate to form a biofilm and hence defending themselves from unfavorable conditions (Lindow and Brandl 2003).

*Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, and *Firmicutes* are major phyla that account for the microbial community in the phyllosphere region (Vorholt 2012). Hence, this core is accepted to be made out of individuals exhibiting a co-developmental history with plant species, with the host physiology being complementary to the features found inside the microbial cells. Microbes such as protists largely act as

predators on the bacterial community (Flues et al. 2017). Environmental conditions such as low nutrients, high UV, changing temperature, and humidity help in selecting consistent biological traits and low functional diversity at the community level for phyllosphere microbiomes as observed in next-generation sequencing (Lambais et al. 2017). The phyllosphere is dominated by oxygen-consuming organoheterotrophs, and metabolic diversity exists with regard to utilizable carbon compounds.

## 3.9 Competitive Interactions Among Plant Microbiota Members

Plant microbiomes show competitive behavior with closely or distantly related microbiota and affect microbial structure, its stability, and homeostasis.

### 3.9.1 Resource Competition

Microorganisms utilize limited resources and therefore compete indirectly with other microbes. For example, using advanced techniques, microorganisms sequestrate iron using the emission of siderophores, thereby affecting the growth of the opponent microbes present in their niche (Little et al. 2008). When advantageous *Pseudomonas* spp. secrete iron-chelating molecules, it suppresses the disease caused by fungal pathogens indicating that nutrient sequestration is a trait of biocontrol agents to outpower pathogens (Mercado-Blanco and Bakker 2007). In tomato plants, resource competition is said to be an essential factor connecting the bacterial network and pathogen attack on plants (Wei et al. 2015). In resource competition, individual microbes in a group share resources, but the ones who use the resources in an uncooperative can evade paying the price of cooperation while reaping the benefits of utilizing the resource, thereby increasing their fitness (Riehl and Frederickson 2016) and bringing the situation of distress to the commons. Nitrogen-deficient soil could harbor plants with rhizosphere having microbes that can capture nutrients for their usage. For instance, actively growing roots could signal for microorganisms that are capable of producing extracellular enzymes releasing nitrogen bound in soil organic matter (Lemanceau et al. 2017). In prokaryotes, mineralization processes are density-dependent and need a quorum of producers to sufficiently enter key nutrients in the soil. Such producers are taxonomically diverse microbiota that can biosynthetically produce the specific enzymes which are secreted into the soil. In this scenario, selection in the rhizosphere could favor microhabitats to promote coordinated group behaviors that enhance plant access to nitrogen or phosphorus upon cell turnover, while the microorganisms benefit from having an abundant supply of carbon and other nutrients from plant roots (Fig. 3.1). Phosphorous is also made available to plants using microbial taxa which could mobilize phosphorus in the soil via the production of extracellular compounds (Alori et al. 2017). Iron is an important plant nutrient which can be obtained through the production of siderophores (Radzki et al. 2013).

### 3.9.2 Contact-Dependent Competition

Plant microbiome participates in direct antagonistic cooperations interceded by the bacterial type VI secretion framework, a molecular weapon used by certain microscopic organisms (generally *Proteobacteria*) to convey effectors/toxins into both eukaryotic and prokaryotic cells (Records 2011). A few examples of contact-dependent competition are discussed as in the case of the plant pathogen *Agrobacterium tumefaciens* that utilizes a puncturing type VI secretion system to convey DNase effectors upon contact with a bacterial competitor in vitro and on the leaves of *Nicotiana benthamiana*. Moreover, the bacterial kind III secretion system can also be used in *Burkholderia rhizoxinica*, which uses this mechanism to control the productivity of its beneficial interaction with the contagious host, *Rhizopus* microspores. Physical parameters bring a change in plant-associated microbes. Soil condition (organic matter, nitrogen, and moisture content) identification helps in changing the macrophage activation potential of *Echinacea purpurea* and determining these changes in activity that relates to the shifts (Haron et al. 2019). Increasing soil organic matter in the root extracts of *E. purpurea* may increase the macrophage activation. Bacterial communities also differed significantly between root materials having varying levels of organic matter. The activity of *E. purpurea* roots is changed by the soil's organic matter level. Use of bacterial preparation (e.g., probiotics) is reported to impact human health; similarly, *Echinacea* too shows therapeutic effects and is impacted by development conditions that change its related bacterial community (Haron et al. 2019).

### 3.9.3 Antimicrobial Compound Secretion

Various plant-related microorganisms appeared to emit chemical compounds that stifle the development of microbial rivals (Raaijmakers and Mazzola 2012). Filamentous eukaryotes are outstanding in delivering a large number of antifungal activity of secondary metabolites that have a low molecular weight spotted against phylogenetically distinct organisms (e.g., acetylgliotoxin and hyalodendrin) (Coleman et al. 2011). The secondary metabolites so obtained become activated in co-culture and remained inactive in pure culture. Netzker et al. (2015) indicate their specific role in competitive interactions. Antagonistic collaborations among microscopic organisms have been reported to be imperative in the organizing of soil-, coral-, or plant-related bacterial networks (Maida et al. 2016).

Strikingly, the investigation of adversarial collaborations among bacterial segregates from the rhizosphere, the roots, and the phyllosphere of the healing plant *Echinacea purpurea* proposes that plant-related microorganisms compete against one another through the discharge of antimicrobials (Maida et al. 2016). The microbiome related to plants has a robust influence on their strength and yield. The bacterial pathogen, *Candidatus Liberibacter asiaticus* (Las), causes Huanglongbing (HLB) disease and lives inside the phloem of citrus plants, including the root system. It has been proposed that Las negatively affects citrus microbiome. At the

same time, the natural microbial flora of citrus also impacts the association between Las and citrus (Riera et al. 2017), i.e., two bacteria closely related to Las *Agrobacterium tumefaciens* and *Sinorhizobium meliloti* were found. Among them, *Burkholderia metallica* strain A53 and *Burkholderia territorii* strain A63 are within the β-proteobacteria class, whereas *Pseudomonas granadensis* strain 100 and *Pseudomonas geniculata* strain 95 are within the γ-proteobacteria class. It was observed that four bacterial strains *Burkholderia territorii* A63, *Burkholderia metallica* A53, *Pseudomonas geniculate* 95, and *Bacillus pumilus* 104 showed antagonistic action against the pathogen *Phytophthora nicotianae* (citrus root) on the basis of dual culture assays. Some of the antimicrobial-producing strains, *Burkholderia metallica* A53 and *Burkholderia territorii* stress A63, from a mandarin rhizosphere, belong to the *Burkholderia cepacia* complex (BCC) and its agricultural applications are restricted because of its high risk to human health (Depoorter et al. 2016). It remains to be determined whether *Burkholderia metallica* strain A53 and *Burkholderia territorii* strain A63 can cause human diseases. Both *Burkholderia metallica* A53 and *Burkholderia territorii* strain A63 can modulate citrus immune system beneath greenhouse situations while applied as a soil drench. Additionally, the *Burkholderiaceae* family changed into determined to be key taxa within the citrus microbiome of healthy trees in comparison to that of HLB-symptomatic trees in the discipline (Zhang et al. 2017).

### 3.9.4 Predation

Bacterial mycophagy comprises of microscopic organisms' capacity to effectively develop at the expense of living contagious hyphae (De Boer et al. 2004). Mycophagous microbes colonize saprotrophic rhizosphere parasites and feed as auxiliary consumers on root-determined carbon (Rudnick et al. 2015). Some oomycetal species of family *Trichoderma* or *Pythium* can parasite or irritate other growths or oomycetes and can be utilized as biocontrol operators (Benitez et al. 2004). Root-related bacteria can prey on other microscopic organisms as described for *Bdellovibrio* spp. Protist predation on microscopic organisms is also well studied, and recent microbiota reconstitution tests in microcosm demonstrate a reasonable impact of *Cercomonads* (*Rhizaria*: *Cercozoa*) on the structure and the capacity of the leaf microbiota (Flues et al. 2017). Their results show that Alphaproteobacteria and Betaproteobacteria are less impervious to grazing and that predation rebuilds the bacterial system in leaves and impacts bacterial metabolic center capacities. Microbial assortment related to aphid inhabitants was characterized at species and intraspecies scales using a methodological structure (Guyomar et al. 2018). Utilizing this approach, on metagenomics read sets, high genomic diversity in different symbiont taxa can be uncovered in both between and within their hosts. The complete functional diversity related with host and microbiota was the first time it can be accessed using metatranscriptomics datasets which also helps in isolating the transcriptome of each member of the holobiont (Meng et al. 2018).

### 3.9.5 Genetic Management of Valuable Plant-Microbe Interactions

Host hereditary qualities add to plant microbiome assembly. Plants identify microorganisms through pattern recognition pattern that binds to the microbe-associated molecular pattern (MAMPs), setting off a basal barrier adequate to stop the development of most pathogenic organisms (Böhm et al. 2014). Plants can probably separate pathogens from nonpathogens and react by opposing microbial development, overlooking it, or effectively supporting it on or inside plant tissues. The transcriptional reaction of *Arabidopsis* leaves varies when vaccinated with various nonpathogenic individuals from its regular microbiota (Böhm et al. 2014). While *Methylobacterium extorquens* actuates no transcriptional reaction, *Sphingomonas melonis* initiates the defense-related genes that somewhat cover with those activated by the pathogen *Pseudomonas syringae* DC3000. This characterizes a mechanism of plant defense priming (Martinez-Medina et al. 2016) driven by the plant microbiome. The reaction example to nonpathogenic microorganisms can vary both crosswise over plant species (Ofek-Lalzar et al. 2014) and crosswise over promotions inside a single species (Haney et al. 2015). While some *Arabidopsis* accessions are colonized by and build up a valuable association with *Pseudomonas fluorescens*, different promotions effectively restrain the development of similar strains in their foundations. Given the basic capacity of defense phytohormones in the invulnerable plant framework, it is not astonishing that the plant microbiome organization is impacted by defense phytohormone flagging. Tests by a set of mutants with transformed protection phytohormone synthesis and notion stated that salicylic acid and salicylic acid-mediated events have an effect on the root microbiome composition at multiple taxonomic levels (Lebeis et al. 2015). The plant microbiome structure changes upon infection (Agler et al. 2016). Antifungal characteristics are enhanced in barley following infection with *Fusarium graminearum*, conceivably using changes in exudate arrangement (Dudenhöffer et al. 2016). An investigation of tomato plants tested with the pathogen *Ralstonia solanacearum* uncovered that the root exudation profile changed upon pathogen infection, expanding the discharge of phenolic mixes. Plant protection systems additionally sway different drivers of plant – organism cooperations, similar to plant sustenance (Hacquard et al. 2015). Present-day molecular methodologies are likewise being connected to understanding nitrogen-fixing symbioses in non-nodulating plants. Utilization of double host-organism transcriptomics depicted that the limit of a nitrogen-fixing *Burkholderia* strain to frame microaerobic biofilms on sugarcane roots is shown to have diminished motility and immunogenicity, trailed by metabolic adjustment to the sugar-rich plant condition. The plant does not enact an invulnerable reaction but, however, changes its root morphology and supplies the bacterium with photosynthates (Paungfoo-Lonhienne et al. 2016), a reaction pattern that is undifferentiated from the procedure of infection by BNF in legumes (Cao et al. 2017). These examples endorse that the coordination of defense and nutrition is crucial to driving microbiome characteristics.

## 3.10 Implication of the Soil Microbiome on Sustainable Agriculture and Food Security

Conveying sustenance security, the way toward expanding nourishment creation, and improving nourishment quality to support populace development without trading off ecological well-being have been known as a worldwide green revolution (Gupta 2012). Sustainable agriculture improvement is expected to relieve these issues. A definitive objective of economical agribusiness, as per the US National Research Council, is to create cultivating frameworks that are gainful, beneficial, vitality saving, and environmentally solid, preserving natural materials, and that guarantee nourishment well-being and quality. This can be achieved by substituting risky agrochemicals (chemical fertilizers and pesticides) with environmentally friendly beneficial microorganisms, which could improve the sustenance of yields and animals and furthermore present protection from biotic (pathogens and pests) and abiotic (pollution and climatic change) stresses. The potential microbial segregates are detailed utilizing different natural and inorganic bearers through either solid or liquid fermentation technologies (outlined in Table 3.1).

**Table 3.1** Marketable products of plant growth-promoting rhizobacteria in plant health and disease management (Lakshmanan et al. 2014)

| Bioagent | Trade name/formulation |
|---|---|
| *Agrobacterium radiobacter* strain K1026 | Nogall |
| *A. radiobacter* strain K84 | Galltrol, Diegall |
| *Azospirillum brasilense/Azotobacter chroococcum* | Gmax Nitromax |
| *A. brasilense* | Azo-Green |
| *B. subtilis* MB1600 | BaciGold, HiStick N/T, Subtilex |
| *B. subtilis* strain FZB24 | Rhizo-Plus, Serenade, Rhapsody, Taegro, Tae-Technical |
| *Bacillus chlororaphis* 63-28 | AtEze |
| *Bacillus cereus* BPO1 | Pix plus |
| *Bacillus pumilus* GB 34 | Concentrate; YieldShield |
| *B. pumilus* QST2808 | Sonata ASO, Ballard |
| *B. subtilis* GB03 | Companion, System 3, Kodiak, Kodiak HB, Epic |
| *Bacillus amyloliquefaciens* GB99 | Quantum 4000 |
| *Bacillus licheniformis* SB3086 | EcoGuard, Green Releaf |
| *Burkholderia cepacia* | Blue Circle, Deny, Intercept |
| *P. fluorescens* A506 | BlightBan A506, Conquer, Victus |
| *Pseudomonas syringae* ESC-100 | Bio-Save 10, 11, 100, 110,1000, and 10 LP |
| *Pseudomonas chlororaphis* | Cedomon |
| *Pseudomonas cepacia* | Intercept |
| *Streptomyces griseovirdis* K61 | Mycostop |
| *B. subtilis* + *B. amyloliquefaciens* | Bio Yield |
| *Pseudomonas* spp. + *Azospirillum* spp. | BioJet |

Further improvement of microbial confines, and the plan procedure is required through broad research to present them in sustainable agricultural practices. Applications of microbial consortia are described in Table 3.2. Aside from the application of individual organisms, distinguishing sound and practically diverse microbiomes and their application for improving harvest yield poses another big challenge to meet.

## 3.11 Conclusions

Several illustrations depict the significance of understanding the multitude of plant microbiome relations that pay to plant versatility in a specified environment. Acknowledgment of the plant microbiome as a coordinated part of the plant genome develops the environmental idea of "feedback." Disproportional accumulation of microbiome parasites (communicated as pathogenic impacts) prompts negative feedback, whereas the disproportional combination of microbiome mutualists stimulates positive feedback. An enhanced information about these interactions and how changes in biodiversity affect ecosystem functions (plant yield, biogeochemical pools, and fluxes) may be a vital feature for explaining plant microbiome boom and gene expression forms. Fast microbial generation time and the prevalence of horizontal gene transfer give probable systems to the improvement of localized genetic differences, or ecotypes, to emerge because of the impacts of local plant species and networks. As the plant-microbiome interaction unfolds, a new emerging methodology incorporates the study of microbial biology, microbiomes, and transcriptomes into plant genetics. The vast diversity documented in the rhizosphere microbiome is linked with the useful genes responsible for important nutrient changes, similar to those involved in $N_2$-fixation. The age of expansive confine accumulations and the investigation of engineered microbial networks in the mix with plant genetic properties will enable us to connect this hole and to direct reductionist, theory-driven tests in progressively complex environmental settings up to handle field tests. These developments can convert our expertise of plant-microbe interactions in nature and agriculture and could make contributions extensively to the next green revolution. The key player(s) regarding microbiome structure have not been recognized. As needs are, there is a major break in the identification of the molecular segments associated with the collaboration among the host plant and the microbial populace. Also, these ongoing microbiome examinations attempted only to distinguish its structure and multifaceted nature instead of to decide how these microbial gatherings are adjusting the plant phenome, which is basic to investigate its usage. Likewise, there would be a cross talk using signal transduction among aboveground and belowground plant tissues that can be modified by an outer biotic or abiotic stress impacting the rhizospheric microbiome.

**Table 3.2** Application of bacterial consortia (Compant et al. 2019)

| Plant and growth conditions | Consortia/origin of bacteria | Stress | Consortia effect | References |
|---|---|---|---|---|
| *Arabidopsis thaliana*, growth chamber, non-sterile soil | *Xanthomonas* sp. WCS2014-23, *Stenotrophomonas* sp. WCS2014-113, *Microbacterium* sp. WCS2014-259/field soil with endemic *Arabidopsis* plants | *Hyaloperonospora arabidopsidis* | Less fungal spores and higher plant fresh weight | Berendsen et al. (2018) |
| *Solanum lycopersicum* cv. Moneymaker, growth chamber | *Bacillus megaterium* SOGA_2, *Curtobacterium ceanosedimentum* SOGA_3, *Curtobacterium* sp. SOGA_6, *Massilia aurea* SOGA_7, *Pseudomonas coleopterorum* SOGA_5, 11, and 12, *Pseudomonas psychrotolerans* SOGA_13, *Pseudomonas rhizosphaerae* SOGA_14 and 19, *Frigoribacterium faeni* SOGA_17, *Xanthomonas campestris* SOGA_20/phyllosphere of field-grown tomato plants | *Pseudomonas syringae* pv. tomato | Fewer pathogen DNA copies on leaf disks | Berg and Koskella (2018) |
| *Solanum tuberosum* cv. Lady Clair, cv. *Victoria*, cv. Bintje, leaf disks in petri dishes | Double or triple combinations of *Pseudomonas* spp. R32, R47, R76, R84, S04, S19, S34, S35, S49/rhizosphere and phyllosphere of field grown potatoes | *Phytophthora infestans* | Reduced fungal sporangiophore development | de Vrieze et al. (2018) |
| Lycopersicon esculentum cv. Jiangshu, greenhouse pots with soil | *Pseudomonas* spp. CHA0, PF5, Q2–87, Q8R1-96, 1M1-96, MVP1-4, F113, Phl1C2/pea, wheat, cotton, tomato, sugar beet, tobacco | *Ralstonia solanacearum* | Reduced disease severity and pathogen abundance | Hu et al. (2016) |
| Blue maize CAP15–1 TLAX/greenhouse pots with vermiculite | *Hyaloperonospora*, *Pseudomonas putida* KT2440, *Sphingomonas* sp. OF178, *Azospirillum brasilense* Sp7, *Acinetobacter* sp. EMM02/unknown | Desiccation | Increase of shoot and root dry weight, plant height and plant diameter | Molina-Romero et al. (2017) |
| *Capsicum annuum*, *Vitis vinifera* cv. Barbera, growth chamber, greenhouse | *Acinetobacter* sp. S2 and *Bacillus* sp. S4, *Sphingobacterium* sp. S6, *Enterobacter* sp. S7 and *Delftia* sp. S8/*Vitis vinifera* rhizosphere and endosphere | Drought | Increased fresh root, aerial biomass, and photosynthesis | Rolli et al. (2015) |
| *Nicotiana attenuate*, field | *Arthrobacter nitroguajacolicus* E46, *Bacillus mojavensis* K1, *Pseudomonas frederiksbergensis* A176, *Arthrobacter nitroguajacolicus* E46, *Bacillus cereus* CN2, *Bacillus megaterium* B55, *Bacillus mojavensis* K1, *Pseudomonas azotoformans* A70, *Pseudomonas frederiksbergensis* A176, *Bacillus megaterium* B55, *Pseudomonas azotoformans* A70/tobacco plants | Natural wilt disease | Less dead plants | Santhanam et al. (2015) |

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# Plant Mycobiome: Current Research and Applications

4

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**Abstract**

Plant mycobiome studies are one of the leading burning aspects of the twenty-first century for ecological management and sustainable agricultural. A plant-associated fungal community plays an important role in maintaining ecological fitness by cycling organic matter and channeling nutrients across the trophic levels. Several reports highlighted the need for plant mycobiome studies for better disease management, ecological practices, and the use of eco-friendly methods for crop production. In this context, plant mycobiome revealed the effect of the fungal community on the composition of other microbial communities associated with the plant, plant growth, and plant responses against the pathogens. Fungal biodiversity, functionality, and associative interaction with other microbiome organisms and plants are revealed by high-throughput sequencing methods that broaden our view on understanding the fungal importance to plants. The present chapter discussed the modern tools and techniques utilized to study fungal diversity and community structure by the use of different kinds of OMICS approaches such as ITS rDNA gene or specific functional gene sequencing, transcriptomics, proteomics, and metabolomics. Here, the chapter focused on the current research, development of new techniques and approaches that can provide an integrative insight of the role of fungal communities in the plant

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microbiome. Plant mycobiome and their diversity are important to predict plant growth and survival against the pathogen and multitrophic interactions between the organisms to identify their functional cores in regard to plant health and forecast which fungal community is likely to affect plant fitness and produce useful secondary metabolites.

**Keywords**
Mycobiome · Sequencing technique · Plant pathogen · Biofertilizer

## 4.1 Introduction

The plant mycobiome represents the plant-associated fungal community that have multiple functional roles, in response to plant, ecological management, and environmental indications (Finzi et al. 2011; Pagano et al. 2017). The plant-associated fungal community is most commonly associated with both terrestrial and aquatic ecosystems by continuous degradation of organic matter and cycling nutrients across the trophic levels of the food web (Delgado-Baquerizo et al. 2013; Toju et al. 2018). These communities are cosmopolitan in different ecological habitats, such as natural soils, decaying wood, and plant material, living plants (endophytes), water-related environments, etc. (Rai et al. 2019; Hardoim et al. 2015). Most of these fungal species are saprophytes, and they are capable to decompose complex polymers such as cellulose, chitin, and lignin by different hydrolytic enzymes (Benitez et al. 2004; Anil and Lakshmi 2010; Błaszczyk et al. 2014). Several factors, like plant host, plant density, environmental conditions, nutrient availability, and interactions with other external microbiomes (e.g., soil fungi and bacteria), are contributed to maintaining the plant mycobiome composition (Bahram et al. 2015; Nilsson et al. 2018).

The plant mycobiome is usually comprised of five main functional groups of fungal communities, viz., saprotrophic, pathogenic, epiphytic, endophytic, and mycorrhizal fungi (ectomycorrhizal fungi, arbuscular mycorrhizal fungi, ericoid mycorrhizal fungi, and orchid mycorrhizal fungi) (Porras-Alfaro and Bayman 2011; Beckers et al. 2016). The plant-associated fungal community is participating in mineral conduction, carbon distribution, water acquisition, tolerance to abiotic and biotic stresses, and interplant competition (Bucher et al. 2009; Druzhinina et al. 2011; Cai et al. 2013). They applied different mechanisms, such as parasitism, stimulation of plant growth, competition for nutrients, cross-protection, microbial compound production, and induction of systemic resistance against abiotic and biotic stresses in plant mycobiome to maintain ecological fitness and fungal diversity (Kubicek et al. 2011; Porras-Alfaro and Bayman 2011; Hardoim et al. 2015; Cai et al. 2015).

Several reports highlighted the increasing interest of plant mycobiome studies to explore hidden mechanisms and reshape the rhizospheric microbiome for biofertilization, which stimulates plant growth, protection from stress, parasitism, antibiosis, induced plant systemic resistance, and rhizoremediation (Ikeda et al. 2010; Hu et al. 2018). The mycobiome research will draw new insights into plant fungi

interaction that can help to understand the complex networking and ecological function of fungi. In this context, molecular identification revealed the cultivable and uncultivable fungal communities of mycobiome that gives information about mycobiome diversity, structure, compositions, and signaling mechanism between the host plant and associated microbiome (Bayman 2006; Chacon et al. 2007; Tellenbach et al. 2010). However, due to limited knowledge of plant-associated mycobiome composition, diversity, and functionality, these subjects need to explore up to depth. The modern molecular approaches such as high-throughput sequencing methods and omics techniques (epigenomics, genomics, transcriptomics, proteomics, and metabolomics) are utilized to identify the function of fungal communities with plant and soil as well as with other microbes in different habitats (Bálint et al. 2016; Gohl et al. 2016; Kchouk et al. 2017; Castle et al. 2018).

This chapter focuses on plant mycobiome compartments (rhizosphere, phyllosphere, and endosphere) and interactions between plants and microorganisms with major emphasis on fungal fractions of the microbiome within each compartment. The chapter discussed the significant application of plant mycobiomes to improve plant health and how artificially engineered mycobiome can help the crop to improve productivity. This study provides a summary of recent and cutting-edge techniques that can help to classify the fungal communities, their biodiversities, and plant-associated functions.

## 4.2 Plant-Associated Microbial Communities

The soil-inhabiting fungal communities play a vital role in plant health management. They suppress plant diseases through physiological restrictions of pathogen establishment in plant tissues (Mendes et al. 2011; Mukherjee et al. 2012; Classen et al. 2015) and help to convey resistance to the system against "invaders" (Pérez-Jaramillo et al. 2018). Several additional factors have been recognized to mycobiome in close association with plants such as ability to provide nutrient mobilization, like phosphorus solubilization and nitrogen fixation, their support in nutrient uptake from the soil, suppression of abiotic and biotic stresses, host health, and ecological fitness (Molla et al. 2012; Oros and Naár 2017). The plant-associated microbial communities usually comprise five main functional groups of fungal communities, saprotrophic, pathogenic, epiphytic, endophytic, and mycorrhizal, which performs different functions in ecological balancing (Mendes et al. 2013). The major studied compartments of plant-associated microbial communities are established microbial cells and perform distinct functions, called rhizosphere, phyllosphere, and endosphere (Herrera et al. 2010; Porras-Alfaro and Bayman 2011).

### 4.2.1 Rhizosphere Fungal Microbiome Communities

Lorenz Hiltner coined the term rhizosphere in 1904 (Curl and Truelove 1986), which refers to the bulk of soil surrounding the plant roots. Rhizosphere provides

opportunities to organisms to competitively colonize plant roots by secreting root exudates, which are active carbon compounds, and uptake of mobile nutrients and water (Hartmann et al. 2008; Singh and Sharma 2012). The biodiversity and community structure of fungal communities are maintained by the coevolutionary process of rhizosphere and plant roots which perform a significant role in soil biological processes, soil carbon sequestration, and nutrient channelization through decomposed matter cycling in natural systems (Hinsinger et al. 2009; Lambers et al. 2009). The plant rhizosphere system generally enhanced the biomass and soil microorganism's activities by releasing root exudates, which respond with chemotaxis and grow faster (Hartmann et al. 2009). The rhizosphere microbial community composition and diversity are affected by plant growth, as root exudates change during the plant's life cycle and seasonal environment responses (Baetz and Martinoia 2014).

The rhizospheric fungal communities are closely interconnected to plant health, fitness, and growth, decaying plant residues, providing nutrients by cycling minerals, and facilitating their roles in antagonizing pathogens (Ehrmann and Ritz 2014). The beneficial fungal microbes include mycorrhizal fungi (Zhang et al. 2018; Turrini et al. 2018), *Trichoderma* strains (Kotasthane et al. 2015; Li et al. 2015), *Penicillium* sp. (Babu et al. 2015), and other endophytic fungi like *Fusarium* sp., *Colletotrichum* sp., *Cladosporium* sp., and *Dendrobium moniliforme* (Chadha et al. 2015; Shah et al. 2019) which are an indispensable component of agroecosystems. In response to microbial activities, plants fundamentally control rhizospheric fungi through the production of carbon and its derivative compounds and bioactive metabolites (Ellouze et al. 2014). Several examples are elucidating the role of fungal communities, as biocontrol, activity against pathogenic microorganisms (Kowalchuk and Veen 2004, Chapelle et al. 2016). *Trichoderma* is a hyper-diverse genus of rhizospheric fungal community that gained importance due to their antagonistic capability to plant pathogen by employing different mechanisms of parasitism, stimulation of plant growth, competition for nutrients, and induction of systemic resistance against abiotic and biotic stresses (Rai et al. 2016). These fungal communities positively influence plant productivity and protect the plants from oxidative stress by synthesizing antioxidant enzymes (peroxidase, catalase, superoxide) and nonenzymatic antioxidants (glutathione, ascorbate, and α-tocopherol). In another context, certain rhizospheric fungal communities can also negatively influence plant productivity by causing disease, for example, *Fusarium* species, *Verticillium* spp., and *Macrophomina* spp., which affects many crops (Tetali et al. 2015). Most studies have focused on rhizospheric bacterial communities. However, very less information is known about the interaction of fungal communities in the plant rhizosphere. Recently developed molecular techniques, metagenomics, transcriptomics, proteomics, and next-generation sequencing studies showed colonization of plant roots by fungal communities that establish information about the changes in expression of plant genes in response to stress, biomolecule synthesis, photorespiration, photosynthesis, and carbohydrate metabolism.

### 4.2.2 The Phyllosphere Fungal Microbiome Communities

The phyllosphere microbiome is associated with a diverse group of microorganisms, such as viruses, bacteria, algae, yeasts, filamentous fungi, protozoa, and nematodes (Lindow and Brandl 2003; Porras-Alfaro and Bayman 2011). Bacteria are the most dominant and abundant microorganisms in the phyllosphere community (Lindow and Brandl 2003; Vorholt 2012), while fungi are comparatively less abundant. The structure of phyllospheric communities is governed by immigration, survival, growth of the microbial colony, and leaf physicochemical properties (Gomes et al. 2018; Yao et al. 2019). The phyllosphere niche has a high significance in sustainable agriculture and an environmental process that provides confirmation for interactions of phyllospheric microbial communities that affect the health of the natural plant and the quality and productivity of agricultural crops (Boddy et al. 2008). Filamentous fungi largely occurring as dormant spores in phyllospheric fungal communities rather than active mycelia and population size range between $10^2$ and $10^8$ colony-forming unit/gram of leaf (Andrews and Harris 2000; de Jager et al. 2001). The most abundant fungal communities found on leaves are considered *Cladosporium*, *Alternaria*, *Penicillium*, *Acremonium*, *Mucor*, and *Aspergillus* (Inácio et al. 2002; Porras-Alfaro and Bayman 2011). Filamentous fungi appear to occur ubiquitously as endophytes, and their diversity is particularly observed in long-lived tropical leaves. Fungal endophytes are the second most abundant phyllospheric fungal communities that protect against pathogens and increase abiotic tolerance (Arnold et al. 2000, 2003; Schweitzer et al. 2006). The diversity of cultured yeasts appears as the genera *Cryptococcus*, *Sporobolomyces*, and *Rhodotorula*, either singly or with multiple species in the phyllosphere (Inácio et al. 2002; Glushakova and Chernov 2004). To explore the phyllospheric fungal species, culture-dependent approaches are applied over 340 genetically distinct taxa of two tropical forests, but still, culture-independent approaches have not yet been reported to characterize fungal diversity.

### 4.2.3 Endosphere Fungal Microbiome Communities

Although less than 100,000 fungal species have been described as an indispensable role of endophytes encompasses a large, hidden component of fungal biodiversity (Arnold 2007; Rodriguez et al. 2009), the dominating classes of fungal endophyte communities included *Sordariomycetes*, *Dothideomycetes*, *Eurotiomycetes*, *Leotiomycetes*, and *Pezizomycetes* (Jumpponen and Jones 2009; Atugala and Deshappriya 2015) which are associated with grasses and many woody plant tissues and roots (Alberton et al. 2010; Bhagobaty and Joshi 2012). Endophytic fungal communities are important components of plant microbiomes that live within plant tissues without causing disease symptoms. Fungal endophytes reside symbiotically

inside the plant tissue or interact with other microbial groups that colonize in the plant tissues, e.g., mycorrhizal fungi, pathogens, epiphytes, and saprotrophs (Porras-Alfaro and Bayman 2011). Some fungal endophytes play an important role in plant growth, resistance to abiotic and biotic stresses, and disease in the plant by producing useful, antagonistic, and signaling molecules (Gao et al. 2010; Khan et al. 2011; Gautam et al. 2013). Endophytes have a significant effect on the plant fitness, growth, and development by modulating different pathways of plant-like phytohormone (Khan et al. 2012, 2015), antimicrobial compound (Prabukumar et al. 2015), and secondary metabolites (brefelcin A, mevinolin, 2-(3,4-dihydroxyphenyl) ethanol, cytochalasins, polyketides, terpenoids, flavonoids, and steroids) producing pathway (Guo et al. 2008; Balbi and Devoto 2008; Aramsirirujiwet et al. 2016).

Because most of the important root endophytes are not culturable, that's why molecular techniques have been important for identification (Roe et al. 2010;) and confirmed that many of these endophytes coexist with other functional groups in the microbiome. Rapid advances in DNA and RNA sequencing technologies and comparative genomics, transcriptomics, and proteomics approach now facilitate us to study fungal communities in an integrative way, including exploring the taxonomic and phylogenetic profiles of fungal communities in the rhizosphere. In this sequence, their functional and ecological attributes put forward an open discussion about the role of fungal endophytes in the plant microbiome composition, structure, and their diversity, important for plant growth and survival and interactions with another plant-associated microbiome.

## 4.3 Manipulation of the Plant Microbiome Toward Improved Plant Health

The rhizosphere, phyllosphere, and endosphere microorganisms both directly and indirectly influence the composition, structure, diversity, and productivity of natural plant communities (Mendes et al. 2018). Microbial species richness provides information about the plant diversity and productivity (Sharma et al. 2015). On purpose manipulation of the plant microbiome may be an alternative way to advance the agriculture sustainability (Bai et al. 2015). This would be done by manipulating rhizosphere, phyllosphere, and endosphere microorganisms with useful traits.

The recent advancement in rhizosphere engineering, manipulation of the rhizospheric microbiome, can aid in the management of soil health and ecological stability (Broberg et al. 2018). The diversity of microorganisms in the soil is increased through crop rotation that promotes high flexibility to plant pathogens (Hwang et al. 2009). The application of certain microorganisms or the introduction of inoculants is one of the emerging strategies for plant microbiome manipulation, and through this approach, a beneficial community effectively replaces the plant pathogenic microbes. The co-inoculation of several beneficial strains, including endophytes, reduces the time of niche (Compant et al. 2010).

Manipulation of the phyllosphere microbiome can provide a new strategy for enhancing plant growth and health. Falk et al. (1995) suggested that the severity of powdery mildew infections caused by *Uncinula necator* on grapevines was reduced by releasing the conidia of the mycoparasite fungus *Ampelomyces quisqualis*. The literature describes several success stories of the use of *Trichoderma* spp. in mycobiome engineering for biotic stress management (Błaszczyk et al. 2014) and to combat with abiotic stresses by multiple beneficial effects on plant growth and stress tolerance (Singh and Sharma 2012; Chepsergon et al. 2014). Perazzolli et al. (2014) showed that the naturally occurring microbiomes of grapevine leaves could reduce signs of powdery mildew on controlled conditions. A number of reports indicated that growth-promoting and stress-managing fungal communities are applied in rhizosphere, as in the form of liquid and solid formulations to produce active and viable conidia of fungal strains, which are comparatively more tolerant to adverse conditions and provide crop growth in terms of root expansion and nutrient uptake (Waghunde et al. 2016).

## 4.4 Modern Molecular Tools to Study Plant Mycobiome

The traditional techniques used to identify plant mycobiome relied upon cultural and morphological based approaches. These methods are often time-consuming, laborious, and species-dependent, require extensive knowledge of classical taxonomy, and have the inability to accurately quantify the pathogen (Goud and Termorshuizen 2003). For this reason, the availability of fast, sensitive, and accurate methods is required for detection and identification purpose. These limitations have led to the development of different molecular approaches with improved accuracy and reliability. A variety of molecular methods have been used to detect, identify, and quantify plant pathogenic fungi. Here, we discuss the modern molecular tools and technique to study plant mycobiome, their applicability, and their implementation (Table 4.1).

Still, most of the studies on plant mycobiome are based on a culturable technique that involved first isolation of organisms into pure culture and then identification of them, but majority of uncultivable organism is overlooked due to their not or slow-growing properties in cultural media. To overcome this constraint, several techniques were developed, viz., direct amplification of DNA from the surface-sterilized plant material and identification of fungal species, multilocus barcode approaches, and next-generation sequencing studies that investigated fungal communities and overall fungal diversity in plant mycobiome. To fingerprint, the most dominant fungal species at different taxonomic levels usually applied denaturing gradient gel electrophoresis (DGGE) or single-strand conformation polymorphism (SSCP). Quantifying fungal biomass in plant tissues can be achieved by real-time PCR techniques that greatly depend on the type of fungus as well as cell size, number, and type and require extensive calibration for each species using pure cultures (Tellenbach et al. 2010). Next-generation sequencing technologies and systems biology allow simultaneous extension and examination of all members of microbial

**Table 4.1** Different molecular tools used in plant mycobiome study

| Method | Plant mycobiome organisms | Purpose of the study | References |
|---|---|---|---|
| Conventional PCR | *Sclerotium rolfsii*, *Colletotrichum capsici* | Diversity analysis by using ITS region | Torres-Calzada et al. (2011) and Jeeva et al. (2010) |
| Cooperational-PCR (Co-PCR) | Grapevine fungi | Sensitive and specific detection of microorganism | Martos et al. (2011) |
| PCR-DGGE | *Phytophthora* species | Detection of multiple species | Rytkönen et al. (2011) |
| Real-time PCR | *Colletotrichum acutatum*, *Fusarium oxysporum* f. sp. *ciceris*, *Discula destructiva*, *F. poae*, *Pythium vexans* | Quantification of a minimal amount of pathogenic microorganisms | Samuelian et al. (2011), Jiménez-Fernández et al. (2011), Zhang, N. et al. (2011), Kulik et al. (2011), and Tewoldemedhin et al. (2011) |
| Restriction fragment length polymorphism (RFLP) | *Pythium myriotylum* | Differentiation of pathogenic and nonpathogenic strains | Gómez-Alpizar et al. (2011) |
| Nested-PCR | Numerous fungi | Used for detection and/or characterization | Hong et al. (2010); Aroca and Raposo (2007), and Grote et al. (2002) |
| Multiplex PCR | *Podosphaera xanthii*, *Golovinomyces cichoracearum*, and *Phytophthora lateralis* | Detection and differentiation | Guglielmo et al. (2007) and Chen et al. (2008) |
| Reverse transcription (RT)-PCR | *Mycosphaerella graminicola*, *Oidiumneo lycopersici* | Detection of viable populations | Guo et al. (2005) and Matsuda et al. (2005) |
| In situ PCR | *Blumeria graminis* | Identification and diversity analysis | Bindslev et al. (2002) |
| PCR-ELISA | *Didymella bryoniae*, *Phoma* species, *Phytophthora*, and *Pythium* | Detection and differentiation at species level | Somai et al. (2002) and Bailey et al. (2002) |
| Magnetic capture hybridization (MCH) PCR | *Nectria galligena* | Detection at species level | Langrell and Barbara (2001) |
| Isothermal amplification methods | *Fusarium graminearum*, *Phytophthora ramorum*, and *P. kernoviae* | Rapid detection and diversity analysis | Abd-Elsalam et al. (2011) and Tomlinson et al. (2007, 2010) |

(continued)

**Table 4.1** (continued)

| Method | Plant mycobiome organisms | Purpose of the study | References |
|---|---|---|---|
| Random amplified polymorphic DNA (RAPD) | *Fusarium* spp. and *Elsinoë* spp. | Genetic diversity analysis | Arici and Koc (2010), Lievens et al. (2007); Hyun et al. (2009), and Hiremani and Dubey (2019) |
| Amplified fragment length polymorphism(AFLP) | *Cladosporium fulvum*, *Pyrenopeziza brassicae*, *Aspergillus carbonarius*, *A. ochraceus*, *Colletotrichum gossypii*, *C. Gossypii* var. *Cephalosporioides* | Differentiate fungal isolates at several taxonomic levels | Schmidt et al. (2004) and Silvar et al. (2005) |
| Microsatellites | *Ascochyta rabiei, Ceratocystis fimbriata*, *Macrophomina phaseolina*, *Puccinia graminis*, *P. triticina*, *Sclerotinia subarctica*, *S. sclerotiorum*, and *Magnaporthe grisea* | Genetic diversity of plant pathogenic fungi within species and genetic map construction | Jana et al. (2005), Bayraktar et al. (2007), Szabo (2007), Szabo and Kolmer (2007), Winton et al. (2007), and Zheng et al. (2008) |
| DNA arrays | *Fusarium* species and *Penicillium* species | Differentiation of toxin-producing and nonproducing species. *Cox* I high-density oligonucleotide microarray identification | Nicolaisen et al. (2005) and Chen et al. (2009) |
| Micro RNA (miRNAs) | *Fusarium* head blight (FHB) on wheat | Genomic study of wheat | Kharbikar et al. (2019) |
| Induced mutagenesis | *Phytophthora nicotianae* | Identification of *Phytophthora* blight-resistant mutants | Kumari et al. (2019) |
| DNA barcoding. | *Fusarium proliferatum* | Identification at the species level | Nayyar et al. (2018) |
| Transcriptome analysis | *Phytophthora capsici*, *P. infestans* | To assess the change in mRNA levels for selected genes and pathogenic variability | Kandel (2014) and Muthuswamy et al. (2018) |

communities and their interactions by using "omics approaches" (metagenomics, transcriptomics, proteomics, and metabolomics).

The discovery of emerging molecular technology helps to gather the information about the uncultivable fungal species, but nutritional modes and ecological associations of that fungal taxa are still challenging for the researchers (Tedersoo and Nilsson 2016). As DNA sequencing technologies progressed from sequencing single specimens to parallel Sanger sequencing in the early 2000s, it brought inspiration to researchers to reveal the unseen mycobiota and their diversity, structure, functioning, and significance. Sanger and Maxam-Gilbert sequencing technologies were considered as first-generation (the 1990s), most common sequencing technologies used by researchers because of its high efficiency and low radioactivity until the emergence of a new era of sequencing technologies opening new perspectives for genome exploration and analysis (Pareek et al. 2011). The second-generation sequencing methods were developed in the 2000s and marked the beginning of high-throughput sequencing (HTS) which were characterized by the need to prepare amplified sequencing banks before starting the sequencing of amplified DNA clones to analyzed the hidden fungal communities (Vezzi 2012; Qiang-long et al. 2014). The second-generation sequencing included pyrosequencing, Illumina sequencing, Ion Torrent semiconductor sequencing, and SOLiD (Supported Oligonucleotide Ligation and Detection) sequencing (Kchouk et al. 2017). NGS technologies continue to improve, and the number of sequencers increases these last years. However, the third-generation sequencing performs at the level of single molecules and produce higher read lengths than the earlier generations that overcome the problem of the necessity to create the amplification libraries. Also, third-generation sequencing can produce long reads of several kilobases for the resolution of the assembly problem and repetitive regions of complex genomes which is useful in metabarcoding and community analysis of mycobiome (Song et al. 2015; Rhoads and Au 2015; Nilsson et al. 2018). This chapter briefly discusses the application of different HTS, which elucidate the study of fungal community associations related to taxonomic profiling of fungal communities and their mycobiome structure, function, signaling mechanism, and ecosystem functioning.

## 4.5 Major Applications of Plant Mycobiome

Recently, plant-associated fungal communities have been taken a greater interest as bioinoculant to enhance plant growth in different kinds of biotic and abiotic stresses. Thus the rhizospheric engineering involves the development of new strategies to reshape the rhizospheric microbiome for biofertilization and induces root growth stimulation, antibiosis, induced plant systemic resistance, and parasitism.

These microorganisms follow several mechanisms to support plant growth which include the production of phytohormones and the mobilization of organic matter and minerals, like carbon, nitrogen, phosphorus, and iron (Tkacz and Poole 2015). The mechanisms of suppression of pathogens demonstrate several direct

interactions with plant pathogens, as well as indirect interaction, which include stimulation of the plant immune system or systemic resistance (Lugtenberg and Kamilova 2009).

### 4.5.1 Plant Growth Promotion

Plant-associated fungal community structure and metabolism are altered during development and environmental changes, as the nutrients are provided in the microbiome by the plants. So in these consequences, secretion of the nutrients exuded by different plants, the specific fungal pathways responding to them, and the mechanisms by which plant-associated fungal community activates or repress the root colonization process in the response of exudate (Contreras-Cornejo et al. 2016; Zeilinger et al. 2016). Fungal community interactions in plant microbiota likely play determinative roles in colonization of the host plant, plant growth promotion, nutrient uptake, microbiome succession over plant development, and the ability of mutualists to suppress pathogen growth (López-Bucio et al. 2015; Guler et al. 2016; You et al. 2016). Several researchers reported *Trichoderma* colonized plants to secrete different bioactive compounds like auxins, ethylene, gibberellins, plant enzymes, antioxidants, etc. for signaling molecule between mycobiome and compounds like phytoalexins and phenols that provide tolerance to abiotic stresses and enhance the branching capacity of the root system (Brotman et al. 2013; López-Bucio et al. 2015).

Application of plant growth-promoting fungi *Trichoderma longibrachiatum* T6 enhances the tolerance of wheat to salt stress through the improvement of the antioxidative defense system and gene expression (Zhang et al. 2016). The plant microbiota also influences the composition of plant secondary metabolites and the resulting development of different metabolites. Studies on the influence of the microbiome on the taste of strawberries (Zabetakis et al. 1999; Verginer et al. 2010) and the production of metabolites in medicinal plants (Köberl et al. 2013; Schmidt et al. 2014) have been reported. In a study on *Arabidopsis thaliana*, the rhizosphere microbiome could be linked to insect feeding behavior, which was most probably a result of microbiome-driven changes in the leaf metabolome (Badri et al. 2013). Peñuelas et al. (2014) showed that the removal of the floral microbiome of *Sambucus nigra* resulted in a reduced floral terpene emission, which plays an important role in pollination and consequently in fruit and seed production.

### 4.5.2 Disease Management

The plant microbiome is effectively involved in pathogen suppression, particularly the plant mycobiome that acts as a protective armor against phytopathogens (Weller et al. 2002). The various mechanisms are involved in direct interactions with phytopathogens as well as indirect interactions via mechanisms of parasitism, competition for nutrients, and induction of systemic resistance against abiotic and biotic stresses (Lugtenberg and Kamilova 2009). The recent research has shown that the

plant mycobiome is involved not only in managing with biotic stress but also in protection against abiotic stress (Bragina et al. 2013). Several reports indicated that *Trichoderma* spp. have beneficial effects on plant growth and biotic stress management in different horticulture crops like radish, cucumber, pepper, bottle gourd, periwinkle, bitter gourd, chrysanthemum, lettuce, and tomato (Donoso et al. 2008; Bae et al. 2009; Brotman et al. 2013; Contreras-Cornejo et al. 2016; Zeilinger et al. 2016). Kotasthane et al. (2015) reported the antagonistic potential of *Trichoderma* spp. against *Sclerotium rolfsii* and *Rhizoctonia solani* and their plant growth promotion response toward the growth of cucumber, bottle gourd, and bitter gourd. The ongoing research is an effort to elucidate the molecular basis of plant mycobiome to gather broad perspectives regarding their functioning and applicability for growth promotion and defense activation of the plant in disease management (Table 4.2).

## 4.6 Conclusion and Future Prospects

This review was undertaken to explore the current information on plant mycobiome management and focused on developing technologies to overcome the constraints related to mycobiome engineering. Recent advancement in molecular technologies and ongoing research have revealed the greater diversity and complexity of plant mycobiome rather than previously imagined, while an amalgamation of biochemical, molecular, and genetic approaches has led to new visions into the exact mechanism of signal induction and transduction processes of secondary metabolites from fungal communities in plants and other organisms. The ongoing research, technological advancement in molecular biology, and high-throughput omics included genomics, transcriptomics, proteomics, and metabolomics that provide information to elucidate the role of the mycobiome gene that aids in improving plant performance. Therefore, the better understanding of metagenomics, genetic sequence information of microbiome, obtained from next-generation sequencing data has substantial potential for the discovery of diversity and functional perspective of the sequenced genes of the microbiome-related organisms. Looking forward toward the potential of NGS data to demonstrate the functional diversity of mycobiome will require an elucidation of basic biology of fungi through traditional culturing approaches and sequencing of individual fungal genomes to demonstrate the evolutionary studies in natural habitat. Here, this chapter reviews omics-based approaches that are driving forward the current understanding of plant-associated fungal gene functions and describes how these technologies have helped unravel key bacterial genes and pathways that mediate pathogenic, beneficial, and commensal host interactions.

**Table 4.2** Beneficial and pathogenic mycobiome association with different crops

| Antagonist | Pathogen | Counter mechanism | Host plant | References |
|---|---|---|---|---|
| *Trichoderma hamatum* and *T. harzianum* | *Fusarium oxysporum* f. sp. *cepae* | Induction of antifungal compounds | Onion | Adèle et al. (2019) |
| *Glomus* sp. and *Trichoderma* sp. | *Cryphonectria parasitica* | Induction of secondary metabolites | Chestnut | Murolo et al. (2019) |
| *T. viride* | *Macrophomina phaseolina* | Antagonist mechanism by mycelial destruction | Mung bean | Shahid and Khan (2019) |
| *Trichoderma* spp., *T. brevicompactum*, *T. gamsii*, and *T. harzianum* | *F. oxysporum*, *Pythium* spp., and *Rhizoctonia solani* (Kuhn.) | Production of the enzymatic machinery | Tomato | Biam et al. (2019) and Bader et al. (2019) |
| *Piriformospora indica* | *Fusarium* spp. | Induction of antimicrobial product, enzymatic mechanisms, and plant growth promotion | Sugarcane, potato, peas, maize, soybean | Aslam et al. (2019) |
| *Trichoderma harzianum* strain *Th22* | *F. graminearum* | Activation of defense-related genes | Maize | Saravanakumar et al. (2018) |
| *T. harzianum,* | *M. phaseolina, Fusarium oxysporum* f. sp. *cumini* | Induction of secondary metabolites | Guar, moth bean, mung bean, and sesame | Mawar et al. (2019) |
| *T. viride* and *Ampelomyces quisqualis* | *Oidium euonymi-japonici* | Antagonistic mechanism by mycelial destruction and mycolytic enzyme production | *Euonymus japonicus* | Ahanger et al. (2018) |
| *T. viride* isolate NRCL T-01 | *F. solani* | Mycolytic enzyme production | Litchi | Kumar et al. (2018) |
| *T. harzianum* | *M. phaseolina* (Tassi) Goid | Induction of secondary metabolites | Mungbean | Thombre and Kohire (2018) |

(continued)

**Table 4.2** (continued)

| Antagonist | Pathogen | Counter mechanism | Host plant | References |
|---|---|---|---|---|
| *Massarina igniaria*, *Periconia macrospinosa*, *Noosia bankssiae*, *Flavomyces fulophazii* | – | Plant growth promotion and phytohormone production | *Oryza sativa* | Vergara et al. (2018) |
| *Acremonium*, *Arthrinium*, *Botryotinia*, *Chaetomium*, *Dictyosporium*, *Humicola*, *Ilyonectria*, *Mucor*, *Myrothecium*, *Penicillium*, *Periconia*, *Thielavia* | *Alternaria panax*, *Fusarium oxysporum*, *Fusarium solani*, *Phoma herbarum*, and *Mycocentrospora acerina* | Induction of antimicrobial product, enzymatic mechanisms, and plant growth promotion | *Panax notoginseng* | Zheng et al. (2017) and Nandhini et al. (2018) |
| *T. harzianum* | *F. graminearum* | Antagonistic activity by mycelial destruction and nutrient competition | Maize | Saravanakumar et al. (2018) |
| *Penicillium chrysogenum* and *P. crustosum* | *Staphylococcus aureus*, *Salmonella typhimurium*, and *Candida albicans* | Induction of antimicrobial product, enzymatic mechanisms, and plant growth promotion | *Teucrium polium* | Hassan (2017) |
| *T. harzianum* | *F. oxysporum* f. sp. *gladioli* and *Meloidogyne incognita* | Interaction and mycolytic enzyme production | Gladiolus | Khan et al. (2017) |
| *Curvularia geniculata* | – | Plant growth promotion and phytohormone production | *Parthenium hysterophorus* | Priyadharsini and Muthukumar (2017) |
| *T. asperellum* ZJSX5003 | *F. graminearum* | Antagonistic and biocontrol potential | Maize | Li et al. (2016) |
| *T. harzianum*, *T. viride*, *A. flavus*, *A. falcatum*, and *A. niger* | *Colletotrichum falcatum* | Mycoparasitism and mycolytic enzyme production | Sugarcane | Suresh and Nelson (2016) |

(continued)

**Table 4.2** (continued)

| Antagonist | Pathogen | Counter mechanism | Host plant | References |
|---|---|---|---|---|
| *T. viride* | *Pestalotia theae* and *F. solani.* | Hyphal swelling and distortion | Tea | Naglot et al. (2015) |
| *A. niger* and *T. harzianum* | *Fusarium* spp., *R. solani* and *M. phaseolina* | Mycoparasitism and mycolytic enzyme production | Cowpea and mungbean | Ikram and Dawar (2014) |
| *Trichoderma* isolates | *F. fujikuroi* | Antibiosis and biocontrol potential | Rice | Bhramaramba and Nagamani (2013) |
| *Trichoderma* isolates (TvO, TvG, ThC, ThR, and ThM) | *R. solani*, *Sclerotium rolfsii*, *M. phaseolina*, *F. oxysporum*, *A. niger* | Induction of secondary metabolites | Okra, maize cauliflower, and groundnut | Pan et al. (2013) and Gajera et al. (2011) |
| *Trichoderma viride* | *Macrophomina* spp. | Antagonistic and hyphal swelling and distortion | Jute | Srivastava et al. (2010) |
| *Trichoderma* spp. | *Lasiodiplodia theobromae* | Induction of secondary metabolites | *Elaeocarpus munronii* | Priya and Nagaveni (2009) |
| *Glomus mosseae*, *T. harzianum*, *and P. simplicissimum* | *Rhizoctonia solani* | Induction of antimicrobial product, enzymatic mechanisms, and plant growth promotion | *Cucumis sativus* | Chandaniea et al. (2009) |

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# Role of Soil Fauna: En Route to Ecosystem Services and Its Effect on Soil Health

5

Apurva Mishra and Dharmesh Singh

**Abstract**

Soils have an amazingly assorted network of soil fauna that contrast in their versatile procedures, and consequently, in the capacities, they satisfy in soils. Soil fauna works as an extremely proficient means, which aids microorganisms in inhabiting and broadening their venture furthermore into the soil skylines. Lives of soil fauna as soil settlers, indicators, and architects have been underlined, but recent research and worldwide natural concerns are urging the scientific community to look further into the controls of soil fauna for sustainable management of soils in these turbulent times. Molecular data alone is insufficient for many investigations about soil fauna, and hence there is a need for sincere efforts in increasing expertise in the classical taxonomy of soil fauna. This specialization, along with data on the soil fauna's biogeography, their relationship to over-the-ground problem areas, and land management schemes, will be acute for accepting how soil fauna will communicate and react to numerous worldwide alterations. This chapter discusses the soil fauna on the soil grounds as well as below grounds, tight associations of abiotic factors with the soil biodiversity, their roles in ecosystem events and functions, and, finally, the arrangement of ecosystem benefits for human prosperity. A proper and researched thought about soil faunal species, their identities, geographic extents, and an understanding of their roles in soil and land management will help in giving balanced forecasts for the working of future biological communities that are under siege due to inevitable environmental climate change.

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Soil fauna · Soil biodiversity · Soil health · Ecosystem engineers · Faunal indicators

## 5.1 Introduction

Soil speaks as a standout among the essential supplies of biodiversity. It reflects the environmental metabolism since all the biogeochemical procedures of distinctive biological systems are consolidated inside it. It is an intricate framework comprising biotic and abiotic segments, which coordinates with the essential habitat and ensures biological activity and diversity, assisting ecosystem services (Robinson et al. 2013). Inside the composite assembly of soil, biotic and abiotic parts communicate in governing the natural debasement of materials and nutrient cycling (Morgado et al. 2018). Soil fauna or the biotic portion of the soil is an imperative store of biodiversity and assumes a basic role in several soil biological system capacities. Soil environmental conditions are degrading due to the increase in human activities, leading to a reduction in the abundance of plants and animal communities responsible for a balanced environment (Morgado et al. 2018). The consequence of this biodiversity loss is a counterfeit environment that needs steady human intervention and requires additional expenses, while regular ecosystems are optimally controlled by a group of plants and animals and a thorough supply of energy and nutrients—a form of control gradually vanishing with land-use change and industrial growth. As a result, agricultural practices that permit a mix of production targets and ecologically amicable administrations work sustainably, ensuring healthy soil biodiversity, which is fundamental to preventing soil fauna networks from declining in agricultural lands and also helping us in understanding their role toward ecosystem services.

## 5.2 Structure of Soil Ecosystem

### 5.2.1 Soil Organization

Amid biological communities, soil stands as the focal organization, incorporating a huge number of geochemical and ecological capacities (Coleman and Whitman 2005; Crawford et al. 2005; Wall et al. 2010, Delgado-Baquerizo et al. 2019). Soils' position exists at the interface media (Fig. 5.1), located at the intersection of the four main life-supporting provinces: lithosphere, hydrosphere, atmosphere, and the biosphere (living matter). This provides exceptionally dynamic and multiphasic character to the soil, where numerous estimated aggregates are connected and balanced out inside a perplexing lattice of solid, fluid, and gaseous parts communicating at different scales (Lal 2016; Parker 2010).

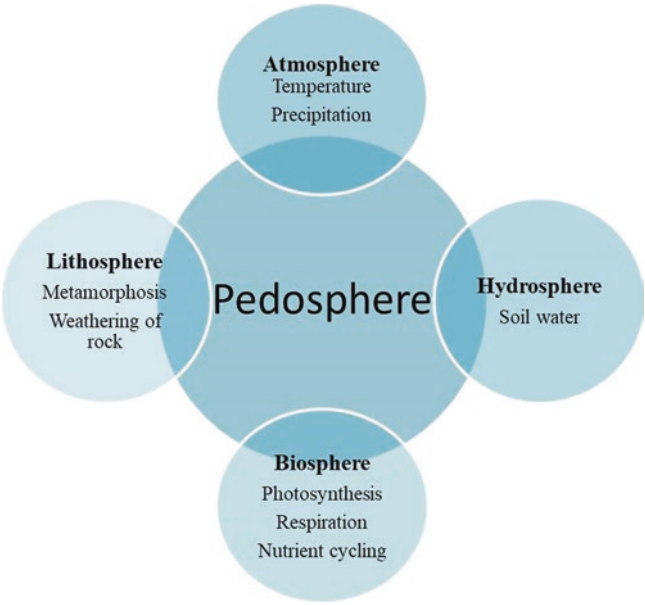


**Fig. 5.1** Pedosphere presenting biotic and abiotic interactions in the soil matrix (Modified from Fitzpatrick [1984] and Coleman and Crossley [1996])

Soil particles do not make a nonstop and conservative mass; instead, the soil volume is framed by pores, chambers, channels, and splits that give an appropriate domain to soil fauna and lead to the development of plant roots. This physical organization in soil additionally directs the motion of gases and fluids inside the soil making different land- or water-capable situations, heterogeneously loaded up with soil "solution" and mostly occupied with gases, which are critical for the soil fauna (Lavelle 2012). A wide variety of dissolved and suspended matter, including inorganic and organic materials, contributes toward soil "solution" composition (Lavelle and Spain 2001). Soil components engaged on the solid stage diffuse to the fluid stage. In this manner, minerals and organic matter end up being accessible to the larger part of soil-living creatures and plants. Soil gaseous stage permits oxygen ($O_2$) utilization and carbon dioxide ($CO_2$) generation amid biological exercises. As the soil $O_2$ gets used up, usually there is a trade-off between the soil and atmospheric $O_2$ with the help of a concentration gradient, with $CO_2$ transition happening in reverse. Relative humidity of terrestrial ecosystems remains near saturation, which is indispensable to most soil fauna, for instance, in the case of springtails. Nematodes, on the other hand, can survive at different levels of relative humidity (Lavelle and Spain 2001)

Notwithstanding the huge predisposition toward the above-ground portion of the soil ecosystem, it is the below ground, the subterranean, where indispensable biodiversity focused on the pore spaces resides (Beare et al. 1995). Pore spaces have an expanded surface territory that makes a large number of active microenvironments

where neighborhood species are exposed to low, competitive exclusion, and concurrence is set between these neighborhood species through resource partitioning (Haynes 2014; Lavelle 1997). For instance, low water potential causes low pore connectivity, hence responsible for the increase in the bacterial abundance (Carson et al. 2010). Competition acts as organizing power for biota consensus in the soil, limited to finer scales, for instance, soil aggregates for microbial networks or the soil pore space and rhizospheric area for microfauna, where prospective contenders may utilize a similar space and accessible resources (MEA 2005).

### 5.2.2 Soil Biodiversity

On earth, soils are the most phylotype-rich environments (Giller 1996). No place in nature is plausible enough to discover such a large number of organisms as in soil ecosystems (Hågvar 1998). Unfortunately, despite the immense efforts by scientists in the past few years to portray and comprehend soil networks, the taxonomic shortage for soil biodiversity is the biggest mysteries (Decaëns 2010) and little is known about the soil's cryptic biodiversity (Bardgett and van der Putten 2014). In spite of this fact, one perspective as of now remains undisputable: soil biodiversity is obligatory for an optimum level of soil functioning that eventually supports all soil-based ecosystems and goods (Barrios 2007). Subsequently, enriching the information about soil biodiversity is foremost to completely comprehend the fundamentals of soil health, adequately oversee soil-based environmental benefits, and foresee future patterns and situations for the recent period (Bardgett and van der Putten 2014).

Soil organisms are hyperdiverse and amazingly unpredictable and incorporate soil communities from all major scientific categorizations (Parker 2010; Wardle 2006). They are typically arranged depending on their body size, whose variety inside soil networks traverses a few orders of size (Barrios 2007; Lavelle 2012; Parker 2010). A greater part of decent soil variety is created by microbiota or microscopic organisms such as bacteria, archaea, and fungi; however, it includes an exceptional range of microfauna, mesofauna, macrofauna, and even megafauna (Bardgett 2002; Lavelle 2012; Orgiazzi et al. 2016; Wurst et al. 2012). Also, it incorporates a tremendous assortment of photosynthetic entities like lichens, bryophytes, and vascular plants, and their root systems have significant roles in soil ecosystem organization (Orgiazzi et al. 2016). Soil microbiota contributes, for the most part, to decay forms, favoring carbon (C), nitrogen ($N_2$), and other biogeochemical cycling, yet also assume a vital role in defeating disease and in plant growth and development (Wurst et al. 2012). These life forms make vital cooperative collaborations with plants, such as enhancing nutrient uptake (e.g., $N_2$ fixation, phosphorus redistribution), as well as manage plant hormones (Wurst et al. 2012). Soil organic matter (SOM) is critical for the biological processes and supply of nutrients, in every variety of soils. Two to ten percent of the soil mass is SOM, which is essential for the comprehended function, including physical, chemical, and biological functions. SOM has been instigated in plants, which can be categorized into "living" and "dead" components during different stages of decomposition and

varies in age from fresh residues to those that are years old in the form of resistant organic matter. Carbon and other organic particles, such as hydrogen ($H_2$), oxygen ($O_2$), and little amounts of $N_2$, phosphorous, sulfur, potassium, calcium (Ca), and magnesium (Mg), constitute SOM. Nearly 5–10% of below-ground SOM contains roots, fauna, and microorganism, that is, living, and this living pool of microbial components is referred to as microbial biomass, which is considered essential for decomposition of organic matter, nutrient cycling, chemical degradation, and stabilization of soil (Ha et al. 2008). During early plant development, phosphorus is required for cell growth. Soil phosphorous can be distributed into three pools, each varying in its accessibility to plants:

1. Soil organic phosphorus bound to organic compounds
2. Inorganic compounds (phosphorus joined with Ca, Mg, iron (Fe), aluminum (Al), or clay minerals)
3. Natural and inorganic phosphorus compounds related to living cells

Soil phosphorus moves between each of these pools using mineralization (separate of natural matter), immobilization, and redistribution of phosphorus between microorganisms, plants, and natural matter. Phosphorus is immobilized (made inaccessible to plants) when it is consolidated into the living microbial biomass. Redistribution of phosphorus happens when phosphorus is discharged from microbial cells and moved into different phosphorus pools. Phosphorus mineralization and immobilization happen at the same time in soil. While mineralization of natural soil phosphorus to inorganic phosphorus expands the accessibility of phosphorus to plants and microorganisms, an extensive amount (between 15 and 80%) of soil phosphorus stays in natural structure and remains inaccessible to plants (Ha et al. 2008).

Soil microfauna incorporates organisms that are ≤100 μm (e.g., nematodes, protozoa, and rotifers) in size. Only one taxon, protozoa, is discovered completely inside this class (Ruggiero et al. 2015). Mainly, mites, nematodes, rotifers, tardigrades, and copepod scavengers fall inside this group (Kennedy and Gewin 1997). Soil microfauna feeds on microscopic organisms such as fungi and algae, yet they additionally represent predator and saprophytic gatherings too. Through their actions they manage: (1) nutrient cycling by enhancing the accessibility of nutrients to different species (e.g., excretion), (2) population and actions of microscopic organisms and fungi, and (3) scattering of pivotal rhizospheric microbiota (Wurst et al. 2012). Their impacts on plants are likewise—when in direct contact with roots, they benefit from the root excretions and modify the plant's defenses or hormones. The role of plant roots in soil processes cannot be separated from the varied group of organisms, which flourish around them. These comprise parasites too, herbivores and predators, nematodes or worms as microbial grazers, and free-living microbes feeding on root exudates. Secondary metabolites produced by plants such as iridoid glycosides are present in root exudates. Other volatile organic compounds (VOCs) are also released by green leaves and above-ground roots that attract pollinators. Below-ground roots also release VOCs that dissuade soil-inhabiting herbivores.

Likewise, plants are not just providers of litter for decomposers but rather they assume a functional job in pulling in valuable soil invertebrates. For instance, plants draw in entomopathogenic nematodes to kill the rhizospheric herbivores, giving innoculum to the rhizospheric bacterial population, aggravating the correspondence between destructive microorganisms, and furthermore, in adjusting rhizodeposition and root architecture. This eventually results in a better communication between soil fauna and plant roots (Briones 2018). All these synthetic signs discharged by the plants are coordinated in a way that profits their very own development and health.

Soil mesofauna ranges from 100 μm to 2 mm (gatherings include taxa like Acari, Collembola, Tardigrada, Protura, Diplura, and Enchytraeidae) in size. Microarthropods, for example, mites and springtails, are the fundamental organisms of this group. They could be classified into herbivores, bacterivores, or fungivores. At times, they can additionally gorge on higher trophic dimension life forms. They live near the air and water existing in soil and are thus exceptionally reliant on soil aeration and humidity. Soil mesofauna helps in nutrient cycling and management of pests and disease control by their preferential mode of feeding, acts as food for other soil organisms, and aids in soil fauna distribution (Wurst et al. 2012).

Soil macrofauna (>2 mm in size) is fundamentally in charge of litter comminution and redistribution, and predation on other soil-abiding organisms frequently called as "ecosystem engineers." Macroarthropods (e.g., isopods, spiders, bugs) alongside annelids and gastropods are the fundamental congregations of soil macrofauna. These creatures are in charge of modifying the structure of their natural surrounding by contributing to various soil capacities, like physical disintegration and transport of litter residues to lower layers of soil that eventually help in nutrient cycling by microfauna and microbes, water penetration (e.g., by burrowing practices), and pest and disease control (rich biodiversity and predation; Wurst et al. 2012). They have an immediate constructive outcome on plant development and yield, yet they could be the reason for some harmful impacts on crops.

Soil megafauna (soil fauna whose size surpasses 20 mm): Individuals from this group incorporate an extensive range of invertebrates (worms, snails, myriapods) and vertebrates (insectivores, terrestrial and aquatic rodents, and reptiles). Moles, voles, gophers, snakes, and burrowing owls are few examples of soil megafauna, which help in the breakdown of the complex substances in decomposing plants and animals so that living plants and other organisms can utilize them. This comprises soil organisms as promoters in biogeochemical cycles, with carbon (C) cycle being the most prominent followed by nitrogen ($N_2$) and sulfur (S) cycles.

Soil life forms can be characterized additionally as per their usefulness, which makes a difference to explain about their environmental roles in soil systems. Turbé et al. (2010) recommended the utilization of three widely inclusive practical gatherings: chemical engineers, biological regulators, and ecosystem engineers (Fig. 5.2). Creatures directly participating in carbon and other biogeochemical cycles, including nitrogen, phosphorus, and sulfur cycles, and helping in soil decomposition and transformation can be classified as the chemical engineers. Biological regulators are in charge of monitoring the changing aspects of soil inhabitants, thereby advancing the strength and constancy of soil biological systems (Fusaro et al. 2018). Ecosystem

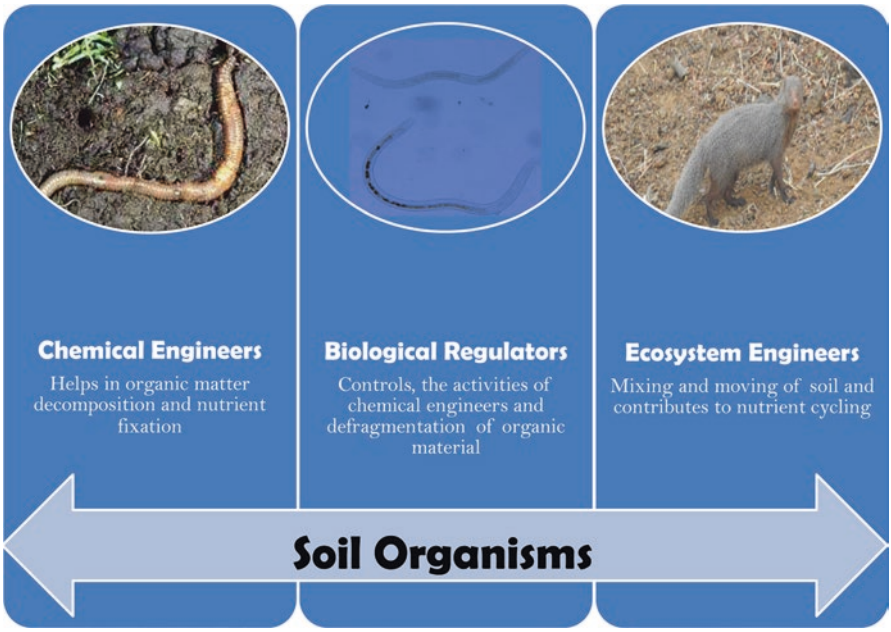


**Fig. 5.2** Classification of soil beneficiaries and their appropriate role in the maintenance of agroecosystem

engineers are in charge of maintaining the soil structure by advancing the formation of stable aggregates (unit of soil structure), the pore network construction, and the advancement of complex biostructures.

In spite of a long history of studies focusing on the enumeration of soil fauna (Menta 2012), it is still extremely hard to give precise biomass estimates for soil fauna. This is somewhat due to their inconsistency in time and space (Menta 2012) and also due to contrasts in the methodologies used for their inspection (Wall et al. 2001). Also, most of the studies are from the temperate zones while other climactic/ecological regions have been genuinely disregarded (Turbé et al. 2010).

## 5.3 Soil Fauna Organization in the Ecosystem

Soil fauna is viewed as an all-inclusive imperative part necessary for reusing organic matter, soil vitality, and nutrient cycling (Jeffery and Gardi 2010). In conjunction, they are the main players in supporting and managing environment services. Hence, soil biologists frequently organize soil fauna in terms of their broad functions divided into three categories: chemical engineers, biological regulators, and ecosystem engineers (Fig. 5.2).

1. Chemical Engineers: They are the key components of the soil food web. Bacteria and fungi make up the largest portion of this group and also include algae and

viruses (viruses could impact C cycling through viral shunt where, by bacterial cell lysis, they build up the concentration of labile C in the soil ecosystem that eventually serves toward increment in microbial generation and respiration in soils; refer to Williamson et al. [2017] and Trubl et al. [2018] for more details on this topic). These microscopic engineers help in the decomposition of organic material and transforming complex forms of carbon and nitrogen into $CO_2$ and simpler nutrients (Coleman and Crossley 1996). For their survival and growth, they depend on optimum soil moisture, suitable atmospheric conditions, and pore spaces between soil particles. Significant quantities of organic matter and animal manure in soil environments certify their prevalence. Moreover, they are key parts of a soil food web or sustenance networks as their action ensures the growth of living organisms, commencing from plants to the animals that feed upon them, responsible for organic turnover. Rough evaluations of soil biodiversity demonstrate a few thousand invertebrate species for every site and additionally ambiguous levels of microbial and protozoan variety that contributes in each of the trophic level (Turbé et al. 2010).

2. Biological Regulators: They are the diverse group of soil organisms that control the activities of the subordinate chemical engineers and form a vital link in the food web. Some act as plant pests and parasites, while others stimulate microflora. Their movement around the soil assists fragmentation of organic material, providing more surface area and hence enhancing the availability of the nutrients to microbes. In this group, protists are the smallest organisms, which live in the water layer surrounding soil particles and control bacterial populations through feeding. Nematodes, microarthropods, and protists are the most abundant biological regulators.
3. Ecosystem Engineers: Of major significance in the ecosystem, soil advancement, and support are the alleged "ecosystem engineers," as these species control, either straightforwardly or in a roundabout way, the accessibility of resources to other co-inhabitants of soil (Davies et al. 2019; Wright et al. 2002). These life forms physically change, keep up, and make new habitat for other life forms. Typically, there are two types of ecosystem engineers—firstly, the allogenic ones that modify the soil background by transforming living or nonliving things from one physical state to another, via mechanical or other means, and, secondly, the autogenic ones that change the soil background using their physical structures, that is, their existing and/or dead tissue (Byers et al. 2006; Lavelle and Spain 2001; Wright et al. 2002). A precedent of physical "ecosystem engineers" is plant roots that make substantial voids (spaces) in the soil through root rot (Byers et al. 2006). Termites and earthworms assume a noteworthy role in moving, blending, and circulating air through the soil through their tunneling.

Soil fauna is a very important entity, and the greater part is exceedingly versatile, stretching from herbivores to omnivores and even include carnivores. Contingent upon the accessible nutrient supply, a good chunk of soil fauna can modify their feeding systems from a more noteworthy to a lesser degree with instances where numerous carnivorous species amid low food accessibility can head toward the

transformation and organic matter turnover (Brose and Scheu 2014). The cooperation between soil fauna and ecosystem are various, and unpredictable, due to the huge diversity and prevalent shifts.

The level of connection amid soil fauna and the soil itself can vary significantly between taxa and is subjected to soil existence cycle (Wall et al. 2001). Specifically, in conjunction with their physical forms and the ecological roles, it is conceivable to arrange soil fauna into four fundamental gatherings: incidentally dormant geophiles, briefly dynamic geophiles, periodical geophiles, and geobionts (Fig. 5.3). It is noticed that this classification does not have any taxonomical importance, yet, rather, they are helpful after contemplating the existential procedures of soil invertebrates.

Incidentally, dormant geophiles are life forms residing in the soil for a certain period of their lifecycle, for example, during hibernation or while experiencing transformation, at a point when security from climatic forces is essential (Menta 2012). Because of their relative dormancy, these life forms affect the natural capacity of the soil, even though they can act as prey for different creatures in this critical

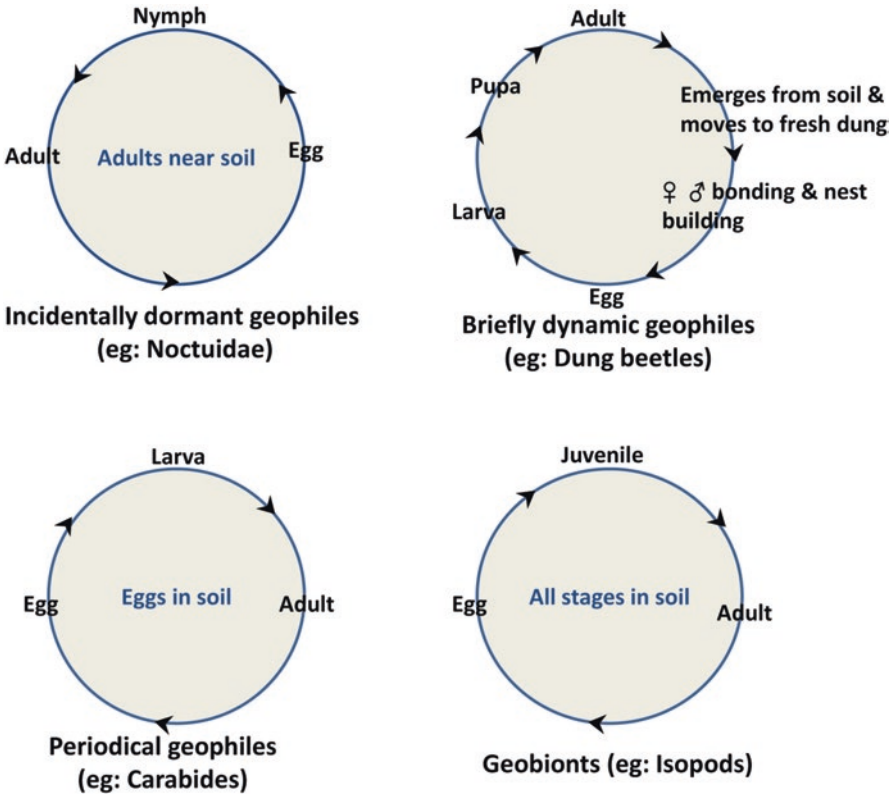


**Fig. 5.3** The primary four fundamental gatherings of soil invertebrates contingent upon their life techniques and how nearly they are connected with the soil

time of their lifecycle. They spend time in the soil to let pass adverse atmospheric conditions, or for metamorphosis; for instance, caterpillars of butterfly families like Noctuidae, Geometridae, or Sphingidae bury themselves in the ground at the time of nymphosis. These incidentally dormant geophiles exert no mechanical force on soil (Menta 2012).

Briefly dynamic geophiles reside in the soil in a steady state for an extensive period of their life (i.e., for at least one or more developmental phases and rise out of the earth as an adult). A large portion of these creatures is insects, for example, Neuroptera, Diptera, Coleoptera, and Lepidoptera. Organisms with "pupal" phase in their life cycle assume a minor role in the soil amid this stage, while the "larval" phase is considerably more imperative for the soil ecology, particularly at times where populace thickness is high (Wall et al. 2001, 2008). Most larvae in the soil can go about as both detritivores and predators.

Periodical geophiles reside for a significant portion of their life in the dirt, for the most part as hatchlings; however, for the duration of their lives, they at times return to the dirt to perform different exercises, for example, chasing, egg laying, or to flee from threats. A few Coleoptera gatherings (e.g., carabides, scarabeids, cicindelids) pass their larval phase in the litter or the upper layers of mineral soil, and when they reach a mature stage, they utilize soil as a nourishment source, a shelter, and for different purposes (Wall et al. 2001, 2008).

Geobionts are living beings that exceptionally spend their entire lifecycle in soil and cannot leave this condition even for a limited period. They have characteristics that counteract survival outside of the soil environment either because they are deficient in ways to secure themselves from desiccation or temperature changes, or are lacking the sensory organs essential to survive above the ground, for example, lacking appendages, necessary for discovering nourishment above ground or for protection from predators. A few types of myriapods, isopods, Acari, mollusks, and the larger part of Collembola, Diplura, and Protura have a place with this gathering (Wall et al. 2001). These distinctive sorts of connections between soil life forms and the soil decide a separated level of weakness among different gatherings and as a result of any conceivable effect on soil condition. In case, if soil pollution happens due to some human interference, geobionts will be the most severely affected group as they cannot leave the dirt and must consume all their time on earth there.

### 5.3.1 Soil Networks

Soil organisms are the principal intermediates of soil working at various scales. The investigation of soil biodiversity began through surveying soil food networks as a feeding web between organisms—one of the major integrating features of soil communities. Natural ecosystems contain numerous species that are associated with their feeding connections over various trophic levels to make a complex food web (Digel et al. 2014). Consumer and producer interactions can be envisioned as a binding principle for food webs in different ecosystems with hierarchical

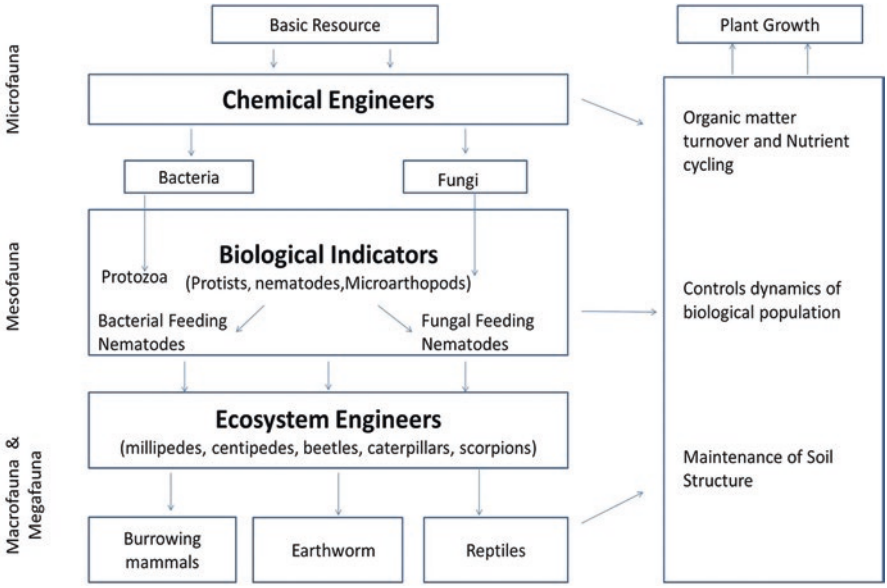


**Fig. 5.4** Soil network showing linkages between the different functional groups of soil fauna, along with their role in different soil biological functions

associations acting as a backbone (Brose and Scheu 2014; Digel et al. 2014; Fontaine et al. 2011). Figure 5.4 delineates the various hierarchical associations of the determinants of soil forms (Beare et al. 1995).

The arrangement of elements influencing soil working is resolved at both spatial and temporal scales (Wu and Wang 2019). Different organisms, including higher plants and creatures, assume considerable roles in this regard.

The working of the soil framework is additionally controlled by:

- The deterioration rates of dead organic materials and the equalization between mineralization (which discharges nutrients accessible to plants and microorganisms) and humification (which frames stores of soil organic matter or SOM and colloidal organic mixes)
- The level of synchronization of nutrient discharge with plant request
- The soil physical structure, which decides the rates and examples of gas trade, soil water movement into and through the soil, and disintegration rates
- The texture of the soil (percent of sand, silt, and clay), which impacts the action of soil life forms and henceforth the soil biological working

Soil food networks are created by two fundamental frameworks: one, herbivory based ("green" food networks) and the other detritus based ("brown" food networks; Briones 2014). In herbivory-based food networks, plant roots establish the fundamental basal reserve, grazed by plant-feeding nematodes and insects,

which in turn are grazed by a predaceous network ranging within a few trophic levels (Parker 2010). The brown food network is composed of recalcitrant, nonliving organic materials, supported by the detritus pool. Two vitality channels have been distinguished inside brown food networks: (1) the bacterial vitality channel, with microorganisms as essential decomposers, and (2) the fungal vitality channel, where they are the decomposers (Ramsden and Kervalishvili 2008). This bacterial–fungal vitality channel idea by and large adopts a sensibly well-separated network of detritivores/microbivores (Coleman 1996). Moreover, expanding confirmations recommend that omnivory and feeding versatility is summed up inside soil food networks (Jordán 2009), not only at higher trophic levels but also inside detritivores/microbivores organizations (Gupta and Malik 1996). This entire system, counting the distinctive vitality channels, is balanced out by a broad number of trophic and nontrophic collaborations at different spatial and temporal scales (Ramsden and Kervalishvili 2008). Interactions between networks happen at predator levels (Parker 2010), as well as among autotrophs and decomposers, either connected by uneven metabolic abilities (Giller 1996) or resource competition (Hågvar 1998; Scheu 2002). Nontrophic interactions, for example, the movement of soil engineers, are additionally thought to advance assorted variety and decrease competitive interactions, thus contributing to balance soil food networks (Thompson et al. 2012).

### 5.3.2 Soil Health and Faunal Indicators

Soil health suggests the limit of soil to work as an indispensable living framework, a dynamic framework. It includes the knowledge of soil as a powerful living organism that works comprehensively relying on its condition or state. Soil health relies upon the joined impacts of three noteworthy connecting parts. These are the chemical, physical, and biological attributes of the soil (Cardoso et al. 2013). Soil health is upgraded by the management and land-utilization choices that consider the various functions of soil. It is weakened by choices that look just on single capacities and momentary arrangements, for example, expanding yet not sustaining crop productivity.

Soil organisms apply a noteworthy power over many soil forms through their impacts on the deterioration of dead natural material, nutrient cycling, the change and transport of soil materials, and the development and support of soil structure (Griffiths et al. 2018). Though occasionally not easily perceived, the biological activity of soils is to a great extent gathered in the topsoil, from a couple of centimeters to a depth of 30 cm. The living segment of SOM comprises of:

- Dissolved organic matter made up of soluble root exudates
- Particulate organic matter, including each of fresh residues and living organisms
- Humus content
- Resistant organic matter

Dead organic matter is at first mostly devoured by macrofauna, comminuting and redistributing it into smaller sections. These partially degraded organic residues are then accessible to meso- and microfauna and microorganisms. Through its tunneling movement, soil macrofauna mixes organic matter profoundly into the soil and stimulates the movement of microorganisms. In this manner, soil organisms take an interest in a variety of procedures fundamental to the working ecosystems. For instance, they assume an imperative role in the cycling of SOM and nutrients in the soil, water purification, agrochemicals detoxification, and in the change of soil structure.

Among the capacities directed by soil organisms that help in maintaining the soil health are:

- Decay: separating litter, making hummus, and nutrient cycling
- Atmospheric $N_2$ conversion into organic forms and reconverting organic $N_2$ into gaseous $N_2$
- Enzyme synthesis, hormones, vitamins, and other organic substances for plant growth and development
- Soil structure modification, influencing porosity, water transitions, and organic matter conveyance, and advancing further root development
- Overpowering as well as feeding on soil-borne plant pathogens and plant-parasitic nematodes

More significance has been accustomed to individuals from soil fauna as pointers of health. This aggregate includes the invertebrate network that lives absolutely or amid something like a period of the existing cycle in the soil (Cardoso et al. 2013; Coyle et al. 2017; Jordán 2009). They assume roles in organizing procedures of terrestrial ecosystems, the disintegration of plant residues, and setting up connections at various dimensions with microorganisms. Hence, they effectively participate in procedures that influence the soil properties and quality, and hence are great indicators of changes in the soil (Lavelle and Spain 2001). For example, nematodes, the simplest metazoan, act as bioindicators of soil health. They demonstrate a high and varied sensitivity to pollutants, and because of their trophic diversity, nematode gatherings do reproduce not only their fate but also the state of the bacterial, fungal, and protozoan communities. Because of such characteristics, they are the potentially remarkable bioindicators for soil disturbance and health (Bongers and van de Haar 1990).

A decent variety, biomass, abundance, and thickness of soil fauna have been utilized as indicators of natural or anthropogenic effects on terrestrial ecosystems since these are entirely associated with physical, chemical, and microbiological soil traits (Eggleton et al. 2005; Wardle et al. 2004), and these characteristics of the soil fauna are considered in any prudent methodology utilized for evaluation of the soil health.

Associations among plants and soil's chemical engineers play a critical role in plant network advancement, assorting plant variety, biogeochemical cycling, and supporting general soil structure. Plant roots and microorganisms interact with one

another through molecular crosstalk, which is useful, impartial, and sometimes detrimental (Kardol et al. 2006). Plants influence the creation of their dirt network, and, consequently, the soil network influences the efficiency and arrangement of the plant network. There are two noteworthy pathways of soil input. One is immediate, using root herbivores, pathogens, and symbionts, and the second pathway is more roundabout, through the impact of the dirt decomposer subsystem on the supply of supplements (Miethling et al. 2000). In many biological communities, plant development is constrained by the measure of nutrients discharged by microbes and parasites, such as ammonium ($NH_4^+$), which rely mainly upon the microbial driven disintegration rate. Plants being nonmotile regularly face nutrient deficiencies in their environment, but they have measures to overcome this and obtain micronutrients required for their development and growth. Plant–soil feedback (PSFs) is one such process in which they change the biotic and abiotic potentials of soil, in which they grow, which then alters the plant ability to grow in that soil in the future. Alteration in the measure of quality and quantities of organic substrates flowing into the soil as exudates and litter affects the substrate availability for the microorganism and, eventually, the macroorganisms residing in the soil. Soil invertebrates, along with micro-, meso-, and macrofauna, contribute to plant–soil feedback function, which can lead to the negative, positive, and neutral effects on plant growth. Plant invasion activities are influenced by the PSF, by their impacts on the plant growth. PSFs are mostly involved in the nutrient availability, litter decomposition transfer, soil pathogen accumulation, and interruptions in mutualistic associations in conjunction with soil micro- and mesofaunal populations (Yang et al. 2018 and Zhang et al. 2019). In such similar ways, soil fauna can modulate the soil health that is directly or indirectly related to the associated plant health and can influence the plant invasions and plant network advancement in natural systems by chance, speed, and other different consequences.

## 5.4 Soil Fauna in Ecosystem Functions and Services

Understanding of how the fauna in soil networks reacts to natural change and how this impacts over-the-ground forms has progressed extensively in the last few decades (Albert et al. 2016; Bragazza et al. 2013; Cheeke et al. 2012; Wurst et al. 2012). A prevalent loss of phylotypes is being observed because of the different management practices, anthropological disintegration, pollution, and widespread urbanization. These have resulting impacts on biological system capacity and ecosystem services, which end up as broadly perceived and identified with bigger concerns such as a reduction in biodiversity, the transformation of habitable lands into deserts, and increased levels of greenhouse gases in the atmosphere (Koch et al. 2013). An examination by Jeffery and Gardi (2010) showcased prospective susceptibility of micro- and mesofaunal biodiversity and ecosystem services to ecological pressures, together with land management practice. Global trials and amalgamations have kept on tending on the evaluation of the role of soil fauna in ecosystem processes and specifically have prompted expanded proof for their commitment to

C cycling. Worldwide tests from different geographical areas demonstrate that soil biota are the main controllers of deterioration rates at biome scale (Wall et al. 2008) and indicate that mainly the invertebrate populations in the soil fauna were in charge for approximately 27% normal improvement of litter decay crosswise over biome scales (GarcíaPalacios et al. 2013; Ma et al. 2019).

The network of organisms living in soil conveys out an extremely wide scope of biochemical and biophysical forms that directs the working of the soil itself and that can likewise influence the neighboring ecosystems (Maltby et al. 2017; Faber and Van Wensem 2012). Huge numbers of these capacities additionally give basic advantages to human society. The vast majority of these services are supporting services or services that are not specifically utilized by people in any case, which underlie the provisioning of every other service. These incorporate, for instance, nutrient cycling and soil arrangement. Furthermore, soil biodiversity is associated with all the fundamental regulatory services—to be specific, the regulation of atmospheric configuration and temperature, water and air quality, pest and disease occurrence in agriculture and natural environments, and human infections. Soil life forms may likewise control or diminish ecological contamination. At last, soil life forms additionally add to provisioning services that straightforwardly benefit individuals; for instance, the genetic resource of soil microorganisms can be utilized for creating novel pharmaceuticals.

Each function adds directly or indirectly up to services. For example, nutrient cycling underlies crop production, while water transfer and storage is affected by soil engineering, and soil biodiversity offers a source of species that may add to pest control, the upgrading of new medicines, or decontamination (Ritz et al. 2012). Different capacities exhibited by soil and soil biodiversity contribute, in a way, to human prosperity; for example, decomposition of soil organic matter, which adds to carbon storage and climate control.

Ecosystem services are characterized as the advantages that individuals get from nature, fundamental for natural wellbeing and human prosperity (MEA 2005). The Millennium Ecosystem Assessment (MA) and the Common International Classification of Ecosystem Services (CICES) classifies ecosystem services into: (1) *provisioning*, which incorporates the generation of products by biological systems (e.g., fibers, water, food, and energy); (2) *regulating*, which incorporates the support of a few procedures identified with climate, water and air quality, and pest and disease management; (3) *supporting*, vital for the execution of all remaining administrations, for example, soil arrangement, nutrient cycling, essential generation, and natural surroundings arrangement; and (4) *cultural*, which incorporates nonmaterial advantages like amusement, ecotourism, and social legacy.

The disintegration of the natural entities by soil fauna is pivotal for the working of a biological community in light of its significant role in giving biological community administrations for plant development and its essential efficiency (Brevik et al. 2019). A comprehensive list of various soil functions performed by soil fauna along with the ecosystem services can be found in Table 5.1.

Soil ecosystem services rely upon soil environmental organization (soil biotic and abiotic parts and the connections inside and among them) and soil environment

**Table. 5.1** Relationship between soil ecosystem functions and services with relevant indicators and responsible soil fauna

| Kind of service | Ecosystem service | Functions of soil biota | Indicators | Soil fauna | References |
|---|---|---|---|---|---|
| Regulating and supporting services | Water quality | Soil organization and its preservation; decomposition and mineralization of organic compounds; resource for predatory microfauna; biofilm formation | Bulk density, porosity, particle size distribution, soil and water retention capacity, water holding capacity | Microfood web; litter transformers; root/rhizosphere biota; N-fixers: mycorrhizae, free-living microbes, microbial pathogens; root herbivores: bacteria, archaea, fungi, actinomycetes; Protista: predators (bacterivores); Nematoda: plant feeders, predators (bacterivores, fungivores); Collembola: saprovores, predators; Acari: saprovores, predators; litter transformers: mesofauna—Enchtriaeids, earthworms, termites | Metcalfe et al. (2014) |
| | Soil erosion control | Regulation of soil hydrological processes; associative nitrogen (N)-fixing with legumes; N fixing | | | |
| | Climate regulation | Gas exchange and carbon sequestration | | | Hammer et al. (2016) and Metcalfe et al. (2014) |
| | Attenuation of pollutant | Soil detoxification; immobilization of nutrients; mutualistic and commensal association; parasite of nematodes and organic matter | Macro- and micronutrients, total organic matter, total organic carbon | Ecosystem engineers: macrofauna—earthworm, macroarthropods, burrowers, saprovores, Arthropoda, Enchytraeids; biocontrollers: predators, parasites (hyper), microbial pathogens, root herbivores | Hope et al. (2014), Baron et al. (2014) and Wu et al. (2010) |
| | Soil structural maintenance (pest and disease control) | Decomposition of organic matter; synthesis of organic compounds; mediate transport of water and ions from soil into the plant root; C allocation below ground and its regulation; suppression of pests and diseases | Emissions of greenhouse gas, phosphorous overflow, nitrate leaching | | Jouquet et al. (2014); Evans et al. (2011); Leal et al. (2014) |
| | Biodiversity conservation | Sources of food and medicine; symbiotic and asymbiotic relationship with plants and their roots; plant growth control (positive + negative) | Soil toxicity testing, soil food webs, soil respiration | | Leal et al. (2014), Pringle et al. (2010), Shi and Shofler (2014), and Maleque et al. (2009) |

| | | | | | |
|---|---|---|---|---|---|
| Provisioning services | Food, water, fiber, and energy supply | Consumption of organic matter; create channels in soil and litter for movement; predators of other soil and surface-dwelling organisms | Food quality and storage, plant physiology, crop productivity, creative landscape, leisure activities | Microfood web; microflora: bacteria, fungi; microfauna: protozoa, nematodes, microarthopods; litter transformers: megafauna—moles, little rodents, reptiles | Rodriguez et al. (2006), Macadam and Stockan (2015), Sehnal and Sutherland (2008) and Dzerefos and Witkowski (2014) |
| Cultural services | Physical or experimental use; intellectual representations | | Beautiful landscape, participation in leisure activities, vital actor awareness | | Vidal et al. (2014) and Morley et al. (2014) |

capacities (regular procedures happening in soil). In the case of soil, water, and air, air compartments are interdependent, and their quality and maintainability are reliant on one another. Soil environment structure is in charge of the adjustments in individual life forms, and hence their role and capacity in soil modification and consequently in the biological community assembly. Soil biodiversity in this way manages soil structure and capacities (Morgado et al. 2018).

## 5.5 Conclusions and Future Studies

Earth environmentalists and soil modelers have generally depicted the occupants of soil as a black box named as "soil fauna" or "decomposers or detritivores," recognizing the fact that they reuse the stored dead material. The soil is a standout among the various living spaces on earth and contains a diverse collection of living organisms; but, the opaqueness of this world has extremely constrained our comprehension of their functional commitments to soil forms and ecosystem flexibility. Conventional scientific categorization, given morphological and anatomical angles, is getting to be supplanted by molecular techniques (e.g., with marker gene-based methodologies). Nonetheless, this might be impracticable in numerous natural or ecological situations, and therefore the larger part of the present learning still contributes little to our comprehension of their roles in ecosystem functioning.

The present chapter has investigated the reasons why soil fauna is considered for their inherent, useful, and functional qualities. Inherent reasons alone legitimize examination into these different and complex networks and their preservation through natural surroundings and suitable land management practices to address the issues for forthcoming generations. It is recommended that the significance of soil fauna remains an oddity in light of the fact that on one side broad research has demonstrated that they have significant consequences for soil biophysical forms at the scales at which the organisms are active, and on the other side these impacts are once in a while obvious at plot, biological community, and ecosystem scales. Three components are proposed to clarify this mystery. Firstly, that in very differing networks the "signal" of specific soil fauna impacts is concealed by the "noise" from other biophysical occasions that add to similar properties and procedures (e.g., carbon and nitrogen mineralization, soil structure). Secondly, that numerous procedures made by soil fauna have "sink" and "source" elements that can cancel the signal of these nearby impacts at bigger spatial scales. Thirdly, that at enormous temporal and spatial scales, biophysical parameters are utilized as rate determinants of biological system forms and the structure of above or subterranean networks is once in a while raised. However, there are conditions in which the environments are in transitional states, or in which natural occasions synchronize with activities of soil fauna when roles of soil fauna end up clear at the plot and environment level. Further examination into these procedures could define circumstances in which soil fauna are key determinants of biological system functions or ecosystem processes and increase acknowledgment of the soil fauna by different disciplines.

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# An Insight into Current Trends of Pathogen Identification in Plants

6

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**Abstract**

Worldwide, plant diseases have caused major economic losses in the agricultural industry. Food security is a major issue to the rising global population. Plant health monitoring and the early detection of pathogens are important in decreasing the propagation of diseases and providing the best strategy. To reduce the damage caused by old and new pathogens, and to speed up the management and reduction of crop loss, a fast and reliable detection method in combination with decision support systems is essential. New tools and technologies were developed for both detection and diagnosis, often substituting old methods to make them quicker, more accurate, and precise. With the rising global population, there is need to boost crop production and protect the crop from seed to market. There is also need to use the latest technology for rapid and precise diagnosis of plant diseases to minimize losses for sustainable production. In addition to the traditional visual screening for symptoms, nucleic acid and serological-based tools will provide precise and rapid detection and diagnosis. Remote sensing technology, along with spectroscopy, will help in the capture of high spatialization of results, and can be very useful to identify primary infections quickly. Handheld antibody-based immuno-biosensor also helps the rapid detection of plant diseases. This chapter enlightens on the various recent technologies in plant

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pathogen identification for sustainable and effective crop production, and the safeguard of the crop produce.

**Keywords**
Pathogen identification · Molecular diagnostics · PCR · Plant pathology · Plant diseases

## 6.1 Introduction

Due to increasing population and globalization, food security, and prevention of the spread of invasive pests/pathogens, accurate identification and diagnosis of plants are very important to protect available crop produce. Plant pathogens causes significant losses in plant yield. Enormous economic losses due to wheat rusts and Fusarium head blight claimed US $5 and US $3 billion/year, respectively (Schumann and D'Arcy 2009). Precise, sensitive, and special diagnoses are necessary to manage plant diseases efficiently and economically. Identification of plant diseases have evolved from visual inspection to the use of high-profile serological tools such as the enzyme-linked immunosorbent assay (ELISA), and molecular methods like polymerase chain reaction (PCR). MLO-7 gene in the grapes against powdery mildew disease, which ultimately increase the precision for the latest technologies such as CRISPR (clustered regularly interspaced short palindromic repeats) in plant disease studies, has become possible through the application of biotechnology and bioinformatics in plant pathology (Malnoy et al. 2016). Although progress has been made about all aspects of plant disease diagnosis, there is a need for increased sensitivity and specificity of molecular studies for rapid detection and diagnosis. This chapter describes briefly the different techniques used for diagnosing plant diseases and their contribution to the current challenges for global food security.

### 6.1.1 Microbiome Diversity

The diversity of the plant microbes is defined as the number of microbe species present in the particular ecological area and relationship between them with their ecological, environmental conditions (Vos et al. 2016).

Nowadays, a different type of molecular methods was used in the detection of microbes present in the sample. Firstly we collect the sample and isolate the DNA/RNA/protein/lipids and then follow the different methods, viz., PCR, qPCR, MALDI-TOF MS, electrospray, and ionization mass spectrometry, for the assessment of diversity present in the sample (Table 6.1). Here, Table 6.1 discusses some molecular techniques for plant disease diagnosis.

**Table 6.1** List of different techniques involved in the detection of plant microbes with their advantages and disadvantages

| Detection methods | | Merits | Demerits | References |
|---|---|---|---|---|
| Visual detection | | Simplest methods | Lack of sensitivity in detecting that occur in low concentration, asymptomatic | Ali et al. (2019), Gomez et al. (2019) and Cooke and Cacciola (2007) |
| Polymerase chain reaction-based methods | Conventional PCR | Specific primer for particular species produces precise and rapid results | More costly and necessitate more labor | Milijasevic et al. (2006) and Walcott and Gitaitis (2000) |
| | Portable rapid cycler PCR | Identification of plant disease at on-site | Pricey and deficient in robustness | Love et al. (2012) |
| | Nested PCR | Utilization of two pairs of primers raises yield and accuracy of desired DNA amplification | More chances of contamination due to two times amplification reactions | Lee et al. (1997) and Bereswill et al. (1995) |
| | Multiplex PCR | Resources and time rescued by using the various set of primers in PCR reactions | Primer and probe interference reduces the sensitivity | Rico et al. (2003) and Fegan et al. (1998) |
| | Reverse transcriptase PCR | It is more accurate than standard PCR and produces the quantitative data | Information about every test is difficult and involves costly materials and chemicals | Liu et al. (2019a, b) |
| | Real-time qPCR | No requirement of post-amplification reaction and it is an automated process | Pricey and difficult in owing to concurrent detection of thermocycling with fluorescence | Schaad et al. (2007) and Salm and Geider (2004) |
| | Serial analysis of gene expression (SAGE) | Reference knowledge of the genome is not required | Accuracy of the tag sequences | Tarasov et al. (2007) |

(continued)

**Table 6.1** (continued)

| Detection methods | | Merits | Demerits | References |
|---|---|---|---|---|
| DNA/RNA probe-based methods | Northern blotting | Identification of size of RNA | It applies to the very specific sample of the gene sequences | Yin et al. (2019) and Boccardo et al. (2019) |
| | In situ hybridization | It required more supply of tissue | It is very complex to detect the target of sequences with low copies of DNA or RNA | Ellison et al. (2016) and Tanaka (2009) |
| | Fluorescence in situ hybridization (FISH) | It can be used in nondividing cells | It is essential to identifying the target DNA sequences | Sidra et al. (2017) and Ratan et al. (2017) |
| Post-amplification technique | Microarray<br>Macroarray | It is high-throughput tool and allows identification of various pathogens | It does not differentiate the living and non-living cells analysis | Osmani et al. (2019), Leborgne and Bouhidel (2014) and Sato et al. (2010) |
| Isothermal amplification-based methods | Loop-mediated isothermal amplification (LAMP) | Fast, accurate, and extremely specific | Primer formation is more careful binding only on the specific pathogen | Almasi et al. (2013) |
| | Rolling circle amplification (RCA) | Simplicity, efficiency, tunability | – | Gomez et al. (2014) |
| | Nucleic acid sequence-based amplification (NASBA) | There is no need for expensive equipment, and also it is better than RT-PCR | Sample reaction temperature is 42 °C, and it cannot be increased because it depends on enzymes | Gabrielle et al. (1993) |
| RNA-sequence based next-gen. sequencing | | Specificity and sensitivity higher | It requires more costly tools and information for the data analysis | Dawei and Peng (2014) and de Jonge et al. (2012) |
| The RNA interference (RNAi) | | It is potential to investigate thousands of genes concurrently | Diversity and incompleteness of knockdowns and latent in nonredundancy of chemicals | Machado et al. (2017) |

(continued)

**Table 6.1** (continued)

| Detection methods | | Merits | Demerits | References |
|---|---|---|---|---|
| Spectroscopy | MALDI-TOF MS | Much faster, rapid turnaround time | Difficulty in distinguishing some organisms that are closely related genetically | Bruker (2016) |
| | Volatile compounds (GC-MS) | Highly indicative, accurate, high specificity | Capable of directly analyzing which are nonvolatile, polar, or thermally labile | Fang et al. (2014) |
| Antibody | Quartz crystal microbalance immunosensors (QCMI) | Real-time analysis possible | Better sensitivity required | Huang et al. (2014) and Zan et al. (2012) |
| | Agriculture nanobiosensors | Real-time analysis possible | Just beginning, trouble of reproducibility, and measurement errors | Etefagh et al. (2013) and Dubas and Pimpan (2008) |
| | Tissue blot immunoassay (TBIA) | Quick and simple to use | Costly | Chang et al. (2011) |
| | ELISA | It can manage a large amount of samples in the same time and produces more accurate results | Pre-enrichment is required for more surface antigens and more highly skilled manpower required | Kanakala and Kuria (2018) |
| | Flow cytometry | Concurrently identification and quantification of various pathogens in a consistent manner | More investment and very less information of this tools | Fang and Ramasamy (2015) and D'Hondt et al. (2011) |
| Quantum dots (QDs) | | High sensitivity, real-time analysis possible | Limits for detection, trouble of reproducibility, and measurement errors | Syed and Ahmad (2013) and Kumar et al. (2007) |

## 6.2 Toolbox for Diagnosing Plant Pathogens

The knowledge of the physiological method of interaction is lacking for many years between host and pathogen for many systems of pathogens (Table 6.2). Recently, quantitative high-performance imaging methods have been developed to phenotype plant growth and development (Mutka and Bart 2015). The following methods help to study the changing physiology of plants due to pathogens that further help in the development of mechanisms for disease symptoms.

**Table 6.2** List of important fungal, bacterial, and viral pathogens affecting crop plants with their diseases, host, and percentage of crop loss

| S.no. | Pathogen | Disease | Host | Crop loss | References |
|---|---|---|---|---|---|
| A | **Fungal** | | | | |
| 1 | *Fusarium oxysporum* | Wilt | 100 different hosts (mostly horticulture crops) | Nearly 80% | Debbi et al. (2018) |
| 2 | *Blumeria graminis* | Powdery mildews | Poaceae crops | Up to 45 | Zulak et al. (2018) |
| 3 | *Botrytis cinerea* | Gray mold | 200 crop hosts worldwide | 50–100% | Rupp et al. (2017) |
| 4 | *Colletotrichum* spp. | Anthracnose | Economically important crops, especially fruits, vegetables, and ornamentals | Up to 100% | Han et al. (2016) |
| 5 | *Magnaporthe oryzae B.C. Couch* | Blast disease | Poaceae crops and their wild relatives | 10–30% | Yoshida et al. (2016) |
| 6 | *Melampsora lini* | Rust | Flax | ~80% | Nemri et al. (2014) |
| 7 | *Fusarium graminearum* | Head blight | Wheat and barley | Up to 70% | Yang et al. (2013) |
| 8 | *Mycosphaerella graminicola* | Blotch | Wheat | 30–50% | Simón et al. (2012) |
| 9 | *Ustilago maydis* | Smut | Corn | <20% | Dean et al. (2012) |
| 10 | *Puccinia* spp. | Rust | Ferns, gymnosperms to highly evolved families of dicotyledons and monocotyledons | >80% | Dean et al. (2012) |
| B | **Bacterial** | | | | |
| 1 | *Agrobacterium tumefaciens* | Crown gall tumor | 140 species of eudicots | Considerable economic loss | Kerpen et al. (2019) |
| 2 | *Xylella fastidiosa* | Pierce's disease of grapevine (PD), citrus variegated chlorosis (CVC), and almond leaf scorch disease (ALSD) | Horticulture crops and especially grapes | Serious losses in grape production | Kyrkou et al. (2018) |

(continued)

**Table 6.2** (continued)

| S.no. | Pathogen | Disease | Host | Crop loss | References |
|---|---|---|---|---|---|
| 3 | *Erwinia amylovora* | Fire blight | Apple, pear, quince, blackberry, raspberry, and many wild and cultivated rosaceous ornamentals | Serious losses in pome production | Aćimović et al. (2015) |
| 4 | *Xanthomonas axonopodis* pv. *manihotis* | Cassava bacterial blight | Tubers | 12–100% | López and Bernal (2012) |
| 5 | *Pseudomonas syringae* pathovars | Blight, canker, brown spot, etc. | Mostly horticultural crops | 40% | Mansfield et al. (2012) |
| 6 | *Pectobacterium carotovorum* (and *P. atrosepticum*) | Soft rot, blackleg | Mostly potato | Serious | Nykyri et al. (2012) |
| 7 | *Xanthomonas oryzae* pv. *oryzae* | Bacterial leaf blight | Poaceae crops | Up to 22.5% | Soto-Suárez et al. (2010) |
| 8 | *Ralstonia solanacearum* | Bacterial wilt | Very wide range of potential plants | 25–75% | Artal et al. (2012) |
| 9 | *Xanthomonas campestris* pathovars | Black rot | 57 families of dicotyledons and mostly cruciferous crops | 20–45% | Hayward (1993) |
| C | **Virus** | | | | |
| 1 | Tomato yellow leaf curl virus (TYLCV) | Leaf curl | Wide host range (>800 plant species) | Up to 60% | Ding et al. (2019) |
| 2 | Tomato spotted wilt virus (TSWV) | Spotted wilt | Around 500 species of crops | Up to 100% | Gupta et al. (2018) |
| 3 | Brome mosaic virus (BMV) | Mosaic of grasses | Poaceae crops | Up to 20% | Ding et al. (2018) |
| 4 | Potato virus Y (PVY) | Necrotic lesions | Solanaceae | 50–80% | Hussain et al. (2016) |
| 5 | Cauliflower mosaic virus (CaMV) | Cauliflower mosaic virus | Brassicaceae family | 25–59% loss | Schoelz et al. (2015) |
| 6 | Cucumber mosaic virus (CMV) | Mosaic | More than 40 families | Up to 60% | Phan et al. (2014) |
| 7 | Tobacco mosaic virus (TMV) | Necrotic local lesions | Many economically important crops | 20–80% | Scholthof et al. (2011) |
| 8 | Plum pox virus (PPV) | Plum pox | Pome fruits | 20–40% | Cambra et al. (2006) |
| 9 | African cassava mosaic virus (ACMV) | Cassava mosaic disease | Tubers | 30–100% | Fargette et al. (1988) |

### 6.2.1 Visual Detection

One of the simplest methods for plant pathogen detection was a plant germplasm visual inspection and subsequent selection of healthy matter. Disease scaling based on visual symptoms is being acceptable with accuracy since the last 80 years (Martinelli et al. 2015). Different methods can be used for the visual inspection including visible illumination, chlorophyll fluorescence imagery, infrared imaging, and electromagnetic spectrum imaging (Cooke and Cacciola 2007; Bock et al. 2010; Li et al. 2014a, b; Odilbekov et al. 2018). The environmental and biological factors have a strong influence on these methods. Traditional methods for measuring the severity of the disease are not reliable for population estimating diseases lack standardization and procedural (Nilsson et al. 2011). In asymptomatic plants and less virulent pathogens, visual inspections may not be helpful; many hosts remain symptomatic (Mutka and Bart 2015; Ali et al. 2019; Liu et al. 2019a, b; Gomez et al. 2019).

### 6.2.2 Serology-Based Diagnostics

Phenotypic observation of plants with pathogenic symptoms would require extensive experience in the diagnosis of plant diseases and isolation of the disease. But for all attempts, the exactness of the same cannot necessarily be the same. Furthermore, the detection of the pathogens in asymptomatic samples or seeds is very likely to be diagnosed properly. The precise identification and an adequate diagnosis procedure would contribute more promisingly to plant health management. Through inventions of precise methods of detection, better management strategies are developed, and then, the rapid detection tool, in particular, an onsite handy one, definitely helps to achieve management success. A handy detection kit for potato viruses has been developed recently, which growers can use easily in the field (Ansar and Singh 2016). No artificial cultivation of plant pathogens like viruses and obligate pathogens causing rusts and powdery mildew and downy mildew diseases was possible. To address this problem, there were developed serologic assays, which are also used to identify other plant pathogens (Caruso et al. 2002). Serological pathogens of plants involve identifying disease based upon color-changing antibodies of the assay. The antibodies consist of immunoglobulin (Ig) proteins produced within the animal body (mammalian), which in general include foreign proteins, complex carbon dioxide, multiple nucleotides, or lipopolysaccharides. As we know, each antigen binds to a particular antibody. The serological method most commonly used is ELISA (enzyme-linked immunosorbent assay) as previously developed for plant virus detection (Clark 1981).

#### 6.2.2.1 Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA is done with polystyrene plates capable of binding enzyme-substrate reaction antibodies or proteins (Corning Life Science 2001; Luminex 2010). The enzyme-substrate response must be optimized in the timing and development

conditions to produce accurate and replicable results. ELISA is a popular test for the detection of plant pathogens present in plant resources and insect vectors (Clark and Adamas 1977; Naidua and Hughes 2001; Webster et al. 2004). Disease levels are observed on the bases of optical density in ELISA (Corning Life Science 2001; Webster et al. 2004). ELISA has benefits because it is more accurate; a big amount of tested materials can also be evaluated using a small number of antibodies to detect illnesses and a semiautomated process (Vemulapati et al. 2014; Naidua and Hughes 2001). Specific antibodies against the target pathogen have been developed (Torrance 1998). This technique is utilized for the identification of the various types of virus, i.e., Citrus tristeza virus (CTV), potato leaf roll virus (PLRV), potato virus X (PVX), and potato virus Y (PVY) (El-Araby et al. 2009; Sun et al. 2001). Since ELISA is a test based on antibody antigens, the availability of antibodies that react properly to the target agent is considered very important. ELISA often provides erroneous diagnostic because of false positive, which results primarily from unspecific or cross-reactive reactions with certain sample factors (Kfir and Genthe 1993). Several strains with a clear different symptom may be responded to by the antibody used in ELISA due to the absence of great specific places to bind.

Consequently, much related to different types of virus are not properly distinguished by ELISA (Boonham et al. 2014). Different type of additives were includes in extraction buffer for increased in ELISA sensitivity (Fegla and Kawanna 2013). When compared to molecular methods, ELISA is generally less sensitive. For these reasons, the utilization of ELISA in the way of diagnosis appears to progressively fall down, even when ELISAs have been used in the most up-to-date diagnostic purposes.

#### 6.2.2.2 Tissue Blot Immunoassay (TBIA)

Tissue Blot Immunoassay (TBIA) is similar to the principle of ELISA in which antibodies are functional; TBIA has a similar consistency to ELISA to detect plant pathogen like that virus (Hančević et al. 2012). The main difference is that polystyrene plate is working as a platform for ELISA, while nitrocellulose and nylon membranes are treated with TBIA; that's why this assay is also known as TIBA or TBIA (Webster et al. 2004). Similar to ELISA, TBIA is also required for the particular antibody to find clear false positive and false negative. Therefore, TBIA has huge advantages over ELISA in different conditions such as time, money, sensitivity, and expediency of detection. In TIBA, we have detected a various number of viral diseases in which some are here, i.e., bamboo mosaic virus (BoMV), bean yellow mosaic virus (BYMV), CTV, *Cymbidium* mosaic virus (CyMV), papaya ringspot virus (PRSV), sweet potato feathery mottle virus (SPFMV), and tomato spotted wilt virus (TSWV) (Bove et al. 1988; Eid et al. 2008; Hančević et al. 2012; Lin et al. 1990; Makkouk and Kumari 2006; Shang et al. 2011; Webster et al. 2004).

#### 6.2.2.3 Flow Cytometry (FCM)

Flow cytometry is a laser-based optical method. The technique is widely used in the fields of cell count, cell sorting, biomarker, and protein detection (O'Donnell et al. 2013). Many samples can be processed simultaneously via electronic detection

devices, and the technique simultaneously measures many parameters (Fang and Ramasamy 2015). Flow cytometry is widely used to evaluate microorganisms in the processing of food in drinking and marine water. FCM is not currently widely used as an instrument for research and detection in plant pathology; however, it can be used to test the total DNA content in bacterial, oomycete, and fungal disease agents and check pathogen viability; FCM can be used in the field of plant pathology for multiplexed path detection (D'Hondt et al. 2011). Flow cytometer rating is highly precise and purifies little or complex populations, but some of them are not fast enough to achieve the desired results even for a high-speed rating, and it costs more than the alternatives such as radioimmunoassay and ELISA.

## 6.3 Molecular Methods

Molecular methods of diagnosis of diseases are one of the remarkable sciences that are fast-growing and have the potential to revolutionize numerous scientific research, innovations, healthcare, and agriculture disciplines.

## 6.4 Genomics-Driven Diagnostics

### 6.4.1 Conventional PCR

The innovation of PCR got an incredible boom in the field of plant pathology. This tool permits amplification of specific DNA sequences in lots of duplicates by utilizing specific primers (Cha and Thilly 1993). At first, PCR was very explicit for the detection of infections brought about by microscopic organisms, i.e., bacteria, fungi, and virus. Presently, this tool is broadly utilized for the detection of the pathogen from the infected plants (Fig. 6.1). Occasionally, efficiency is influenced by sampled inhibitors (Fang and Ramasamy 2015). To resolve this problem in plants, cetyltrimethylammonium bromide (CTAB) can be used to add particular treatments with different chemicals and enzymes. A few different techniques are available to decrease PCR inhibitor effects. In the case of DNA isolation through the phenol-chloroform method, as a substitute for ethanol precipitation and silica bed, recovery of DNA can be done by purification (Mancini et al. 2016). Also, it can detect plant pathogens; PCR technology requires first to start the DNA replication procedure that might decrease the convenient application for disease field sample technology. Sometimes a single pair of primer does not give accurate results so that its limiting effect is overcome by DNA samples and nested primers. (Compton 1991). The detection of *P. granati* was established using nested PCR (Yang et al. 2017). Presumably, this methodology gives exceptionally explicit outcomes. However, it is a lot very expensive and required much effort (Sidra et al. 2017).

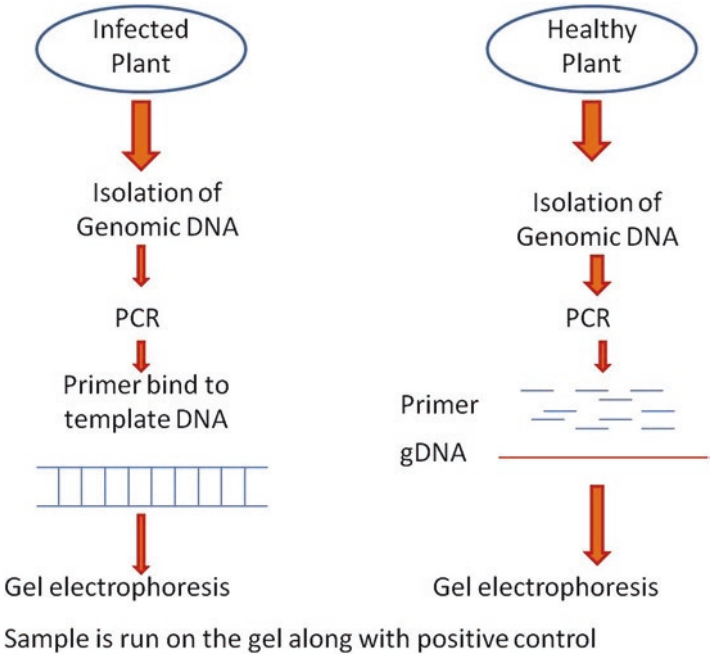


**Fig. 6.1** PCR-based method for identification of microbes

### 6.4.2 Nested PCR

A high degree of specialty and sensitivity can be found using nested PCR (Sidra et al. 2017). For example, "Grote et al. (2002) reported that relative study on sensitivity and specificity of *phytophthora nicotianae* using both simple and nested PCR" revealed that the nested PCR sensitivity is 1000 times the detection of a single PCR. In this procedure, primers can be utilized for the amplification of large DNA sequence through the two continuous cycles, the afterthat sequence of amplified product works act as a specific sequence for second times by the use of two internal primers. In nested PCR, tremendous risk of contamination is assessed even though, in separate tubes, two amplification cycles should be performed. There is, therefore, the probability of false adverse population outcomes, and heavy labor is a significant defect (Rahman et al. 2013). Laboratories must take certain tough measures for the use of different instruments and space for each PCR cycle to conquer such borders (Trtkova and Raclavsky 2006). Two *Phytophthora* species *P. palmivora* and *P. parasitica* have been identified using this PCR (Tsai et al. 2006). Likewise, *P. cactorum* was particularly identified in strawberry infected plants through utilizing this technique (Bhat and Browne 2010), as well as rapid detection of *Cylindrocladium scoparium* on eucalyptus (Qiao et al. 2016). For the identification of *Candidates Liberibacter asiaticus* attached with citrus Huanglongbing (Hong

et al. 2019), another report says that nested PCR can be used for detection of Lyme disease spirochete, *Borrelia burgdorferi*, in ticks (Wills et al. 2018).

### 6.4.3 Multiplex PCR

This method is utilized to demonstrate in less period as well as less money by utilizing few sets of preliminaries for a similar response to permits synchronous recognition of various focused on successions of DNA (Hyun et al. 2017). The method has such extraordinary significance of enormity in plant disease when crops are contaminated with many pathogens. Distinctive parts are clearly to target pathogenic parasites where at the same time enhanced and identified based on their molecular weight on agarose gel electrophoresis. DNA combining accuracy was unequivocally influenced by amplicon measure. To maintain a strategic distance from this entanglement, groundworks must be structured cautiously along with the comparative center and strengthening (Dasmahapatra and Mallet 2006). At present, lock tests (padlock primers) are utilized for the detection of pathogenic growths. This system has been utilized to the concurrent ID of parasitic pathogens, for example, *F. oxysporum*, *B. cactivora*, *P. nicotinae*, and *P. cactorum*, that key illness in joined cacti (Cho et al. 2016). Detection of various citrus disease-causing viruses in Jeju Island (Hyun et al. 2017), multiplex reverse transcription PCR assay for simultaneous detection of six main RNA viruses in tomato plants (Wu et al. 2018).

### 6.4.4 Portable PCR Systems

Fast PCR can manufacture a fully mobile configuration that will not just facilitate plant pathologists to carry out great efficiency with consistent experiment testing, but it might be significantly similar to downstream methods, i.e., those mandating in situ genomics detection tools. Palm PCR, manufactured in Korea by Ahram Biosystems, is easy to use, accurate, and high-competence thermal cycler (Love et al. 2012). Although it's small in size, this control tool provides tremendously well-defined and rapid amplification for different types of DNA materials (Monis and Giglio 2006). The amplification of the DNA to a sufficient amount is less than 25 minutes for the optimal detection in the agarose gel electrophoresis. The transferable way provides a greatly efficient easy-to-handle way for the learner and experienced academician to perform a different type of PCR tests. Koo et al. (2013) reported the successful identification of the six diverse fungal and bacterial plant pathogens in the sample.

Twista quantitative and portable real-time fluorometer are personalized tools produced to examine reactions to recombinase polymerase amplification (RPA) comparable with a different type of detection systems and the latest technology in DNA identifications, very high speed, easy to carry, and user-friendly with super quality. Twista RPA fluorometer provides urgently rapid diagnosis, enabling

appropriate on-time therapy compared to usual microbiological test requiring at the minimum an hour and molecular assays typically requiring consolidated tools (Surrette et al. 2018).

### 6.4.5 *Real-Time* Polymerase Chain Reaction (RT-PCR)

Real-time PCR invention has developed more amount of modification in its protocol. A portion of maximum changes has extended the utility and detection quality of this PCR in numerous natural as well as therapeutic fields (Tang et al. 1997). These tools are insufficient to differentiate the living and dead parasites. PCR cannot differentiate between dead and living parasites. The DNA can be obtained from the liver and circulating DNA from other cells or bodies. The addition of a PCR test aimed at Leishmania-specific mRNA in a householder gene should make a difference between living and dead parasites (Bretagne et al. 2001). The development of RT-PCR overpowers this limitation. Contamination in mRNA is present in the dead cell; therefore, RT-PCR can detect the presence of mRNA in the cell (Capote et al. 2012). In this regard, RNA is conversely deciphered into cDNA by irregular groundworks and real-time compound and after that intensified by various PCR methods. In this way, RT-PCR is utilized to recognize and analyze the RNA-containing (*retroviruses*) contaminations. The finding of RNA-containing infections can help make or check the practicality of antimicrobial inoculations or treatment. For example, it can be utilized for evaluation of *Fusarium graminearum* growths to reason *Fusarium* early curse sickness within grains, for example, wheat, grain, and oat (Brown et al. 2011). *Cryphonectria parasitica* can be detected early using RT-PCR (Chandelier et al. 2019). Concurrently, it can detect the various pathogens of potato (Nikitin et al. 2018).

Recently, quantitative real-time PCR is a technique which affects microbial ecology greatly. The qPCR is a very sensitive technique to quantify the microbial population within the ecological sample (Trung et al. 2011). In short, qPCR amplified the specific sequence of nucleic acids and also calculates the sample amount of DNA. The fluorescent marker (SYBR Green) present at the end of the reaction detected by the PCR instrument. SYBR Green I and high-resolution melt dyes (LC Green, EvaGreen, BEBO, and SYTO9) can link between the dsDNA and emit the fluorescence at 494 to 521 nm wavelength.

Similarly, another way used in the qPCR analysis, i.e., TaqMan probes, works on a third oligonucleotide during annealing. The probe is made up of two molecules: (1) reporter and (2) quencher. When these two come together, then they repress the fluorescence of reporter in the reaction of DNA amplification. Also it generates the signal intensity with the amount of target DNA amplification. Detection of the pathogen such as *Phytophthora infestans* from the potato (Hassain et al. 2014; Li et al. 2014a, b; Clement et al. 2013) and *Verticillium dahliae*, *Colletotrichum acutatum*, and *C. gloeosporioides* from the strawberry (Bilodeau et al. 2012; Garrido et al. 2009) is through qPCR.

### 6.4.6 *Serial Analysis of Gene Expression* (SAGE)

Sequential examination for quality articulation was thorough based on grouping strategy for the quantitative quality articulation data that permits recognizable proof in numerous transcripts (Moreno 2003). SAGE is based on sequencing data of 15 bp or more nucleotides and comparability of successions beside accessibility of genome groupings to locate the relating communicated qualities (Velculescu 1995). It can utilize two examples that are bound and signed with the different preliminaries, and it's intensified. At that point, concatemers are shaped by the sticky finishes through the expulsion of groundwork. In sequencing of the cloned vector using the computational investigation, SAGE has a few downsides. In the first place, it needs mRNA in an extensive amount. Second, now and then the 15 bp tag isn't sufficient to explicitly recognize the quality of birthplaces with the more mind-boggling genomes. Thomas et al. (2002) reported that in the profiling of transcript of mildew pathogen *Blumeria graminis* in barley, SAGE is used, and another scientist also reported that gene expression of *Nicotiana tabacum* in *Rhizoctonia solani* is through SAGE (Portieles et al. 2017).

### 6.4.7 Sequencing-Independent Methods

The probe of DNA/RNA strategies is utilized to analyze plant infections caused by the microorganisms, for example, fungi with extraordinary sensitivity and speed (Sidra et al. 2017). This innovation is considered as the spine to a large portion of the present information. In these techniques, the probe shall be used without its amplification for the testing of nucleic acid. Samples are the shortest single-stranded DNA sequence that is named with chemiluminescent molecules or radio isotopes, for example, $^{32}$P, $^{33}$P, and $^{35}$S (Sidra et al. 2017). For instance, engineered zinc finger proteins do not require targeted amplification for the detection of specific pathogen DNA (Kim and Kim 2016). It is utilized for the recognition of similar sequencing present on specific DNA. In traditional techniques, DNA probes is utilized for the most part as an option to PCR for the recognizable proof of microorganisms such as fungi. Be that as it may, in ongoing techniques, these are for the most part utilized related to PCR (McCartney et al. 2003; Khater et al. 2019). Engineered zinc finger proteins and pathogen targeted DNA immobilized on a chip of polymer (Ha et al. 2018; Guixia et al. 2019).

### 6.4.8 Northern Blotting or Northern Blot

This technique is also called as RNA smear technology and is generally used for the exchange of RNA onto a bearer for the recognizable proof of pathogenic growths utilizing the quality articulation (Zimmers-Koniaris 2001). The northern blot is equivalent to the southern smear apart from RNA material is utilized rather than DNA (Qi and Yang 2002). Right off the bat, the RNA from each tissue ought to be

purged be inspected the statement of the quality of intrigue. RNA molecule was separated on the agarose gel electrophoresis. In this gel, litter molecule moves faster than a higher molecule; in this way, higher molecules present well as compared with the litter molecule (Kim et al. 2010). Hence every RNA molecule keeps its position regarding every single another particle (Berg 2007). The way is present to do a radioactive test to hybridize its related grouping molecule. After that, the channel is put for autoradiography to build up the film. At last, a band ought to show up on it, if both tests have hybridized a section of RNA atom on the channel. To start with, the situation of groups at smudge gives RNA approximation, but a measure of RNA is known; then they will give a guess to coding limit of transcripts as well as the extent of protein to which it recognized. Right from the beginning, the danger mRNA corruption amid electrophoresis is the primary impediment in the smudging system. Besides, recognition with numerous tests is hazardous. The affectability of the northern smudging strategy is low contrasted with RT-PCR. *Magnaporthe grisea* was perceived in rice plants through utilizing ongoing PCR and northern blot technique (Qi and Yang 2002). *Agrobacterium*-infiltrated place can be detected with the presence of more amount of GFP mRNA and siRNA through northern blot techniques (Yin et al. 2019; Boccardo et al. 2019).

### 6.4.9 In Situ Hybridization

In 1969, exploration of nucleic acids by in situ hybridization was first revealed (Gall and Pardue 1969). In situ hybridization is likewise called the hybridization histochemistry. It gives precious data to recognizing and listing the parasites. In these method, the ssRNA test is utilized known as riboprobes and 35S. It has an extraordinary closeness to northern blotting. These two methods rely upon hybridization of marked tests of DNA/RNA to the comparative groupings of mRNA molecule. These strategies vary in the utilization of beginning material. On account for northern smudge, the part of tissue digest is utilized as beginning material, while in situ hybridization histological area is utilized. Notwithstanding utilizing the immediate hybridization, the recognizable pieces of proof utilizing signal hybridization are most productively acquired after the organism's development or its natural intensification (Jensen 2014).

The preferred primary standpoint of this innovation is the most extreme utilization of tissue, for example, clinical biopsies and refined cells. Several unique hybridizations can be done in a similar tissue. Tissue libraries can be arranged by putting away in cooler for future use. There are various techniques for performing in-situ hybridization, for instance, testing with counterfeit oligonucleotides copy of DNA and RNA. These strategies give the most powerless and efficient outcomes. Tests may be named according to radioactive or nonradioactive nucleotides; after these tests, 35S riboprobes are a very delicate strategy for the ID of mRNA (Hayden et al. 2002). ISH was used to locate specific chromosome DNA sequences using radioisotope-labeled samples (Gall and Pardue 1971; Zeller et al. 2001). It was later used for detection of viral particles and mRNA high copy in cultivated cells and

sectional tissue, so that gene expression patterns could then be located (Brahic and Haase 1978; Zeller et al. 2001). It is used for pathogen visualization in host plant tissue, using ISH technology (Tanaka 2009).

In situ hybridization has a few entanglements. Right off the bat, radiolabel tests are in all respects expensive and risky materials. It must be dealt with, transported, and discarded in all respects cautiously. A drawback of utilizing in situ hybridization procedure is the trouble in distinguishing focuses on with contains a low copy of DNA and RNA molecules (Qian and Lloyd 2003). It can be used for identification of various parasites, i.e., *Blastomyces dermatitidis*, *Coccidioides immitis*, *Cryptococcus neoformans*, *Histoplasma capsulatum*, and *Sporothrix schenckii* (Hayden et al. 2001). This type of hybridization can be used to imagine the difficulty in plant tissue of the rust organism, as well as it can be utilized for confinement region that is present in plant partition in rust fungi (Ellison et al. 2016).

### 6.4.10 Fluorescence In Situ Hybridization (FISH)

Because of the disadvantage of radiolabeled probe-based hybridization, the FISH technique has emerged, which is used for the speedy characterization of microorganism such as fungi (Sidra et al. 2017). This technology offers superior speed, resolution, and security and smoothens the way for simultaneous and quantitative phylogenetic analysis of multiple targets (Tsui et al. 2011). Besides this, FISH is also an essential type to identify break down in the spatial association of fungal networks.

Specific DNA sequence on chromosomes is detected using this cytogenetic technique (Ratan et al. 2017). The fluorescent probe is used in FISH which single binds those parts of the chromosomes, which shows a higher quantity of similarity. Fluorescent samples are established throughout the sample length by an enzymatic fuse of altered fluorophore base (Baschien et al. 2001). Conventional systems for detection of microorganism necessitates that cells should be effectively isolating (Ainsworth et al. 2006). However, for non-diving cells, FISH can be used, making it a highly resourceful technique. It is possible to recognize non-dividing cells by their low fluorescence intensity level. It is possible to use a different type of samples, for instance, an allele-specific sample, a centromeric repeat sample, and an entire chromosome sample. Many of them have various implementations. In FISH probe, preparing ready procedure is confounded because it is important to tailor probe to recognize the particular sequence of DNA (Volpi and Bridger 2008).

### 6.4.11 Microarray

A microarray is a technique that is used simultaneously to detect the expression of thousands of genes. It is a microscopic slide that is printed in defined positions with thousands of tiny spots with each spot referring to the known DNA sequence or gene, and these slides are known as DNA chips. It is used to detect potato viruses, cucurbit-infecting tobamoviruses, and grapevine viruses (Bystricka et al. 2003; Lee et al.

2003; Nicolaisen 2011). It is not quite the same as the above method in this point of view because it gives expression estimations of characterized genes of the set (Eshaque and Dixon 2006). Within a small surface area, thousands of DNA probes are displayed on a matrix that consists of glass chip or nylon filters in this technology. Sample position is called spot on the chip (Robinson et al. 2000). On the support matrix, these probes are immobilized, and focused cDNAs are useful as a hybridization chip. The Quantification encircled cDNA quantification is quantified using radio-labeled probes only after that signal is generated by hybridizing the probe to the focused mRNA that the specific software can identify and integrate. The Keen Software gives gene expression trend for every natural living sample (Russo et al. 2003). The microarray can detect a very large range of fungal cell. In the microarray techniques, large-scale DNA sequencing is not used. Even though this method can detect any changes in gene expression, noteworthy issues are noted. Initially, microarray required a lot of mRNA. Besides, this investigation is constrained by the expense and right to use (Singh and Kumar 2013). Because of the abundant error steps in microarray technique, it is minimized by the repetition of the experiments. Microarrays are referred to as critical testing because it requires physical cell trouble to gain accesses to its patterns of gene expression; it is remembered that false data can be generated by the mRNA degradation (Singh and Kumar 2013). This technique can detect bacteria, viruses, parasites, fungi, viroids, and phytoplasmas (Hadidi and Barba 2006). Postnikova and Nemchinov (2012) used microarray-based analysis for the comparative study of the various viruses in *Arabidopsis*. Leborgne and Bouhidel (2014) studied plant-microbe interaction using microarray. Osmani et al. (2019) used microarray analysis to detect responses of various viruses of potato.

### 6.4.12 Macroarray

It is also known as the hybridization of the DNA array or the plot of the reverse dot. It utilizes the feasibility of DNA amplification while radioisotopes are not required (Singh and Kumar 2013). In a growing number of labs around the world, macroarray is a quick molecular technique for the diagnostic of greenhouse plants (Le Floch et al. 2007; Lievens et al. 2003), ginseng (Punja et al. 2007), and potatoes (Fessehaie et al. 2003). It's working according to concurrent amplification of the linked species by the PCR. Also, in one hybridization reaction, it simultaneously analyzes several amplified sequence. It is a technique that is more sensitive and sophisticated than other PCR (Taoufik et al. 2004). In this evaluation amplification of PCR is joined with hybridization that builds affectability up to thousand folds or higher than PCR only technique, and it has a quick rotation time 1–2 days in contrast with radioactive culture techniques that involve 2 months for their conclusion (Taoufik et al. 2004). In this technique, the inward probe is considered to separate the variety. These probes are attached to the hold of the nylon membrane. By ultraviolet cross-linking, oligos are eternally bound to the membrane. The amplified PCR products are firstly hybridized and then spotted on the strips where species-specific interaction takes place (Tsui et al. 2011; Leinberger et al. 2005). To identify the species level of

*Mycobacterium*, the combination of the PCR and microarray technique is used (Leinberger et al. 2005). It is also used to detect *Alternaria alternata*, *Fusarium solani*, *Candida albicans*, *Aspergillus fumigates*, and *Cladosporium herbarum* (Sato et al. 2010) and identification of fungal as well as oomycete of the pathogen that causes the illness in solanaceous plants (Zhang et al. 2008).

### 6.4.13 *Loop-Mediated Isothermal Amplification* (LAMP)

LAMP has a vigorous and new approach for amplifying nucleic acid as a substitute for PCR. LAMP amplified specific nucleic acid with high specificity under the isothermal situation. It never requires PCR to produce changes in temperature but requires a single Tm for amplification of DNA molecule (Tsui et al. 2011). This technique is also based on auto-cycling and strand dislocations. LAMP utilizes polymerase of Bst DNA and two sets of internal and external primers. This reaction works at 650C for an hour while being put on either a dry or water bath, and after that, SYBR Green can be used for the detection of the amplified product. The end product of the LAMP has numerous reverse repeat with several loops which show cauliflower-like structure. LAMP is ten times greater exact and accurate than standard PCR (Ren et al. 2009). In LAMP, thermal changes are not needed; as compared with PCR, LAMP reaction requires only one tube (Fakruddin 2011). The sensitivity of LAMP reaction is affected by various factors, i.e., utilization of the DNA polymerase. This is significant for LAMP effectiveness. For recognition and diagnosis, LAMP is useful but it cannot be utilized for cloning purposes. While, the major disadvantage of LAM is, it cannot used for the direct assessment different dyes, for example $Mn^{2+}$ (dye), SYBR Green dye, hydroxyl naphthol blue dye etc. that cannot be distinguished among the required specific amplified product size and non specific product size, So, lead to false positives result. It can be resolved due to the utilization of molecular beacons (MBs) by producing the fluorescence signals while it combines with specific DNA sequences. Therefore, MBs can detect the amplified product. The LAMP finds a suitable situation for MBs (25–45 bp beacon length, 60–65 °C reaction temperature, and 0.6–1 pmol/μL beacon concentration) for the evaluation technique. An original MB-LAMP-based method has proven direct identification of the LAMP product. MBs are the nucleic acid fluorescent sample with hairpin structure. The structure of the hairpin left the fluorescence as quencher that is near to a fluorophore (Liu et al. 2017). LAMP detects *Ascochyta rabiei* fungi that cause *Ascochyta* blight disease in chickpeas. The contrast of standard PCR and LAMP not only reveals superior accuracy, sensitivity, and specificity to detect *A. rabiei* but also utilizes simple apparatus and takes less time to operate (Chen et al. 2016). LAMP was successfully used to identify *Paracoccidioides brasiliensis*, a thermal-reliant dimorphic fungus (Endo et al. 2004). This method also detects the pathogenic fungus, i.e., *Ochroconis gallopava* (Tsui et al. 2011). The use of lamp technology has also efficiently diagnosed *Penicillium marneffei* (Sun et al. 2010). Recently, LAMP is used to identify *Ophiostoma clavatum*, main blue stain fungus (Villari et al. 2013), *Colletotrichum gloeosporioides* (Wang et al. 2017), and whitefly *Bemisia tabaci* (Blaser et al. 2018).

### 6.4.14 Sequencing of the Next Generation Based on RNA-Seq

RNA sequencing is a recently developed technique for deep sequencing. A huge populace of RNA are generally transformed into a library of cDNA with adapters that bind to single or both ends. After that, every sequence having with or without amplification is sequenced for obtaining minute sequence from one end as in single-end sequencing or both ends as in pair-end sequencing in a high-throughput way. Based on DNA, sequencing tools can be used for reads which are commonly up to 30,400 bp. Prepared library to observe how strongly the results of the RNA sequencing unveil that most of the unique RNA transcripts are identified in the step of preparation of the library. To build up an RNA sequence library, it is necessary to fragment RNA or DNA moreover to permit processing by sequencing of the next generation. In the real-time reaction, mRNA can be prepared using either oligo or random primers. The benefit for the utilization of oligonucleotide is the significant production of polyadenylated mRNA proportion of cDNA. Therefore most of the acquired sequence can be insightful (Mortazavi et al. 2008). The three very generally RNA-Seq technologies of next generation are SOLiD and Ion Torrent, which are produced by Life Technologies, and HiSeq by Illumina (Dawei and Peng 2014).

After that sequencing, we found the maximum reads that are either aligned with reference genome whose sequences are known or constructed with de novo sequence without genomics sequence to develop a transcriptional map consisting of both expression level of every gene and transcription structure. Although RNA sequencing data obtained that can be used for alignment with the referred genome data, Annotator and Trinity assembler that assemble the RNA sequencing data without reference genome data using the assemble of adjacent identification of shorts reads. These methods enable new transcripts to be discovered and numerical transcript to be detected fairly. They are allowing more efficient use of the RNA sequencing to detect transcripts and classify the transcriptome in a nonmodel organism (GrabHerr et al. 2011). The *Magnaporthe oryzae* fungus is detected using this technology, which induces rice blast disease (Soanes et al. 2012). *Verticillium dahliae* fungus in tomatoes that induces vascular wilt disease is also identified using this technique (de Jonge et al. 2012). RNA sequencing would be used to detect pathogens in plants. The prospective suitability of mRNA sequencing data to recognize nucleotide divergences can disclose fungal pathogenicity genes of the plant in their protein-coding transcriptome that is mutant. The technique allows us to diagnose plant disease using RNA measurements (Metzker 2009).

### 6.4.15 Lateral Flow Microarrays

Nucleic acid is rapidly detected using lateral flow microarrays (LFM) based on hybridization technique with colorimetric signals that can be easily visualized (Carter and Cary 2007). LFM based on lateral flow nitrocellulose membrane chromatography, hybridized in very less time, have detection limits and might decrease the requirement for high-price lab tools. Skill is dependent for accessibility of

useful and reliable biomarkers of host and pathogen revealed by transcriptomics perspective (Martinelli et al. 2012a, 2013a). While we can use metabolomics for the detection of primary and secondary metabolites; it can be utilized as a biochemical marker for a variety of pathogenic diseases (Rizzini et al. 2010; Tosetti et al. 2012; Martinelli et al. 2012b, 2013b, 2014; Ibanez et al. 2014). Early pathogenic infections like Huanglongbing disease in citrus can be identified by an included omics approach (Dandekar et al. 2010). More highly synergistic proteins are necessary markers for plant health status, i.e., heat shock protein (HSP) or dehydrins, upregulated by various ecological ways (Natali et al. 2007).

## 6.5 Remote Sensing (RS)-Based Diagnostics

Spectroscopy is one of the main useful RS methods, including sensors such as VIS, NIR, SWIR, and imaging or nonimaging. Due to their possibility as a functioning instrument, elasticity, efficacy, and cost-effectiveness, these tools hold exciting promise for crop disease monitoring. Below are discussed the most applicable and recent developments in spectroscopy depending on techniques.

### 6.5.1 Nonimaging Spectroscopy

It is based on the natural things of leaf pigments, chemical factor, properties, and structural characteristics (Jacquemoud and Ustin 2001). Laboratory or field-collected leaf spectra determined spectral area of visibility for identification of diseases, i.e., leaf gall disease in sugarcane through remote sensing (Purcell et al. 2009), powdery mildew disease in wheat (Graeff et al. 2006), curl mite (Stilwell et al. 2013), yellow leaf virus in sugarcane (Grisham et al. 2010), also yellow rust in wheat during winter (Zhang et al. 2014), and grapevine viruses in grapes (Naidu et al. 2009). Yuan et al. (2014) experimented with the separation of winter contamination from pathogens, insects such as wheat aphids, and virus, i.e., yellow rust and powdery mildew. Methods for early identification of disease are of faithful interest (Malthus and Madeira 1993; Delalieux et al. 2007; Rumpf et al. 2010), while their actual use is incoherent across crops for crop management. Studies available are crop definite, and results cannot be comprehensive with comparable accuracy to other crops and places. When Huang et al. (2012) compared both, firstly canopy scale detection of leaf and severity determination of rice leaf folder disease, the best concurrence of recognition rates using VIS and NIR reflection by linear regression method and the NIR plateau has a very high negative correlation (737–1000 nm) and severity of infestation.

Many authors are working with radiometry to determine the harshness of the damage of crop (Nutter 1989). Yang et al. (2007) reported that rice plants are infested with brown plant hopper and leaf folder. Mirik et al. (2006) studied spoil from green bugs to winter wheat, Chen et al. (2008) Accessed loss of cotton from *Verticillium* wilt and studied leafhopper disease from Prabhakar et al. (2011). Also utilized for fruit value evaluation was spectroscopy, often connected with additional

resources, i.e., e-nose data; booming integration of remote sensing depends on the way with VOC analysis (Costa et al. 2007).

### 6.5.2 Imaging Spectroscopy

Hyperspectral imaging tools have recently been integrated to evaluate and observe plant illness. Lab experiments detect various diseases, i.e., head blight disease in wheat, a fungal disease with *Fusarium* (Bauriegel et al. 2011), sugar beet disease (Mahlein et al. 2012) on leaves, rust, and powdery mildew (Mahlein et al. 2013). Diverse infections and its stage of maturity are of particular interest for effective intervention (Mahlein et al. 2012). This report utilized a wide range of statistical instruments for image investigation, such as linear regression, principal component analysis (PCA), spectral angle mapper (SAM), and support vector machine (SVM) categorization with elevated accuracy of detection of disease. However, these trials concentrated on one or a few plants with little probability of generalization. On the basis of field survey of wheat plant yellow rust disease (Bravo et al.) and attempts to distinguished among wheat disease and abiotic stress (Moshou et al. 2004), Reynolds et al. (2012) and Huang et al. (2007) used the hyperspectral field and aerial information for evaluating the seriousness of *Rhizoctonia* crown and root rot disease in sugar beet and yellow wheat rust, respectively. Airborne hyperspectral data is best suited for farm and regional-scale remote sensing (RS) applications.

## 6.6 Protein-Based Diagnostics

### 6.6.1 MALDI-TOF MS

Bacterial product isolates were subcultured on LB agar media (Thermo Fisher Scientific, UK) and incubated at 37 °C for a 24–48 h and recognized with a Bruker MALDI biotyper system (microflex LT, Bruker) as directed by the manufacturer. The calibration of the mass spectrometer was performed by an automatic calibration operation with Bruker bacterial test standard (BTS), which is a modification of *E. coli* BH5α, spiked using two extra enzymes to allow calibration with 4–17 kDa mass ranges. After 6x40 laser shots have been implemented, a sum spectrum is automatically acquired from separate locations on BTS control. The minimum amount of peaks reverted to seven after the calibration phase (Fig. 6.2).

Using a sterile toothpick, an individual colony from a new culture of each isolate was selected and smeared on the specified places on normal MALDI target plate, and the drying plates were put on RT (25 °C) for 5 min after loading the sample. 1 µl HCCA matrix (saturated solution of α-cyano-40hydroxycinnamic acid in 50% acetonitrile and 2.5% trifluoroacetic acid) was put on the top of a spot not more than 10 min of drying the samples. Before loading plate into a mass spectrometer, sample was air-dried again for 5 min. Every bacterial colony has been screened in duplicate. MALDI Biotyper Real-time Classification (MBT-RTC) software (Bruker) acquired data set. It compares unknown peak from the reference database (MBT

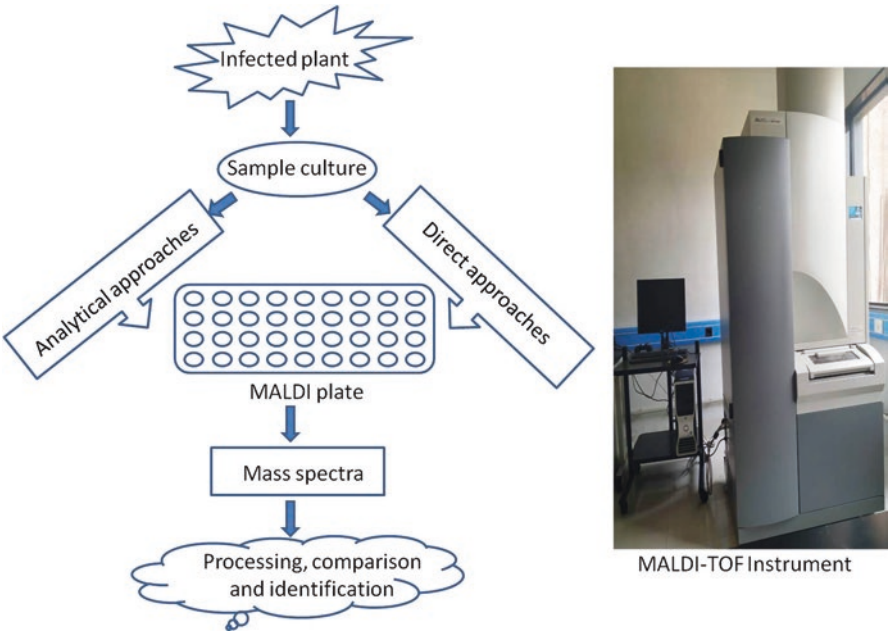


**Fig. 6.2** MALDI-TOF method for the identification of the microbes from the infected plant

Compass 4.1, Bruker) as well as used statistical algorithm to produce a log score ranging from 0.000 to 3.000. The classification score was considered to be suitable for species-level recognition (>=2.000 confident species identification), although recognition of the genus level (1.700–1.999 confident genus identification), below <1.700 no reliable identification (Bruker 2016).

Bacterial proteins were obtained by the process of ethanol/formic acid extraction outlined by the manufacturer for isolates, which could not be identified directly by transfer of colonies to the MALDI target plates. A bacteria-packed loop was briefly moved and resuspended with a 10 µl inoculation loop in 0.3 mL of deionized water and then added with 0.9 mL 100% ethanol, through blending and centrifugation at 16,000 × g for 2 min. Bacterial pellets were shortly drained, blended sequentially with 70% formic acid and 50 µl acetonitrile with 50 µl (20 µl for tiny pellets), and centrifuged to 16,000 g for 2 min. 1 µl supernatant was put on the spot on a plate of MALDI, dried, and overlaid using 1 µl HCCA matrix solution; this can be detected using Bruker MALDI Biotyper System (Bruker 2011).

### 6.6.2 Quartz Crystal Microbalance Immunosensors (QCMI)

QCMI analysis is based on concurrent mass vibration and its frequency variations and extensively utilized to conclude little vacuum, gas, and liquid mass (Kurosawa et al. 2006; Mecea 2005, 2006). Transducer device has a mass sensitive, the immunological mixture with QCM resultant in QCMI (Owen et al. 2007).

Both the antigen and antibody compatibility reaction in a positive reaction affects the reduced frequency of quartz crystal oscillation. QCMI, which offers certain advantages, high accuracy, real-time output, portability, label-free entity, and low operating, manufacturing, and protection costs, is an attractive option to a conservative tool of analysis (Chen and Tang 2007; Lee and Chang 2005; Tang et al. 2006). The diagnostic signal is very less to identify specific material; sensitivity to identification can be bigger through the introduction of the signal enhancement step (Kurosawa et al. 2006). QCMI is highly sensitive to detection of pathogen sample with viruses (Bachelder et al. 2005; Eun et al. 2002; Kleo et al. 2011; Lee and Chang 2005; Owen et al. 2007; Su et al. 2003; Uttenthaler et al. 2001). Since a QCM detection tool is potable, and QCM covered with virus-specific antibodies to identify plant viruses has a finite life span, it may also be utilized for the identification of plant viruses on-site (Becker and Cooper 2011; Eun et al. 2002). QCMI has been clearly detecting plant viruses, i.e., *Cymbidium* mosaic virus (CMV), TMV, turnip yellow mosaic virus (TYMV), *Odontoglossum* ringspot tobamovirus (ORSV), and maize chlorotic mottle virus (MCMV) (Dickert et al. 2004; Eun et al. 2002; Huang et al. 2014; Zan et al. 2012).

## 6.7 Nanotechnology-Driven Diagnostic

### 6.7.1 Agriculture Nanosensors

The nano sensors are results in collective advances to biology and nanotechnology (Yang et al. 2008). These nanosensors have the ability for improved sensitivity and also increased sensitivity with those can hold the potential in sensors. Therefore they considerably decrease the reaction time to detect prospective illness challenges in plants (Small et al. 2001), and therefore they can contribute to improving efficiency and food safety in agriculture. Hashimoto et al. (2008) developed the latest biosensor system that contains two types of biosensors for the speedy diagnosis of soilborne diseases. The system was built utilizing two different microbes in equal quantities, each being separately immobilized on an electrode. The optical properties of the silver nanoparticles can be differentiated or evaluated between sulphuration ethyl herbicide and silver nano particles, taking into account the specific optical properties of silver nanoparticles (Dubas and Pimpan 2008). They found that silver nanoparticles in a solution are sensitive to improved herbicide concentration and induced a difference in nanoparticle color converted from yellow to orange and then finally giving the violet color. It is a useful identification of contamination in water bodies and the environment, like organic pollutants and microbial pathogens (Dubertret et al. 2001). Combined with antibody molecules, fluorescent silica nanoparticles (FSNP) effectively identify plant pathogens like *Xanthomonas axonopodis* pv. vesicatoria in tomatoes and peppers that cause bacterial spot disease (Yao et al. 2009). Solgel and spray pyrolysis strategies were used to analyze nanoparticles and nanolayers of copper oxide (CuO). The detection of *A. niger* was conducted using both CuO nanoparticles and nanostructure layer biosensors

(Etefagh et al. 2013). In contrast, silver nanoparticles (AgNPs) are frequently used in soil and water bodies to detect toxins and microbial pathogens. The utilization of nanosensors has enabled plant disease prediction and disease control in agriculture (Bogue 2008).

## 6.8 Other New Technologies

### 6.8.1 Quantum Dots (QDs)

QDs are nanoparticles with a semiconductor that fluoresce while it stimulates through a cause of excitation light. In addition, QDs are inorganic fluorophore with foremost advantages compared to conventional organic fluorophore (Alexa Fluor 488, Alexa Fluor 514, BODIPY FL, carboxyrhodamine 6G, Cy 5.5, Cy 7, fluorescein, etc.) used as sign-on nucleic acids or visual proteins (Wang et al. 2006; Arya et al. 2005). Ferrari and Bergquist (2007) compared QDs and organic fluorophore for the detection of C*ryptosporidium* parasites. Different microbes make the semiconductor nonmaterials through mycosynthesis in single cell yeast (Dameron et al. 1989) and are also used for Cadmium sulfide (CdS) biosynthesis; through, some reports reported its luminescent values. The fungus *F. oxysporum* produced QDs when combined with $Cdcl_2$ and $Secl_4$ at normal temperature (Kumar et al. 2007). A proficient myco-mediated synthesis of highly fluorescent CdTe QDs was accomplished by the F. oxysporum isolates when reacted with a combination of $CdCl_2$ and $TeCl_2$ at room temperature (Jain 2003). Biosynthesized CdTe nanoparticles were described using electron microscopy and electron diffraction technique (Syed and Ahmad 2013).

The CdTe QD units were widely present in yeast cells, particularly in the cytoplasm and nucleus, while nothing was present in the cell membrane. The CdTe QD units are only spread into the cytoplasm of the yeast cells, and none in the cell membrane are found. The organic semi-compatible crystals are formed by a core and a shell which enables the ligands to bind and thus the fluorescent marker to be attached to the pathogen. A biosensor, also known as quartz microbalance crystal biosensor, is the best example. It can vibrate under electrical stimulation, and a mass change with the connection of every compound to its surface can be detected by reducing the vibration rate. A modified shape and distributed resonant frequency of quartz and crystal biosensor significantly decrease when a nucleic acid sample is attached to the surface of a quartz crystal biosensor and instead subjected to a supplementary PCR product. The hybrid forms and significantly reduces the resonance frequency of QC biosensor even if a nucleic acid probe is inserted on the surface of a quartz crystal microbalance and subjected to existing PCR product. This scheme decreases the time needed for particular environmental pathogens in conjunction with quick PCR methods (Sharon et al. 2010).

## 6.9 Conclusion

An alternative to traditional methods may be molecular methods such as PCR and qPCR as well as serological methods and flow cytometry. These methods are very sensitive and specific and take very little time, while simultaneously, it is necessary to optimize this method to fulfill the specificity and sensitivity requirements. Furthermore, standardized protocols are needed as a standard protocol for quarantine purposes for the global acceptance of the method. The portable diagnostic instrument, nanoparticle-based, bio-barcoded DNA sensor, and QD have prospective utilization in numerous identifications of various plant pathogens and toxic fungi. It can serve as an analysis to establish identification of plant illness easily and it can be used to stop epidemics. These diagnostic kits depend on nanomaterials and not just enhance the speed of pathogen detection as well as increase diagnostic accuracy. These tools have experienced notable technological changes since their innovation, resulting in them remaining important characteristics of research methods devoted to exploring diversity and all of its microorganism variations. Microscopy is progressively augmented with molecular technologies to show data on relative species availability and existence of noncultural microbes in certain environmental fields. Researchers and business will expand and take up these techniques as expenses are lowered in a manner comparable to desktop or cell phones. Harshness and robustness of specifications and precise attributes are key to the production of any current diagnostic as well as detection of various technologies. Ideally, fresh tools should be resilient to detect distinct entities using air sampling devices merely by removing product such as primers, antibodies, and biosensors. Ultimately, the use of sophisticated but inexpensive diagnostic methods is expanded to automated mobile collecting instruments. Further, in the future automated collecting instruments will also assist plant health inspection monitoring the development of fresh pathogens. This could threaten both agricultural and environmental systems.

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# Linkages of Microbial Plant Growth Promoters Toward Profitable Farming

7

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**Abstract**

Soil and plant health are linked with each other, and both are badly affected by the excess application of inorganic fertilizers and pesticides. The negative impact of chemical fertilizers toward the environment forced the scientific community to find out an alternative strategy that can improve crop yield and quality in an eco-friendly manner. The modern agriculture system is well furnished with microbial inoculants and plant defense elicitors. However, the application of microbes to manage plant growth and fitness needs to improve. Microbial inoculants play an important role in soil mineralization, energy mobilization and channelization, and also nitrogen fixation. This chapter aims to review the microbial plant helpers and their interlinks toward plant and human health. Microbial inoculums improve crop quality and yield, plant and soil health, and profit to farmers and reduce pollution. Proper utilization of microbial inoculants could help to improve the economic condition of the farmers and the country.

**Keywords**

Economic development · Human health · Microbial linkage · Sustainable agriculture

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## 7.1 Introduction

Population growth and food demand both are expanding with time, and alleviation of fertile land and polluted natural resources place distinctive stress on current agriculture system (Lotze-Campen 2011). Moreover, increased demand for quality food without harming the environment and human health has become a global challenge for the farmers and agriculture researchers (Amundson et al. 2015). A smart agriculture system needs to develop for the country—as the country's economic growth is particularly based on agriculture—and this system can be helpful to solve the problem of malnutrition and hunger. The modern agriculture system needs to project well now to balance higher crop production and environmental safety. Global food demand might be increased by more than 9 billion in 2050 as the projected population will be increased more than the present time (Cole et al. 2018; Hunter et al. 2017).

Currently, eco-friendly farming is receiving attention globally because it offers the likelihood to organize our agricultural requirements with environmental and human concern (Amundson et al. 2015; Hunter et al. 2017; Morawicki and Díaz González 2018). This system accustoms a special farming technique whereby the environmental resources will be completely consumed without damaging the substratum that provides a healthy agricultural product. Agro-ecosystems are significantly managed by microorganism populations and soil fauna that play a critical role in soil fertility. Microbes are the major gadgets of topsoil and plant that drive stability and productivity by the multiple microbial processes such as nutrient cycling, biodegradation, biostimulation, restoration, and disease management (Jacoby et al. 2017; Kumari et al. 2019).

Biofertilizers are often delineating as constituents that incorporate with living microorganisms that are within the rhizosphere, or inside or outside of the plant (Patil and Solanki 2016). These microbes stimulate plant growth through fixing nitrogen, improve nutrient uptake, and work as a growth promoter when applied to seed, plant surfaces, or soil (Muraleedharan et al. 2010; Kumari et al. 2019). Presently, researchers are interested in eco-friendly and safe farming practices (Harish et al. 2009a; Harish et al. 2009b; Kavino et al. 2010; Schütz et al. 2018), and most of the scientists have characterized rhizospheric and endospheric microbes that cause beneficial impact to the plant are mostly utilized as microbial inoculants (Glick 1995; Harish et al. 2009b; Gouda et al. 2018; Patil and Solanki 2016). Phytobiome-associated microbes are synergistically interacting with plants and their community. These interactions regulate the microbiome structure, and it is not only influenced by the environmental factors where they reside but also have widespread effects of the host metabolism and the location of the host. For example, Grice and Segre (2012) proved that the composition of the human gut microbiome is directly linked with physiological and psychological health of the host.

Similarly, Chaparro et al. (2012) reported that the soil microbiomes are interconnected with plant health. Therefore, due to the great influence of the microbiome on ecosystems, proper attention is needed to look out for the beneficial insights into

those different elements that contribute to plant soil health. Herein, this chapter presents the current insights of microbial linkages toward the soil and plant fitness and deliberations about the valuable microbes.

## 7.2 Effect of Micronutrients to Sustain Plant Growth

Many Asian countries are facing the problem of micronutrient deficiency, and to solve this problem they are utilizing chemical fertilizers or pesticides in an excess amount that is damaging the native beneficial soil activity that poses major constraints such as low or high pH, fewer minerals, less organic matter, contaminated water with high bicarbonate, drought, and salinity (Malakouti 2008; Fageria 2012). Deficiency of micronutrients, increases plant stress that destructively affects the crop yield and quality by causing hindrance to internal plant tissues such as xylem vessels, causing the spread of the pest and disease faster than a healthy plant, reducing the fertilizer use efficiency, and deactivating the phytosiderophores and phytostimulation that helps plants grow. Malakouti (2008) proved that Calcareous soil has Zn deficiency that is responsible for the yield loss and other important micronutrients such as Fe, B, Mn, Cu, and Mo that plays a significant role in the plant growth. Application of micronutrients can intensify grain yield up to 50% of durum wheat (*Triticum durum* L.), and mostly micronutrients are applied as a soil application, foliar spray, or seed treatment that improves the crop yield and quality, as well as macronutrient uptake efficiency (Malakouti 2008). The micronutrients play an essential role in crop production and quality that directly influences the crop and human health by providing sufficient nutrients (Table 7.1). Zohaib et al. (2018) reported that application of micronutrient mepiquat chloride enhanced the cotton yield and nutritional quality under boron-deficient and adequate boron conditions by nutrient accumulation in the seed tissues. Recently, a study proved the positive interaction between potassium and nitrogen advising that higher application of K helped to alleviate ammonium stress although growth vigor enhanced during the application of nitrate nutrition that improved the nutrient uptake and enhanced the growth of wheat plants (Guo et al. 2019).

## 7.3 Microbial Linkage for Sustainable Agriculture and Environment

Poor coordination of energy conversion is directly linked with the low agricultural production that frequently gets affected by physiological factors such as pH, temperature, and beneficial and pathogenic microbes (Souza et al. 2015). Plant-associated microbes that exist in either soil or plant rhizosphere improve the plant growth and accelerate plant defense against the plant pathogens. Beneficial plant growth-promoting (PGP) microbes mostly produce the secondary metabolites and solubilize the minerals that play a significant role in the plant rhizosphere (Olanrewaju et al. 2017). These kinds of beneficial microbes have a primary effect

**Table 7.1** Essential plant nutrient elements and their primary forms utilized by plants

| S. No. | Essential plant element | | Symbol | Primary form |
|---|---|---|---|---|
| 1. | Non-mineral elements | | | |
| | | Carbon | C | $CO_2$ (g) |
| | | Hydrogen | H | $H_2O$ (l), $H^+$ |
| | | Oxygen | O | $H_2O$ (l), $O_2$ (g) |
| 2. | Mineral elements | | | |
| I | Primary macronutrients | | | |
| | | Nitrogen | N | $NH_4^+$, $NO_3^-$ |
| | | Phosphorus | P | $HPO_4^{2-}$, $H_2PO_4^-$ |
| | | Potassium | K | $K^+$ |
| | | Calcium | Ca | $Ca_2^+$ |
| ii | Secondary macronutrients | | | |
| | | Magnesium | Mg | $Mg_2^+$ |
| | | Sulfur | S | $SO_4^{2-}$ |
| | | Iron | Fe | $Fe_3^+$, $Fe_2^+$ |
| | | Manganese | Mn | $Mn_2^+$ |
| | | Zinc | Zn | $Zn_2^+$ |
| iii | Micronutrients | | | |
| | | Copper | Cu | $Cu_2^+$ |
| | | Boron | B | $B(OH)_3$ |
| | | Molybdenum | Mo | $MoO_4^{2-}$ |
| | | Chlorine | Cl | $Cl^-$ |
| | | Nickel | Ni | $Ni_2^+$ |

Source: Parikh and James (2012)

on the soil and plant quality (Kumari et al. 2019). Beneficial microbes promote the plant growth through two different kinds of mechanism such as indirect and direct. In indirect growth promotion, microbes inhibit plant pathogen by different ways such as by antagonism or mycoparasitism, while direct mechanism projected by microbes involves the nutrient solubilization that improves the plant nutrient uptake from the environment (Glick 1995; Kafle et al. 2019; Kashyap et al. 2019). Moreover, microbes possess an antibiosis character, that is, antagonism for food and space, which induces systemic resistance of plants against the pathogens (Verma et al. 2017b). Microbes regulate various growth parameters/yields of crop/fruit plants that have been listed in Table 7.2.

## 7.4 Potential Microbes Act As Biofertilizers

Microbial plant growth helpers enhance the plant growth attributes and crop yield considerably. Ribeiro et al. (2018) reported that endophytic *Bacillus* could solubilize iron phosphate (Fe-P), produce siderophores and indole-acetic acid (IAA), and enhance the pearl millet plant biomass and nitrogen and phosphorus content of plant under a no-phosphorus-added condition. Bargaz et al. (2018) reported that

**Table 7.2** Various crop diseases regulated by plant growth promoting microorganisms (PGPM) as biocontrol agents

| S. No. | Disease | PGPM | References |
|---|---|---|---|
| 1. | *Cucumber mosaic virus* (CMV; of genus *Cucumovirus* of tomato (*Lycopersicon esculentum*) | *Bacillus pumilus* strain SE34; *Kluyvera cryocrescens* strain IN114; *Bacillus amyloliquefaciens* strain IN937a; *Bacillus subtilus* strain IN937b | Rabie et al. (2017); Rendina et al. (2019); Wang et al. (2018); Zehnder et al. (2001) |
| 2. | *Tomato mottle virus* | *Bacillus amyloliquefaciens* 937a; *B. subtilis* 937b; *B. pumilus* SE34 | Ambros et al. (2017); Gill et al. (2019); Gong (2018); Murphy et al. (2000) |
| 3. | Bacterial wilt disease in cucumber (*Cucumis sativus*) | *Bacillus pumilus* strain INR7 F | Park et al. (2013); Rojas et al. (2011); Zehnder et al. (2001) |
| 4. | Sheath blight disease and leaf folder insect in rice (*Oryza sativa)* | *Pseudomonas fluorescens* based bioformulation | Commare et al. (2002); Kumar et al. (2016); Seenivasan et al. (2012) |
| 5. | Blue mold disease of tobacco (*Nicotiana*) | *Bacillus pumilus* strain SE34 | Sahoo et al. (2014); Wu et al. (2015); Zhang et al. (2002) |
| 6. | Downy mildew in pearl millet (*Pennisetum glaucum*) | *Bacillus subtilis* strain GBO3; *Bacillus pumilus* strain INR7; *Bacillus* pumilus strain T | Jogaiah et al. (2014); Mahatma et al. (2011); Raj et al. (2003) |
| 7. | CMV in cucumber | *Bacillus subtilis* strain IN937a | Borriss (2011); Jetiyanon et al. (2003); Zhang et al. (2010) |
| 8. | Foliar diseases of tomato | *Bacillus cereus* strains B101R, B212R, and A068R | Koné et al. (2010); Silva et al. (2004); Zodape et al. (2011) |
| 9. | Blight of bell pepper (*Capsicum annuum*) | *Bacillus* strains BB11 and FH17 | Díaz-Pérez (2014); Jiang et al. (2006); Oh et al. (2011) |
| 10. | Saline resistance in groundnut (*Arachis hypogea*) | *Pseudomonas fluorescens* | Asif et al. (2011); Saravanakumar and Samiyappan (2007) |
| 11. | Maize (*Zea mays*) rot | *Burkholderia* strains MBf21 and MBf15 | Hernández-Rodríguez et al. (2008); Löffler et al. (2010) |
| 12. | Soil-borne pathogens of cucumber and pepper (*Piper*) | *Bacillus subtilis* ME488 | Chung et al. (2008); Pliego et al. (2011) |
| 13. | Significantly reduce the Banana bunchy top virus (BBTV) incidence | *P. fluorescens* strain CHA0 + chitin bioformulations | Kavino et al. (2008); Niyongere et al. (2013) |
| 14. | Rice blast | *Bacillus* sp.; *Azospirillum* strains SPS2, WBPS1, and Z2–7 | Shan et al. (2013); Zakira (2009) |
| 15. | Rice sheath rot (*Sarocladium oryzae*) | Fluorescent *Pseudomonas* spp. | Hittalmani et al. (2016); Saravanakumar et al. (2009) |
| 16. | Blight of squash | *Bacillus* strain | Ji et al. (2012); Zhang et al. (2010) |

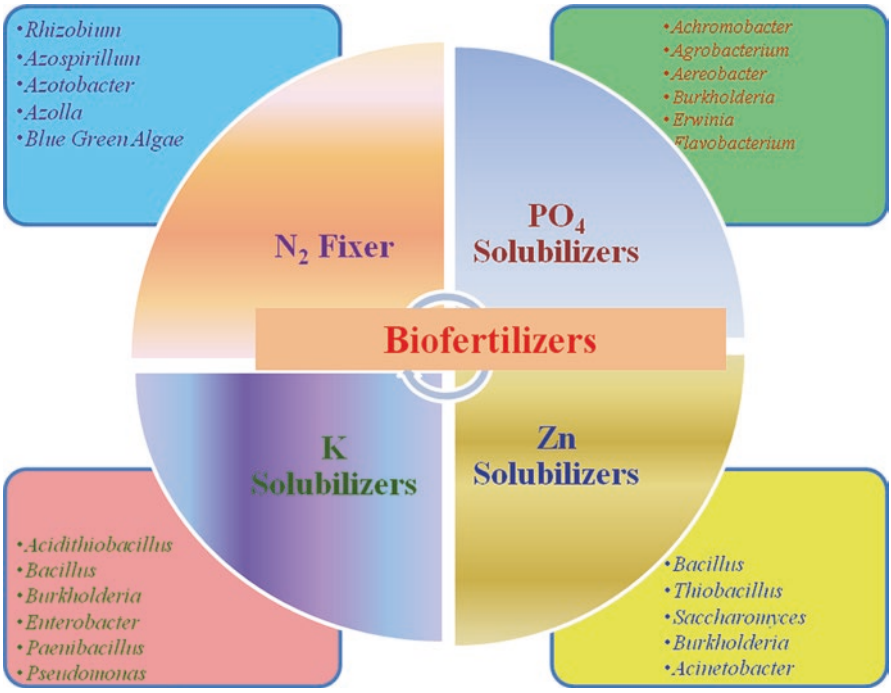


**Fig. 7.1** Potential microbial linkage to improve agriculture and human health

nitrogen-fixing and P-solubilizing/mobilizing microbes are an especial element of soil fertilization for improving crop productivity and fertilizer efficiency. Moreover, these groups of microbes possess multitrophic interaction that helps in plant mineral uptake. The plants that are inoculated with appropriate microbial strains have been found to be more proficient in acquiring nutrient in even nutrient-deficient conditions (Ribeiro et al. 2018). Several beneficial plant growth-promoting microbes used as biofertilizers are represented in Fig. 7.1.

## 7.4.1 Nitrogen Fixers

### 7.4.1.1 *Rhizobium*

Among all plant growth-promoting bacteria, *Rhizobium* bacteria are heterotrophic soil bacteria that are known to be the most prominent nitrogen fixers. *Rhizobium* fixes approximately 50–100 kg/ha nitrogen in the leguminous plants (Checcucci et al. 2017). It belongs to family Rhizobiaceae, for example, the well-known symbiotic bacteria of the legumes that are present in the nodules and fix the atmospheric nitrogen for the plant. It is mostly detected from useful pulses, like black gram, red-gram, pea, chickpea, and lentils, and also isolated from oil-seed legumes like soybean and peanut, and some important forage crops like berseem and

lucerne. Recently, Clúa et al. (2018) discussed the importance of legume and *Rhizobium* strain compatibility that largely depends on the availability of compatible strain for a particular legume. Moreover, rhizobia get signal molecules through the plant to infect the root tissue and colonize the nodule and depend on the plant growth stages and plant metabolism to get successful nodulation (Clúa et al. 2018; Zhao et al. 2018).The nodule is a bulb-like structure that acts as a nitrogen fixation factory (Geetha and Joshi 2013). It assists in converting atmospheric nitrogen by symbiosis with leguminous plants, and in certain cases also with the nonleguminous plants like *Parasponia* (Santi et al. 2013). Leguminous plants improved the *Rhizobium* population in the soil and to maintain the *rhizobium* population crop rotation and artificial inoculation methods used mostly that enhance the rate of nitrogen in the soil.

#### 7.4.1.2 *Azospirillum*

After *Rhizobium*, *Azospirillum* (family Spirilaceae) is the next diazotrophs that is globally recommended as important nitrogen-fixing microbes, and it can fix about 20–40 kg/ha nitrogen by biological nitrogen fixation (BNF) process in legumes and nonleguminous plants (Pankievicz et al. 2015; Zeffa et al. 2019). It is capable of improving plant growth by various ways like amino acids formation and release, improving root growth that helps plants to uptake more water and nutrient, and working as plant growth stimulant via biosynthesis of phytohormones such as indole-acetic acid, gibberellins, cytokinins, and different forms of polyamines (Pereg et al. 2016; Mehnaz 2015; Vejan et al. 2016). The *Azospirillum* forms symbiosis in several plants species mainly with the $C_4$-dicarboxylic pathway of photosynthesis (Hatch and Slack pathway), because they mostly grow and fix nitrogen on salts of organic acids such as malic or aspartic acid (Arun 2007). Bashan and Levanony (1990) reported "Multiple Mechanism Theory" that mostly worked with the plant system, and it varied as per the host and growth stages (Bashan and de-Bashan 2010). Recently, Zeffa et al. (2019) proved that *Azospirillum brasilense* Ab-V5 inoculated maize plants improved the biomass and yield under nitrogen deficit condition. Thus, it is mainly endorsed for these cereal crops such as maize, sugarcane, sorghum, and pearl millet. Among all species of this genera, *Azospirillum lipoferum* and *A. brasilense* are extensively used as biofertilizer throughout the world (Zeffa et al. 2019).

#### 7.4.1.3 *Azotobacter*

*Azotobacter* is a free-living aerobic soil bacteria that belongs to the family Azotobacteriaceae and is a known plant growth-promoting rhizobacteria (PGPR; Wani et al. 2016; Viscardi et al. 2016; Van Oosten et al. 2018). These bacteria play an eminent role in the BNF and fix an average of 20 kg N/ha per year. *Azotobacter* is easily colonized in the soil and plant tissues and improves the plant growth through the production of auxins, gibberellins, cytokinins, ammonia, vitamins, and beneficial metabolites that improve seed germination, provide protection to the plant root against pathogens, and work as growth stimulator of beneficial rhizospheric microbiomes that improve the crop yield. It can secrete 1-aminocyclopropa

ne-1-carboxylate (ACC) deaminase enzyme that helps the plant to grow in extreme environments, which makes these bacteria most vital for the stress management of the *plant (*Kukreja et al. 2004; Van Oosten et al. 2018). However, the real mechanism that exactly helps the plant is not fully understood. *Azotobacter* population is influenced or regulated by various factors of soil such as soil physicochemical (e.g., organic matter, pH, temperature, soil moisture) and microbiological properties. Major *Azotobacter* species are *A. vinelandii*, *A. beijerinckii*, *A. insignis*, and *A. macrocytogenes* (Subba Roa 2001; Tippannavar and Reddy 1993), and most of the strains can restrict several plant pathogens such as *Alternaria*, *Fusarium*, and *Helminthosporium.* Higher abundance of *Azotobacter* has been reported from the rhizosphere of various crop plants such as rice, maize, sugarcane, bajra, vegetables, and plantation crops (Arun 2007; Wani et al. 2016; Viscardi et al. 2016; Van Oosten et al. 2018).

#### 7.4.1.4 Blue-Green Algae (Cyanobacteria)

Photosynthetic prokaryotes that are mostly associated with both marine and freshwater environments and fix the environment nitrogen are known as blue-green algae (BGA). BGA are filamentous vegetative cell chains that are heterocyst, which are mostly symbiotically associated with different hosts such as fungi, liverworts, ferns, and flowering plants, but the most common symbiotic association has been found between a free-floating aquatic fern and rice paddy. BGA can fix 20–30 kg N/ha and are able to produce plant growth substances such as indole acetic acid, auxins, cytokinins, gibberellic betaines, amino acids, vitamins, and polyamines (Ronga et al. 2019). Several microalgae families such as Chlorophyceae, Trebouxiophyceae, Ulvophyceae, and Charophyceae are able to produce auxin and cytokinin that are utilized as plant growth stimulants (Stirk et al. 2013). Moreover, the major species of BGA available commercially are: *Isochrysis* spp., *Chaetoceros* spp., *Chlorella* spp., *Arthrospira* spp., and *Dunaliella* spp. (Priyadarshani and Rath 2012). *Arthrospira* spp. and *Chlorella* spp. are the most common BGA species that are cultivated and utilized commercially all over the world (Ronga et al. 2019). Several past reports underline a beneficial impact on plant nutrient uptake, biomass, and crop yields when BGA are applied as biofertilizers (Shaaban 2001a, b; Hall and Williams 2003), and due to these multiple characteristics, BGA could play a significant role in sustainable agriculture practices.

#### 7.4.1.5 *Azolla*

Genus *Azolla* contains several species of aquatic ferns also known as floating plants. They are native to the tropics, subtropics, and warm temperate regions of Africa, Asia, and America (Costa et al. 2009). *Azolla* is habitually detected in different water resources such as stagnant waters, ponds, ditches, canals, or paddy fields. *Azolla* mats cover these water resources in a suitable condition. Generally, *Azolla* is associated with other free-floating plant species such as water lettuce (*Pistia stratiotes* L.), watermeal (*Wolffia* Horkel ex Schleid), water caltrop (*Trapa natans* L.),

duckweed (*Lemna minor* L.), water purslane (*Ludwigia palustris* L.), knotweed (*Polygonum arenastrum)*, and mud-rooting species such as hornwort (*Ceratophyllum demersum* L. [Kannaiyan and Kumar 2006; Mosha 2018]). *Azolla* is known as a potential source of organic manure and nitrogen source for the crops, particularly for rice. The dry biomass of *Azolla* contains 4–5% N and 0.2–0.4% wet biomass. *Azolla* biofertilizer is applied on rice crop as a nitrogen source; after application, it decomposes very fast and provides the available nitrogen to rice plants. Moreover, it also contains substantial quantities of P, K, S, Zn, Fe, Mb, and other micronutrients (Mosha 2018). *Azolla* is recommended as a green manure that is incorporated to the rice field before showing. *Azolla pinnata* is most commonly used in India on a commercial scale. However, *Azolla caroliniana*, *A. microphylla*, *A. filiculoides*, and *A. mexicana* are also utilized for biomass production and used as biofertilizer.

## 7.5 Phosphate Solubilizers

Soil is a reservoir of numerous biochemical reactions performed by microbes to transport minerals from the soil to the plant. Microbes that are involved in the solubilization of insoluble inorganic phosphate compounds, such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite, and rock phosphate, into available phosphorus are known as phosphate (P) solubilizers. These microbes improve the microbial activity of soil and enhance plant growth (Alori et al. 2017; Raj 2014). Among all bacterial genera, *Achromobacter*, *Aereobacter*, *Agrobacterium*, *Bacillus*, *Burkholderia*, *Flavobacterium*, *Erwinia*, *Microccocus*, *Pseudomonas*, and *Rhizobium* are known as prominent P solubilizers and provide the available phosphorus to the plants (Rodríguez and Fraga 1999). Rhizosphere soil of plant mostly contains considerable amounts of P solubilizers that play an indispensable role in the P mineralization (Kasiamdari et al. 2002). Phosphate-solubilizing bacteria (PSB) are aerobic and anaerobic strains that exist in the soil or plant, with a prevalence of aerobic strains in submerged soils. *Pseudomonas* and *Bacillus* are widely accepted as major P solubilizers, and they mobilize the insoluble P compounds by the production of organic acids that enhance the acidic nature of substratum (Chen et al. 2016). The organic and inorganic acids help to convert tricalcium phosphate to di- and monobasic phosphates so that plants can easily absorb the available P. Moreover, different microbes produce different kinds of organic acid to solubilize P. Tri- and dicarboxylic acids are able to solubilize P more effectively as compared to the monobasic and aromatic acids. Similarly, aliphatic acids have better ability to solubilize P as compared to phenolic, citric, and fumaric acids (Chen et al. 2016; Khan et al. 2010; Manzoor et al. 2017). Panhwar et al. (2011) assessed that Christmas Island Rock Phosphate application with a phosphate-solubilizing bacteria of *Bacillus* spp. improved P uptake and plant biomass in aerobic rice (*Oryza sativa* L.). Oteino et al. (2015) proved the ability of an endophytic *Pseudomonas* bacteria to solubilize P by releasing gluconic acid (GA) and stimulate the growth of *Pisum*

*sativum* L. plants. Chen et al. (2016) reported that phosphate-solubilizing activity of *Pseudomonas* sp. PSB12 was associated with the release of organic acids, specially gluconic acid, formic acid, butyrate, and propanedioic acid, and it varied as per the different phosphate forms, and phenol could be used as the carbon source to dissolve insoluble phosphorus. Manzoor et al. (2017) revealed that the integrated use of P solubilizers with insoluble rock phosphate enhanced the P availability in soil pool and improved the maize plant growth significantly. Ahmad et al. (2018) concluded that two P-solubilizer bacteria (*Bacillus subtilis* strain Q3 and *Paenibacillus* sp. strain Q6) could promote cotton growth under alkaline conditions by supplying P nutrients. Prakash and Arora (2019) discussed the importance of an integrated application of Tricalcium phosphate with P-solubilizer *Bacillus* sp. that could be used to increase menthol production and oil yield of *Mentha arvensis*.

## 7.6 Phosphate Absorbers

Phosphate is a major element of plant nutrients. Plant root associated symbiotic microbes play a significant role to absorb phosphate from the soil. These symbiotic fungal microbes are known as arbuscular mycorrhiza (AM; fungal roots) that basically colonize in two kinds of environment, host plant (inside the root, or in rhizoids or thalli) and in surrounding soil of the plant (Jansa and Gryndler 2010). Mycorrhizal hyphae helps to uptake plant nutrients such as phosphorus, zinc, copper, and sulfur. AM fungi enhance the plant phosphorus uptake by increasing the absorbing surface area of root that helps to mobilize available phosphorus. AM-associated plants display a greater volume of phosphorus invasion than the non-mycorrhizal controls. Yang et al. (2012) assessed that rice + AM improved 70% phosphorus uptake. Moreover, colonized mycelia of vesicular-arbuscular mycorrhiza (VAM) protects the host root from the fungal pathogens and nematodes. Moreover, the fungal mycelia that colonize inside the host plant give a stable environment to the plant even in extreme conditions, through the extended root system that provides greater volume of water and nutrients to the host (Pavithra and Yapa 2018; Rask et al. 2019; Wang et al. 2017). VAM is ubiquitous and widely colonized in the plants that even exist in the arctic, temperate, and tropical regions, which shows the broad ecological range of this microbe and its ability to proliferate from aquatic to desert environments (Jansa et al. 2013; van der Heijden et al. 2015). However, only a few fungi have the ability to form a mycorrhizal association with the plant, for example, order *Glomales* of class *Zygomycetes* have 150 fungal species, and, among them, a small number of fungi are presumed to be mycorrhizal. Two genera, *Glomus* and *Sclerocytis*, are able to produce chlamydospores, whereas four genera can form zygospores: they are similar to *Gigaspora*, *Scutellospora*, *Acaulospora*, and *Entrophospora* (Maia et al. 1994). Mycorrhizal fungi have a wide range of applications in the agriculture system such as in seedling growth enhancement (van der Heijden and Horton 2009), decomposition of plant and crop residues (Lindahl et al. 2007), soil aggregation, and soil formation (Rillig and Mummey 2006).

## 7.7 Potassium Solubilizers

Potassium (K) is recommended as the third most important macronutrient for plant fitness and is involved in several biochemical and physiological processes. Low level of soluble potassium (up to 90% exists as insoluble rocks and silicate minerals) in the soil impacts negatively on plant metabolism and plant development and grain quality; it plays a significant role in the synthesis of cells, proteins, enzymes, cellulose, starch, and vitamins in normal or in stress condition (Shahzad et al. 2019; Wakeel et al. 2017). Macronutrient K not only regulates nutrient by transportation and absorption but also modulates the plant defense system under abiotic and biotic stresses and thus improves the crop production and quality (Oosterhuis et al. 2013; Zahoor et al. 2017). Sheng and He (2006) reported that Nanjing feldspar and Suzhou illite are two potassium (K)-bearing minerals that are used with *Bacillus edaphicus* through soil application and bacterial inoculation, which enhances the K uptake significantly in plant components.

Verma et al. (2015a) described a gram-positive plant growth-promoting bacterial strain *Bacillus amyloliquefaciens* that showed a significant level of K solubilization in in-vitro and in-vivo conditions. Verma et al. (2016) reported that most of the bacilli-solubilized potassium, such as *B. aerophilus*, *B. atrophaeus*, *B. cereus*, *B. circulans*, *B. horikoshii*, *B. licheniformis*, *B. megaterium*, *B. mojavensis*, *B. pumilus*, *Lysinibacillus sphaericus*, *Exiguobacterium antarcticum*, *Paenibacillus amylolyticus*, *P. dendritiformis*, *P. polymyxa*, *Planococcus citreus*, and *Planococcus salinarum*. However, K-solubilizing bacteria may have been used in the amelioration of K-deficient soil in agriculture. The most important K-solubilizing microbes (KSM) *Acidithiobacillus ferrooxidans*, *B. circulans*, *B. edaphicus*, *B. globisporus*, *B. mucilaginous*, *B. subtilis*, *Burkholderia cepacia*, *Enterobacter hormaechei*, *Paenibacillus kribensis*, *P. mucilaginous*, and *Pseudomonas putida* are used as K solubilizers and solubilize K in an eco-friendly manner. Therefore, these efficient K-solubilizing microbes (KSM) should be applied for solubilization of a fixed form of K to an available form of K in the soils (Verma et al. 2017a).

## 7.8 Zinc Solubilizers

Zinc (Zn) is an essential element of soil and Zn deficiency is widely reported in Indian soils (>50%) that exhibit the critical level of 1.5 ppm of available zinc (Katyal et al. 1994). The plant alone cannot absorb Zn from the soil, and hence farmers apply a huge amount of zinc sulfate ($ZnSO_4$) as an external fertilizer application. However, external fertilizer application also does not work well because the plant utilizes a very low percentage (1–4%) of the total available Zn and 75% of the applied Zn is transformed into different mineral fractions (Goteti et al. 2013). Two core mechanisms are applied to fix Zn: one is cation exchange, which mostly operates in acidic soils, and the other operates in alkaline conditions, where fixation

occurs using chemisorption (chemisorption of zinc on calcium carbonate [$CaCO_3$] forms a solid-solution of Zn by complexation of organic ligands; Alloway 2008). Microbe-based solubilization of Zn plays a significant role in the soil, and major bacterial inoculants that fix Zn are *Bacillus subtilis*, *Thiobacillus thiooxidans*, and *Saccharomyces* sp., *Burkholderia* sp., and *Acinetobacter* sp., and these microbial inoculants are used as biofertilizers (Azooz and Ahmad 2013; Vaid et al. 2014). Mahdi et al. (2010) reported that Zn-solubilizing microbes are mostly applied in soils where native Zn is higher or in conjunction with insoluble cheaper zinc compounds like zinc oxide (ZnO), zinc carbonate ($ZnCO_3$), and zinc sulfide (ZnS) instead of costly zinc sulfate ($ZnSO_4$)). Kamran et al. (2017) reported that Zn-solubilizing microbes include *Rhizobium* sp. (LHRW1), while *Enterobacter cloacae* (PBS 2) improved Zn uptake in wheat plants and enhanced the root and shoot biomass. Dinesh et al. (2018) concluded that Zn-solubilizing *Bacillus megaterium* ZnSB2 strain played a significant role in Zn dissolution in soil, and it would allow reducing the utilization of inorganic Zn application rates. Gontia-Mishra et al. (2017) assessed that Zn-solubilizing bacteria such as *Pseudomonas aeruginosa*, *Ralstonia picketti*, *Burkholderia cepacia*, and *Klebsiella pneumoniae* improve the growth of rice seedlings and these bacteria could be used as Zn mobilizers for profitable farming.

## 7.9 Microbes Play a Role in Biofertilization

In the past several decades, chemical fertilizers have been applied as a source of major plant nutrients such as nitrogen, phosphorus, and potassium. These fertilizers improved the crop production but enhanced the farming cost and also polluted the natural resources that increased the environmental risks (Gong et al. 2011; Machado et al. 2017; Mullin et al. 2010; Vitousek et al. 2009). The plants themselves cannot utilize the insoluble forms of P and $N_2$. Therefore, plant growth-promoting microbes that can fix $N_2$ and solubilize the P are used as biofertilizers (Zahir et al. 2004; Schütz et al. 2018; Zaidi and Khan 2006; Alori et al. 2017). These microbe-based biofertilizers are environment-friendly and less expensive as compared to the chemical fertilizers. These biofertilizers improve the nutrient accumulation in the plant root by a different mechanism, such as mobilization, solubilization, and absorption, and enhance P uptake and $N_2$-fixation. Moreover, biofertilizers also play a significant role in the uptake of micronutrients like Fe, Cu, Zn, B, Mn, Co, and Mo (Bargaz et al. 2018). Several reports also concluded that mixed inoculation of $N_2$-fixing and phosphate-solubilizing bacteria had provided more balanced nutrition to different agriculture crops such as sorghum, barley, black gram, soybean, and wheat (Abd-Alla et al. 2001; Alagawadi and Gaur 1992; Galal 2003; Tanwar et al. 2003)

*Rhizobium* is one of the prominent diazotrophs that is mostly used as a biofertilizer alone in India, but the use of *Rhizobium* in combination with phosphate-solubilizing bacteria (PSB) needs to be explored more extensively. Nevertheless, application of combined microbe inoculum to enhance the soil fertility is getting the attention of researchers (Basak and Biswas 2010; Dash et al. 2018; He et al. 2019; Kant et al. 2016; Sharma et al. 2019). Recent findings specified that combined

inoculation of plant growth-promoting rhizobacteria (PGPR) in arable soils enhanced the crop yield significantly (Harish et al. 2009a; Harish et al. 2009b). The PGPR strains *Pseudomonas alcaligenes* PsA15, *Bacillus polymyxa* BcP26, and *Mycobacterium phlei* MbP18 had positive impact on maize plant health and the uptake of N, P, and K in nutrient-deficient Calcisol soils (Egamberdiyeva 2007; Verma et al. 2017a). These PGPRs have a strong ability to produce growth-promoting phytohormones and play a significant role in phosphate mobilization, production of siderophore and antibiotics, inhibition of plant ethylene synthesis, and induction of plant systemic resistances to pathogens that helps to enhance the crop yield (Cakmakçi et al. 2006; Han and Lee 2006; Kohler et al. 2006; Turan et al. 2005; Zahir et al. 2004; Zaidi and Khan 2006). PGP endophytes also play a significant role in plant growth regulation (Suman et al. 2016b). *Rhizobium*, *Azotobacter*, *Azospirillum*, *Cyanobacteria*, *Azolla*, Phosphate, and potassium-solubilizing microorganisms are the most applied microorganisms that are considered as beneficial for sustainable agriculture and used as biofertilizers. Silicate-solubilizing bacteria also help to promote plant growth and are available as liquid biofertilizers (Parewa et al. 2014; Suman et al. 2016a; Verma et al. 2014a; Zhang and Kong 2014). Microbes and their beneficial association with crop plants are listed in Table 7.3.

## 7.10 Economic Development in Agriculture

Microbe-based biofertilizer development is accepted as a relevant approach to increase food production (Schütz et al. 2018). Global population pressure as well as climate change have become serious constraints for the economic growth of several countries and food security (Cole et al. 2018). Fundamentally, to improve the soil and plant health, two major strategies need to be followed: (1) extensive application of microbe-based biofertilizer, and (2) the manipulation of naturally existing microbial populations (Patil and Solanki 2016; Schütz et al. 2018). Mostly, PGPRs that are grown as either saprophytic or endophytic symbionts are protagonists of applied microbial biotechnology in agriculture (Backer et al. 2018). Especially, microbial formulation, quality control, and modes of application of microbial inoculants are getting the attention of researchers (Singh et al. 2014; Velivelli et al. 2014). Several mechanisms underlying the plant–microbe interactions in the rhizosphere and plant still need to be explored in depth. Researchers are facing difficulties, mostly regarding how to utilize the large microbial structure, especially unculturable microbes, to boost the soil and plant health. Secondary, a plethora of culture-independent molecular techniques is becoming accessible and is presently being applied either to interpret the hidden networks of microorganisms inhabiting soil and rhizosphere microenvironments or to outline the molecular bases of the plant–microbiome interactions (Patil and Solanki 2016). Multitrophic factors, including nutrient deficits, salt, low water, air contamination, diseases, and pests, cause negative impacts on the functionality/productivity of agricultural products and plant systems (Fig. 7.2).

Agriculture plays an indispensable role in the Indian economy, and biofertilizers are essential to improve crop yield in an eco-friendly manner (Kesavan and

**Table 7.3** Microbial linkage promoting various types of growth in plants to improve crop yield

| S. No. | Types of growth promotion in plants | Microbial inoculants | References |
|---|---|---|---|
| 1. | Growth enhancement of canola and lettuce | *Rhizobium leguminosarum* | García-Fraile et al. (2012); Noel et al. (1996); Singh (2013) |
| 2. | Early developments of canola seedlings | *Pseudomonas putida* G 12-2 | Glick et al. (1997); Hwang et al. (2011); Zhang et al. (2015) |
| 3. | Growth enhancement of wheat and maize plants | *Azospirillum brasilense* and *Azospirillum irakense* strains | Couillerot et al. (2013); Dobbelaere et al. (2002); Fukami et al. (2016) |
| 4. | Growth enhancement of pearl millet | *Pseudomonas fluorescens* strain | Fukami et al. (2016); Karnwal (2012); Raj et al. (2003) |
| 5. | Growth stimulation of tomato plant | *Pseudomonas putida* strain | Gravel et al. (2007); Mariutto et al. (2011); Pastor et al. (2014) |
| 6. | Growth and productivity enhancement of canola | *Azotobacter* and *Azospirillum* strains | Naderifar and Daneshian (2012); Naseri et al. (2013); Yasari and Patwardhan (2007) |
| 7. | Enhanced uptake of N, P, and K by maize crop in nutrient-deficient Calcisol soil | *Pseudomonas alcaligenes* PsA15; *Bacillus polymyxa* BcP26; *Mycobacterium phlei* MbP18 | Egamberdiyeva (2007); Saharan and Nehra (2011); Shrivastava and Kumar (2015) |
| 8. | Growth and yield enhancement of chick pea (*Cicer arietinum*) | *Pseudomonas*, *Azotobacter*, and *Azospirillum* strains | Joseph et al. (2012); Rokhzadi et al. (2008); Rokhzadi and Toashih (2011) |
| 9. | Improvement in the yield and phosphorus uptake in wheat | *R. leguminosarum* (Thal-8/SK8); *Pseudomonas sp.* strain 54RB | Afzal and Bano (2008); Mehboob et al. (2012); Shaikh et al. (2016) |
| 10. | Improvement in seed germination, seedling growth, and yield of maize | *P. putida* strains R-168 and DSM-291; *P. fluorescens* strains R-98 and DSM-50090; *A. brasilense* DSM-1691; *Azospirillum lipoferum* DSM-1690 | Gholami et al. (2009); Karunakaran et al. (2013); Shen et al. (2010) |
| 11. | Improvement in seed germination, growth parameters of maize seedling in greenhouse, and also grain yield of field-grown maize | *P. putida* strain R-168 | Gholami et al. (2009); Noumavo et al. (2013); Singh et al. (2011) |

(continued)

**Table 7.3** (continued)

| S. No. | Types of growth promotion in plants | Microbial inoculants | References |
|---|---|---|---|
| 12. | Increase in growth, leaf nutrient contents, and yield of banana cv. Virupakshi (Musa spp. AAB) plants | *P. fluorescens* strain R-93; *P. fluorescens* DSM 50090; *P. putida* DSM291; *A. lipoferum* DSM 1691; *A. brasilense* SM 1690; *P. fluorescens* strains CHA0 and Pf1 | Kavino et al. (2010); Patil (2013); Selvarajan and Balasubramanian (2014) |
| 13. | Improvement in seed germination, growth parameters of wheat seedling in pots, and also grain yield of field-grown wheat | *Bacillus amyloliquefaciens* IARI-HHS2-30 | Verma (2013, 2015, 2016); Verma et al. (2014a, b, 2016) |

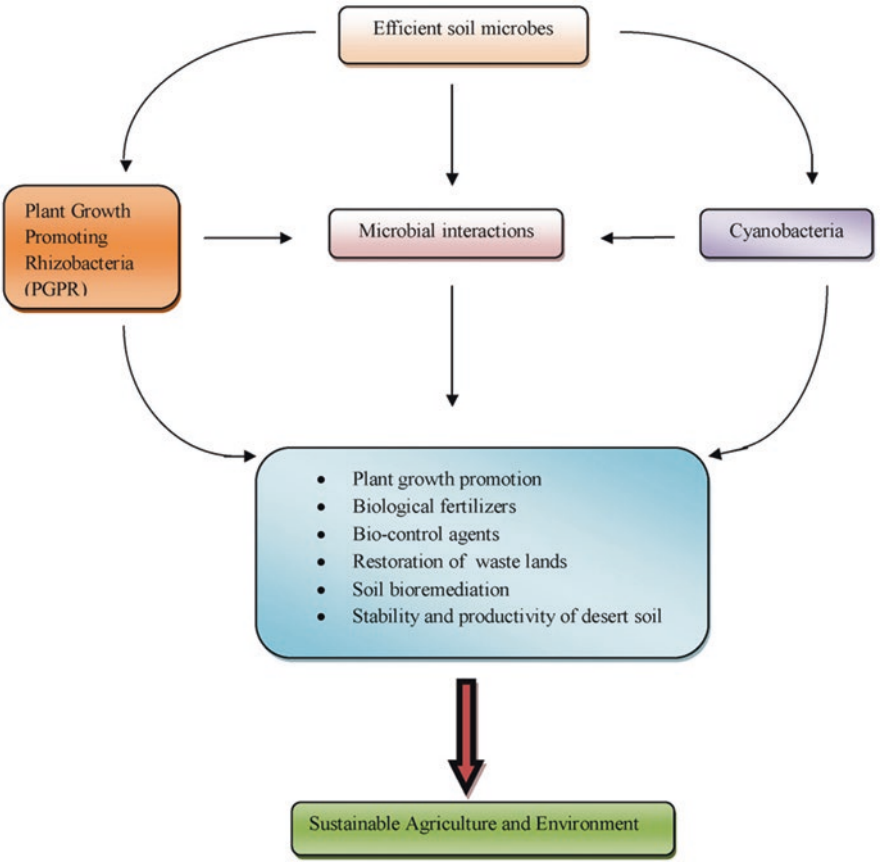


**Fig. 7.2** Microbes and their role in the intensification of sustainable agriculture

Swaminathan 2008). Out of India's total geographical area (329 million hectares), about 114 million hectares are under farming (Raghuwanshi 2012). To gain better yield, farmers vaccinate the soil with fertilizers. These fertilizers belong to two classes. They are either inorganic chemical or organic biofertilizers. Increasingly high inputs of chemical fertilizers during the last 150 years have not only negatively influenced the fertility of the soil but also polluted the water sources. Soil fertility degraded significantly, and polluted resources have also posed severe health and environmental hazards (Schütz et al. 2018).

In contrast, microbe-based biofertilizers not only give a better yield, but are also harmless to the ecosystem (Patil and Solanki 2016). Biofertilizer-based organic farming methods would help solve these concerns and boost the ecosystem (Shukla et al. 2016). In the present time, organic biofertilizer market has reached around US$ 30 billion globally and with an excellent growth rate (8%). Approximately, 22 million hectares of agricultural land is now cultivated organically by the use of microbe-based fertilizers. However, organic farming represents less than 1% of the world's conventional agricultural production and about 9% of the total agricultural area (Verma et al. 2014b). To have an environmentally safe technology, microbial product-based agriculture system needs to be used efficiently.

## 7.11 Microbial Engineering Gets Better Agriculture and Human Health

A microbial structure is composed of and influenced by host location and environmental factors that generate undesirable phenotypes in the hosts. The disturbance of microbiome negatively affects the associated ecosystems that cause diseases and disorders in the host. Microbiome engineering can be used to modify or restore the microbial community to improve the plant and human health. Foo et al. (2017) discussed the vitality of microbiome engineering toward agriculture prospects and human health. Plant microbiome can be planned with known microbial inoculants with desired functions to get significant production in an eco-friendly manner. Santhanam et al. (2015) reported an example of artificially developed microbiome by using five root-associated bacteria that significantly reduced the sudden-wilt disease in *Nicotiana attenuata*.

Additionally, Glick (2012) assessed co-inoculation of rhizobacteria had shown higher growth and yield in various crops as compared to the single microbes. Soil microbiome engineering is usually flourished by hiring different agricultural practices such as intercropping, crop rotation, and tillage (Fageria 2012; Solanki et al. 2017, 2019). Application of green organic manure, farmyard manure, is also one of the important agriculture practices that enhance the soil microbiomes significantly (Chaparro et al. 2012; Abebe and Deressa 2017). All these agricultural practices targeted to modify the soil microbial diversity, mineral cycles, and biological activity, and these significantly helps to reduce the off-farm inputs, for example, chemical fertilizers and herbicides.

In the present scenario, microbial engineering is most extensively utilized to modify the human microbiome to improve immunity and disease resistance. Advanced biotechnological tools help to manipulate human microbiome to treat diseases such as diabetes and cancer; modified microbiota influence the host metabolism that helps to regulate diseases (Grice and Segre 2012). Meat industry badly suffered from antibiotic-resistant bacteria, and it has become the most common problem for livestock farming. Thus, microbiome alteration of livestock by using feed enzymes, prebiotics, and probiotics is a suitable alternative as compared to the overdose of antibiotics that also negatively affect human health. To promote gut health in swine and poultry, feed enzymes like phytase, amylase, non-starch polysaccharide (NSP)-degrading enzymes (e.g., xylanase, $\beta$-glucanase, and $\beta$-mannanase), proteases, and lysozyme are used that enhance the substrate digestion, improve the production of prebiotics from dietary NSPs, and reduce the antinutritive factors (Archna et al. 2015; Kiarie et al. 2013; Verma et al. 2015b).

## 7.12 Conclusion and Future Prospects

During the past several decades, microbial research has made significant progress in sustainable agriculture. Subsequently, advanced microbiological tools offer to use microbial engineering to alter microbiota of human and plant to improve health and increase production. However, plant microbiome and different environmental stress need to be explored to reveal a unique signaling network among microbes that help the plant to survive. Characterization of these signaling molecules can help to design advanced biotechnological strategies that unlock the plant adaptation mechanisms. Similarly, soil health can be flourished by using the crosstalk of soil microbes. Moreover, different microbial tools and agriculture technologies are being established, providing deeper insights into the plant/soil microbiomes, and these tools widely help to accelerate the microbial engineering efforts. With nonstop hard work in understanding and manipulating microbes, the microbial link will be a mainstream approach for refining human health and agriculture output.

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# Wheat Microbiome: Present Status and Future Perspective

8

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**Abstract**

Wheat microbiome harbors a diverse array of microbial communities and play a vital role in maintaining wheat physiology as well assist in offering protection from biotic and abiotic stresses. Several research findings indicated that wheat microbiome encompasses predominantly fungi, bacteria, viruses, actinomycetes, cyanobacteria, protozoa, archaea, etc. which performed myriads of advantageous activities including bio-management of crop pathogens, abiotic stress amelioration, as well as plant growth promotion under adverse conditions. In this chapter, attempts have been made to provide comprehensive and up-to-date insights on wheat microbiome research with major emphasis on emerging microbiome-based sustainable solutions for profitable and quality wheat production under every changing climate.

**Keywords**

Abiotic stress · Bio-antagonist · Microbiome · PGPR · Wheat

## 8.1 Introduction

Wheat (*Triticum aestivum*) is the largest cultivated crop in the world occupying an area of 222.35 million hectares with an estimated production of 753.89 million tons in 2016–2017 (Jasrotia et al. 2018). It is a staple food in more than 40 countries and

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finds a significant share of about 35% in the consumption basket of millions across the world. It is estimated that global population depends on wheat for 85% and 82%, respectively, for calories and protein (Chaves et al. 2013). Moreover, wheat meets up 21% of the world's food demand and is grown on 200 M ha of farmland globally (Tsvetanov et al. 2016). By 2050, the world will have the challenge to meet the food demand of an estimated population of 9.6 billions. Feeding for growing population at the era of climate change requires the use of optimized, reliable and existing resources which have less impact on the environment (Busby et al. 2017). Now research topics mainly highlighted on the issue of not only quantity but also quality of food product with minimal uses of existing earth resources. Under developing countries like India, which has self-sufficiency in wheat production, there is a need to give emphasis on quality wheat production by understanding plant microbiome and its interactions with environment and stress regimes.

Moreover, the future global climate scenarios predicted an increase in the occurrence of exceptionally hot days, together with an increase in average global temperatures and its implications on global food production. Asseng et al. (2015) reported that the global wheat production is influenced by the increase of temperature and becomes more variable over space and time. In Indian perspective, temperature changes in the last 30 years had a bigger impact on national wheat production, where over 90% of wheat is irrigated (Singh and Mustard 2012), than changes in precipitation (Lobell et al. 2011). One of the most important cropping patterns in South Asia is rice-wheat cropping system, facing immense pressure because of heat stress and degraded soil health due to high cropping intensity and tillage for growing rice and over-exploitation of the natural resources (Joshi et al. 2007). The most affected locations of South Asia are eastern Gangetic plains, central and peninsular India and Bangladesh. Besides, a substantial proportion of cultivated land under wheat in South Asia is salt affected. The salt-affected land under wheat in India is 4.5 m ha, whereas 6.0 m ha in Pakistan amongst the other South Asian countries (Singh and Chatrath 2001). Although soil reclamation and provision of proper drainage may be more effective solution, it does not seem possible in the near future due to huge acreage affected by salt. Another constraint is deficiency of macro-nutrients like zinc, sulphur, iron, manganese and boron which are being observed in some pockets of northern India, Bangladesh and Nepal due to imbalanced fertilization, overmining of essential plant nutrients and burning of crop residues (Chatrath 2004). Water is also becoming scarce as the water table is going down due to overmining of groundwater in intensive rice-wheat cultivation and comparatively less water recharge from monsoon rains.

Amongst the biotic stresses, rusts continue to be the major threat (Khan et al. 2017; Savadi et al. 2018; Singh et al. 2006; Kumar et al. 2019; Kashyap et al. 2019). Out of three rusts prevalent in Indian subcontinent, leaf rust is the major disease which affects almost whole of India, parts of Bangladesh and Nepal. Spot blotch caused by *Bipolaris sorokiniana* (Sacc.) Shoem is also considered an important disease in the eastern part of South Asia (Joshi et al. 2007; Devi et al. 2018). In addition, other diseases, viz. Karnal bunt, powdery mildew and wheat blast also affect wheat crop to some extent (Singh 2017; Kashyap et al. 2011, 2018, 2019;

Nallathambi et al. 2019; Kumar et al. 2020). The use of microbiomes increases crop productivity, reduces the cost of cultivation and sustains the health of soil as well as environment. Use of microbiome in wheat cultivation has the ability to change the entire scenario of the current agricultural and food security.

The plant microbiome is a key determinant of plant health and productivity and has received substantial attention in recent years. There is an expanded version of the tree of life dominated by bacterial diversification due to their ability to perform lateral gene transfer across disparate phylogenetic groups (Mcdonald and Currie 2017; Hug et al. 2016). Recent advance researches in high-throughput sequencing technique and the increasing number of microbial culture libraries, they can quickly proliferate and have high mutation rates (Kibota and Lynch 1996; Boe et al. 2000; Denamur and Matic 2006). Individual microbes of the same species could potentially bear different genetic endowments and thus functional characteristics (Sergaki et al. 2018). Amplicon sequencing has been invaluable in determining general patterns of microbial diversity within the plant microbiome (Bulgarelli et al. 2012; Lundberg et al. 2012; Peiffer et al. 2013; Tkacz et al. 2015).

However, use of microbiome on wheat is very limited. Most of the previous studies mostly based on identifying microbes in the root's rhizosphere (Hartmann et al. 2014; Mahoney et al. 2017; Ofek et al. 2013; Yin et al. 2017) and limited research work is focused on aboveground organs (Granzow et al. 2017; Huang et al. 2016; Karlsson et al. 2017). To our knowledge, till date no detailed published works are available on entire wheat microbiome, including both above- and belowground plant organs, mode of actions, how they work under stress conditions and what are the easy and fast way of identification with high-throughput sequencing techniques. Here we classify the microbiome in a detailed way and also use fullness of those microbiomes especially for wheat crop. But still microbial communities were dependent on type of plant organ, growth stages of the plant, environmental conditions, acceptability amongst the farmers and community composition, etc. and therefore need to be checked before their successful intervention and execution.

## 8.2 Plant Microbiome: Concept and Definitions

Microbes are associated with majority of living species and are omnipresent in nature. However, majority of them are noticed in harsh environments. The microbial diversity consists of various types of microbes such as archaea, bacteria, cyanobacteria, fungi and protozoa (Vessey 2003; Verma and Suman 2018). The plant microbiomes include rhizospheric, endophytic and epiphytic microbes which help in plant growth and in adaptation even in extreme environments. In our planet, researchers have recorded the presence of microbiomes amongst 1.7 million living species and noticed that 1–10% bacterial species are present amongst 5000 species of prokaryotes (Federhen 2014; Moore 2014). The microbiomes are useful in industry and

medical processes too in addition to agriculture, and they act as abundant reservoirs of bioresources. Thus in keeping good soil health, enhancing productivity and improving the growth of plants, these plant microbiomes play vital role and are found helpful in providing a sustainable biosphere.

The following definitions are found for plant microbiome in literature.

1. Bulgarelli and Schlaeppi (2015) have defined microbiota or microbiome as a set of genomes of the microorganisms in some habitat.
2. Several researchers have defined that microbial communities are the ones associated with any plant that can live, thrive and interact with various tissues such as roots, shoots, leaves, flowers and seeds (Turner et al. 2013; Haney and Ausubel 2015; Mueller and Sachs 2015; Haney et al. 2015; Nelson 2018). It is also found in literature that microbiome includes all the microbes of a community.

## 8.3 Wheat Microbiomes: Concept and Types

The wheat microbiome is mainly composed of various kinds of organisms such as archaea, protozoa, bacteria, fungi and virus (Mueller and Sachs 2015). Research done on the microorganisms so far found that they are present in healthy crops, and their findings are limited to species of wild *Triticum* and of wheat (Marshall et al. 1999).

It is a well known fact that nematodes, ants, moles and several fungi and bacteria take common shelter in soil. They play an essential role in protecting the plant from potential plant pathogens and also in improving plant growth, health, and production (Berg et al. 2014; Haney et al. 2015). There is a closer association between plants and microbial communities and normally found at phyllosphere (above the ground), in rhizosphere (below the ground especially on roots and surrounding area of roots) and in endophytes (inside the root intercellular spaces) (Hirsch and Mauchline 2014). Relationship of plants with microbes is beneficial in improving disease resistance, in increasing stress tolerance levels and also in nutrient uptake. It is not always beneficial; rather, sometimes interactions of plants with microbes may be harmful too. This is dependent on kinds of bacteria involved, their characteristics and finally the ways of interactions.

Mainly, the interactions between plants and microbes are classified as rhizospheric, endophytic and epiphytic (as shown in Fig. 8.1). Wheat plants interact mostly with either common microbes or niche-specific microbes (Fig. 8.2). Common microbes are *Arthrobacter nicotianae*, *Bacillus amyloliquefaciens*, *B. sphaericus*, *B. subtilis*, *Paenibacillus amylolyticus*, *P. polymyxa*, *Micrococcus luteus*, *Pseudomonas aeruginosa* and *P. azotoformans*, and most predominant species were reported from various parts of the plant such as phyllosphere, rhizosphere and internal tissues (Bhattacharyya and Jha 2012; Verma and Suman 2018). Further, the wheat bacterial microbiomes belong to different taxonomic positions of phyla, namely, *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Proteobacteria* and *Gemmatimonadetes* (Verma and Suman 2018). Some novel microbiomes like acidophiles, alkaliphiles,

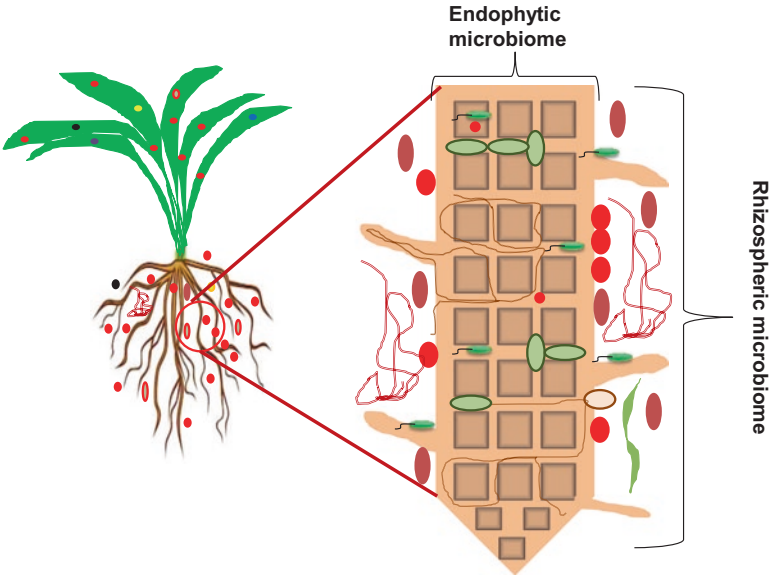


**Fig. 8.1** Assemblage of microbes in and around wheat plant

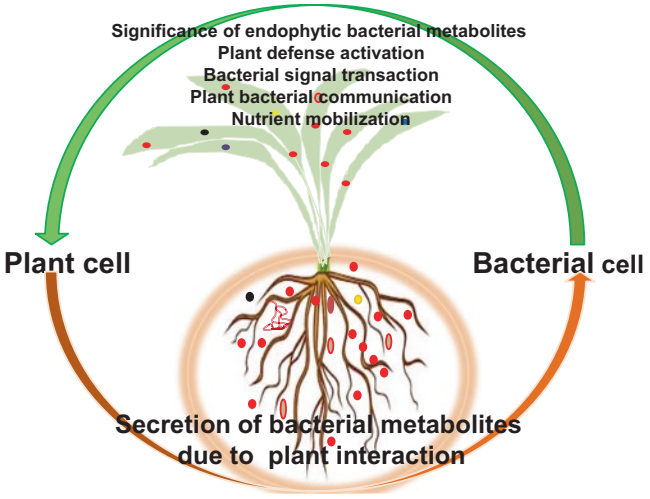


**Fig. 8.2** Relative distribution amongst different microbes isolated from phyllospheric, endophytic and rhizospheric sample of wheat

halophiles, psychrophiles, thermophiles and xerophiles are also found in wheat (Narula et al. 2006). According to Verma and Suman (2018), bacterial microbiomes, viz. *Actinobacteria* (12%), *Bacteroidetes* (7%), *Firmicutes* (32) and *Proteobacteria* (49%), which occupy a major portion, and three classes of bacteria, namely,

*Alphaproteobacteria* (6%), *Betaproteobacteria* (8%) and *Gammaproteobacteria* (35%) are present in wheat.

### 8.3.1 Endophytes

Wilson (1995) and Brader et al. (2014) observed that the bacteria or fungi during their life cycle attack the plant tissues. This invasion causes infections in plant tissues which are not apparent to the naked eye. However, these infections do not cause any symptoms of disease. Endophytic microbiomes get into plants through wounds and root hairs to benefit plants by colonizing them and preventing effects of pathogenic organisms. They may systematically colonize the plants (Compant et al. 2010; Kushwaha et al. 2020a). Requisite chemicals are produced by them to prevent the growth of plant pathogenic competitor organisms. Bacterial endophytes help plants in their growth (Kushwaha et al. 2019, 2020b). Carroll (1988) has done research on endophyte associations and shown that they enhance the survival chances against fungal pathogens.

The specific endophytic species are *Achromobacter piechaudii*, *A. xylosoxidans*, *Delftia lacustris*, *D. acidovorans*, *Acinetobacter lwoffii*, *Ochrobactrum intermedium*, *Pantoea dispersa*, *P. eucalypti*, *Staphylococcus epidermis*, *Pseudomonas monteilii* and *Variovorax soli* and *Serratia* (Liu et al. 2010; Hallmann et al. 1997). Carroll (1988) has observed that host benefits is a common phenomenon related with fungal endophytes.

Endophytic fungi are used as genetic vectors and as abundant source of secondary metabolites (Fisher et al. 1986; Stierle et al. 1993; Strobel and Daisy 2003). Further they act as biological control agents (Clay 1989; Bacon 1990; Schardl et al. 1991; Dorworth and Callan 1996). Hence, biotechnological interest is shown very much on endophytic fungi (Murray et al. 1992). It is also studied that fungal endophytes cause water loss in leaves. Some kinds of fungal endophytes help plants to survive in drought conditions and extreme temperature climates (El-Daim et al. 2014; Naveed et al. 2014; Khalafallah and Abo-Ghalia 2008). Thus, focused research has been done on it in general and on grass endophytes in particular (Farooq et al. 2009).

Direct antagonism of microbial pathogens is one way that endophytic bacterial strains fuel plant growth. As biocontrol agents, endophytes induce resistance in plants to disease-causing organisms and allow plant to be healthy (Pleban et al. 1995). Figure 8.3 described the isolation of host-associated endophyte and their vital function.

The anti-microbial property of endophytic microbes generates secondary metabolites, and thus they act against pathogenic microbes (Tan and Zou 2001). There are many bioactive compounds different from endophytes, and such compounds belong to classes like alkaloids, flavones, peptides, phenols, quinines, steroids and terpenoids (Yu et al. 2010).

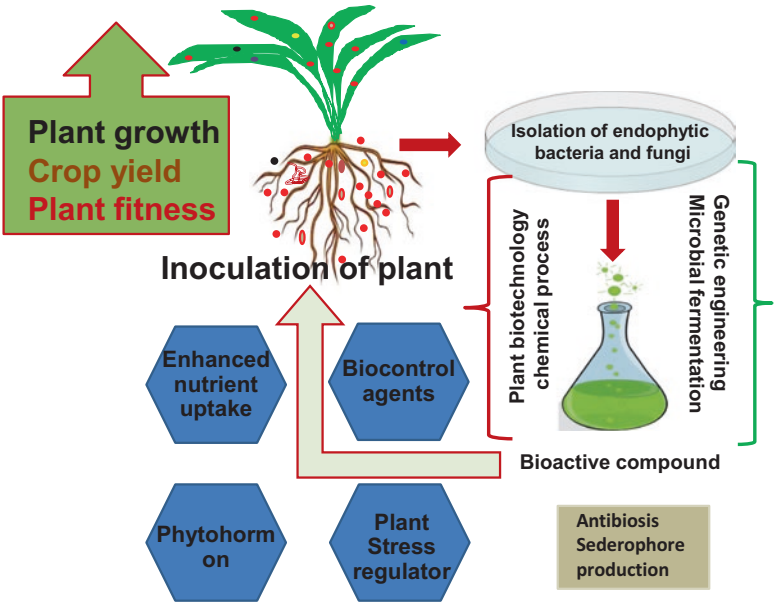


**Fig. 8.3** Schematic representation of isolation, purification and function of endophytes in host plant

### 8.3.2 Epiphytic Microbiomes

Synergy between plant and bacteria is due to the presence of epiphytic microbes on phyllosphere. Microorganisms found on leaf surfaces are identified as extremophiles due to their capacity to survive even at high temperature (40–55 °C). Further they tolerate ultraviolet radiation during the day and even cool temperature (5–10 °C) during the night (Kushwaha et al. 2020b). The association between the microorganisms in the phyllosphere influences the plant growth in natural habitat and the productivity of agricultural and horticultural crops for human consumption.

The aerial parts of plants are normally exposed to air and dust. This helps typical microbiomes to get attached to the surface. Hence, survival and proliferation of the phyllospheric microbiomes on leaves depend on the leaf diffuses or exudates. Amino acids, glucose, fructose and sucrose are some nutrient factors found in the leaf exudates. Hence, the plant growth is accomplished by the essential process like nitrogen fixation (Iniguez et al. 2004; Venieraki et al. 2011).

In the phyllosphere of wheat, researchers have observed the presence of microbes, viz., *Achromobacter*, *Corynebacterium*, *Agrobacterium*, *Haemophilus*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Azotobacter*, *Enterobacter*, *Lysinibacillus*, *Micrococcus*, *Brevundimonas*, *Paenibacillus*, *Methylobacterium*, *Micromonospora*, *Pseudomonas*, *Micrococcus*, *Streptomyces*, *Stenotrophomonas*, *Pantoea* and *Psychrobacter* (Sharma et al. 2011a, b; Verma et al. 2016).

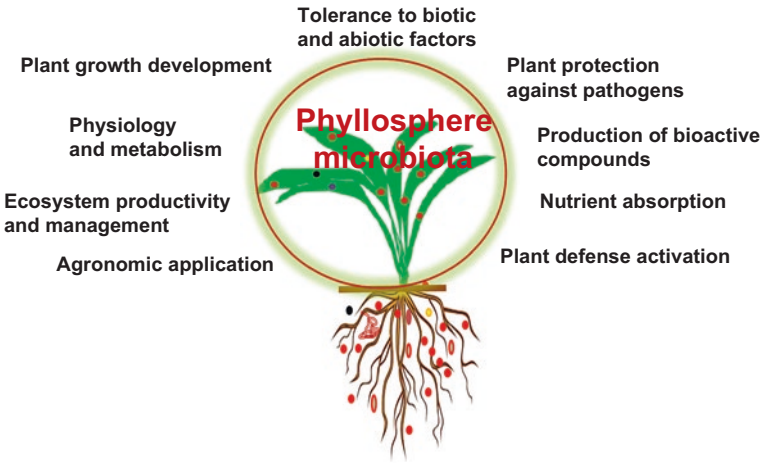


**Fig. 8.4** Function of phyllosphere microbiota

Production of bioactive compounds, tolerance to biotic and abiotic stress, immune response, agronomic applications, nutrient acquisition, ecosystem productivity and management, physiology and metabolism, plant protection against pathogen are some of the benefits happen due to phyllosphere microbiota and responsible for improved plant growth (Roat and Saraf 2017) (Fig. 8.4).

### 8.3.3 Rhizospheric Microbiome

The microbiomes in rhizosphere predominantly gained importance due to attraction of different types of root exudate substrates, and this zone is considered a hotspot for microbial survival and its diversity compared to other plant parts (Arkhipova et al. 2005; Kuzmina and Melentev 2003). The rhizospheric microbiomes also play a vital role in growth of the plant. They are closely attached between roots and soils and help the plants in nutrient uptake. However, rhizospheric microbiome is greatly affected by several factors like soil types, pH values, soil moisture and presence of micronutrients in soil etc. (Ahemad and Kibret 2014; Khalid et al. 2004).

Rhizospheric microbes live in soil near roots, and due to gradient of root closeness they utilize metabolites from the surrounding roots as carbon and nitrogen sources and they inhabit spaces between cortical cells, they colonize rhizoplane and also they live in the specialized root structures like root nodules. Specific species of the rhizospheric are *Pseudomonas extremorientalis*, *P. rhizosphaerae*, *Arthrobacter nicotinovorans*, *Azotobacter tropicalis*, *Bacillus atrophaeus*, *B. horikoshii*, *B. mojavensis*, *B. siamensis*, *B. thuringiensis*, *Enterobacter asburiae*, *Exiguobacterium acetylicum*, *Serratia marcescens*, *Planomicrobium okeanokoites*, *Rhodobacter capsulatus* and *Rhodobacter sphaeroides* (Hassan and Bano 2015; Zahir et al. 2003).

Different genera related to the wheat rhizospheric microbiomes are *Arthrobacter*, *Alcaligenes*, *Acinetobacter*, *Azospirillum*, *Methylobacterium*, *Bacillus*, *Burkholderia*, *Erwinia*, *Flavobacterium*, *Enterobacter*, *Lysinibacillus*, *Paenibacillus*, *Rhizobium*, *Serratia* and *Pseudomonas* (Khalid et al. 2004; Verma and Suman 2018).

## 8.4 Wheat Microbiome Detection and Diagnosis

Both culturable and un-culturable techniques have been employed to wheat-associated microbes to study diversity of their association with wheat crops and also to know their distribution (Fig. 8.5). Particularly, surface sterilization and serial dilution techniques are used to isolate wheat endophytic and rhizospheric microbes (Forchetti et al. 2007; Suman et al. 2016a, b). The epiphytic microbes are isolated with the imprinting method and, alternatively, serial dilution, and then pour or spread plate methods are used in sequence to isolate the same (Akbari et al. 2007; Yadav et al. 2015a, b; Suman et al. 2016a, b). For isolation and enumeration of wheat microbiomes (eubacteria, archaea, fungi), the following specific methods are employed along with specific medium.

The specific medium for isolation of heterotrophic microbes is nutrient agar; for *Pseudomonads* isolation, King's B agar medium is used. *Arthrobacter* is isolated by growing it in trypticase soy agar media; soil extract agar is used for the isolation of

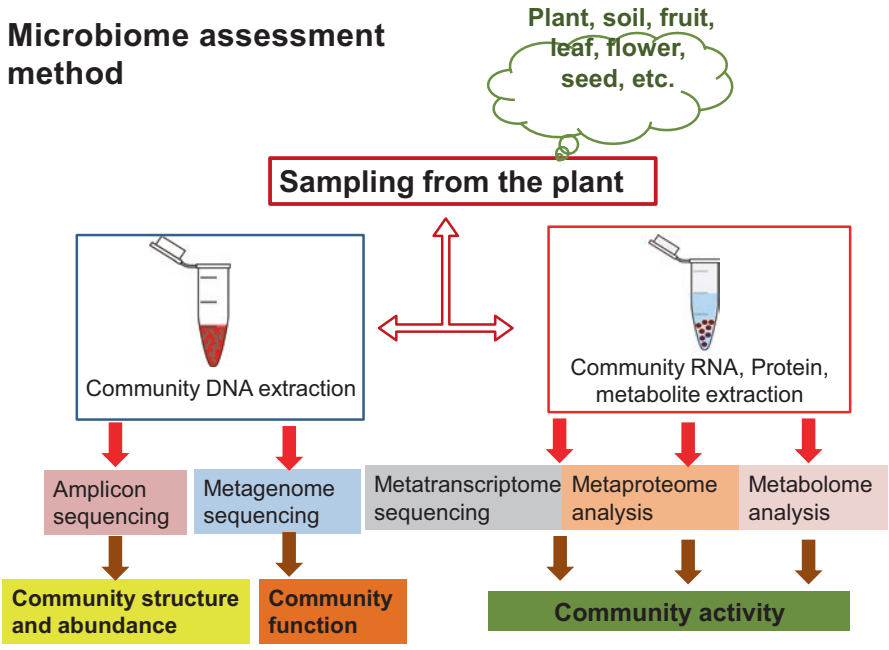


**Fig. 8.5** The flowchart of the assessment of microbiomes

soil-specific microbes. *Bacillus and Bacillus*-derived genera can be isolated by T3A with heat treatment methods. Any chemically defined and complex medium is used for the isolation of archaea. Rose Bengal media and potato dextrose agar is used for isolation of fungi (Forchetti et al. 2007; Verma and Suman 2018).

Microbiome samples can be assessed either through community DNA extraction or community RNA extraction. Community DNA extraction is sequenced through PCR amplification (16S–18S rRNA, ITS, cpn60) or amplicon sequencing and metagenome sequencing through which species, number, abundance and its composition are analysed; and the function of the community is assessed, respectively. Community RNA, protein and metabolite extraction can be done through metatranscriptome sequencing, meta-proteome analysis and metabolome analysis from which the activity of the community is known (Liu et al. 2014).

## 8.5 Role of Wheat Microbiomes

The wheat microbiomes stimulate plant growth, soil health and fertility (Canbolat et al. 2006; Singh et al. 2007; Khan et al. 2007). Further, diverse abiotic stresses are ameliorated directly through the following: $N_2$ fixation, production of siderophore and phytohormones (auxin, cytokinin and gibberellins) and solubilization of potassium, phosphorus and zinc (Sachdev et al. 2009; Kumar et al. 2001; Zaidi and Khan. 2005; Kudoyarova et al. 2014; Rroco et al. 2003). The same stresses are ameliorated indirectly through the following: production of ammonia, hydrogen cyanide, iron-chelating compounds, hydrolytic enzymes, antibiotics and antagonistic molecules for suppression of soilborne pathogens (Jankiewicz 2006; Scavino and Pedraza 2013; Upadhyay et al. 2012).

Plant growth-promoting microbes produce phytohormones or plant growth regulators (Glick 2015) for better growth of the plants. Phytohormones are metabolites derived from bacteria such as *Azotobacter*, *Bacillus*, *Azospirillum* and *Pseudomonas* which show benefits such as promotion and spread of root development; with these benefits, roots of the plants increase their ability to uptake water and nutrients from soil in an efficient manner (Mehnaz 2015).

Nitrogen-fixing microbe *Azospirillum brasilense* produces small amount of phytohormones like gibberellins, indole-3-acetic acid (IAA) and substances like cytokinin. PGP microbial strains like *Azospirillum*, *Pseudomonas* and *Bacillus* produce phytohormone like auxin which modulates shoot elongation and similar plant physiological processes and further promotes root development in plants (Verma et al. 2016). IAA and related compounds are also present, and these are demonstrated in many diazotrophs such as *Acetobacter diazotrophicus*, *Azospirillum*, *Azotobacter*, *Paenibacillus* and *Polymyxa* sp. (Timmusk et al. 2014; Aarab et al. 2015).

Plants produce soluble organic compounds such as chelators and phytosiderophores which help to bind iron ($Fe^{3+}$) and are made available in solution (Sarode et al. 2009; Rroco et al. 2003). Further, chelators' produced ferrous ion ($Fe^{3+}$) is reduced to ferric ion ($Fe^{2+}$) and absorbed immediately on the root surface. Phytosiderophores are low molecular weight and are ferric ion-specific ligands.

Thus, by production and discharge of phytosiderophore, PGPR are able to prevent the proliferation of phytopathogens which in turn facilitates plant growth (Mehnaz 2015; Sarode et al. 2013; Solanki et al. 2014; Jog et al. 2014).

## 8.6 Plant Growth-Promoting Bacteria (PGPB)

Plant growth-promoting substances such as rhizospheric or endophytic bacteria show beneficial effects in plant growth (Santoyo et al. 2016; Kushwaha et al. 2020b). Plant growth-promoting substances extend over plant roots and the soil in vicinity and show direct or indirect effect on plants (Glick 2012; Santoyo et al. 2012, Gupta et al. 2015).

The elements such as nitrogen, phosphorus and iron are available by plant growth-promoting bacteria, which are very much needed for plant growth and development (Calvo et al. 2014) (Fig. 8.6). Further, PGPB modulates level of hormones in the production of phytohormones like auxins, cytokinins and gibberellins (Yadav et al. 2017a, b). PGPB are also able to reduce the levels of the ethylene phytohormone by synthesizing an enzyme, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, that slices the ACC compound. ACC is a precursor compound of ethylene in higher plants (Glick et al. 1998; Yadav et al. 2016).

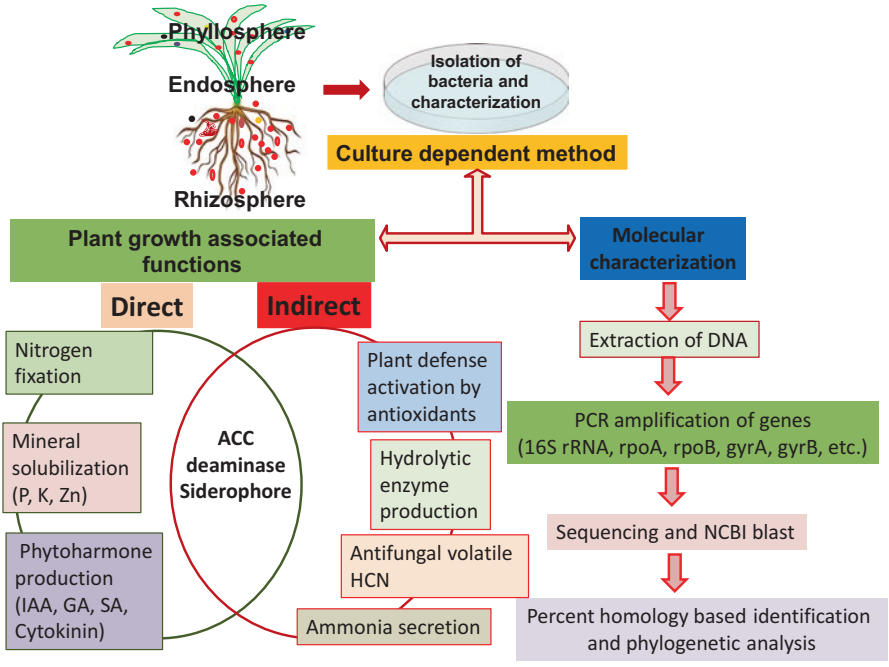


**Fig. 8.6** A schematic representation of the isolation and characterization of plant growth-promoting bacteria

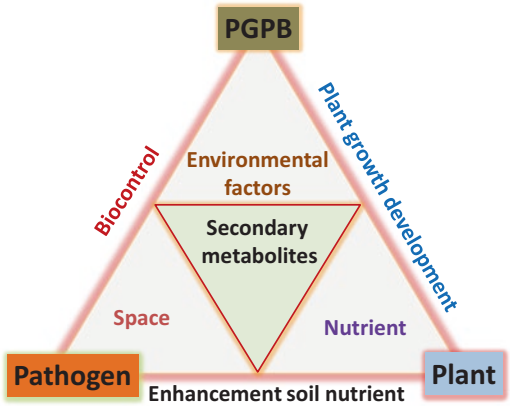


**Fig. 8.7** Plant-microbe interactions and their action of plant growth promotion

After plant pathogen infection, PGPB decreases plant damage by indirect mechanism and thus promotes plant growth (Fig. 8.7). This is being demonstrated because of the inhibition of the pathogens which is induced by PGPB (Santoyo et al. 2012, 2016). Generally, the mechanisms are synthesis and release of antibiotics (such as 2,4-diacetylphloroglucinol); bacteriocins; chitinases; lipopeptides like iturin A, bacillomycin D and mycosubtilin; proteases; siderophores; and volatile organic compounds (Santoyo et al. 2012; Glick 2012) (Table 8.1).

As already stated earlier, wheat microbiomes (phyllospheric, endophytic and rhizospheric) such as *Azotobacter*, *Achromobacter*, *Alcaligenes*, *Azospirillum*, *Enterobacter*, *Herbaspirillum*, *Methanospirillum*, *Klebsiella*, *Pantoea*, *Burkholderia*, *Bacillus*, *Paenibacillus*, *Lysinibacillus*, *Methylobacterium*, *Pseudomonas*, *Rhodosporidium*, *Serratia*, *Staphylococcus*, *Penicillium*, *Streptomyces* and *Thermomonospora* are isolated and characterized for plant growth promotion (Cakmakci et al. 2017).

## 8.7 Phosphate Solubilization and Mineralization

Phosphorus is an important plant major nutrient which follows nitrogen. Microorganisms undergo a sequence of processes that transform soil phosphorus (P), and thus they are considered as integral part of soil phosphorous cycle. But, application of huge amount of soluble inorganic phosphate into soil is fixing it as insoluble phosphate, and hence they become unavailable to the plants (Yadav et al. 2015a, b; Sharma et al. 2011a, b).

Soil microbes have the capacity to change insoluble forms to soluble forms which are desired by plants for absorption. This is done by producing organic acids such as acetic acid, lactic acid, glycolic acid, formic acid, propionic acid, succinic acid and fumaric acid. Plants absorb only inorganic P so that bacteria take action to hydrolyse the organic P compounds with the help of the phosphatase enzyme that originates from their roots (Yadav et al. 2015a, b). This enzyme also helps in

**Table 8.1** Microbes from different wheat niche involved in plant growth regulation

| Wheat microbe | Source and its application towards plant | References |
|---|---|---|
| *Bacillus, Azospirillum, Azotobacter* | Rhizosphere<br>Nutrient and water uptake from soil | Verma and Suman (2018) |
| *Azospirillum brasilense*<br>*Klebsiella pneumoniae* | Nitrogen-fixing microbe<br>Gibberellin and IAA production | El-Razek and El-Sheshtawy (2013) |
| *Pseudomonas, Azospirillum, Bacillus* | PGPB<br>Cytokinin production | Verma and Suman (2018) |
| *Paenibacillus, Polymyxa, Acetobacter* | Diazotrophs<br>IAA and related compounds | Timmusk et al. (2014) and Aarab et al. (2015) |
| *Pseudomonas* sps. | Rhizosphere<br>Phosphorous solubilization, siderophore, IAA, DAPG | Roesti et al. (2006) |
| *Providencia* sp. PW5 | Rhizosphere<br>HCN, IAA, P solubilization, Zn solubilization | Rana et al. (2012) |
| *Acinetobacter calcoaceticus* | Rhizosphere<br>P solubilization, siderophore, IAA | Prashant et al. (2009) |
| *Pseudomonas putida* | Produces several types of antibiotics, siderophores and slight quantity of hydrogen cyanide (HCN) | Flaishman et al. (1996) |
| *Azotobacter chroococcum*<br>*Pantoea agglomerans* | Gibberellic acid (GA)<br>IAA | Narula et al. (2006) |
| *Azorhizobium caulinodans* | N fixation | Sabry et al. (1997) |
| *Bacillus* sp. (AW1)<br>*Providencia* sp. (AW5)<br>*Brevundimonas diminuta* (AW7) | P solubilization, N2 fixation, ACC deaminase, siderophore, ammonia, HCN | Rana et al. (2011) |
| *Bacillus thuringiensis*<br>*Azotobacter chroococcum*<br>*Paenibacillus ehimensis*<br>*Pseudomonas pseudoalcaligenes* | Higher heavy metal resistance<br>Siderophore, indole acetic acid, HCN, P solubilization | Kumar et al. (2015) |
| *Pseudomonas* spp. | IAA, P solubilization, rhamnolipids, siderophores | Mishra et al. (2009) |
| *Bacillus* sp. | Indole-3-acetic acid<br>Antioxidant defence system<br>SOD shoots and roots<br>Shoot POD and CAT | Wang et al. (2013) |
| *Pseudomonas denitrificans*<br>*Pseudomonas rathonis*<br>*Azotobacter chroococcum*<br>*Pantoea agglomerans* | Gibberellic acid (GA), IAA, and auxin | Narula et al. (2006) and Egamberdiyeva and Höflich (2003) |

mineralization of organic phosphorous compounds. Through production of organic acids, chelation and exchange reactions process, the phosphorous-solubilizing bacteria convert insoluble inorganic P to a form available to plants (Yadav et al. 2015a, b). Mostly in soils, one can find a range of percentage of required amount of phytates which are organic P forms roughly 10–50% of phosphorous, and phytases should be mineralized to make ready supply of P to the plants (Singh et al. 2014). Reports showed that P solubilization is positive during their study on *Alcaligenes* sp., *Providencia* sp., *Bacillus* sp. and *Brevundimonas* (Verma and Suman 2018). Role of PGP microbes is very much observed in producing phosphatase, β-gluconase, dehydrogenase and antibiotics. Phosphate and other nutrient solubilization improved soil structure with stabilized soil aggregates (Verma and Suman 2018).

## 8.8 Biological Nitrogen Fixation

Through biological nitrogen fixation, 60% of available nitrogen on this earth is fixed (Verma et al. 2010). This fixation is alternative to chemical fertilizers and hence an economically beneficial and environmentally friendly alternative (Islam et al. 2002, Venieraki et al. 2011). The above-said process of nitrogen fixation is done by *nif* genes, i.e. coded form of the nitrogenase enzyme. In the case of nitrogen-poor soils, *Azospirillum* sp. is isolated, and it is diazotroph for nitrogen fixation (Hegazi et al. 1998; Verma and Suman 2018). Members of these bacterial genera have the ability in fixing atmospheric nitrogen. Symbiotic $N_2$-fixing *Rhizobium* and others like *Azospirillum* are some microbial groups which fix nitrogen by colonization of root zones. *Actinomycetes* in non-leguminous trees and free living $N_2$ fixers as blue green algae, *Bacillus*, *Acetobacter*, *Klebsiella*, *Azotobacter* and *Pseudomonas* are also helping nitrogen fixation (Prasanna et al. 2012).

## 8.9 Biological Control

To prevent the proliferation of plant pathogens, a variety of antibiotics are identified and are having compounds such as amphisin, 2,4-diacetylphloroglucinol (DAPG), phenazine, oomycin A, pyoluteorin, tensin, pyrrolnitrin, tropolone, cyclic lipopeptides produced by *Pseudomonads* and oligomycin A, kanosamine, zwittermicin A and xanthobaccin produced by *Bacillus*, *Streptomyces* and *Stenotrophomonas* sp. (Verma and Suman 2018).

Many bacterial genera such as *Agrobacterium* (Hammami et al. 2009), *Arthrobacter* (Banerjee et al. 2010), *Azotobacter* (Kannan and Sureender 2009), *Bradyrhizobium* (Akhtar et al. 2010; Mishra et al. 2009), *Bacillus*, *Enterobacter* (Wang et al. 2012; Solanki et al. 2012; Kushwaha et al. 2019; Singh et al. 2014), *Burkholderia* (Santiago et al. 2014) and *Pseudomonas* (Solanki et al. 2015; Goswami et al. 2013) have shown their potential in biocontrol under in vitro and in vivo conditions, and they are able to resist soilborne fungal pathogens (Table 8.2).

**Table 8.2** Microbes involved in biological control

| Microbiome genera | Antagonist against | References |
|---|---|---|
| *Bacillus, Enterobacter* sps. | Rusts of wheat | Wang et al. (2012) and Li et al. (2013) |
| *Verticillium lecanii, Erwinia herbicola*, *Pseudomonas aurantiaca* | | Alien (1982), Srivastava (1985), Kempf and Wolf (1989), and Wang (2011) |
| *Verticillium lecanii* + *Paecilomyces fumosoroseus, Beauveria bassiana* | | Hall (1981) |
| *Pseudomonas putida* | | Flaishman et al. (1996) and Peng et al. (2014) |
| *Trichoderma harzianum, Streptomyces viridosporus, Bacillus subtilis, Saccharomyces cerevisiae* | | Eldoksch et al. (2001), Kalappanavar et al. (2008) and El-Sharkawy et al. (2015) |
| Combined application of arbuscular mycorrhizal fungi and *Azospirillum amazonense* | | Ghoneem et al. (2015) |
| *T. harzianum* | Tan spot, spot blotch and *Helminthosporium* leaf blight, *Fusarium* head blight of wheat and septoria blotch | Perelló et al. (1997), Monte (2001), Hossain et al. (2015), and Mahmoud (2016) |
| *B. subtilis* NJ-18 | Sharp eye spot | Peng et al. (2014) |
| Combined application of yeasts like *Rhodosporidium kratochvilovae* strain UM350, *Cryptococcus laurentii* strain UM108 and *Aureobasidium pullulans* strain LS30 | Powdery mildew of wheat | Curtis et al. (2012) |
| *Bacillus subtilis* strain E1R-j | Fusarium head blight disease of wheat and powdery mildew of wheat | Gao et al. (2015) and Mahmoud (2016) |
| *Bacillus subtilis* BTS 3, *B. amyloliquefaciens* BTS 4, *Staphylococcus saprophyticus* BTS 5 and *B. amyloliquefaciens* BTLK6A | Wheat blast and black point complex of wheat | Surovy et al. (2017) and El-Gremi et al. (2017) |
| *B. subtilis* D1/2 (DAOM 231163) and *B. subtilis* RC 218, *Brevibacillus* sp. RC 263, *Bacillus amyloliquefaciens* | *Fusarium* head blight of wheat; take all disease of wheat | Chan et al. (2003), Nasraoui et al. (2007), Crane et al. (2014), Palazzini et al. (2016) and Liu et al. (2009) |
| *Lysobacter enzymogenes* strain C3 | *Fusarium* head blight of wheat | Jochum et al. (2006) |
| *Cryptococcus*, *Brevibacillus* sp. RC 263 | | Schisler et al. (2002, 2006, 2014) |
| *Clonostachys rosea* strain ACM941 (CLO-1) FHB | | Xue et al. (2014) |

(continued)

**Table 8.2** (continued)

| Microbiome genera | Antagonist against | References |
|---|---|---|
| *Pseudomonas fluorescens* (strains MKB 158 and MKB 249) and *P. frederiksbergensis* (strain 202) | Karnal bunt of wheat | Khan and Doohan (2009) and Vajpayee et al. (2015) |
| *Aureobasidium pullulans* | | Wachowska and Głowacka (2014) |
| *Trichoderma viride*, *T. harzianum* and *Gliocladium deliquescens* | | Sharma and Basandrai (2000) |
| *Trichoderma pseudokoningii*, *T. lignorum*, *T. koningii*, *G. deliquescens* and *G. virens*, *Azotobacter chroococcum* | | Amer et al. (2000) |
| *Trichoderma viride*, *Gliocladium deliquescence, T. harzianum*, *Pseudomonas fluorescence* and *Bacillus subtilis* | Loose smut of wheat and black point complex of wheat | Agarwal and Nagarajan (1992), Singh and Maheshwari (2001), Monaco et al. (2004) and El-Meleigi et al. (2007) |
| *Bacillus megaterium* B5, *B. amyloliquefaciens* B28, *T. harzianum* T37 and *Epicoccum* sp. E52 | Black point complex of wheat | El-Meleigi et al. (2007) |
| *Streptomyces*, *Bacillus*, *Pseudomonas fluorescens*, *P. putida*, *Pseudomonas chlororaphis* MA 342, *Gliocladium* and *Trichoderma harzianum* | Common bunt disease of wheat | Borgen and Davanlou (2000), McManus et al. (1993) and Kollmorgen and Jones 1975 |
| *Phialophora radicicola* var. *radicicola* | Take all disease of wheat | Wong and Southwell (1980), Wong et al. (1996) and Mathre et al. (1998) |
| *Trichoderma koningii*, *T. harzianum*, *T. viride* | | Simon and Sivasithamparam (1989) and Zafari et al. (2008) |
| *Pandoraea apista* (S18 and S19) and *Cylindrocarpon destructans* S22 | *Rhizoctonia* root rot disease of wheat | Barnett et al. (2017) |

*Bacillus amyloliquefaciens* is understood for lipopeptide and polyketide production for biological management and plant growth promotion activity against soilborne pathogens. Some bacteria also are capable of producing volatile compound referred to as hydrogen cyanide (HCN) for biocontrol of black root rot of tobacco, caused by *Thielaviopsis basicola*; also reported is the production of DAPG and HCN by *Pseudomonas* contributing to the biological management of bacterial canker of tomato (Sacherer et al. 1994; Lanteigne et al. 2012).

Wheat microbiome bacteria can reduce virulence of a plant pathogenic fungus by altering histone acetylation (Chen et al. 2018). The molecular mechanisms between *Pseudomonas piscium* bacterium isolated from the wheat head microbiome and the plant pathogenic fungus *Fusarium graminearum* have revealed that a compound secreted by the bacteria (phenazine-1-carboxamide) directly affects the activity of fungal protein FgGcn5, which is a histone acetyltransferase of the

Spt-Ada-Gcn5-acetyltransferase (SAGA) complex which led to deregulation of histone acetylation in *F. graminearum* and subsequently suppression of fungal growth and virulence and biosynthesis of mycotoxin. Thus, it has been proven that an antagonistic bacterium can inhibit growth and virulence of a plant pathogenic fungus by manipulating fungal histone regulation (Chen et al. 2018).

According to Liu et al. (2017), effects of jasmonic acid signalling on the wheat microbiome differ between body sites. The influence of jasmonic acid (JA) on the diversity and function of wheat microbiome and whether there is any specificity of the influence with respect to plant part were determined. By exogenous application of methyl jasmonate, JA pathway was activated and was confirmed by significant increases in the abundance of JA signalling-related gene transcripts. Phylogenetic marker gene sequencing revealed that JA signalling reduced the diversity and changed the composition of root endophytic, but the composition of shoot endophytic or rhizospheric bacterial communities was unchanged. But the total enzymatic activity and substrate utilization profiles of rhizosphere bacterial communities were not affected by JA signalling. Therefore, the conclusion drawn after this study was that JA signalling on the wheat microbiome is specific to individual plant parts.

## 8.10 Wheat Microbiome Under Different Stress Conditions and Advance Research

Till date, the status of wheat microbiome research is at budding stage. However the research in the following aspects has provided insights, and the work presented here provided a way forward to study fundamental microbiome research with the aim of better understanding of identification of potentially beneficial microbes, their dynamics and their role in reducing the pathogen pressure and improving plant yield. To successfully reach the goals in the microbial research, the composition of plant-associated community that can fulfil the required need in plant disease management and in sustainable management has to be determined.

### 8.10.1 Performance of Microbiome Under Nutrient Stress Status

Very recently Page et al. (2019) described the microbiome profiling of *Triticum aestivum* under nitrogen- and phosphorus-starved situations. As wheat farming consumes approximately 20% of the worldwide production of inorganic N and P fertilizers and keeping in view the fact that plants have natural partnerships with microbes that can enhance their nitrogen (N) and/or phosphorus (P) acquisition, it is essntial to evaluate the association between wheat and its microbes in boosting N/P availability. They have tested whether N/P-starved *Triticum aestivum* showed microbiome profiles that are similarly different from those of N/P-amended plants in their own bulk soils. The conclusions drawn that six N/P starvation and plant specific microbial communities that may represent the attraction of microbes towards *T. aestivum* when it experiences N/P starvation. However, additional research will be needed to validate this interpretation under different climatic conditions. But the

work done helps to keep a step forward. Identification of the potential *T. aestivum* partners give us a target list for subsequent relationship-assessing microbioata for boosting yields of the crop and therefore need to be standardized in farmers field with common package of practices. Microbiome enhances N and P uptake and experimently proved by Panwar and Singh (2000). *A. chroococcum* and *P. agglomerans* like microbe not only enhance P and K uptake but also increase plant growth and plant dry matter (Narula et al. 2007, Islam et al. 2002). Application of biofertilizers also reported to enhance the accumulation of nutrient in wheat crop by different scientists (Khan and Zaidi 2007; Abbasi and Yousra 2012). Not only macronutrient, but microbiomes also provide positive impact on the availability of essential micronutrients of wheat (Jog et al. 2014; Mishra et al. 2011; Yasin et al. 2015).

### 8.10.2 Performance of Microbiome Under Drought Condition

In the introduction, it is already mentioned that areas in South Asian countries are suffering from drought. Here microbiomes could be a good supplement to overcome this stress as they counteract with damage due to water stress. Rhizosphere biology mainly influences on the productivity of plants (Watt et al. 2006; Richardson et al. 2009 and Berendsen et al. 2012). Plant growth-promoting (PGP) strains of *Azospirillum* and *Herbaspirillum* have been reported to colonize *Brachypodium* roots and enhance growth of some *Brachypodium* genotypes under low or no nitrogen conditions (Amaral et al. 2016). Another group of scientists also used the PGP strain *Bacillus subtilis* B26 to increase *Brachypodium* biomass and improve plant drought resistance (Gagne et al. 2015). Also some microbiome provided a better water status in osmotic stress condition on wheat plant (Chakraborty et al. 2013; Pereyra et al. 2012). Artificial inoculation of *Azospirillum brasilense* sp. 245-primed wheat seed can sustain under water stress condition by increasing the apoplastic water function in both shoot and root compared to non-inoculated wheat plant (Creus et al. 2004).

### 8.10.3 Performance of Microbiome Under Stress Cultivation System

Nowadays governments are gearing towards conservation agriculture. So another selection criteria for microbiome that they can survive under stress cultural practices or minimal management approaches. A long-term field experiment demonstrates the influence of tillage on the bacterial potential to produce soil structure-stabilizing agents such as exopolysaccharides and lipopolysaccharides (Cania et al. 2019). The non-metric multidimensional scaling (NMDS) ordination plot showed a difference between the composition of bacterial families from the deepest sampled soil layer and the uppermost soil layers. It was revealed that there is no clear separation of the tillage treatments like the effects of tillage, depth and

their interaction on the general microbial community structure. Even salicylic acid signalling in wheat microbiome also depends on soil type and on strategic tillage (Liu et al. 2016).

Another study by Hartman et al. (2018) revealed that cropping practices manipulate abundance patterns of root and soil microbiome members. The soil and wheat root microbiomes under conventional and organic managements with different tillage intensities were conducted to analyse the effects of management type and tillage on microbial communities as well as their infulence on various ecosystem processes and regulations. The study revelaed that the microbial communities play an important roles in nutrient cycling via decomposition of organic matter, and transformation and fixation of soil nutrients like nitrogen and phosphorus. It was revealed that there was pronounced influence of cropping patterns on microbial community composition which were specific for the respective microbiomes. In roots, management type was the important factor for bacteria when compared with fungi, and this is generally determined by changes in tillage intensity. There were many taxonomically diverse cropping-sensitive microbes, in which their response practices are specific. Cropping practices may allow manipulation of influential community members in which members co-occurring with many other microbes in the community. Understanding the patterns of cropping-sensitive microbe abundance helps in developing microbiota management strategies for smart farming.

Even the soil microbiomes perform better in non-suppressive soil than suppressive soil (Hayden et al. 2018). The comparative metatranscriptomic approach was applied to assess the taxonomic and functional characteristics of the rhizosphere microbiome of wheat plants grown in adjacent fields which are suppressive and non-suppressive to the plant pathogen *R. solani* AG8. Soils collected prior to sowing showed similar inoculum levels of the pathogen *R. solani* AG8 in both the suppressive and non-suppressive fields, as determined by quantitative PCR. The inoculum was observed in both fields throughout the cropping season, in particular during the initial 8 weeks of crop growth. The inoculum potential was more in the non-suppressive soils compared to that in the suppressive soils, resulting in significantly higher *R. solani* AG8 DNA in the non-suppressive soils, and the disease index was higher in non-suppressive soil compared to suppressive soils.

The status of wheat microbiome under different management strategies and potentiality of endophytes in disease protection were studied by Gdanetz and Trail (2017) in detail. They analysed the fungal and bacterial communities of leaves, stems and roots of wheat throughout the growing season across growth stage and four crop management strategies like organic, low input, no till and conventional using 16S and fungal internal transcribed spacer region gene amplicon sequencing, and endophytes were isolated from plants and are tested for antagonistic activity against wheat pathogen *Fusarium graminearum*, and also antagonistic strains were assessed for plant protective activity in seedling assays. But contrary to the expectations based on the previous studies on different management strategies, management strategy does not show a strong influence on the plant microbial communities.

A meta-analysis was done to determine whether plant domestication affects the composition of the root-associated microbiome (Jaramillo et al. 2018). The significant and consistent differences in the abundance of *Bacteroidetes*, *Actinobacteria* and *Proteobacteria* for different plant species were observed. The higher relative abundance of different bacterial families on the roots of wild relatives of the different plant species was observed. For conducting this study, it is emphasized that the same computational pipeline was used by adopting a rarefaction of the operational taxonomic unit (OTU) table to the same sequencing depth and also found similar differences between the prokaryotic composition of the rhizosphere microbiome of wild and domesticated plant species with a significantly higher abundance of *Bacteroidetes* in the roots of wild plant relatives. However, the reasons behind the relative abundance of *Bacteroidetes* in the root and rhizosphere compartments of wild relatives of various crop plant species are yet unknown. Therefore, their prevalence in the root compartments of wild plant species may be a phylogenetic signal associated with the presence of complex biopolymers in the root exudates (Table 8.3).

### 8.10.4 Role of Microbiome to Trigger Flowering

Sometimes microbiomes have strong influence on the crop physiology like timing of flowering (Lu et al. 2018). The rhizosphere microbial communities can modulate the timing of flowering of *Arabidopsis thaliana*. The abundance of rhizosphere microorganisms and prolonged nitrogen bioavailability by nitrification, delayed flowering by converting tryptophan to the indole acetic acid (IAA), thus downregulating genes that trigger flowering, and stimulating plant growth. Lu et al. (2018) also observed a novel metabolic network in which soil microbiota influenced plant flowering time, thus providing a water font on the key role of soil microbiota on plant functioning and thus helping to mitigate the effects of climate change and environmental stress on plants using microbiomes.

## 8.11 Future Prospectives and Conclusion

Over the last 150 years, plant pathologists only dealt with individual microbes that have adapted to specific niches on their hosts. Now we need to think more to perform high-throughput sequencing of these niches to explore microbial communities that can affect disease outcomes. A fundamental understanding of the plant microbiome is necessary for their successful execution amongst the farmers.

The rhizospheric microbiomes are regulated by root exudates, selection of plant genotype and adoption of environment. So, plant microbiome must be a part of future breeding programmes so that next-generation plant genotypes have enhanced ability to interact with beneficial microbes either of natural soil microbiota or of microbial inoculants. Worldwide many wheat cultivars that are biofortified, for

**Table 8.3** Microbes involved in amelioration of various stresses

| Wheat microbes | Stress | References |
|---|---|---|
| *Azospirillum lipoferum* | Drought | Naveed et al. (2014) |
| *Bacillus thuringiensis* | Heavy metal | Kumar et al. (2015) |
| *Bacillus safensis, Ochrobactrum pseudogregnonense* | Drought | Chakraborty et al. (2013) |
| *Bacillus* sp. | Cold stress | Sezen et al. (2016) |
| *Bacillus aerophilus, Lysinibacillus sphaericus* | Acid and alkalinity stress | Verma et al. (2016) |
| *Azospirillum brasilense* INTA Az-39 wheat roots | Drought | Diaz-Zorita and Fernández-Canigia (2009) |
| *Bacillus amyloliquefaciens* and *Azospirillum brasilense* | Temperature | Abd-Alla et al. (2013) |
| *Pseudomonas fluorescens, Pantoea agglomerans, Mycobacterium* sp. | | Egamberdiyeva and Höflich (2003) |
| *Pseudomonas fluorescens* 153, 169, *Pseudomonas putida* 108 | Salinity | |
| *Achromobacter xylosoxidans*, *Serratia marcescens* | | Barra et al. (2014) |
| *Pseudomonas putida* N21, *Pseudomonas aeruginosa* N39 and *Serratia proteamaculans* M35 | | Zahir et al. (2009) |
| *Azospirillum* sp. | | Nabti et al. (2010) |
| *Pseudomonas putida, Pseudomonas extremorientalis, Pseudomonas chlororaphis* and *Pseudomonas aurantiaca* | | Abbaspoor et al. (2009) |
| *Bacillus thuringiensis, Azotobacter chroococcum, Paenibacillus ehimensis, Pseudomonas pseudoalcaligenes* | Heavy metal | Kumar et al. (2015) |
| *Bacillus megaterium* M3, *Bacillus subtilis* OSU142, *Azospirillum brasilense* Sp245, *Raoultella terrigena* | Cold | Turan et al. (2012) |

example, rich in iron, rich in zinc, rich in anthocyanin, etc., are already released and also popular. In this scenario not only the microbial but also the host part of the interactions need to take more attentions, since the associated microbiome is emerging as a fundamental trait for controlling plant biotic and abiotic stress and optimizing plant growth promotion (Bulgarelli et al. 2013; Sclaeppi and Bulgerelli 2015). In the case of managing pathogen burden, the combined deployment of beneficial services of the plant microbiome (agricultural probiotics) and innate immune functions (resistance genes) is expected to deliver durable and sustainable protection from disease (Dangl et al. 2013). Identification of the genetic components of the host-microbiome control will be key factor for its ultimate inclusion into breeding programs.

Developing the next-generation agricultural tools and practices will be dependent on integration of all covariates present in agro-ecosystem. Under a specific agro-climatic condition, the performance of native soil microbiome and inoculated microbiome sometime interact with one or both with individual plant species and

genotype, and this interaction drastickly influence the performance of the crop. Hence, we can exploit the use of plant microbiome at work and next-generation agriculture in reality to solve the aforesaid multivariate equations.

The ultimate goals of the researchers for the fate of the farmers are to minimize the use of fertilizers, protect plants from chemicals as well as reduce the cost of cultivation in the era of sustainable agriculture. But to reach this goal, we need to know the fundamental relationships between microbes and microbes and wheat plants and microbes and how much longer this relation may work under changing climatic conditions. Knowledge gain from this chapter will help to understand the selection criteria of microbes for alternative use of microbiomes as fertilizers, biocontrol agent, growth promoters and in efficenet soil nutrient cycling. But need more depth of researches for long duration under different cropping systems to translate microbiome concept in agriculture. Cropping practice and microbiome engineering would help only in sustainable agriculture system. Due to intensive cultivation, soil just become empty of nutrients and the physical and biological health of the existing soil hamphered badly. The microbiome is a fundamental part of basic processes such as plant development or growth of essential organs such as the root and for improved acquisition of nutrients and water; the mentioned capabilities make the microbiome an important component for the plant to carry out physiological functions. Analysis of metagenome and comparison with plant-associated communities will lead to novel phylogenetic and functional insight. The application of microbial inoculants to the field presents one of the several conceivable next-generation agricultural tools or practices.

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# 9 Entomopathogenic Fungi: A Potential Source for Biological Control of Insect Pests

Anjney Sharma, Ankit Srivastava, Awadhesh K. Shukla, Kirti Srivastava, Alok Kumar Srivastava, and Anil Kumar Saxena

**Abstract**

Insect pests with high population level are inimical and cause enormous damage to agricultural crops and economy as well as decrease the food security for the growing human population. For controlling insect pests, the use of entomopathogenic fungi (EPF) is very much useful, and it was developed as eco-friendly mycopesticide that is useful in the regulation of insect pests. These entomopathogenic fungi have a unique mode of infection on different orders of insects. Recently, it was investigated that in addition to insect pest control, these entomopathogenic fungi also act as endophytes and biocontrol agents of plant pathogens and promote plant growth as rhizosphere fungi. Numerous environmental abiotic and biotic factors are recognized to inhibit or enhance the fungal efficacy against the insect pathogens. Advanced research in the genome biology of pathogenic insect fungi has shown that genetic features of these organisms are developed for fungal adaptation with various host insects. The efforts toward genetic engineering of entomopathogens, the knowledge of its virulence and tolerance to adverse conditions will potentiate cost-effective applications of mycoinsecticides for pest control in the agricultural fields. The studies suggest that the exploitation of ecological, genetic and functional diversity of these fungi increases our potential for integrated pest management. Consideration of the insect microbiome in fungal insect pathology represents a new frontier which may contribute in deciphering the obscure pathological aspects of the biology of entomopathogenic fungi and its ecology. Taking these into account, in the present chapter, we highlight the importance, classification, mode of action, factors affecting its virulence effi-

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cacy, the role of genetic engineering in strain improvement, and ground level of entomopathogenic fungi for integrated pest management (IPM), and plant growth promotion with commercial available entomopathogenic products is critically discussed.

**Keywords**
Entomopathogenic fungi · Genetic engineering · Insect pests · Integrated pest management · Virulence

## 9.1 Introduction

Due to the dependency of the market at a global scale to agricultural merchandise, leading-edge technology in agriculture is increasingly used that is required to develop new agricultural practices and has potential to alleviate the adverse negative effects on the ecosystem and consequently results in the production of valuable safe products for human consumption. However, there is a major constraint in increasing agricultural production yield due to loss caused by insect pests, plant diseases, weeds, etc. (Oerke et al. 1994). In evolutionary agricultural practices, pest problems are unavoidable and arise largely due to simplified agroecosystems and additionally because of the creation of less stable natural ecosystems. It is investigated that insect pests such as locusts, grasshoppers, termites, and cattle ticks have caused huge economic and agricultural production losses, that is, about 40%, in many parts of the world (Thacker 2002; Chandler et al. 2011). Although the use of pesticides has increased marginally, crop losses have remained relatively stable (Oerke 2006). Various industrialized nations are trying to shift their strategy to using transgenic plants that express particular traits like resistance to insects, fungi, or viruses for pest management. Several agrochemicals pesticides are still being used by farmers for the control of insect pests and diseases in their agricultural practices. They have been responsible for maintaining and increasing the quality and quantity of food and fiber worldwide. The extensive use has resulted to adverse effect on nontarget organisms, deposition on edible food crops, groundwater pollution, and resistance of insects against the chemicals, and negative effect on human health has forced scientists to focus on the development of alternative environmentally safe strategies that are cost-effective and reliable. Integrated pest management (IPM) is a comprehensive approach to crop production (Chandler et al. 2011). This is a shift from the traditional individual pest-centered strategies that relied heavily on chemical pesticides to a more holistic approach of viewing the entire crop production system as a whole and managing rather than eradicating the pests. In line with this point, several microbial agents have been developed to manage insect pests. Entomopathogenic fungal biological plant protection plays a key role in the program of sustainable pest management. For integrated pest management, ready-made use of entomopathogenic fungus as biocontrol agents to control the insect density and increase the crop yield has several benefits compared with conventional insecticides. These

encompass low expenses, high performance, safety for beneficial organisms, reduction of residues within the environment, and accelerated biodiversity (Asi et al. 2013; Gul et al. 2014). Entomopathogenic fungus has been known as important inhibitory factor of plant bugs for more than a century and is continuously used in pest biocontrol management throughout the world (Jaber and Enkerli 2017). Entomopathogenic fungi fill an extremely important niche and are well suited for development as microbial biopesticides for control of the harmful insect pests.

Almost all orders of insects are susceptible to fungal sicknesses. Entomopathogenic fungi are potential eco-friendly biocontrol agents especially because of their excessive reproductive abilities, goal-specific activity, minimal impacts on beneficial and other nontarget organisms, brief era time, and resting stage or saprobic phase—generating competencies which can make sure their survival for an extended time when no host is present (Mudgal et al. 2013). Entomopathogenic fungi can be found in a wide range of environmental conditions (including arid to tropical settings, terrestrial to aquatic habitats, and arctic to temperate climates), possess potential to colonize a wide variety of plant species, and infect a broad array of insects (Meyling et al. 2012; Mantzoukas et al. 2015). Researchers have stated that approximately a thousand species of fungi are identified as causal agents of disease in arthropods (Goettel et al. 2010; Vega et al. 2012). The majority of entomopathogenic fungi used for biological control of insects and mites are in the orders Hypocreales and Entomophthorales. Most of the biological control fungi produced commercially are included in Hypocreales. Various other fungi such as *Metarhizium* spp., *Beauveria bassiana*, *Lecanicillium* spp., and *Isaria fumosorosea* are being used against a large number of pests in different variety of crops. About 170 fungal formulations from at least 12 species of fungi have been developed (Faria and Wraight 2007). It is believed that there are about more than 750 species of fungi that have been identified to cause various infections in insects. Most of the entomopathogenic strains or species are obligate pathogens that show specific ecomorphological adaptations of their host's life cycles, such as the production of infective spores that are produced on insect cadavers during the night. These fungi often cause natural epizootics in insect and mite populations. Natural epizootics of entomophthoralean fungi are being used as a natural form of pest control. Pioneering work of Inglis et al. (2001) conducted with *Beauveria bassiana* (Balsamo) and *Vuilleminia* (Ascomycota: Hypocreales), as entomopathogenic endophytes and ubiquitous soilborne fungus and one of the most commercialized fungal biopesticides, reveals that they infect >700 insect species. A number of recent studies reveal that entomopathogenic fungi, often entirely taken into consideration as insect pathogens, play extra roles in nature, such as endophytism, plant disease antagonism, plant growth promotion, rhizosphere colonization, and management of various abiotic stresses (Vidal and Jaber 2015). Moreover, most studies investigating the interactions among plants and endophytic entomopathogens have up to now centered on the advantages of such interactions to the host plant through multiplied tolerance and resistance to biotic elements including pests and illnesses (Lopez and Sword 2015). Due to low virulence and inconsistencies of their performance within various field environmental stresses, currently, fungal pathogens have a small market share and small chances of

killing insects in comparison to chemical insecticides (Fang et al. 2012). Low virulence may be incorporated as an evolutionary balance between microorganisms, and their hosts may have adapted to the pathogen to avoid rapid killing even at high doses, in which cost-effective biocontrol will require genetic modification of the fungi (Gressel 2007). Genetic engineering is an effective green device to improve the efficacy of mycoinsecticides by enhancing their virulence and tolerance to environmental stresses. Modern biotechnological tools that now enable genetic improvement of entomopathogens for myco-biocontrol of fungi are now feasible. Molecular biology techniques, coupled with the cloning of genes to express insect proteins and insecticidal proteins/peptides from insect pathogens, will create more effective strains for pest management. Further research is desirable under variable environmental conditions to monitor the environmental fate of recombinant fungal strains. Comparative genomics has facilitated the identification of fungal fitness characteristics and the selective forces acting on them to enhance our knowledge of why and how entomopathogenic fungi interact with insects and other additive components of their environment. So far, there may be plenty of extra-genomic facts on ascomycete insect pathogens, as sequences are available from *Metarhizium* strains, *Beauveria bassiana*, *Cordyceps militaris, Ophiocordyceps sinensis* (anamorph, *Hirsutella sinensis*), *Ophiocordyceps unilateralis, Tolypocladium inflatum*, and *Hirsutella thompsonii* (Zheng et al. 2011; Xiao et al. 2012; Bushley et al. 2013; Pattemore et al. 2014; de Bekker et al. 2015; Agrawal et al. 2015). Molecular studies provided valuable information on the phylogenetic relationships with various other fungi. Hence, sequence data can, therefore, provide crucial information on how these organisms reproduce and persist in different environments (Wang and St. Leger 2014). It is important to understand the ecology of fungal entomopathogens. Further, it is reported that various entomopathogenic fungi play important roles in nature, such as endophytes, useful rhizosphere associates, antagonists of plant pathogens, and possibly even plant growth promoters. A growing quantity of research has currently established the ability of several entomopathogenic fungi after endophytic establishment to promote plant growth (Sasan and Bidochka 2012; Lopez and Sword 2015). Increased plant growth, mediated through colonization by fungal endophytes, is the outcome in the suppression of numerous abiotic and biotic stresses, consisting of plant diseases (Kuldau and Bacon 2008). Hence, it is an urgent need to review and understand how entomopathogenic fungi solely act as biopesticides and also promote plant growth. Keeping the above facts, we are trying to summarize the recent information regarding the role of entomopathogenic fungi in biocontrol and plant growth promotion. Additionally, the factors affecting the activities of entomopathogenic fungi are also being discussed.

## 9.2 Classification and Type of Entomopathogenic Fungus

In eukaryotes, the kingdom Fungi is a major group with approximately 700 described entomopathogenic fungi species (Roberts and Humber 1981) which represent <1% of the total fungal species (McLaughlin et al. 2009). Entomopathogenic

fungi are found in three main groups (Entomophthoromycota, Blastocladiomycota, and Microsporidia) and 12 different classes in six phyla of fungi. These pathogenic fungi are found in the divisions Ascomycota, Zygomycota, Deuteromycota, Oomycota, and Chytridiomycota (Humber 1997). Many of the potential entomopathogenic fungi genera known so far belong to either the order Entomophthorales in the Zygomycota or the class Hyphomycetes in Deuteromycota (Jaber and Enkerli 2017). The detailed descriptions of the classification are illustrated in Table 9.1. Among the different phyla and orders, the species that belong to genus *Metarhizium*, *Beauveria*, *Nomuraea*, *Verticillium*, and *Hirsutella* of different environmental habitats are most prominently agriculturally important entomopathogenic fungus which is commercially used successfully in the field level. The detail of this genus is illustrated in Table 9.2 and Fig. 9.1.

## 9.3 Entomopathogenic Fungus Infection

Entomopathogenic fungi have a unique mode of infection. The infection on different orders of insects (Lepidoptera, Coleoptera, Diptera, Hymenoptera, Homoptera) becomes started when insects come into contact with entomopathogenic fungal spores. The life cycle of insect pathogenic fungi starts with the spore germination on the cuticle of the host (Fig. 9.2). Attachment can be passive in spores that are covered with a sticky or slimy substance (e.g., *Lecanicillium* spp., *Entomophthorales* spp., and *Hirsutella* spp.). Adhesive processes involve physical, chemical, and enzymatic interactions. Different hydrolytic and detoxifying enzymes like protease, chitinase, lipase, catalyzes, cytochrome P450, and their secondary metabolite are generated during final insect adaptive responses. Two adhesion genes, namely, Mad1 and Mad2 in *M. anisopliae*, have been characterized (Wang and St. Leger 2007). Under favorable environmental condition such as optimum temperature, humidity, nutritional and chemical cue, and cuticular secreted material of the host, spore's germination occurs (Tanada and Kaya 1993). Through penetration hook, the germinating spores produce germ tubes which enzymatically and physically penetrate the cuticle and into the thinner softer part of the insect's body by sensory pores or wounds (Gabarty et al. 2014). Samuels and Paterson (1995) reported that a variety of cuticle-degrading proteases are produced by both insects and entomopathogenic fungi. An extensive range of proteases have been recognized, consisting of trypsin, chymotrypsin, esterase, collagenase, and chymoelastase (Sanchez-Perez et al. 2014). Once inside the body, the fungus spore starts multiplying by taking internal nutrient material of the insects, which create nutrient deficiency and disruption of biological functions, produce toxic compounds, invade insect's tissues, block the vessels, and ultimately cause host death (Goettel et al. 2000). After the insect host is killed and all nutrition has been consumed, hyphae grow out of the cadaver, particularly at the margins of the intersegmental regions, and produce resting or infective spores that promote the spread of the fungus. Finally, they must be transmitted to new hosts (Boomsma et al. 2014). Entomopathogenic fungi such as *Beauveria, Metarhizium Hirsutella, Paecilomyces, Isaria, Lecanicillium,* and

**Table 9.1** Classification of the entomopathogenic fungus

| Phylum | Order | Description | Genus/species | Infected insect host |
|---|---|---|---|---|
| Oomycota | Lagenidiales, Leptomitales | Cellulose in their coenocytic hyphae (without chitin) and they have biflagellate zoospores, reproduced by a thick-walled oospore, at the cellular level; they possess mitochondria with tubular cristae | *Lagenidium giganteum, Leptolegnia chapmanii* | Mosquitoes, crabs, and other aquatic crustaceans, arthropods |
| Chytridiomycota | Blastocladiales, Chytridiales, Blastocladiales | Coenocytic hyphae; chitin is a major constituent of cell wall, and zoospores are with a single flagellum | *Coelomomyces, Myriophagus, Coelomycidium, Myriophagus (Chytridiales), Coelomycidium (Blastocladiales)* | Hemipterans and dipterans, mosquitoes and flies |
| Zygomycota | Entomophthorales | Mycelium is multicellular, nonseptate gametangia that after fusion form zygospores | *Batkoa apiculata, Batkoa major, Entomophaga grylli, Entomophaga calopteri, Entomophaga maimaiga, Pandora neoaphidis, Pandora delphacis, Pandora blunckii, Pandora bullata, Zoophthora radicans, Conidiobolus thromboids, Neozygites parvispora* | Aphids, hemipterans, flies, lepidopterans, grasshopper, gypsy moth larvae, homoptera, lepidoptera, diptera, leafhoppers, psyllids, leaf rollers, clover leaf weevil |
| Ascomycota and Deuteromycota | Hypocreales | Septate mycelia, ascospores (sexual spores) formed in the fruiting body called ascus, haploid | *Aspergillus, Metarhizium, Hirsutella, Beauveria, Aschersonia, Culicinomyces, Lecanicillium, Paecilomyces, Tolypocladium,* etc. | Lepidoptera, hymenoptera (wasps), whitefly, pine caterpillar, potato Beetle, corn borer, coding moth, grasshoppers, chinch bug, boll weevil, cowpea curculio, pecan weevil, mosquitoes, lygus bug, granary weevil, brown plant hopper, termites, spider mite, fire ants, European cockchafer, sugarcane borer, brown planthopper, citrus rust mite |

(continued)

**Table 9.1** (continued)

| Phylum | Order | Description | Genus/species | Infected insect host |
|---|---|---|---|---|
| Basidiomycota | Septobasidiales, Atheliales | Produce sexual spores called basidiospores, formed outside the specialized reproductive cells called basidia. These spores are forcibly discharged by specialized structures Unique traits for this group are clamp connections | *Septobasidium and Uredinella, Fibularhizoctonia* | Scale insects (Hemiptera, Diaspididae), termite eggs |
| Entomophthoromycota, | Entomophthorales | Somatic state consisting of a well-defined mycelium, coenocytic or septate Protoplasts either hyphoid or amoeboid and changeable in shape; cystidia or rhizoids formed by some taxa Conidiophores branched or unbranched. Primary spores true conidia, uni-, pluri-, or multinucleate | *Pandora neoaphidis and Entomophthora planchoniana, Entomophaga maimaiga* | Different aphid species, certain lepidopteran larvae |

*Verticillium* are known as commercially used and sold biopesticides in multiple formulations around the world (Anchez-Rodríguez et al. 2015; Lacey et al. 2015).

## 9.4 Enzymatic Virulence Mechanisms of the Entomopathogenic Fungus

Virulence can be defined as a process involved in insect death during pathogenesis (Mondal et al. 2016). The production of cuticle-degrading enzymes has been proposed as an important attribute of the pathogenic fungi determining the virulence of the entomopathogenic fungi that cause insect death during pathogenesis (Samuels et al. 2011). The insect cuticle is a highly heterogeneous structure that can vary

**Table 9.2** Some agriculturally important entomopathogenic fungi and their commercially available bioproducts against the insect pests

| Genus | Spp. | Characteristics | Plants | Host insects/pests | Toxins against the insect pests | Commercial products manufacturer |
|---|---|---|---|---|---|---|
| *Metarhizium* | *M. anisopliae*, *M. hiemalis*, *M. acridum*, *M. album*, *M. flavoviride*, *M. brunneum*, *M. pingshaense*, *M. robertsii*, *M. guizhouense*, *M. lepidiotae*, *M. majus*, and *M. globosum* | Initially, the colony color is white which turns to green at later stage. The hyphae are hyaline, septate, branched. Conidiophores are short, erect, hyaline, septate, simple, or branched, terminating in single or a cluster of phialides. Synnemata absent. Conidia may be hyaline, lilac, brown or green, smooth and form chains. Size ranges from 4.0–14.5 × 0.0–5.0 µm; shape may be cylindrical, globose, ellipsoidal. Conidia are hydrophobic due to the presence of cysteine-rich proteins called hydrophobins in the cell wall | Rice, maize cotton, sugarcane, coconut | Brown plant hopper (*Nilaparvata lugens*), western corn rootworm (*Diabrotica virgifera*), *Dysdercus peruvianus*, Scirpophaga incertulas, green leafhopper (GLH), brown planthopper (BPH), coconut rhinoceros beetle, groundnut cutworm, rice brown planthopper, diamondback moth and early shoot borer, top shoot borer and internode borer of sugarcane white grub, red cotton bug, rhinoceros beetle | Destruxins (DTXs), cytochalasins, and ribotoxin anisoplin | **India:** M/s Pest Control (India) Pvt. Ltd., Bengaluru; M/s Sri Biotech; M/s Viswa Mithra Bio-Agro P. Ltd., Guntur; M/s Microplex (India), Wardha; M/s Varsha Bioscience & Technology, Hyderabad, M/s Agri Life, Secunderabad, AP; M/s Sri Venkateshwara Chemicals, Bangalore; M/s Biotech International Ltd, New Delhi; M/s DVS BioLife; M/s International Panacea Ltd; M/s T. Stanes & Co. Ltd; M/s Khodke Agro Products Pvt. Ltd.; M/s Ruchi Oyster Mushroom, Gondia (MS); M/s Advance Cropcare (India) Pvt. Ltd., Indore; M/s Adiraj Agro Industries, Pune (MS); M/s Nirmal Seeds Pvt. Ltd., Jalgaon (MS); M/s Arya Biotech & Research Laboratory, Amravati **USA:** Novozymes Biological Inc**.** **Spain:** Trichodex S.A. **Australia:** Becker Underwood Inc. |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Beauveria* | *B. bassiana,*<br>*B. velata,*<br>*B. brongniartii,*<br>*B. amorpha*, and<br>*B. caledonica* | A filamentous fungus. Grows naturally in soils. The colonies are white with cottony aerial mycelium. Conidiophores are single or branched, oblong, cylindrical, or flask shaped and are bearing laterally or at extremity and vesicles giving rise to sporogenous cells (phialides). Phialides generally are globose, sometimes cylindrical, flask-like, and curved or straight. Hydrophobic properties of conidia, due to the presence of cysteine-rich proteins called hydrophobins in the cell wall. Conidia are globose (1–4 μ) to oval shape (1.5–5.5 × 1–3 μ) | Rice,<br>black gram,<br>red gram,<br>groundnut,<br>sugarcane,<br>mango,<br>castor,<br>maize,<br>potato,<br>pine | Pales weevils (*Hylobius pales*),<br>black bug (*Scotinophara lurida*),<br>zigzag leafhopper (*Recilia dorsalis*),<br>spotted pod borer (*Maruca testulalis*),<br>aphids,<br>*H. armigera*<br>(American boll worm),<br>yellow hairy caterpillar,<br>shoot borer,<br>leaf webber,<br>hairy caterpillar,<br>stem borer,<br>western corn rootworm,<br>ladybird beetle, Colorado potato beetle,<br>bark beetle | Beauvericin,<br>bassianolide,<br>isarolides, and<br>beauverolides | **India:**<br>M/s T. Stanes & Company Ltd.;<br>M/s International Panacea, New Delhi;<br>M/s Sri Biotech;<br>M/s. Romvijay Bio-Tech (P) Ltd;<br>M/s Multiplex Bio-Tech Pvt. Ltd;<br>M/s Viswa Mithra Bio-Agro P. Ltd., Guntur;<br>M/s Metroplex (India);<br>M/s Pest Control (India) Pvt. Ltd;<br>M/s Varsha Bioscience & Technology, Hyderabad;<br>M/s Sri Venkateswara Chemicals, Secunderabad;<br>M/s DVS BioLife Ltd;<br>M/s Jai Biotech Industries;<br>M/s Vaibhav Lakshmi Bio-control Laboratories;<br>M/s Bio-Agro Ferticon, Pune;<br>M/s R.B. Herbal;<br>M/s Jai Kisan Agro;<br>M/s Avishkar Bio-farm Pvt. Ltd.;<br>M/s Khodke Agro Products Pvt. Ltd.;<br>M/s Choudhary Agrotech (I);<br>M/s Govinda Agro Tech. Ltd;<br>M/s Om Agro Organics, Yavatmal, Maharashtra;<br>M/s INORA, Pune;<br>M/s Ellor Bio Tech & Agro Services, Aurangabad;<br>M/s Super Pesticides & Agro (India) Pvt. Kolkata<br>**USA:**<br>Jabb of the Carolinas Inc.,<br>Laverlarn International Corporation,<br>Mycotech Corp.,<br>Troy Bioscience Inc.,<br>**Spain:** Trichodex S.A.<br>**South Africa**: Biological Control Products SA (Pty) Ltd.<br>**Colombia:** Live System Technology S.A. |

(continued)

**Table 9.2** (continued)

| Genus | Spp. | Characteristics | Plants | Host insects/pests | Toxins against the insect pests | Commercial products manufacturer |
|---|---|---|---|---|---|---|
| *Verticillium* or *Lecanicillium* | *V. lecanii,* *V. chlamydosporium,* *V. dahlia,* *V. albo-atrum* or *L. lecanii,* *L. attenuatum,* *L. longisporum,* *L. muscarium,* *L. nodulosum* | Conidiophores are formed in verticillate whorls, on which conidia are borne in slime or mucus balls. Conidiospores are ovoid/ellipsoid, single-celled; branching is verticillate, i.e., the branches arise in whorls on the upper portions of conidiophores. Hydrophilic conidia vary in shape and size, with a mean length of 5.0 ± 1.0 μm and width of 1.9 ± 0.5 μm. They have/form an ascus (and may, therefore, be described as asci fungi). They form resting structures, long phialides. The vegetative mycelium has the following characteristics: hyaline, multinucleate, and septet | Groundnut, guava, potato, castor, maize | *Aphis craccivora*, *Mylabris pustulata*, aphids, whiteflies, grasshoppers, *Icerya seychellarum*, *Myzus persicae*, *Pericallia ricini*, thrips, brown planthopper, scale insects, groundnut pests, hairy caterpillar, stem borer | VD toxins | **India:** M/s T. Stanes & Company, M/s. Romvijay Bio-Tech (P) Ltd, M/s Viswa MithraBio-Agro P. Ltd., M/s Indian Institute of Horticultural Research, M/s Microplex M/s Agri Life, M/s Varsha Bio Science & Technology, M/s Jai Biotech Industries, M/s T. Stanes & Co. Ltd., M/s Sri Biotech, M/s Avishkar Bio farm (P) Ltd., M/s DVS Bio Life Ltd., M/s Bio-Agro Ferticon, M/s Pravara Agro Biotech, M/s R.B. Herbal, M/s Institute of Natural Organic Agriculture, M/s Khodke Agro Products Pvt. Ltd., M/s Choudhary Agrotech (I), M/s Om Agro Organics, M/s Sai Agrotec, M/s Sri Venkateswara Chemicals, M/s Junna Life Sciences, M/s Shri Ram Solvent Extractions Pvt. Ltd., M/s Mitcon Biotechnology, M/s Pruthvi Fertilizers Organics, M/s Nirmal Organo Bio Tech, M/s Sun Agro Biosystem Pvt. Ltd., M/s Sri Dutta Gro-Tech Equipments **Russia**: Biodron **United Kingdom:** Koppert Biological Systems **Spain:** Trichodex **Brazil:** Natural Rural |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Paecilomyces* | *P. lilacinus,*<br>*P. marquandii,*<br>*P. farinosus,*<br>*P. amoeneroseus,*<br>*P. fumosoroseus,*<br>*P. tenuipes,*<br>*P. javanicus,*<br>*P. variotii* | The colonies are white, red, or yellow. Well-developed synnematous conidiophores are found in many species, septate bearing whorls of divergent branches and phialide, the conidiogenous cells. The phialides are flask to oval shaped or subglobose with a distinct neck, borne singly or in whorls; conidia are unicellular, hyaline to light colored. Phialides occur as divergent loose group | Groundnut, castor | *Aproaerema modicella*, *Pericallia ricini*, Yellow and red mites, whiteflies, etc. | Leucinostatins | **India:**<br>M/s Indian Institute of Horticultural Research,<br>M/s T. Stanes and Co. Ltd.,<br>M/s RPC Balaji Crop Care Pvt. Ltd;<br>M/s Nico Orgo Manures,<br>M/s Shree Shiva BioTech,<br>M/s Agriland Biotech Ltd.,<br>M/s Kanbiosys Pvt. Ltd.,<br>M/s. Devi Biotech (P) Ltd.,<br>M/s Varsha Bioscience & Technology,<br>M/s Gujarat Life Sciences (P) Ltd.,<br>M/s. Chitra Agri Organics,<br>M/s Agri Life,<br>M/s Jyothiraditya Bio Solutions Ltd.,<br>M/s Viswa Mithra Bio-Agro (P) Ltd.,<br>M/s Excel Crop Care Ltd.,<br>M/s Romvijay Bio Tech Private Ltd.,<br>M/s Jhass Agro Industries,<br>M/s Camson Bio Technologies Ltd.,<br>M/s Agriva Agro Tech Kappallur,<br>M/s Poabs Biotech Pvt. Ltd.<br>**USA:** Certis |

(continued)

**Table 9.2** (continued)

| Genus | Spp. | Characteristics | Plants | Host insects/pests | Toxins against the insect pests | Commercial products manufacturer |
|---|---|---|---|---|---|---|
| *Hirsutella* | *H. thompsonii*, *H. gigantea*, *H. citriformis* | Fungal colonies show fluffy mycelial growth, gray with a brownish to grayish-green substratum color. The hyphae are 1.5–2.0 μm wide and smooth. The conidiogenous cells arise singly at intervals from the vegetative hyphae, mono- or polyphialide, unevenly verrucous, with a conical- to flask-shaped base and a narrow neck. The neck may be unbranched or branched, bearing enteroblasts conidia singly at the tip of the branch | Rice, citrus plants | *N. lugens*, *N. virescens*, and *S. furcifera*, citrus rust mite, (*Phyllocoptruta oleivora*) hoppers and bug pests, whiteflies, red mites BPH, GLH, and WBPH | Hirsutellin A (HtA) | **India:** M/s International Panaacea Ltd. |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Nomuraea* | *N. rileyi*, *N. atypicola* | Colonies of the fungus are white initially and later turn to green in color (malachite green). Hyphae are 2–3 μ in diameter, smooth, septate, hyaline, and slightly pigmented. Conidiophores are long (160 μ) and consist of dense, compacted clusters of phialides and branches in whorls on the upper section, short and swollen. Phialides are short and cylindrical to globose, with very swollen base tapering abruptly to a narrow neck. The conidia are 3–4 and 2.5 μ and pale green | Sorghum, soybean, groundnut, rice | Tobacco caterpillar (*S. litura*), American bollworm (*H. armigera*), *Helicoverpa*, *Trichoplusia ni*, *Heliothis zea*, *Plathypena scabra*, *Bombyx mori*, *Pseudoplusia includens*, Anticarsia gemmatalis, ear head caterpillar (*Helicoverpa armigera* Hubner), pod borers, cutworms, cabbage borers, leaf folder | Diketopiperazine | **India:** M/s Nirmal Seeds Pvt. Ltd. |

Sources: Mathew et al. (1998), Sandhu and Vikrant (2006), Inglis and Tigano (2006), Sandhu et al. (2012), Sujeetha and Sahayaraj (2014), Lacey et al. (2015), Vega (2018), and Reddy et al. (2013), http://ppqs.gov.in/divisions/cib-rc/registered-products

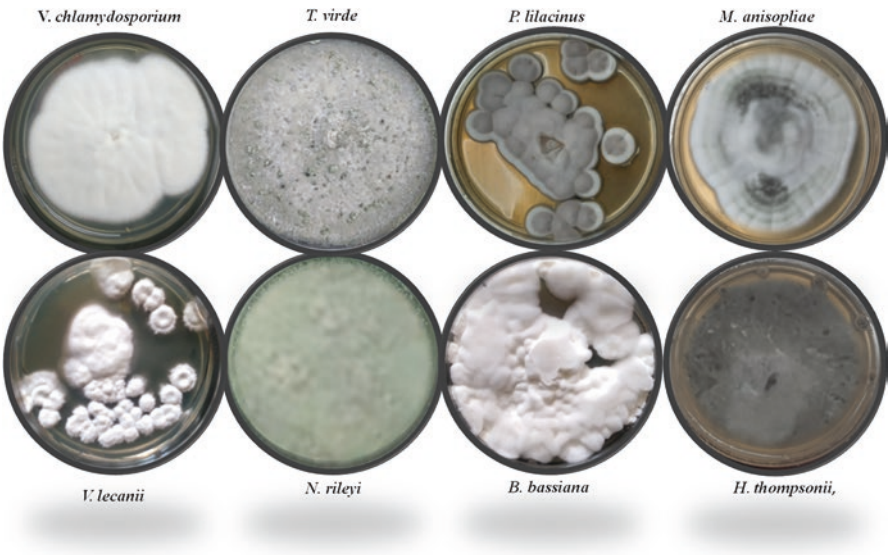


**Fig. 9.1** Agriculturally important entomopathogenic fungi


**Fig. 9.2** Life cycle or mode of action of entomopathogenic fungi against the insect pests

greatly in composition and composed of wax, protein, and chitin associated with lipids and phenolic compounds; these represent a significant barrier to the invading fungus during the various life stages of a particular insect (Ortiz-Urquiza and Keyhani 2015. The entomopathogenic fungus can produce diverse enzymes like lipase, chitinase, and proteases as a virulence factor in response to different insects. During pathogenesis, degradation of the cuticular polymers in insect cell wall takes place due to these enzymes, which helps in penetrating the insect exoskeleton and getting nutrients for fungal growth; thus, they control insect pests and pathogens that attack productive crops and provide potential economic benefit to agribusiness (Petrisor and Stoian 2017). Most studies were focused on cuticle-degrading enzymes produced by entomopathogenic fungi and on extracellular activities of them (Charnley 2003; Ali et al. 2009). Several studies have carried out the biochemical characterization of cuticle-degrading enzymes such as chitinase, protease, and lipase, the key enzymes involved in the process of pathogenesis, and have been carried out to understand the host-pathogen interaction (Mondal et al. 2016; Cristina and Stoian 2017).

### 9.4.1 Proteases

Proteins and proteases that constitute the majority of insects' cuticle are a large group of hydrolytic enzymes that break the peptide bonds of cuticle proteins into small peptides and amino acids.

Proteases (endopeptidase, aminopeptidase, carboxypeptidase) attack insect cuticle before chitinases, because this masks cuticular chitin microfibers, followed by epicuticle that has been broken down through lipases. Entomopathogenic fungi produce a variety of endo- and exo-acting proteolytic enzymes in culture. Many of the proteases of entomopathogens are classified as collagenases or chymoelastases that show strong homology with the subtilisin family of proteases (Chrzanowska and Kolaczkowska 1998). The invading fungi produce great quantities of virulence indicator subtilisin-like serine protease Pr1 and trypsin-like protease Pr2, which degrade the proteinaceous material. It was validated that protease Pr1's extracellular involvement in cuticular penetration is initialized by cuticular infection (Mustafa and Kaur 2009). Pr1 and Pr2's extracellular activities were determined in *B. bassiana, M. anisopliae, Lecanicillium lecanii, Metarhizium flavoviride,* and *Nomuraea rileyi* (Liu et al. 2007). Also, degradation by amino peptidases and exopeptidases of solubilized proteins into amino acids is done to provide nutrients for entomopathogenic fungi (Mondal et al. 2016).

### 9.4.2 Lipase

Due to the great potential of lipases in commercial fields, studies on lipases, mainly of microbial origin, have increased in recent years. The epicuticle, the outer layer of the cuticle of the insect, is hydrophobic and acts as the first microbial attack barrier.

The main constituent of insect cuticles is a heterogeneous mixture of lipids, long-chain alkenes, esters, and fatty acids. Lipases are responsible for the hydrolysis of glycerol ester bonds, lipoproteins, and long-chain fatty acids found at the interior part of the insect integument (Mondal et al. 2016). Extracellular lipase activity is extra as compared to protease and chitinase; therefore, lipase can be taken into consideration as a crucial enzyme in the metabolic activities of *B. bassiana* (Hegedus and Khachatourians 1988). Secretion of pathogenic fungal lipases that significantly penetrate the cuticle may be linked to invasion of an insect host and then ease of nutrient absorption from host's nutrient source. The importance of lipases in the tegument penetration and breaking down process and defense mechanism in the insect has already been demonstrated (Silva et al. 2010). In *B. bassiana*, the Bbcyp52x1 gene and MrCYP52 gene cluster from *Metarhizium robertsii* encoded the lipase activity with an enzymatic complex (Zhang et al. 2012). Microorganism's lipase production varies not only from the source of the lipid but also from its concentration. In the initial stages of insect adhesion and penetration, the lipase secreted by entomopathogenic fungi was involved (Silva et al. 2005). Also, Dhawan and Joshi (2017) showed that the *B. bassiana* MTCC 4495 strain was more virulent to third instar larvae of *P. brassicae* that exhibited the highest levels of lipolytic activity. Apart from the role of fungal lipase in insect morality, fungal lipase also catalyzes numerous distinctive reactions which might be widely applied in more than one industry, which includes dairy and meals manufacture, leather-based and detergent industries, and manufacturing of cosmetics and pharmaceuticals, and natural synthesis reactions, mainly reactions in nonaqueous media (Akoh et al. 2007).

### 9.4.3 Chitinase

Chitin is a combined polymer of b-1,4 N-acetyl glucosamine and a principal structural component of cell walls of entomopathogen and invertebrate exoskeletons (Seidl 2008). Chitinases hydrolyze the b-1,4 bonds of chitin polymer, producing a foremost N,N′-diacetylchitobiose. That is accomplished via the breakdown of N-acetyl glucosamine (GlcNAc) monomer by using chitobiose. They collaborate with proteases to degrade the insect's cuticle (St Leger 1991) and are related with various stages of the life cycle of entomopathogenic fungi which includes germination, hyphal growth, morphogenesis, nutrition, and defense against competitors (Adams 2004). The genome of filamentous fungi consists of chitinases liable for diverse physiological functions along with (a) chitin degradation in the exoskeletons of arthropods used as nutrient assets; (b) remodeling of cell partitions at some stage in hyphae growth, branching, hyphae fusion, autolysis, and competence; and, (c) additionally, safety from different fungi placed within the equal ecological niche (Adams 2004; Yang et al. 2007). From *M. anisopliae*, the chit1 gene that encoded chitinase was first molecularly characterized (Bogo et al. 1998). Enzyme chitinases play a vital role in the process of insect cuticle degradation. Fungal virulence is often determined with the extracellular chitinases (Hegedus and Khachatourians 1996). From the growth media of *M. anisopliae*, *M. flavoviride*, and *B. bassiana*

supplemented with insect cuticle, production of enzymes chitinolytic, N-acetyl-b-D-glucosaminidase, and endochitinases was reported (St Leger 1991).

## 9.5 Factors Affecting Entomopathogenic Fungus Activity

Numerous environmental elements are recognized to inhibit fungal efficacy. The extensive version in the susceptibility to individual abiotic elements has been determined among and within fungal species and genera. Abiotic stresses together with ultraviolet (UV) radiation, humidity, high temperature, pH, and low water pastime result in inconsistent performances, restricting the effectiveness of the entomopathogenic fungi, delay pest mortality, and contribute to their constrained use in agricultural manufacturing. Sunlight and ultraviolet (UV) rays are probably the most detrimental environmental factor that affects the viability of entomopathogenic fungi and can reduce the process of infection. Radiation harms entomopathogenic fungi performance (Fang et al. 2012). UV radiation causes DNA damage through the induction of chemical base modification. It has also been found that UV-A radiation inactivates and delays the germination of certain fungi's conidia (Braga et al. 2008), while the exposure of UV-B radiation damaged or killed the spores of many entomopathogenic fungi (Goettel et al. 2001). A high temperature is another adverse factor in reducing the performance of the entomopathogenic fungi by reducing virulence and persistence in field conditions. It has been found that generally most of the entomopathogenic fungi germinate, grow, and sporulated at optimal temperatures between 20 and 30 °C. Fluctuation in the temperature range may influence the germination, growth, and performance of the fungus (Goettel et al. 2000; de Crecy et al. 2009). Further, for developmental stages of entomopathogenic fungi, high humidity is required. Low humidity has been implicated in failures of germination or field trials, by timing the application of fungi when humidity levels are naturally higher; these low-moisture problems can be addressed. Similarly, soil moisture, organic matter in the soil, and pH also are essential in figuring out infection level. Rainfall also can cause speedy and full-size loss of inoculum. For instance, 15 min of mild rainfall in a simulator resulted in approximately 90% elimination of *B. bassiana* conidia from foliage (Inglis et al. 2001). The diversity of entomopathogenic fungi varies according to the nutritional mode of insects from biotrophy to necrotrophy.

Apart from abiotic factors, a range of biotic factors such as the host stage of the pest, competitive microbial organisms, and antagonistic enzymes and compounds on the plant or host surface can also impose negative impact on the efficacy of entomopathogenic fungus. Immature stages of the insects tend to be more sensitive to fungal infection than the mature stage (Brownbridge et al. 2010). Insect behavior also can reduce the effectiveness of a fungal infection. Some termites avoid coming in contact with infected individuals within the colony and thereby escape infection (Chouvenc et al. 2008). The termite *Coptotermes lacteus* showed an avoidance response, walling off tunnels with *M. anisopliae*, thus protecting the colony from infection. Aphids and mites are sometimes able to escape infection by molting before the fungus enters the body (Alavo et al. 2002).

## 9.6 Genetic Engineering to Improve Virulence of EFP

Due to low virulence and inconsistencies in their performance compared to the chemical insecticides, entomopathogenic fungus currently has a small market share (Fang et al. 2012). The inconsistencies in performance within the field are mainly due to the sensitivity to environmental stresses of entomopathogenic fungi (Lovett and St. Leger 2015). Barriers to the large-scale application of fungal biocontrol agents still exist due to their slow killing speed and environmental stability issues. With growing public concern about the continued use of synthetic chemical insecticides and increasing public acceptance of genetically modified organisms, new types of genetically engineered biological insecticides offer a range of environmentally pleasant alternatives for cost-effective insect pest control. Genetic engineering has been demonstrated to enable the virulence efficacy of mycoinsecticides to be substantially advanced by improving their tolerance to damaging environmental stress. To improve virulence, genetic engineering has focused on reducing both lethal conidial dosage and killing time. Virulence enhancement has been carried out by engineering fungi to express insect proteins and insecticidal peptides from insect predators and other insect pathogens or by overexpressing the pathogen's genes. For genetic improvement in fungal virulence, the first trial was performed by engineering *Metarhizium* to overexpress the gene encoding the endogenous cuticle-degrading protease Pr1, and the resulting transformant took 25% less time to kill insects (Hu and St. Leger 2002). In another similar study in constitutive overproduction of chitinase, CHIT1 improved virulence by 23% in *B. bassiana* (Fang et al. 2005). An insect-selective scorpion neurotoxin peptide gene (AaIT) was synthesized and used to transform *M. anisopliae* for targeted expression in insect hemolymph. The expression results of this gene decreased the killing time with the aid of 40% lethal spore dose in mosquitoes, caterpillars, and beetles by up to 22-fold (Pava-Ripoll et al. 2008). The current information of molecular mechanisms for *B. bassiana* and *Metarhizium* spp. pathogenesis has enabled many gene-related pathogenicity like Pr1A, Bbchit1, Mr-Npc2a, ATM1, Mr-Ste1, and BbBqrA responsible for subtilisin-like protease, chitinase, sterol carrier, trehalase, esterase, and benzoquinone oxidoreductase, respectively, to be characterized, and these genes may be used as a genetic enhancement resource for entomopathogenic fungi. The virulence of *B. bassiana* was increased through the expression of endogenous insect genes, such as those for a diuretic hormone, neuropeptide, and serpin, to disrupt or inhibit normal hormone levels, electrophoretic behavior, or phenoloxidase activation in insects (Fan et al. 2012; Yang et al. 2012). The events of horizontal gene transfer (HGT) revealed that *Metarhizium* species, like other fungi, acquired various genes from bacteria and archaea and even arthropods, plants, and vertebrates (Zhao et al. 2014). By transferring the sterol carrier gene into *B. bassiana*, this evolutionary event was reproduced, lacking an endogenous Mr-NPC2a homologue, and enhances its pathogenicity (Ortiz-Urquiza et al. 2013).

### 9.6.1 Improve Virulence Against the Abiotic Stresses

Among the abiotic stresses, UV radiation, high temperature, and low water activity cause prominent damage to molecular and metabolic functions of the pathogenic fungus that results in inconsistent limiting performances in the field. The range of genes and metabolic pathway genes like trxA (thioredoxin), Try (tyrosinase), MrPhr1 (CPD photolyase), BbSOD1 (superoxide dismutase), HsPHR2 (CPD photolyase), and heat shock protein 25 (HSP25) have been found suitable for improving virulence in *Metarhizium, Beauveria*, and other insect pathogens against the abiotic stresses (Zhao et al. 2016). The engineering of *Beauveria* or *Metarhizium* to overexpress a DNA repair photolyase also increased fungal resistance to solar radiation (Fang and Leger 2012). Expression of thioredoxin (trxA) also improved the tolerance of *B. bassiana* against heat, UV-B irradiation, and oxidation (Ying and Feng 2011). In another study, a look at genetically engineering *B. bassiana* with an exogenous tyrosinase gene expanded fungal production of melanins for stepped forward conidial tolerance to ultraviolet radiation and elevated virulence in opposition to diverse insects (Shang et al. 2012). The integration of a PKS gene cluster for melanin biosynthesis from a plant pathogen to *M. anisopliae* enabled the fungus to resist UV irradiation and improved fungal virulence. To improve the virulence against high temperature, *M. robertsii* was genetically transformed to overexpress an endogenous heat shock proteins (HSPs)-encoding gene (Hsp25), and the results showed that mutant gene not only survived extreme temperatures but also showed resistance to oxidative stress and osmotic agents (Liao et al. 2014). Thus, genetic engineering is an effective way to improve fungal environmental stability and, therefore, the efficacy of field applications. Combining the available genomes of *Bassiana* and several *Metarhizium* species in the future, with robust genetic manipulation technologies, will make it possible to characterize the full range of pathogenicity and host-specific genes by which novel combinations of insect specificity and virulence will be created (Gao et al. 2011; Xu et al. 2014). Depending on the regulations in different countries, developing these genetically modified strains will potentiate the cost-effective application of mycoinsecticides for the control of different insect pests or disease vectors.

## 9.7 Entomopathogenic Fungi and Integrated Pest Management Strategies

For plant protection from harmful pests and insects, chemical insecticides are commonly used which impose a negative impact on the ecosystem and increase the resistance of insects to various chemical substances. Therefore, this issue forces to seek an alternative new effective eco-friendly approach of reducing outbreaks of insects or pests. In current years, more interest is paid to the opportunity of the usage of natural insect enemies, which include entomopathogenic fungi, in biological control or inhibition of insect pests (Sahayaraj 2014). Entomopathogens may be a vital tool in IPM techniques in both natural and conventional manufacturing

systems. There are numerous examples of entomopathogen-based bio-insecticides that have performed a critical role in pests control. EPF has been extensively investigated for the biological control of pests as well as human and animal disease-causing arthropod vectors (Blanford et al. 2011; Paula et al. 2013; Nana et al. 2015). Entomopathogens may be used alone or in combination with chemicals, botanical pesticides, or other entomopathogens depending on the crop, pest, and environmental conditions.

The most prominent instance of EPF inoculation biological control is the use of *Beauveria brongniartii* against *Melolontha melolontha*, European cockchafer beetles, that's a critical pest of pastureland, orchards, and wooded area and trees in Central Europe. The fungal application can provide useful control levels for up to 9 years (Keller et al. 1997). An IPM research in Californian strawberries validated the capacity function of entomopathogenic fungi for handling the western tarnished plant bug (*Lygus hesperus*) and other insect pests (Dara 2016). The combination of azadirachtin and *B. bassiana* reduced root aphid of rice and populations of honeysuckle aphid by 62% in natural celery in California (Dara 2015). Development of formulations can greatly improve the activity and performance of entomopathogenic fungi. Since the 1960s, worldwide, among the 171 products, a considerable number of potential formulation have been developed of which 33.9% is from *Metarhizium* spp., 33.9% from *Beauveria* spp., 5.8% from *Isaria fumosorosea*, and 4.1% from *B. brongniartii*, in which three-quarters are currently commercially available for the targeted Hemiptera, Coleoptera, Lepidoptera, Diptera, Orthoptera, and Acari pests. Some commercial formulations are illustrated in Table 9.2. These organic formulations used in augmentation, classic, and conservation biological control pose a minimum threat to useful organisms and hence are in all likelihood to be categorized as "low-danger" substances by authorities regulators.

Also, beneficial EPF is known to elicit induced systemic resistance (ISR), which emerged as another potential mechanism through which entire plant accelerates own defense system against a wide range of insects/pest pathogens (Pieterse et al. 2014). *Beauveria bassiana*-inoculated plant showed significantly reduced bacterial blight disease on the leaves compared with the noninoculated control plants. Similarly, in another study, *Lecanicillium*-pre-inoculated cucumber plants significantly induced systemic resistance against *P. ultimum* and powdery mildew *S. fuliginea* (Benhamou and Brodeur 2001; Hirano et al. 2008). Later *B. bassiana* and *Lecanicillium* spp. colonization of date palm plants showed upregulation of defense-related proteins for alleviation against the plant biotic stress (Gómez-Vidal et al. 2009). Hartley et al. (2015) and Khan et al. (2012) reported that fungal endophytes and *M. anisopliae*-inoculated plants secret isoflavonoid phytoalexin's bioactive secondary metabolites which play a major role in the adaptation of plants to different adverse environmental condition. In a recent study, colonization of *B. bassiana* in tomato plants has shown a significant positive effect on terpenoid accumulation in tomato plants compared with arbuscular mycorrhizal fungus *Rhizophagus intraradices* colonization (Shrivastava et al. 2015).

By enhancing plant growth, fungal entomopathogens can contribute to protecting their host plant against disease pathogens. A developing variety of research has

recently proven the ability of several fungal entomopathogens to promote plant growth following endophytic establishment (Sasan and Bidochka 2012; Lopez and Sword 2015; Jaber and Enkerli 2016). Kuldau and Bacon (2008) reported that EPF-primed plants increased plant growth with suppression of various abiotic and biotic stresses. Jaber and Salem (2014) state that when plant is challenged with zucchini yellow mosaic virus (ZYMV), *B. bassiana*-inoculated plants not only reduced the severity of disease incidence but also significantly showed faster growth and development in plants compared to control. The same has been demonstrated in *M. robertsii*-inoculated plants that reduced disease indices and enhanced plant growth upon challenge inoculums of *F. solani*, through the production of plant growth-promoting attributes like phytohormones or siderophores (Sasan and Bidochka 2013). This evidence is supported by the study of Khan et al. (2012), where *M. anisopliae* significantly supports soybean plant's health and growth through the production of phytohormones in inoculated plants. Furthermore, Sánchez-Rodríguez et al. (2015) reported that entomopathogenic-fungi-induced plant growth promotion might be due to the increased uptake and transportation of nutrients (Behie and Bidochka 2014).

## 9.8 Conclusions, Future Opportunities, and Challenges

Due to increasing insect pest disease problems in agriculture and environmental concerns, myco-biocontrol of insects has been viewed as a substantial significant substitute for synthetic chemical pesticides and a key component of eco-friendly pest management. Understanding the ecological and environmental parameters associated with insect-pathogenic fungi is important in advancing our basic knowledge about the evolution and maintenance of pathogenesis of these organisms as well as in field applications for biological control of insect pests. Entomopathogenic fungi are a unique and enormously specialized group of microbial agents that possess several desirable traits favoring their development as biopesticides. However, the majority of the commercially produced fungi are handiest based on a few species of *Metarhizium, Beauveria, Lecanicillium, Isaria,* etc. In addition to regulatory issues relating to the premarket authorization of new active substances, the availability of entomopathogens bio-processed products is still restricted to certain crop pests. Indeed, a better understanding of the ecology of fungal entomopathogens would induce the development and uptake of more commercially available biopesticides based on these fungi in mainstream farming. Furthermore, to encourage the extensive use of fungal entomopathogen-based biopesticides, there is a need for microbial products with activity against multiple pests in addition to their genetically modified insecticidal efficacy, improved delivery methods, and increased persistence. Following these trends, as a result, researches and industrial interest on entomopathogenic fungi are required, and new discoveries with increased compatibility, maximized efficacy, increasing investments, and expansion of entomopathogens formulations for integrated pest management are expected in the near future.

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# Role of Microbiotic Factors Against the Soil-Borne Phytopathogens

10

Nasreen Musheer, Shabbir Ashraf, Anam Choudhary, Manish Kumar, and Sabiha Saeed

**Abstract**

Phytopathogenic association with beneficial microbiotic factors influences rhizospheric soil as well as plant growth parameters. Rhizospheric microbiotic factors check nutrients to supplement the lethal sensitivity against soilborne phytopathogens. All microbes besides harming plant growth are also able to reduce or check infection or disease caused by phytopathogens. Each microorganism showed specific antagonistic mechanisms against specific phytopathogens. This chapter discussed the importance of nematodes belonging to order Aphelenchida and Tylenchida which proved to be good management model organisms to inhibit or kill phytopathogens just like plant growth, promoting bacteria and fungi. Beneficial microbes protect plants from a greater extension of damage and induced plant vigor, growth, and development. Besides the beneficial role of microbiotic factors interacting with plants against soilborne phytopathogens present in soil ecology, it can also helps to develop products for agricultural biotechnology, biofertilizers, plant strengtheners, phytostimulation, and biopesticides. This chapter appraises the importance of microbiota factors and their mechanisms against soilborne phytopathogens.

**Keywords**

Arbuscular mycorrhiza · Biocontrol · Microbiome · Plant pathogens · Soil

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## 10.1 Introduction

Soil ecosystem is full of microbiotic communities that help to improve plant sustainability and control soilborne phytopathogens through different kinds of mechanisms (Ahemad et al. 2009; Patil and Solanki 2016a, b; Solanki et al. 2016). Some of the beneficial effects observed are the following:

1. Stimulates plant growth
2. Increased nutrient mobilization through plant's root from surrounding soils
3. Induced plant growth regulators
4. Activated defense system of plants against phytopathogenic infections
5. Improved topsoil properties through bioremediation by sequestering soil pollutants such as toxic heavy metal and xenobiotic residues

Beneficial plant–microbe interaction was found to be of great importance for both plant- and soil-ecosystem functions over plant breeding (Smith et al. 1999). Soil ecosystem association with rhizo-microbiotic factors helps to reduce plant pathogenic disease stress over plants and promotes growth by enhancing nutrient uptake facilities (Thakore and Ehlers 2006; Solanki et al. 2019b). The improved soil ecosystem approach helps in the management of soilborne phytopathogens sustainably by increasing disease resistance, crop production, and quality (Ahemad et al. 2009; Solanki et al. 2012a, b; Singh et al. 2014). *Trichoderma* species were found to be used most widely as fungal biocontrol agents against many phytopathogens and played a significant role in bioremediation and soil restoration (Solanki et al. 2011, 2018, 2019a; Rai et al. 2019). Under field conditions, *Trichoderma* spp. applied as conidia and mycelia biomass showed great stability and viability (Rosane et al. 2008). Several plant growth-promoting rhizobacteria (PGPR) flourished rhizospheric soil and plant root and stimulate various mechanisms against soilborne pathogens to prevent plants from infection and diseases (Patil and Solanki 2016a; Kashyap et al. 2019; Kumari et al. 2019). It includes competitions, phytohormones, secondary active metabolites which increased nutrient solubilization and mobilization, availability, and activation of physical, chemical, and active defense responses in the host against their antagonists (Hammerschmidt and Kuc 1995; Vessey 2003; Solanki et al. 2012a, b, 2014, 2015, 2017; Kumar et al. 2012; Wang et al. 2017). The present chapter enlightens the importance and role of microbiotic factors against soilborne pathogens causing various plant diseases and the definition of the microbiome, biocontrol agents (bacterial, fungal, and nematodes), soilborne phytopathogens, and mode of action of biocontrol agents, mechanisms, and future approaches.

## 10.2 Microbiome

Microbiome or rhizosphere is the specific microenvironment that includes plant roots, soil niche, and plant microbes, viz., protozoans, filamentous fungi, viruses, bacteria, actinomycetes, and nematodes, which formed an association (Hiltner 1904; Saunders et al. 2010). Microbes compete for water, nutrients, and space and

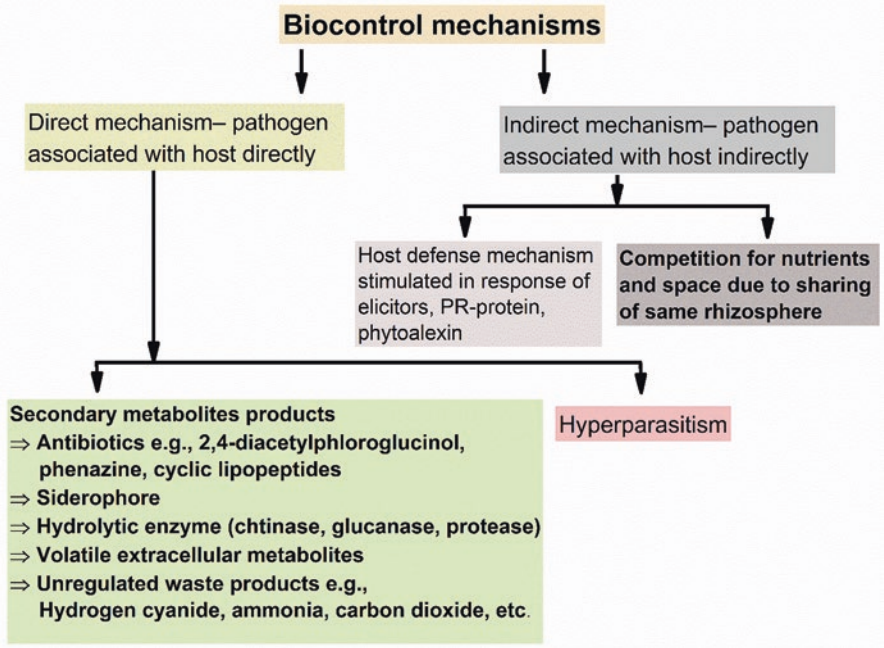


**Fig. 10.1** Different mechanisms of biocontrol that are involved in plant disease management

sometimes improve microbial attractiveness toward plant roots more than phytopathogens (Cook and Baker 1983; Whipps 2001). Microbe colonization is established generously in all plant-associated microenvironments, especially rhizospheric region and root (Malviya et al. 2019; Solanki et al. 2019b). Berg et al. (2006) performed an in vitro assessment of plant-associated microbial isolates between 1% and 35% exhibited in antimicrobial properties. The narrow zone of soil around the plant's root is rich in nutrients due to the secretion of a variety of chemical constituents by plants. Root exudates may contain some protein and sugars. It promotes bacteria growth around the nutrient-rich zone (Gray and Smith 2005). Therefore, rhizospheric microorganisms are responsible for increasing plant vigor in an environmentally and eco-friendly manner (Leach et al. 2017; Igiehon and Bbaloal 2018). Solanki et al. (2018) assessed that tomato plant root extract played a significant role in the antagonism of *Bacillus*, *Streptomyces*, and *Trichoderma* against *Rhizoctonia solani* in the soil microecosystem (Fig. 10.1.).

## 10.3 Soilborne Phytopathogens and Their Causes in Plants

Soil ecosystem is a sophisticated resident of microflora, and their effect may be neutral, positive, or negative toward plants (Thrall et al. 2007). Complex microbial interactions form between (1) soilborne pathogens and their host and (2) pathogens and soil health. Mostly plant microbiota known as "phytobiota" survive in or near the soil and affect plant vigor, for example, cotton seed association with *Epicoccum*

*nigrum* or its exudate improves the seed germination and seedling growth against *Pythium* damping off and root rot (Hashem and Ali 2004; Nelson 2017). Moreover, soilborne diseases are often much influenced by soil conditions, even in the absence of their host. Well-known soil-borne pathogens, e.g., *Phytophthora* spp., *Pythium* spp., *Rhizoctonia* spp., *Sclerotinia* spp., *Fusarium* spp., etc., affect the number of important crops, including wheat, cotton, vegetables, and temperate fruits (Mihajlovic et al. 2017; Patil and Solanki 2016b; Thaware et al. 2017). These pathogen inoculums survive in soil as microsclerotia, sclerotia, chlamydospores, or oospores. World agriculture production is mostly affected by microbes and caused yield losses of approximately 20% and 40% (Oerke 2006; Wild and Gong 2010). Fusarium wilt pathogen was estimated to induced 50–75% yield loss in many crops (Tiwari et al. 2018). Soilborne phytopathogens initiate specific symptoms when shown compatible association (Singh et al. 2019). Various symptoms are produced by pathogens on plants which included decaying and flaccid root, withering and drooping of plants, and flaccid, yellowing, stunting, bark cracking, twig or branch dieback, etc. (Horst 2001; Patil and Solanki 2016b) (Table 10.1 and Fig. 10.2).

**Table 10.1** Soilborne pathogen association with host crop and caused yield loss

| Host | Pathogen | Yield loss | References |
|---|---|---|---|
| Cereals (wheat barley, oats, rye, rice) | *Heterodera avenae* (cereal cyst nematode) | 10% | Ali et al. (2019) |
| Ginger | *Ralstonia solanacearum* (bacterial wilt) | 51.4–51.9% | Guji et al. (2019) |
| Wheat | *Gaeumannomyces graminis* var. *tritici* (take all disease of wheat) | 2–92% | Ramanauskienė et al. (2019) |
| Soybean | *Rhizoctonia solani* | 48–52% | Chang et al. (2018) |
| Chickpea | *F. oxysporum* f. sp. *ciceri* | 34–57.33% | Murali et al. (2018) |
| Lentil | *Fusarium oxysporum* f. sp. *lentis* (wilt) | 50% | Tiwari et al. (2018) |
| Mung bean | *Fusarium oxysporum* | 80% | Kelly (2017) |
| Tomato | Root knot nematode (*Meloidogyne* spp.) | 11–35% | Manjunatha et al. (2017) |
| Tomato | *Fusarium oxysporum* f. sp. *lycopersici* | 10–50% | Ghazalibiglar et al. (2016) |
| Brinjal | *Sclerotium rolfsii* (foot and root rot) | 30–50% | Siddique et al. (2016) |
| Chickpea | *F. oxysporum* f. sp. *ciceri* | 48.29% | Thaware et al. (2015) |
| Turmeric | *Pythium* spp. | 74.50% | Anoop et al. (2017) |
| | *Fusarium* spp. | 30.51% | |
| | *Rhizoctonia* spp. | 28.80% | |
| Tomato | *Rhizoctonia solani* | 67–87% | Solanki et al. (2012a, 2014) |
| Bean | *Fusarium decline disease* | 52% | Saremi et al. (2011) |

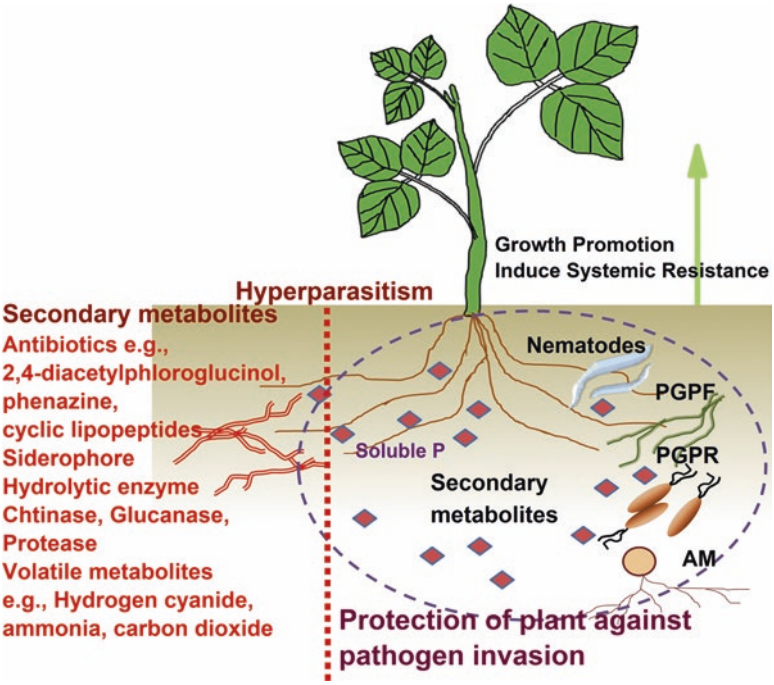


**Fig. 10.2** Rhizosphere shows the interaction between beneficial microbes (plant growth-promoting fungi (PGPF), plant growth-promoting rhizobacteria (PGPR), and arbuscular mycorrhiza (AM)) and protection of plant against the pathogens, and under the rhizosphere microbes perform several activities that played a significant role in disease management such as nutrient competition, mycoparasitism, siderophores, antibiotics, and phytohormones

## 10.4 Biocontrol Agents Mechanisms Employed Against Soilborne Phytopathogens

Biological control agents (BCA) are natural enemies that are used to control the activity of virus, bacteria, fungi, and protozoans (Saunders et al. 2010). Functional interaction or association between bioagents and pathogenic soil microorganisms led to reduced root infection by destroying various infection propagules such as conidia, chlamydospores, zoospores, mycelium, and egg mass (Beneduzi et al. 2012; Hassani et al. 2018). Therefore, the additive effect of antagonistic biocontrol agents was found to be more efficient in declining of multiple soilborne diseases (Mazzola and Freilich 2016). Biocontrol agents are a nonpathogenic and an environmentally alternative safe method. Some bioagents show dominance over chemical for plant protection against their pathogens below or up to threshold level (Neshev 2008). However, commercialization of BCAs followed Central Insecticide Board (CIB) regulatory that provide registration and permit their commercial use after reviewing the submitted reports for registration. It is an important point about the bioagents that can reduce harmful effects of some pathogens below a certain

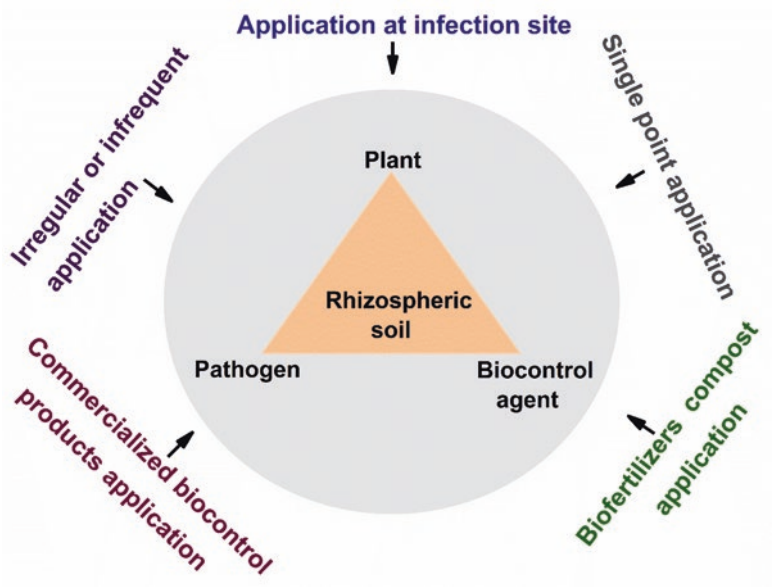


**Fig. 10.3** Rhizospheric soil association with plant, pathogen, and biocontrol agents

threshold with no substantial changes in soil microbiological balance, something that does not occur when chemicals are applied (Neshev 2008) (Fig. 10.3 and Table 10.2).

## 10.5 Biocontrol Agent's Mechanisms and Their Mode of Application

Soil rhizosphere and the plant' root exudates attracts a group of the mixed microbial community including *Trichoderma* spp., yeast, PGRP, nematodes, etc. (Druzhinina et al. 2011; Lombardi et al. 2018). *Trichoderma* is a free-living ubiquitous antagonistic filamentous fungi that is mostly found in soil ecosystem (Harman et al. 2004). It is capable of producing different antimicrobial compounds that help in plant growth promotion and defense management. Biocontrol agents have strong survival ability and high reproductive rate than phytopathogens (Singh et al. 2016). *Trichoderma* shows bioefficacy against several fungal seeds or soilborne pathogenic fungi such as *Fusarium*, *Pythium*, *Macrophomina*, *Verticillium*, *Rhizoctonia*, etc. (Peteira et al. 2001; Smitha et al. 2014). Among various fungal bioagents, yeast is also found commonly in soil ecosystem with variable soil texture, composition, humidity, pH, and diverse climatic conditions (Yurkov 2018). Yeast was found in association with certain plant roots and exhibited as biostimulants against many soilborne pathogens, e.g., *Arabidopsis thaliana*, stimulate a defensive response against *Botrytis cinerea* and increase soil sporulation of arbuscular mycorrhiza

**Table 10.2** Biocontrol agents and their agro-products applied to different crops to protect against the pathogens

| Biocontrol agents | Commercial name | Target organism | Crop | Manufacturer company name | References |
|---|---|---|---|---|---|
| *Bacillus subtilis* FZB24 | Rhizo-Plus Rhizo-Plus Konz | *Rhizoctonia*, *Fusarium*, *Verticillium* and *Streptomyces* | Vegetables and ornamental plants | FZB Biotechnik, GmbH | Shukla et al. (2019) |
| *Pseudomonas chlororaphis* | Cedomon | Soilborne pathogenic fungi | Barley and oat | BioAgri AB | Shukla et al. (2019) |
| *Burkholderia cepacia* | Deny | Soilborne pathogenic fungi | Vegetables | Stine microbial products | Shukla et al. (2019) |
| *Pseudomonas aureofaciens* strain TX-1 | Bio -jet, spotless | *Rhizoctonia solani*, *Pythium* | Vegetables and ornamentals in greenhouses | EcoSoil system | Tsegaye et al. (2018) |
| *Trichoderma viride* | Trichostar | Collar/root/ stem rot, wilt, damping off | Vegetables and pulses | Super Agro India, | Kumar and Sarma (2016) |
| *T. harzianum* | Tricha | *Pythium* rot, *Rhizoctonia* rot, *Fusarium* wilt, *Botrytis* rot, and *Sclerotium*, *Sclerotinia*, *Ustilago* | Food and ornamental crop | Balaji Crop Care Pvt. Ltd., India | Kumar and Sarma (2016) |
| *P. fluorescens* | Greenmax | *Rhizoctonia solani*, *Pythium ultimum* | Paddy, oilseed, pulses, and vegetables | GreenMax AgroTech, India | Kumar and Sarma (2016) |
| *B. subtilis* strain GB34 | GB34 | *Rhizoctonia*, *Fusarium* | Soybean | Gustafon, USA | Junaid et al. (2013) |
| *B. subtilis* strain GB 03 | Kodiac, companion | *Rhizoctonia*, *Aspergillus* | Wheat, barley, peas | Growth products, USA | Junaid et al. (2013) |
| *P. aureofaciens* strain TX-1 | Bio -jet, spotless | *Rhizoctonia solani*, *Pythium* | Vegetables and ornamentals greenhouses | EcoSoil system | Junaid et al. (2013) |
| *T. harzianum* T-22 | Root shield, plant shield | Soil-borne pathogens | Greenhouse nurseries | Bio works, USA | Junaid et al. (2013) |

(continued)

**Table 10.2** (continued)

| Biocontrol agents | Commercial name | Target organism | Crop | Manufacturer company name | References |
|---|---|---|---|---|---|
| *Gliocladium catenulatum* strain JI446 | Prima stop soil guard | Soilborne pathogens | Vegetables, herbs, spices | Kemira Agro Oy, Finland | Junaid et al. (2013) |
| *B. pumilus* GB34 | Yield shield | Soil-borne fungal pathogens | Legumes, cereals grains, soybean, cotton, peanuts | (Gustafson); Bayer Crop Science | Berg (2009) |
| *P. syringae* | Bio-save | Yeast and bacteria | Apple, pear, potato, lemon | JET Harvest, Longwood, FL, USA | Janisiewicz and Peterson (2004) |
| *T. harzianum* T-22 | Trianum-P | *Pythium* spp., *Rhizoctonia* spp., *Fusarium* spp., and *Sclerotinia* spp. | Vegetables, soft fruits, cereals, corn | Koppert Biological Systems partners with nature | |

(Ferreira-Saab et al. 2018). *Yarrowia lipolytica* (yeast) is nonpathogenic and enables to clean the environment from oil spill contamination (Goncalves et al. 2014). Yeast helps in nitrogen, sulfur oxidation, and phosphorus solubilization near the growing plants. Oxidation and solubilization of macronutrients easily intake by plants that help to enhance the health and also protect from the soilborne pathogen infection (Fu et al. 2016; Alonso et al. 2008). El-Tarabily (2004) studied the sugar beet root rot caused by *Rhizoctonia solani* and documented that the disease is biologically controlled by using yeasts, like *Rhodotorula glutinis*, *Trichosporon asahii*, and *Candida valida*. El-Mehalawy et al. (2004) reported that various yeast species, e.g., *Candida*, *Trichosporon*, *Rhodotorula*, *Saccharomyces*, etc., establish antagonistic activity against soilborne fungal pathogens. These all were used either independently or in combination to reduced late-wilt disease occurrence on maize and bean caused by *Cephalosporium maydis*. *Kazachstania exigua*, *Saccharomyces cerevisiae*, *Clavispora*, and *Candida* strains exhibited antimicrobial activity against *Penicillium italicum* (Perez et al. 2016). Beneficial fungal microbiota shows specific mechanism against target pathogens present in or near the soil and in or on the infected host. Several mechanisms are shown by fungal antagonist microbes such as competitions, antibiosis, mycoparasitism or hyperparasitism, lytic enzymes, hydrogen cyanide, defense response, and plant growth hormone (Zaidi and Singh 2013; Al-Naemi et al. 2016). Protists were found to enhance plant growth by increasing plant root's nutrient absorption and maintaining the soil microbiomes by parasitizing soil bacteria, fungi, and other eukaryotic organisms (Geisen et al. 2018). Macias et al. (2018) demonstrated that sucrose-mediated tomato root association with *Trichoderma atroviride* promotes growth and stimulates antagonistic biota against *Phytophthora cinnamomi* (Table 10.3).

**Table 10.3** Biocontrol agents producing secondary metabolites against pathogens

| Biocontrol agents | Target organisms | Secondary metabolites | References |
|---|---|---|---|
| *Bacillus velezensis* | Soilborne phytopathogens | Lipopeptides (i.e., surfactin, bacillomycin-D, fengycin, and bacillibactin) and polyketides (i.e., macrolactin, bacillaene, and difficidin). | Rabbee et al. (2019) |
| *Bacillus velezensis* | *Ralstonia solanacearum and Fusarium oxysporum* | Lipopeptide compounds (surfactin, iturin, and fengycin) | Cao et al. (2018) |
| *Trichoderma harzianum* | *Rhizoctonia solani* | Harzianic acid | Manganiello et al. (2018) |
| *Bacillus amyloliquefaciens* | *Fusarium graminearum* | Bacillomycin D | Gu et al. (2017) |
| *Trichoderma asperellum* ZJSX5003 | *Fusarium graminearum (corn stalk rot of maize)* | Cell wall-degrading enzymes (chitinase, protease b-glucanases, and peptaibols) | Li et al. (2016a, b) |
| *Bacillus amyloliquefaciens* SQR-9 | *Ralstonia solanacearum* | Volatile organic compounds (VOCs) | Raza et al. (2016) |
| *Trichoderma harzianum* | *Aspergillus flavus* | 2-Phenylethanol | Chang et al. (2015) |
| *Streptomyces vinaceusdrappus S5 MW2* | *Rhizoctonia* root rot in tomato | Chitinase | Yandigeri et al. (2015) |
| *Streptomyces glauciniger* | *F. oxysporum* | Chitinase | Awad et al. (2014) |
| *Bacillus subtilis* (B-CM191, B-CV235, B-CL-122) | *Fusarium oxysporum* f. sp. *ciceri* (wilt) | Indole acetic acid, siderophore, phosphate solubilization, and hydrolytic enzymes | Singh et al. (2014) |
| *Pseudomonas fluorescens* MPF47 | *Rhizoctonia* root rot in tomato | Indole acetic acid, siderophore, phosphate solubilization, and hydrolytic enzymes | Solanki et al. (2014) |
| *Arthrobacter agilis* UMCV2 | *Botrytis cinerea and Phytophthora cinnamomi* | Dimethyl hexadecylamine (antibiotics) | Velázquez-Becerra et al. (2013) |
| *Bacillus alvei* NRC 14 | *Fusarium oxysporum* | Lytic enzymes | Abdel-Aziz (2013) |
| *Bacillus subtilis* strain BS-99-H | Gaeumannomyces graminis var. tritici | Phenazines | Recinosa et al. (2012) |

(continued)

**Table 10.3** (continued)

| Biocontrol agents | Target organisms | Secondary metabolites | References |
|---|---|---|---|
| *Lysinibacillus fusiformis B-CM18* | Growth inhibition of *Fusarium oxysporum* f. sp. *ciceri*, *F. solani*, *F. oxysporum* f. sp. *Lycopersici*, and *Macrophomina phaseolina* | Chitinase | Singh et al. (2012) |
| *Bacillus amyloliquefaciens* MB101 | *Rhizoctonia* root rot in tomato | Mycolytic enzyme production and plant defense activation | Solanki et al. (2012a, b) |
| *Pseudomonas fluorescens* F113 | Soilborne phytopathogens | 2, 4-Diacetyl phloroglucinol | Couillerot et al. (2011) |
| *Trichoderma asperellum* UTP-16 | Growth inhibition of *Fusarium* spp. | Chitinase | Praveen Kumar et al. (2012) |
| *Trichoderma/Hypocrea* spp. | *Rhizoctonia* root rot in tomato | Hydrolytic enzymes | Solanki et al. (2011) |
| *Trichoderma* spp. | *Botrytis cinerea*, *Rhizoctonia solani*, *Phytophthora citrophthora* | Hydrolytic enzymes (β-1,6-Glucanases) | Druzhinina et al. (2011) |
| *Bacillus subtilis* strain BS-99-H | *Botrytis cinerea* and *Rhizoctonia solani* | Iturin A | Lin et al. (2010) |
| *Rhizobacteria* | *Fusarium* wilt | Chitinase | Siddiqui et al. (2009) |
| *Bacillus licheniformis* | *Gibberella saubinetii* and *Aspergillus niger* | Chitinase | Xiao et al. (2009) |
| *Pseudomonas* PGC2 | *Rhizoctonia solani* and *Phytophthora capsici* | Chitinase, β-1,3-glucanase, protease, etc. | Arora et al. (2008) |
| *Bacillus* and *Pseudomonas* | Phytopathogens | Chitinase | Yu (2008) |
| *Trichoderma viride* | *Sclerotium rolfsii* | Antibiotic pyrone (Viridepyronone) | Evidente et al. (2003) |

### 10.5.1 Biocontrol Agent Produces Active Secondary Metabolites Against Phytopathogens

Biocontrol agents produce active secondary metabolites against several soilborne phytopathogens such as *Botrytis cinerea* and *Rhizoctonia solani* (Kloepper et al. 2004). Plant root exudates are the main attractants that induced signals and later recognized by beneficial microbes to increase microbial colonization. Beneficial microbes antagonized directly to the soilborne phytopathogens by some mechanisms include (1) production of antibiotics, toxins, and cell wall-degrading enzymes such as chitinases and $\beta$-1,3-glucanase; (2) competition for establishment and supplement of macronutrients and micronutrients, e.g., increase siderophore iron-chelating microbes that help in iron uptake of phytosystems; and (3) toxin production

degrades pathogenicity factors of pathogens (Thangavelu and Mustaffa 2012; Zaidi and Singh 2013; Hassani et al. 2018; Yandigeri et al. 2015). A recent study by Van Agtmaal et al. (2018) shows confirmation about the microbial community of rhizosphere-induced natural volatile organic compounds that cause suppression of phytopathogens. *Trichoderma* spp. are found to produce various metabolites: (a) volatile antibiotics and toxins like trichothecene and a sesquiterpene, trichodermin, (b) hydrophilic compounds heptelidic acid or koningic acid, and (c) peptaibols (Musoni et al. 2015; Marik et al. 2018; Gebarowska et al. 2019).

#### 10.5.1.1 Antibiosis

Many microbes produce and secrete a simple or multiple forms of toxic compounds with antimicrobial properties at low concentrations (Homma et al. 1989). Lanteigne et al. (2012) reported that *Pseudomonas* sp. produced HCN and DAPG antibiotics which help to suppress the infection of black root rot bacterial canker disease of tobacco caused by *Thielaviopsis basicola* and *P. fluorescens;* BL915 inhibited the tomato bacterial canker caused by *Clavibacter michiganensis*. Iturin-A is a lipopeptide poisonous antibiotic and can kill or inhibit the growth of numerous pathogens at low concentration (Meena and Kanwar 2015). Biosynthesis of antibiotics demonstrated the antagonistic association between microbes and soilborne pathogens (Raaijmakers and Mazzola 2012; Schulz-Bohm et al. 2017). *Coniothyrium minitans* sclerotia are producing phenazines antibiotics attack on fungal hyphae of *Pythium oligandrum* (Thomashow et al. 1990; Perez et al. 2016). For instance, *Sporothrix flocculosa* and *Sporothrix rugulosa* liquid culture produced heptadecenoic and methyl-heptadecenoic acid antibiotics with antimycotic and antibacterial activity (Choudhury et al. 1994; Benyagoub et al. 1996). Both *Botrytis* sp. and *Fusarium oxysporum* sp. spore germination and biomass production were reduced in the presence of antibiotic produced by *Salvia flocculosa* (Hajlaoui et al. 1994). In vitro studies showed that yeasts *Saccharomyces* and *Zygosaccharomyces* were found to inhibit the growth of soilborne fungal plant pathogens such as *Rhizoctonia fragariae*, *Sclerotinia sclerotiorum*, and *Macrophomina phaseolina* due to the release of volatile antifungal constituents (Zakaria 2018).

#### 10.5.1.2 Siderophores

Siderophores are iron carrier called iron-chelating compounds that are produced by rhizospheric microbes. Siderophore scavenging bound form of iron ions and solubilize around the host plants that facilitate the growth and vital physiological functions of plants, e.g., photosynthesis, respiration, translation, and transcription under stress conditions (Ahmed and Holmstrom 2014; Sah and Singh 2015). Fungal siderophores like fusigen, fusarine A, and ferricrocin stop the availability of iron for various pathogenic microorganisms but for plants scavenging more iron in a solubilized form that help to promote plant growth (Verma et al. 2011; Vinale et al. 2014). There are some reports which showed that most of the fungal species are also capable of producing iron-chelating siderophore under aerobic conditions except the *Saccharomyces* species (Andrey 2018). Fungi belonging to class zygomycetes produced hydroxamate-type (coprogens, fusigen, fusarine A, and ferricrocin) and

carboxylate-type (rhizoferrin) siderophores (Lehner et al. 2013; Sah and Singh 2015). Moreover, some yeast species, *Candida* sp. and *Rhodotorula* sp., produced siderophores named as rhodotorulic acid that have an inhibitory effect on spore germination of *B. cinerea* that caused gray mold disease on apple wound (Sansone et al. 2005). Yeast antimicrobial compounds were tested against the *Corynespora cassiicola* and *Botrytis cinerea* fungal pathogens of *Theobroma cacao* fruits (Ferreira-Saab 2018).

### 10.5.2 The Enzymatic Action of Biocontrol Agents Against Phytopathogens

Transgenic plants carry genes for endochitinase enzyme activity. In such type of plants, resistance develops against attacking fungal phytopathogens as well as environmental stress (Smith and Osburn 2016; Toufiq et al. 2017). *Trichoderma* spp. and yeast species are capable of producing β-1,3-glucanase and chitinase enzymes that initiated lysis in the cell wall of pathogen and thus leads to cytoplasmic leakage and cause death (Srivastava et al. 2014). Yeast strains, e.g., *Pichia* sp., *Rhodotorula* sp., *Cryptococcus* sp., *Aureobasidium* sp., and *Tilletiopsis* sp., exhibited to produce β-1,3-glucanase enzyme against fungal test pathogens such as *Botrytis cinerea*, *Penicillium expansum*, *Rhizoctonia stolonifer*, *Aspergillus niger*, *Sphaerotheca fuliginea*, and *Puccinia xanthii* (El-Tarabily 2004; Hartmann et al. 2010). Chatterton and Punja (2009) stated that *Clonostachys rosea* f. sp. *catenulate* produced both chitinase and β-1,3-glucanase enzymes and showed a deterioration of mycelia of *Fusarium* and *Pythium* pathogens which caused root rot, stem rot, and damping-off diseases in cucumber plants. *Aureobasidium pullulans* 1WA1 stimulated secretion of both endo- and exo-β-1,3-glucanases in the presence of *Botrytis cinerea* which caused gray mold disease of grapevine (Bauermeister et al. 2015). *Bacillus subtilis*, *Bacillus pumilus*, *Bacillus megaterium*, and *Agrobacterium radiobacter* had showed antifungal activity by secreting chitinase, glucanase, and protease enzyme against citrus and blue mold incited by *Penicillium digitatum* (Mohammadi et al. 2017). Chitosan exhibited a suppressive effect on spore germination and hyphae growth of *Fusarium oxysporum* f. sp. *radicis lycopersici* and *Verticillium dahliae* (Palma-Guerrero et al. 2008). Chitosan shows antifungal activity against pathogenic yeasts, e.g., *Candida* spp. and *Cryptococcus* spp. (Camacho et al. 2017; Garcia et al. 2018).

#### 10.5.2.1 Detoxification of Pathogen Toxin

Detoxification is a mechanism that reduced the virulence of pathogenic toxins by binding with some amino acids (Aliashkevich et al. 2018). Both *Alcaligenes denitrificans* and *P. dispersa* help in detoxifying *Xanthomonas albilineans*' virulent compound albicidin (Basnayake and Birch 1995; Walker et al. 1988). Similarly, fusaric acid toxicity was neutralized by *Burkholderia cepacia* and *Ralstonia solanacearum* (Toyoda et al. 1988). *T. viride*, *T. hamatum*, and *T. virens* were produced compound gliotoxin against *Rhizoctonia solani* (Reino et al. 2008). The yeast showed

antagonistic properties against pathogens by biosynthesis of toxins called mycotoxins (Hatoum et al. 2012).

#### 10.5.2.2 Phytohormones

Phytohormones such as indole-3-acetic acid, ethylene, cytokinins, and gibberellins play an important role against biotic and abiotic stresses (Khan et al. 2011; Shabir et al. 2016; Xu et al. 2018; Li et al. 2016a, b, 2017). Contreras-Cornejo et al. (2009) reported that *Trichoderma* spp. play a key role for auxin phytohormones secretion to promote plant the growth and development. *Aspergillus fumigatus* was found to secrete gibberellins and regulate the production of other phytohormones like abscisic acid (ABA), salicylic acid (SA), and jasmonic acid (JA) (Khan et al. 2011). Cosme et al. (2016) observed that *Piriformospora indica* is a mycorrhizal fungus-induced gibberellin hormone against root herbivory and biotic stress. *T. asperellum* has increased the indole's three acetic acid (IAA) content in maize plants and promoted their growth by activating the plasma membrane H+-ATPase (Coria et al. 2016). de Zelicourt et al. (2018) recently reported that ethylene hormone induced by endophyte *Enterobacter* sp. SA187 helped to improve the yield of alfalfa crops and growth *Arabidopsis*. Wang et al. (2018) reported that an endophytic *Streptomyces chartreusis* WZS021 has enhanced the phytohormones such as indole acetic acid, abscisic acid, and ethylene accumulation against drought stress in sugarcane plants.

### 10.5.3 Hyperparasitism Found in the Presence of Antagonistic Microbiota

Hyperparasitism is an interaction between specific microbes and target pathogens to either kill its propagules or suppress their growth and reproduction; it is also called parasitism. *Trichoderma harzianum* produced anthraquinone pachybasin that recognizes the host and increases the number of coils around *Rhizoctonia solani* (Lin et al. 2012). Some biocontrol fungi show predatory behavior in the production of enzymes that causes cell wall lysis of pathogens and helps to intake nutrient contents of pathogens (Junaid et al. 2013). *Trichoderma* sp. feed on sclerotia of *R. solani* by producing chitinase (Abbas et al. 2017). Lettuce *Sclerotinia* diseases were effectively controlled by using *Coniothyrium minitans* and *Sporidesmium sclerotivorum* (Jones et al. 2004). *Verticillium dahliae* was mycoparasitized by *T. harzianum* in vitro (Ruano-Rosa et al. 2016). *Trichoderma harzianum* parasitized the fungal hyphal of *Rhizoctonia solani*, causing black scurf and canker potato under in vitro conditions (Brewer and Larkin 2005; Ibrahim 2017). Arbuscular mycorrhizal fungi (AMF) has showed a symbiotic association with *Solanaceous* crops and helped to improve the plant health and defense system against *Potato virus* Y (PVY) (Sikora et al. 2019).

### 10.5.4 Competitions

Competition is a basic process that exhibited interconnections between nonpathogenic microorganisms and pathogens in rhizospheres (Brien 2017). Host plant hinders root surface from being exposing to pathogens and drawing nutrients from rhizosphere quickly, thus they reproduce faster than soilborne pathogens. Yeast has special antagonistic ability to colonize root rapidly before root exposed to the pathogens, e.g., 6 days preinoculation with *Candida valida* and *Trichosporon asahii* showed higher roots colonization after radicle emergence, while *Rhodotorula glutinis* pre-interaction colonization was initiated after 8 days. Although both pathogens and bioagents present in same rhizosphere but their association lead to competition (Duffy 2001). Abdallah et al. (2015) reported that nine isolates of *Aspergillus* sp. had showed antagonistic activity against *Fusarium sambucinum* (dry rot) and *Phytophthora erythroseptica* pink rot of potato tubers. *Bacillus* and *Pseudomona*s species are most commonly used as biocontrol agents against soilborne phytopathogens due to their strong compatibility with moist soil and high organic manure, e.g., *Fusarium* wilt of chickpea (Abed et al. 2016; Singh et al. 2014). Bubici et al. (2019) recently reported that the fusarium wilt of banana (FWB) caused by *Fusarium oxysporum* f. sp. *cubense* found to be managed by using *Pseudomonas* spp. up to 79% and *Trichoderma* spp. up to 70%. Mycophagous nematodes, for example, *Aphelenchus*, *Filenchus*, *Tylenchus*, and *Iotonchium*, have been found to feed on fungi and to be used as biocontrol agents for controlling phytopathogenic fungi (Tarique et al. 2017). Lagerlof et al. (2011) reported the *Aphelenchoides* spp. and *A. avenae* reduced the infection of *R. solani* inciting the damping-off disease in cauliflower seedlings. *Aphelenchoides besseyi* caused fungal cell wall degradation by feeding on it (Wang et al. 2014). Cetintas et al. (2018) examined the plant growth-promoting rhizobacteria strains which significantly reduced the *Meloidogyne incognita* infection on tomatoes. *Pseudomonas fluorescens* and *Rhizobium leguminosarum* were found to suppress the *Meloidogyne javanica* infection and enhanced growth of lentil, bean, chickpea, lentil, pea, and tomato (Saeedizadeh 2016; Tabatabaei and Saeedizadeh 2017). Xiang et al. (2017) demonstrated the antagonistic behavior of plant growth-promoting rhizobacteria (PGPR) by forming spore on *Heterodera glycines* cuticle in soybean. *Rhizobium* showed inhibitory effect on causing black rot disease of fava bean (Tamiru and Muleta 2018). *Trichoderma citrinoviride* (T33) and *H. semiorbis* (T15) used to control *Fusarium* wilt severity up to 19.77% under greenhouse condition (Al-Mekhlafi et al. 2019).

### 10.5.5 Biocontrol Agents Induced Host Resistance

Systemic acquired resistance (SAR) is facilitated by chemical compounds salicylic acid (SA) that led to produced pathogenesis-related proteins (PR protein) (Agrios 2005). Induced systemic resistance called secondary mediated pathway, which

induced by signal compounds like jasmonic acid (JA) and ethylene secreted in the presence of antagonistic and non-pathogenic rhizomicrobes (Nie et al. 2017). PR proteins usually synthesize a variety of enzymes in host directly against the infection caused by invading pathogens via (1) lysing of invading host cells, (2) reinforcing host boundaries to resist infections, and (3) host-induce localized cell death (Vallad Goodman 2004). Various *Trichoderma* spp. can produce several plant defense elicitors such as xylanases, swollenins, peptaibols, and cerato-platanins (Harman et al. 2004; Druzhinina et al. 2011; Saldajeno et al. 2014). Microbiota-containing fungi belonging to genus *Trichoderma* are found most effective to stimulate plant growth and eliminate plant pathogens by producing specific and nonspecific antibiotics (Bhattacharjee and Dey 2014; Gebarowska et al. 2019). Beside these microorganisms showing antagonistic activity, they are also able to stimulate plants to defend themselves against phytopathogens by inducing systemic resistance (ISR) (Van Loon 2007). A nonpathogenic *Fusarium solani* can be used as a biological control agent by inducing defense response against pathogens (Vallad and Goodman 2004). Chitosan is a bioactive polymer found in fungal cell wall. Chitosan acts as an elicitor to stimulate defense reactions in plants against various soilborne pathogenic fungi, e.g., *Penicillium digitatum*, *Macrophomina phaseolina*, *Fusarium solani*, *Fusarium fujikuroi*, *Fusarium. oxysporum* f. sp. *lycopersici*, *Fusarium oxysporum* f. sp. *cubense*, *Phomopsis asparagi*, *Colletotrichum gloeosporioides*, *Rhizopus stolonifer*, *Sclerotium rolfsii*, *Rhizoctonia solani*, *Candida* spp., and *Cryptococcus* spp. (Ali et al. 2010; Al-Hetar et al. 2011; Oliveira Junior et al. 2012; Bhattacharya 2013; Long et al. 2014; Kim et al. 2016; Camacho et al. 2017; Garcia et al. 2018).

## 10.6 Mode of Application of Biocontrol Agents Against the Soilborne Phytopathogens

Pure and additive forms of biocontrol agents were used against soilborne phytopathogens. Additive biocontrols and surfactants have increased the efficacy of biocontrol agents as much as with fungicides to control many pathogenic plant diseases, e.g., 10% PelGel (polyox-N-10) is a binder which is used to coat the seed with *Trichoderma* sp. and enhance their growth and bioefficacy against *Pythium* sp. (Lo et al. 1997). Biocontrol must be applied in an active form in plant or soil ecosystem present around plant roots at the time when conditions become favorable for pathogenic disease cycle. The plant root surface is colonized rapidly by antagonistic microbes and unable to come in contact with pathogens infection. Therefore, the plant is protected via biofungicidal applications. Babeva and Belyanin (1966) reported that the culture filtrates of isolated nine yeast strain of *Torulopsis* sp. from cabbage rhizosphere were used to increase presoaked cabbage seed germination. *Trichoderma* species have some antimicrobial gene. Moreover, these genes can be isolated and cloned for commercial application on a large scale, as shown in Table 10.4.

**Table 10.4** The beneficial gene of biocontrol agents associated with antagonism of soilborne phytopathogens

| Biocontrol agent | Gene code for the lytic enzyme | Gene function against pathogen | References |
|---|---|---|---|
| *Pseudomonas* spp. and *Streptomyces* spp. | NRPS (non-ribosomal peptide-synthetase) | NRPS induce antibiotic (vancomycin and gramicidin) and lipopeptide (surfactin, iturin A, and bacillomycin) production against *Fusarium* wilt of banana | Zhao et al. (2018) |
| *Clonostachys rosea* | prs6 (serine protease) | Protease parasitized the plant pathogenic fungi *F. graminearum* | Iqbal et al. (2018) |
| *Trichoderma virens* | Tv-cht1 | 33-kDa endochitinases mycoparasitized the *R. solani* | Abbas et al. (2017) |
| *T. harzianum* | ChiB1 (chitinase) | Chitinase catalyze the transglycosylase of *Aspergillus fumigatus* | Andres et al. (2017) |
| *T. brevicompactum* IBT40841 | Tbtri5 (trichodermin) | Trichodermin showed antimicrobial activity against *Saccharomyces cerevisiae* and *Kluyveromyces marxianus* | Tijerino et al. (2010) |
| *T. virens* | Tv-ech1 (endochitinase) | Endochitinase reduce the infection caused by *A. alternata* and *R. solani* in transgenic cotton plant | Emani et al. (2003) and Kumar et al. (2009) |
| *T. harzianum* | chit36 (chitinase) | Chitinase repress the *Alternaria radicina* and *Botrytis cinerea* in carrot and increase tolerance | Baranski et al. (2008) |
| *T. atroviride* | ech42, nag70 (chitinases) and gluc78 (β-1,3 glucanase) | Chitinase and β-1,3 glucanase inhibit the growth of *Rhizoctonia solani* and *Magnaporthe grisea* in rice | Liu et al. (2004) |

## 10.7 Plant Growth-Promoting Rhizobacteria

Gray and Smith (2005) classified plant growth-promoting rhizobacteria into two categories based on their presence outside and inside the contact region. These included (1) extracellular plant growth-promoting rhizobacteria (ePGPR) such as *Agrobacterium*, *Azotobacter*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Pseudomonas*, *Burkholderia*, *Caulobacter*, *Chromobacterium*, *Erwinia*, *Flavobacterium*, *Micrococcus*, and *Serratia* and (2) intracellular plant growth-promoting rhizobacteria (iPGPR) generally found inside cells of root nodule and belongs to family of Rhizobiaceae which includes *Allorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, and *Rhizobium*, endophytes, and *Frankia* species. *Rhizobium* and *Frankia* species associated symbiotically with higher plants and can fix atmospheric nitrogen (Bhattacharyya and Jha 2012). Deshmukh and Shinde (2016) reported that PGPRs had been proven as vital biocontrol agents available in the form of natural enemies to protect plants against various soilborne phytopathogens. Rhizobium formed a

symbiotic mutualistic relationship with leguminous plants. Rhizobium can be used considerably to improve the growth of legume plants through nitrogen fixation in nodules (Mus et al. 2016). PGRP is also used as biocontrol agents to induce host defense mechanism and suppress the disease produced by pathogen (Ahemad and Kibret 2014). Many antagonistic bacterial species, e.g., *Pseudomonas fluorescens*, *P. putida*, *P. aerofaciens*, *Burkholderia cepacia*, *Bacillus subtilis*, *B. Polymyxa*, *B. carries*, *Azotobacter chroococcum*, *Azospirillum lipoferum*, have been found to suppress or control cotton rhizospheres diseases (Kloepper et al. 2004; Heydari 2007; Ahemad and Kibret 2014).

## 10.8 Plant Growth-Promoting Rhizobacteria

PGPR help to promote and enhance the plant vigor under diverse environmental condition by various mechanisms. PGPR can increase nutrient uptake (nitrogen, phosphorus, potassium, and essential mineral elements) (Bargaz et al. 2018). PGPR community showed the direct or indirect antimicrobial mechanism in rhizosphere niche and included (1) direct promotion of plant growth by increasing biofertilizer activity and (2) indirect growth stimulation by biopesticide activity.

## 10.9 Microbivorous Nematodes

Nematodes are elongated, vermiform threadlike organisms abundantly found in soil and have a great impact in the maintenance of soil biodiversity (Yeates and Bongers 1999). Nematode appears as biocontrol agents showing a wide range of feeding types on the protoplasm of plants, fungal hyphae, bacterial cell contents, and protozoans (Yeates et al. 1993). Nematodes establish by antagonistic mechanism, viz., parasitoids, predators, pathogens, competition for space, and nutrients, by sharing same space habit. Nematodes belong to order Aphelenchida (*Aphelenchus*, *Aphelenchoides*, *Rhadinaphelenchus*, *and Bursaphelenchus*), and some belong to order Tylenchida (*Ditylenchus* spp.) which are called fungivorous nematodes (Yeates et al. 1993). They play a critical role in controlling soilborne phytopathogenic microbial communities (Yeates 2003). Further studies on nematode *Aphelenchus avenae* significantly reduces plant pathogens like *Fusarium moniliforme*, *Pythium butler*, and *Fusarium oxysporum* in soil (Gupta 1986; Okada 2006). Nematode feed on fungi, bacteria, diatoms, etc., *Aphelenchoides hamatum*, *A. composticola*, and *Ditylenchus*, feed on the spores of pathogenic fungi *Agaricus*, *Verticillium*, *Botrytis*, mushroom fungi, etc., while *Panagrolaimus* and *Poikilolaimus* species feed on numerous bacteria (Muschiol and Traunspurger 2007). Preinoculation of *Meloidogyne incognita* was protective against the bean root rot fungus caused by *Rhizoctonia solani* due to the competition of nutrient and space. Wolfarth et al. (2013) demonstrated that mutualistic interaction of fungivorous collembolans *Folsomia candida* and nematodes *Aphelenchoides saprophilus* was responsible for reducing mycotoxin deoxynivalenol released by *Fusarium culmorum* on infected

wheat straw remaining on the soil surface compared to treatment with *Collembolans* and nematodes being separated. *Pochonia chlamydosporia* mycoparasitized the eggs of *Meloidogyne javanica* by producing chitosan and proteolytic enzyme (Martinez et al. 2016; Escudero et al. 2015). Fungivorous or bacterivorous nematodes were associated with microbiomes due to the presence of soilborne pathogen by feeding on their body (Morris et al. 2016; Elhady et al. 2017). Nematodes associated with microbes reduced the severity of apple replant disease (ARD) (Kanfra et al. 2018). Strom et al. (2019) reported that soybean cyst nematode *Heterodera glycines* increased population caused change in soil microbial community and correlated with yield loss. Soybean cyst nematode density attracted the nematode-trapping fungi and reduced the crop loss.

## 10.10 Conclusions

Beneficial microbiotic factors improve soil life. Generally, soilborne phytopathogens are the major constraint of the agriculture ecology and ecosystem by degrading soil fertility, causing human health hazard and groundwater contamination, and consequently disrupting the environment. Beneficial microorganisms present in microbiome system is promising aid and help to preserve soil fertility in a sustainable and eco-friendly manner. Moreover, various impacts of microbiotic factors have been discussed in this chapter. One of the most important mechanisms is competition in which beneficial organisms feed on soilborne pathogen propagules and thus reduce disease-causing potential. Bioremediation potential of microbiota detoxify pollutants like heavy metals and agrochemicals as biopesticides. These all have potential to control soilborne phytopathogens, stimulating defense response to resist pathogen infection, increase plant growth promoting factors, and allow to withstand under adverse environmental conditions.

## 10.11 Future Approaches

Rhizosphere shows great importance for the plant growth as it is interrelated with biotic and abiotic factors of the environment. The rhizosphere is full of viable microbes; some show noxious behavior toward the plant, and some show the beneficial effect that will cause to suppress many soilborne pathogen populations and help to stimulate defense response. Thus, beneficial phytomicrobiome overcomes the noxiousness of harmful microbes and promotes plant growth. Hence, natural microorganisms will offer the best safe pest management module and are the cheap source that we can easily get from the surrounding rhizosphere. Today farmers are more reliable on pesticides for disease management. Therefore, there is a need to manufacture large-scale important phytobeneficial biocontrol at minimum cost, is safe, has long shelf-life, and can easily be handled by farmers. Biocontrol agents applied as to coat the seed or too deep the seedling root in the biocontrol agent formulations before sowing. Biocontrol-treated planting material will help to improve the growth

and provide strong protections against both abiotic and biotic stress. The microbiome is full of natural constituents that help to overcome the phytopathogenic potential by various mechanisms involved like mycoparasitism, competition, antibiosis, and self-defense system activation.

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# 11 Zinc-Solubilizing Microbes for Sustainable Crop Production: Current Understanding, Opportunities, and Challenges

Prity Kushwaha, Prem Lal Kashyap, K. Pandiyan, and Ajay Kumar Bhardwaj

**Abstract**

Zinc (Zn) is an essential and prime micronutrient needed in diminutive amount by agricultural crops for complete growth and development. It acts as an essential and key constituent of a variety of enzymatic reactions, carbohydrate metabolism, synthesis of proteins and auxin, and maintenance of cellular membrane veracity in plants. Zn is also an essential element in human diet as its deficiency affects normal development and functioning of nervous, immune, and skeletal systems. Crop plants and their consumable parts serve as major sources of Zn in human diet. Plants can uptake Zn as divalent cation, but a major portion of it exists in insoluble form in the soil and very little Zn becomes available to the plants. There are wide varieties of microbes which employ myriads of biological processes to make Zn available to plants from unavailable sources. These zinc-solubilizing microbes (ZSM) can be utilized as prospective alternatives to conventional less-efficient fertilizer application for enhancing Zn availability in soils. Owing to the naturally available source of Zn in soil and high cost of synthetic Zn fertilizers, the demand of ZSM is escalating with time. The injudicious application of chemical fertilizers can be minimized by using ZSM in crop production that can lead to environmental and agricultural sustainability. At the global level, several researchers have recognized the importance of ZSM for crop growth, health, and development. The current article illustrates the role of ZSM in improving plant production in an economical, environment-friendly, and sustainable manner. The mechanisms used by ZSM for Zn solubilization have been

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explained. An attempt has been made to provide a comprehensive global overview of research initiatives made in the field of sustainable crop production through ZSM, and further opportunities and challenges for use of ZSM-based technology in agriculture have been discussed.

**Keywords**
Agriculture · Bacteria · Fungi · Microbes · Nutrients · Sustainability · Zinc

## 11.1 Introduction

Zinc (Zn) is an essential and prime micronutrient needed in small amounts (5–100 mg $Kg^{-1}$) by agricultural crops for complete growth, development, and nutrition (Singh et al. 2019; Khan et al. 2019; Yuvaraj and Subramanian 2015; Tripathi et al. 2015; Broadley et al. 2007). In general, Zn deficiency in plants slows down the biological processes of photosynthesis and nitrogen metabolism, reduces complete growth and development of blossoms and fruits, diminishes the production process of carbohydrates and phytohormones, hinders crop ripening duration, and ultimately reduces final plant yield and nutritional grain quality (Mandal and Das 2013; Sadeghzadeh 2013; Nielsen 2012; Alloway 2008; Alloway 2004). Several reports have indicated that at global scale approximately half of the land under agriculture cultivation is deficient in available Zn content and as a consequence resulted in a significant reduction in both nutritional quality as well as the potential yield of agriculturally important crops (Mumtaz et al. 2017; Cakmak 2008; Welch and Graham 2004). Due to the inadequate quantity of Zn contents in the arable soil, Zn deficiency has been reported as one of the most prominent and insidious micronutrient deficiencies in several parts of the globe including India, Bangladesh, and Nepal (Jasrotia et al. 2018). It is estimated that in India, approximately 48% area of arable land is Zn deficient (Singh 2009). Further, Venkatakrishnan et al. (2003) have reported that within a week exogenously applied water-soluble Zn fertilizers are rapidly converted to 96–99% of insoluble Zn forms, although Zn conversion rate to insoluble forms varied with soil type and soil physicochemical properties. To overcome Zn deficiency, several approaches have been developed and evaluated. Zn-EDTA and zinc sulfate ($ZnSO_4$) are the most commonly employed fertilizers in the agricultural fields (Doolette et al. 2018; Karak et al. 2005; White and Broadley 2005), but their rapid transformation (within 7 days) into insoluble complex forms after application makes them uneconomical and environmentally unsafe (Rattan and Shukla 1991). Besides this, several other agronomic interventions (e.g., crop rotations and intercropping strategies), traditional crop breeding, and transgenic and genetic engineering have been exploited for boosting plant Zn uptake (Gunes et al. 2007; Zuo and Zhang 2009; Gustin et al. 2009; Cakmak et al. 2010; Mhatre et al. 2011; Tan et al. 2015). Unfortunately, these technologies are costly, laborious, and slower (Hussain et al. 2018). Therefore, it becomes utmost important to devise a strategy for enhancing availability of native soil Zn which is abundant but

unavailable to plants. In this context, biofortification of agricultural crops by employing zinc-solubilizing microbes (ZSM) is advocated as a novel strategy not only to fulfill the optimum requirement of micronutrient in food crops but also to enhance crop production on less fertile soils.

Recently, zinc solubilization by microbes (Fig. 11.1) has drawn significant attention in the agriculture sector. Microbial strains with such capabilities are generally termed as zinc-solubilizing microbes (ZSM). These microbes, either of fungal or bacterial nature, are reasonably supportive in augmentation of Zn availability in the soil and also play a crucial role in mobilizing Zn to the edible parts of the plants by enhancing Zn uptake capabilities of the plant. More importantly, these microbial strains have the capability to facilitate rapid solubilization of insoluble Zn compounds [zinc sulfate (ZnS), zinc carbonate ($ZnCO_3$), and zinc oxide (ZnO)] in soil by producing organic acids that confiscate the $Zn^{2+}$ cations and alter the soil pH in close proximity of plant rhizosphere (Alexander 1997). Besides this, the anions also have the capability to augment Zn solubility by chelation phenomenon (Jones and Darrah 1994). Other mechanisms associated with Zn solubilization include effective production of siderophores, chelated ligands, proton, and oxidation-reduction systems on plant cell membranes (Saravanan et al. 2011; Chang et al. 2005; Wakatsuki 1995). Several microbes associated with different kinds of agricultural crops have been observed to boost growth and Zn contents of plants when applied exogenously (Table 11.1). Among them, prominent bacterial genera documented as potential Zn solubilizers include *Agrobacterium*, *Azospirillum*, *Burkholderia*, *Bacillus*, *Gluconacetobacter*, *Enterobacter*, *Microbacterium*, *Rhizobium*, *Serratia*, *Pseudomonas*, and *Thiobacillus* (Vidyashree et al. 2018a, b; Jamali et al. 2018; Khanghahi et al. 2018; Khande et al. 2017; Naz et al. 2016; Pawar et al. 2015; Hussain et al. 2015; Abaid-Ullah et al. 2015; Vaid et al. 2014; Ramesh et al. 2014; Deepak et al. 2013; Subramanian et al. 2009; Saravanan et al. 2007; Whiting et al.


**Fig. 11.1** Principal microbial genus associated with Zn-solubilization and uptake by plants

**Table 11.1** Effect of ZSM on crop growth, yield, and uptake

| Crop(s) | Microbes | Experimental condition(s) | Effect on crops | References |
|---|---|---|---|---|
| Maize | *Azotobacter*, *Azospirillum* | Greenhouse | Increase in Zn contents of grain | Biari et al. (2008) |
| Maize | *B. aryabhattai*, *B. subtilis*, and *Bacillus* sp. | Growth chamber | Enhancement in the root length, root fresh biomass, and root dry | Mumtaz et al. (2017) |
| Maize | *Gluconacetobacter diazotrophicus* | Pot | Increase the available zinc content in the soil | Sarathambal et al. (2010) |
| Maize | *Enterobacter cloacae* | Pot assay | Increase in dry weight and Zn uptake | Omara et al. (2016) |
| Maize | *Bacillus* sp. | Growth room | Enhancement in growth and physiology of plants | Hussain et al. (2015) |
| Maize | *Pseudomonas* sp. P29, *Pseudomonas* sp. P33, and *Bacillus* sp. B40 | Pot assay | Increase in total dry mass and uptake of Zn | Goteti et al. (2013) |
| Rice | *Sphingomonas* sp., *Enterobacter* sp. | Greenhouse | Increase in Zn bioavailability in rhizosphere soils and grain yields and Zn densities in grains | Wang et al. (2014) |
| Rice | *Acinetobacter* sp. and *Serratia* sp. | Pot assay | Increase in plant growth and root development | Othman et al. (2017) |
| Rice | *Burkholderia* and *Acinetobacter* | Greenhouse | Enhancement in the total Zn uptake and reduction in phytate | Vaid et al. (2014) |
| Rice | *A. lipoferum*, *Pseudomonas* sp., *Agrobacterium* sp. (consortium) | Field | Increase in growth, physiology, and yield | Tariq et al. (2007) |
| Rice | *A. lipoferum*, *Pseudomonas* sp., *Agrobacterium* sp. (consortium) | Field | Increase in growth, physiology, and yield | Hafeez et al. (2002) |
| Rice | *Pseudomonas aeruginosa*, *Ralstonia pickettii*, *Burkholderia cepacia*, *Klebsiella pneumonia* | Pot | Increment in shoot and root lengths as well as higher dry weights of root and shoot | Gontia-Mishra et al. (2017) |
| Rice | *Bacillus* sp. AZ6 (consortium with ZnO) | Pot | Improved growth, physiology, and yield parameters of rice; improved the quality of rice grains and bioaccumulation of Zn in various parts of rice | Zeb et al. (2018) |

(continued)

**Table 11.1** (continued)

| Crop(s) | Microbes | Experimental condition(s) | Effect on crops | References |
|---|---|---|---|---|
| Soybean | *Bacillus firmus* KHBD-6 and *Bacillus amyloliquefaciens KHBAR-1* | Microcosm | Increase in the zinc concentration in soybean seeds | Sharma et al. (2012) |
| Soybean and wheat | *Bacillus aryabhattai* | Microcosm | Improved mobilization of zinc and its concentration in edible portion, yield of soybean and wheat | Ramesh et al. (2014) |
| Tomato | *Bacillus aryabhattai*, *Pseudomonas taiwanensis*, *Bacillus* sp., *Enterobacter oryzae*, and *Bacillus aerophilus* | Pot assay | Increase in growth, yield, and better quality parameters | Vidyashree et al. (2018a, b) |
| Wheat | *Pseudomonas fragi*, *Pantoea dispersa*, *Pantoea agglomerans*, *E. cloacae*, and *Rhizobium* sp. | Pot assay | Enhancements in total Zn uptake by plant | Kamran et al. (2017) |
| Wheat | *Providencia* sp., *Calothrix* sp., and *Anabaena* sp. | Field | Improvement in the nutritional quality of wheat grains, in terms of protein content | Rana et al. (2012) |

2001; Fasim et al. 2002). Similarly, fungal strains belonging to genera *Absidia, Penicillium*, *Paxillus*, *Hymenoscyphus*, *Oidiodendron*, *Suillus*, *Emericella*, *Beauveria*, and *Trichoderma* have been identified as promising zinc-solubilizing fungi (ZSF) (Pawar et al. 2015; Gadd 2007; Fomina et al. 2005a, b; Schöll et al. 2006; Martino et al. 2003). Therefore, exploitation of these microbial inoculants for boosting available Zn content in the soil as well as its enhanced plant uptake could be one of the prospective options. Hence, this chapter provides a comprehensive global overview of research initiatives made in the field of sustainable crop cultivation through ZSM and brings forth the opportunities along with the challenges that exist for the use of ZSM-based technology in agriculture.

## 11.2 Role and Function of Zn in Plant

Zinc (Zn) is an essential element for plant growth and sustainable crop production, as the plants need all the essential nutrients in appropriate proportion for proper growth functions and optimum yield (Gupta et al. 2016; Hafeez et al. 2013; Sadeghzadeh 2013). Most importantly, in more than 1200 proteins, such as zinc-finger proteins, RNA polymerases, and DNA polymerases, existing inside the plant system, Zn works as a cofactor (Lucini and Bernardo 2015; Figueiredo et al. 2012;

Lopez-Millan et al. 2005). Further, it is required for the efficient functioning of carbohydrate and auxin metabolism in plants (Alloway 2008). Besides this, Zn-finger transcription factors also regulate the growth and development of leaf, flower, fruit, and fertilization (Epstein and Bloom 2005). Zn is also involved in the regulation of various plant physiological processes like repair processes of photosystem (PS)-II complex during photo-inhibition, maintenance of carbon-dioxide concentration in the mesophyll, hormone secretion and mitogen-activated protein kinases (MAPK)-based signal transduction, etc. (Hansch and Mendel 2009; Lin et al. 2005; Bailey et al. 2002). It has been observed that in the majority of crop plants, Zn deficiency results in stunted stem growth, reduced leaf size, and chlorosis and also negatively influences the root development, water uptake and transport inside the plant, pollen formation, and grain yield (Hefferon 2019; Tavallali et al. 2010; Alloway 2004). More specifically, in barley and wheat, a noteworthy decline in crop growth and yield has been observed in Zn-deficient soils (McDonald et al. 2001). Moreover, Zn deficiency also diminishes the amount of Zn contents in consumable parts of crops and resulted in poor nutritional quality of food (Costerousse et al. 2018; Welch and Graham 2004). In the case of rice, Zn deficiency causes multiple deformations in rice seedlings. For instance, rice leaves develop brown blotches and streaks over leaves and result in stunted plant growth. Moreover, under severe conditions, death of plants may occur, while those plants that recuperate will show considerable hindrance in maturity and will lead to substantial grain yield loss (Hafeez et al. 2013).

## 11.3 Zinc-Solubilizing Microorganisms (ZSM)

### 11.3.1 Zinc-Solubilizing Fungi (ZSF)

Zinc solubilization is performed by a wide range of fungi. The significance of Zn in the fungal growth and nutrition was noticed for the first time in *Aspergillus niger*, which was unable to grow without Zn content (Raulin 1869). Strains belonging to *Absidia cylindrospora*, *A. spinosa*, *A. glauca*, *Penicillium aurantiogriseum*, *P. brevicompactum*, and *P. simplicissimum* efficiently solubilized ZnO and $Zn_3(PO_4)_2$ forms of Zn present in the soil (Coles et al. 2001). Among the mycorrhiza, *Beauveria caledonica*, *Hymenoscyphus ericae*, *Oidiodendron maius*, *Paxillus involutus*, *Suillus bovinus*, and *S. luteus* showed prospective role in the solubilization of insoluble Zn compounds (Fomina et al. 2005a, b; Schöll et al. 2006; Gadd 2007). Solubilization of Zn compounds of water-insoluble nature by *O. maius* isolated from heavy-metal-polluted locations has been reported by Martino et al. (2003). Anitha et al. (2015) identified *Emericella rugulosa* ZSF-2, *Penicillium citrinum* ZSF-5, *Aspergillus candidus* ZSF-7, *A. terreus* ZSF-9, and *A. niger* ZSF-16 as potent candidates for efficient solubilization of three insoluble sources of zinc [ZnO, $ZnCO_3$, and $Zn(PO_4)_3$]. In another similar study, Pawar et al. (2015) studied solubilization of insoluble zinc sources [ZnO, $ZnCO_3$, and $Zn_3(PO_4)_2$] by *Trichoderma viride* and *T. harzianum* and further confirmed their prospective utility as ZSF.

### 11.3.2 Zinc-Solubilizing Bacteria (ZSB)

Bacteria is one of the most prospective alternatives that could assist plants in fulfilling its optimum requirement of Zn by solubilizing complex Zn forms occurring in the soil. A wide range of bacterial species belonging to genera *Acinetobacter*, *Bacillus*, *Burkholderia*, *Gluconacetobacter*, *Klebsiella*, *Microbacterium*, *Pantoea*, *Pseudomonas*, *Ralstonia*, *Serratia*, and *Thiobacillus* have been reported to solubilize Zn (Fig. 11.1). Hutchins and associates (1986) noticed that facultative thermophilic iron oxidizers, *Thiobacillus thiooxidans* and *T. ferrooxidans*, have the potential to solubilize Zn from complex sulfide ore (sphalerite) forms. Similarly, Simine et al. (1998) reported zinc phosphate solubilization potential of *P. fluorescens* isolated from a forest soil. They noticed production of high concentration of gluconic acid when *P. fluorescens* 3a was cultivated in a medium containing zinc phosphate. Similarly, ZnO solubilizing strains of *Microbacterium saperdae*, *Enterobacter cancerogenus*, and *Pseudomonas monteilii* were screened and identified in the Zn hyperaccumulating rhizosphere of *Thlaspi caerulescens* plant (Whiting et al. 2001). Fasim et al. (2002) reported solubilization of Zn salts by *Pseudomonas aeruginosa* CMG 823 derived from the ambient environment of a tannery. In their study, they noticed that the solubilization of zinc oxide by bacterium occurred due to a rapid gain in the $H^+$ concentration by brisk synthesis of 2-ketogluconic acid. A similar conclusion was reached by Saravanan et al. (2007) in their studies where *Gluconacetobacter diazotrophicus* was identified as a potential agent for effective solubilization of insoluble Zn compounds. Hussain et al. (2015) revealed the ZnO solubilizing capabilities of *Bacillus sp.* AZ6 and *Bacillus sp.* AZ6 strains. Othman et al. (2017) identified and established *Acinetobacter* sp. TM56 and *Serratia* sp. TM9 as prospective zinc solubilizer inoculants for rice growth promotion. Recently, *E. cloacae* PBS 2, *Pseudomonas fragi* EPS 1, *Pantoea dispersa* EPS 6, *P. agglomerans* EPS 13, and *Rhizobium* sp. LHRW1 were identified and classified as promising zinc solubilizers (Kamran et al. 2017). Similar studies of Khande et al. (2017) also identified *B. anthracis*, *B. cereus*, *B. tequilensis*, *B. thuringiensis*, and *B. subtilis* subsp. *inaquasorum* as promising ZSB for enhancing seed yield and Zn fortification in the seeds of soybean and wheat crops. Gontia-Mishra and associates (2017) identified Zn solubilizing capabilities of *Burkholderia cepacia*, *Klebsiella pneumonia*, *P. aeruginosa*, and *Ralstonia pickettii*. Similarly, *B. aryabhattai* S10, *Bacillus* sp. ZM20, *Bacillus aryabhattai* ZM31, and *Bacillus subtilis* ZM63 and these strains were ascertained as potential zinc-solubilizing candidates for biofortification in maize (Mumtaz et al. 2017).

### 11.3.3 Mechanism of Zn Solubilization by Microbes

The principal mechanisms of Zn solubilization by microbes include (i) acidification, (ii) chelation, and (iii) chemical transformation. Majority of research articles documented that different kind of microbes including bacteria excrete diverse kinds of organic acids (Table 11.2) and extrude protons to reduce the rhizosphere pH (Wu

**Table 11.2** Organic acids produced by ZSM

| ZSM(s) | Organic acid | References |
|---|---|---|
| *Aspergillus terreus* | Gluconic acid | Anitha et al. (2015) |
| *Bacillus aryabhattai*, *Pseudomonas taiwanensis*, and *Bacillus* sp. | Lactic acid, malonic acid, and citric acid | Vidyashree et al. (2018a, b) |
| *Bacillus* sp. | Cinnamic acid, ferulic acid, caffeic acid, chlorogenic acid, syringic acid, and gallic acid | Hussain et al. (2004) |
| *Gluconacetobacter diazotrophicus* | 5-ketogluconic acid | Saravanan et al. (2007) |
| *Pseudomonas aeruginosa* | 2-ketogluconic acid | Fasim et al. (2002) |
| *Pseudomonas fluorescens* | Gluconic acid and 2-ketogluconic acid | Simine et al. (1998) |
| *Pseudomonas* sp. | Acetic acid and gluconic acid | Jaivel et al. (2017) |
| *Streptomyces cinnamonensis*, *Streptomyces scabiei*, and *Streptomyces netropsis* | Formic acid, malonic acid, lactic acid, citric acid, and propionic acid | Poovarasan et al. (2015) |
| *Pseudomonas chlororaphis* strain 44, *Pseudomonas moraviensis* strain 106, *Pseudomonas syringae* strain 24, *Stenotrophomonas rhizophila* NZSB, *Curtobacterium oceanosedimentum* strain 81, *Streptomyces narbonensis* strain 68, *Plantibacter flavus* strain 5, and *Plantibacter flavus* strain 42 | Gluconic acid, citric acid, fumaric acid, malic acid, 2-oxoglutaric acid, and succinic acid | Costerousse et al. (2018) |

et al. 2006). Particularly, *Bacillus* and *Pseudomonas* have the capacity to generate organic acids which decrease the soil pH, thereby ensuring sufficient Zn availability to the plant (Saravanan et al. 2004). It has been observed that rhizobacteria produce gluconate or the derivatives of gluconic acids (e.g., 2-ketogluconic acid and 5-ketogluconic acid) for Zn solubilization (Saravanan et al. 2011; Tariq et al. 2007). Hussain et al. (2004) reported that *Bacillus* sp. AZ6 has the capability to solubilize insoluble source of Zn by synthesizing various types of organic acids (e.g., gallic acid, caffeic acid, syringic acid, cinnamic acid, chlorogenic acid, and ferulic acid). All of the above-described mechanisms facilitate enhanced Zn availability in soil and its uptake by plants. Other indirect effects on plant growth have also been reported. For example, 5-ketogluconic acid synthesized by *G. diazotrophicus* stimulated the Zn solubilization route, and the available $Zn^{2+}$cations have been noticed to enhance the nematicidal activity of *G. diazotrophicus* against *Meloidogyne incognita* in tomato (Saravanan et al. 2007).

In case of fungi, Martino et al. (2003) reported that mycorrhizal fungi produced organic acids to convert insoluble zinc phosphate [$Zn_3(PO_4)_2$] and zinc oxide [ZnO] into water-soluble Zn. Similarly, Subramanian et al. (2009) reported that

inoculation of arbuscular mycorrhizae reduced the pH of rhizospheric soil and facilitated quick release of Zn from mineral fractions. However, the level of rhizosphere pH sinking differed among microbes (Giri et al. 2005). Wu et al. (2006) recorded a significant decline of 0.47 units in pH, owing to the fact that bacterial inoculation helped in the release of organic acids and $H^+$, which eventually led to the rapid Zn solubilization and in turn improved plant Zn uptake efficiency. Entomopathogenic strains of ectomycorrhizal fungi like *Beauveria caledonica* displayed excellent potential to solubilize chemical forms of Zn (e.g., zinc phosphate) in contrast to mineral forms (e.g., pyromorphite) by employing acidolysis and complexolyis mechanisms. Besides this, oxalic acid production has also been found related to Zn solubilization (Fomina et al. 2004). The role of the siderophores in Zn solubilization has been reported in the case of *Fusarium solani* (Hong et al. 2010).

Zinc chelation is another mechanism employed by *E. cancerogenus*, *Microbacterium saperdae*, and *P. monteilii* for enhancing Zn bioavailability and its uptake efficiency by plant roots (Whiting et al. 2001). Generally, bacterial metabolites form complexes with $Zn^{2+}$ and diminish their response within the soil system (Tarkalson et al. 1998). Subsequently, these newly formed Zn chelates travel toward the roots and liberate chelating ligands ($Zn^{2+}$) at the root surface, making them independent to chelate with other $Zn^{2+}$ ions from the soil solution. In another independent study, Tariq et al. (2007) observed that a biofertilizer comprised of *Agrobacterium* sp. Ca-18, *Azospirillum lipoferum* JCM-1270, *A. lipoferum* ER-20, and *Pseudomonas* sp.96-51 helped to discharge fixed Zn quickly and also made it bioavailable to rice seedlings for a longer period by discharging Zn-EDTA as a chelating molecule. Plant inoculation with *Penicillium bilaji* has also been reported to improve Zn bioavailability to plants via chelating mechanisms (Kucey 1987). The results of a metal mobilization experiment performed by Li et al. (2010) demonstrated that despite the presence of glucose in the growth medium, *Burkholderia cepacia* released oxalic, tartaric, formic, and acetic acids which facilitated Zn solubilization. Recently, Costerousse et al. (2018) explained a range of Zn solubilizing processes adopted by bacteria presented in the wheat rhizosphere. They reported proton extrusion and organic acid synthesis by bacterial strains as the major mechanisms linked with Zn solubilization. Further, they observed that in ZnO liquid solubilization assays, *Curtobacterium*, *Plantibacter*, *Pseudomonas*, *Stenotrophomonas*, and *Streptomyces* triggered the rapid synthesis of organic acids, and as a consequence medium acidification took place, which further assisted in effective ZnO solubilization in the presence of glucose. Interestingly, they observed that Zn solubilization by *Streptomyces* and *Curtobacterium* occurred due to a rapid production of six and seven different organic acids, while other strains involve only gluconic, malonic, and oxalic acids for Zn solubilization. Similarly, *Plantibacter* strains performed ZnO dissolution by proton extrusion via ammonia consumption in the absence of glucose. On the other hand, *Curtobacterium* strains employed complexation processes by involving glutamic acid (Costerousse et al. 2018). Therefore, it is clearly evident that solubilization mechanisms vary among ZSM.

## 11.4 Techniques for Identification and Determining Zinc-Solubilizing Capabilities of Microbes

Zinc-solubilizing microbes can be screened for their ability to solubilize Zn from mineral salts in agar medium [Glucose-10 g, $(NH_4)_2SO_4$-1.0 g, KCl-0.2 g, $K_2HPO_4$-0.1 g, $MgSO_4$-0.2 g, and $H_2O$-1000 ml with pH 7.0]. Petri plates supplemented with either insoluble zinc oxide (ZnO) or zinc carbonate ($ZnCO_3$) or zinc phosphate in the concentration of 0.1% have been commonly used. Spot inoculation with actively growing culture (5 µl) onto the medium and incubation at 25–28 °C for 3–5 days has been found suitable. The appearance of transparent and clear zone reveals the zinc-solubilizing capability of spotted strain. The zone diameter is measured and zinc-solubilizing index (ZSI) is calculated using the following formula:

ZSI = Colony diameter + Diameter of clear and transparent halozone / colony diameter

Generally, atomic absorption spectrophotometer (AAS) is used to estimate the available Zn in the supernatant. In general, the microbial solubilization of Zn is strongly influenced by pH, oxygen, microbial strains used, and kind of Zn-bearing minerals, and therefore, optimal conditions for Zn solubilization by microbes need to be standardized for appropriate results.

## 11.5 Application of ZSM in Sustainable Crop Production

Several research reports highlighted that ZSM in soil health restoration and sustainable crop production is gaining significant importance day by day. Whiting et al. (2001) studied the efficiency of ZSB strains derived from the rhizosphere of Zn hyperaccumulating plant (*Thlaspi caerulescens*). In their study, they observed that ZSB enhanced the water-soluble Zn fraction in the soil as well as there was 22–67% increment in Zn content in shoots and roots when inoculations were performed in the rhizosphere of the germinating seeds of *Thlaspi* plants.

Sarathambal et al. (2010) demonstrated that the application of *G. diazotrophicus* with ZnO in maize showed better uptake of the nutrient, irrespective of soil types. Further, they noticed that *G. diazotrophicus* solubilized Zn better in unsterile Zn-deficient soil. Similarly, a pot experiment conducted by Goteti et al. (2013) revealed the impact of seed treatment with zinc solubilizing, plant growth promoting bacteria (P29, P33, and B40) on maize. They observed significant improvement in plant biomass as well as in mineral nutrient (N, K, Mn, and Zn) uptake when seed bacterization was performed with P29 strain @ 10 g $kg^{-1}$ maize seed.

Vaid et al. (2014) reported that effects of individual or combined inoculation of rice plants with ZSB (*Acinetobacter* sp. SG2, *Acinetobacter* sp. SG3, and *Burkholderia* sp. SG1) resulted in significant increase in the number of panicles (13.3%), productive tillers per plant (15.1%), number of grains per panicle (12.8%), straw yield (12.4%), grain yield (17.0%), and mean dry matter yield per pot (12.9%), over the control. Further, they also noticed that bacterial inoculation enhanced the grain methionine concentration (38.8%) as well as total Zn uptake efficiency per pot

(52.5%). In a similar study, Shakeel and colleagues (2015) documented that inoculation with *Bacillus* sp. provided effective Zn movement to rice grains and resulted in rice grain yield gain of 22–49% and 18–47% in the case of basmati-385 and super basmati rice varieties, respectively. Similarly, a significant gain in Zn accumulation in rice grains as well as an increase in seedling growth of rice as a result of inoculation with endophytic strains of *Burkholderia* sp. SaZR4, *Burkholderia* sp. SaMR10, *Enterobacter* sp. SaCS20, *Sphingomonas* sp. SaMR12, and *Variovorax* sp. SaNR1 was obtained for *Sedum alfredii* plant by Wang et al. (2014). Further, they observed that inoculation with SaMR12 and SaCS20 under hydroponic conditions resulted in the elevation of Zn concentration by 73.6% and 83.4% in roots, respectively, and by 44.4% and 51.1% in shoots, respectively. Similar results with endophytic inoculation using SaMR12 and SaCS20 strains were observed in the case of polished rice. In the same study, a significant gain in grain yields and Zn concentrations have been observed in the case of brown rice (20.3% and 21.9%, respectively) and polished rice (13.7% and 11.2%), respectively. Moreover, inoculation with SaMR12 and SaCS20 resulted in the accumulation of 10.4% and 20.6% more DTPA-Zn in the rhizosphere soil of rice seedlings, respectively (Wang et al. 2014).

Ramesh et al. (2014) conducted a pot experiment with soil containing low levels of plant available Zn to reveal the effectiveness of seed inoculation with different *Bacillus aryabhattai* strains on Zn solubilization and plant growth promotion. They observed that *B. aryabhattai* inoculations resulted in the elevation of Zn concentration in wheat grains from 42 to 61 mg $kg^{-1}$, compared to control. They further explained that this elevation was due to the capability of the inoculated ZSB to convert insoluble Zn in the soil by producing organic acids and decrease in the pH (Ramesh et al. 2014). On parallel lines, Hussain and associates (2015) also noticed the significant increment in plant growth attributes, mainly fresh and dry biomass of roots, fresh and dry biomass of shoots, shoot length and root length, etc. as a result of the inoculation of ZSB (*Bacillus* sp. AZ6). Recent research findings of Mumtaz et al. (2017) also revealed that inoculation with *Bacillus* sp. ZM20, *B. aryabhattai* S10, *B. aryabhattai* ZM31, and *B. subtilis* ZM63 enhanced maize growth significantly. They observed that all these isolates have excellent multifarious plant growth promoting attributes and able to colonize plant roots effectively and, therefore, could be utilized as a potential microbe-based eco-friendly alternative for enhancing the productivity as well as nutritional status of maize grains.

The improvement in crop growth and soil fertility of maize plants was observed when seedlings were inoculated with *Enterobacter cloacae* with ZnO (Omara et al. 2016). However, the available Zn content was greater in non-sterile than in the sterile soil conditions, which pointed to the fact that microorganisms other than *E. cloacae* may also be involved in the solubilization of insoluble zinc sources. The research findings documented by Othman et al. (2017) revealed the potential of ZSB (*Acinetobacter* sp. TM56) with zinc sulfate @ 0.2 mg $L^{-1}$ for improving the various plant growth parameters as well as root development of rice. This bacterium had the capacity to solubilize a considerable fraction of insoluble form of Zn under field conditions.

The enhanced content of Zn in the seeds of wheat and soybean crops with effective inoculations of *B. cereus* has been reported by Khande et al. (2017). Under microcosm conditions, they observed that seed priming with the alone application of *B. cereus* BBU-5, *B. cereus* KMR-5, and *B. cereus* DBU-1 significantly enhanced Zn contents in both seed and straw of soybean. Similarly, wheat seed inoculation with *B. cereus* KMR-5, *B. cereus* KBY-5, *B. cereus* BHKD-6, and *B. cereus* DBU-1 provided a high amount of Zn in both seed and straw in contrast to uninoculated control plants. They further explained that the inoculation of ZSB significantly declined phytic-P content in the seeds of soybean and, as a consequence, enhanced bioavailability of Zn in seeds. Similarly, in another experiment, Kamran et al. (2017) studied the effect of ZSB (*E. cloacae* PBS 2, *P. dispersa* EPS 6, *P. fragi* EPS1, *P. agglomerans* EPS 13, and *Rhizobium* sp. LHRW1) on the seedling growth, development, and Zn uptake. They observed that one month after ZSB inoculation, highest shoot and root dry weight and shoot length were observed in seedlings inoculated with *Rhizobium* sp. LHRW1, whereas increased shoot and root length was noticed in *E. cloacae* PBS 2-inoculated plants. Maximum Zn concentration was recorded in shoots of *E. cloacae* PBS 2-inoculated plants and in roots of *P. agglomerans* EPS 13-inoculated plants followed by sole zinc carbonate ($ZnCO_3$) amended control. Interestingly, even after 3 months post ZSB inoculation, a significant elevation in shoot dry weights was detected in plants inoculated with *P. dispersa* EPS6, *P. agglomerans* EPS13, and *E. cloacae* PBS2. Significant increment in terms of root dry weight and maximum Zn content was obtained in the case of *P. fragi* EPS1-inoculated plants derived from the wheat rhizosphere. Interestingly, highest Zn content for roots was obtained in the control plants demonstrating the plant's incapability to mobilize Zn from roots to grains (Kamran et al. 2017).

Gontia-Mishra and associates (2017) demonstrated that the inoculation of rice seedlings with ZSB (*Klebsiella pneumonia* Zn8, *P. aeruginosa* Zn2, and *R. pickettii* Zn3) resulted in a significant increase in plant biomass in contrast to only water-inoculated control seedlings. Additionally, they also reported that treatment of rice plants with individual strain or with bacterial consortium and grown in soils containing an abundant source of an insoluble form of Zn or in Zn-deprived soils with added insoluble Zn compounds enhanced the plant Zn uptake efficiency.

Zeb et al. (2018) demonstrated that compost enriched with *Bacillus* sp. AZ6 inoculum and ZnO enhanced Zn availability and showed a positive effect on growth, physiology, and yield of rice, in comparison to $ZnSO_4$. Further, they observed that Zn-enriched compost with *Bacillus* sp. AZ6 (Zn-EC60:40), in comparison to $ZnSO_4$, significantly enhanced growth, physiology, and yield parameters of rice through a slow and steady release of Zn from ZnO. Additionally, the microbial inoculation also resulted in the bioaccumulation of Zn in various parts of rice as well as rice grain quality.

Based on the basic and applied research knowledge generated till date, it emerges that the application of ZSM can be a promising technique to solubilize the unavailable Zn reserves in the soil and facilitate easy Zn accessibility to the plants, resulting in enhanced plant growth with the minimum application of Zn fertilizers.

## 11.6 Opportunities and Challenges

Zinc-solubilizing microbes (ZSM) could be an excellent option and practical technology to solubilize insoluble Zn into a soluble form; however, their application in the agricultural sector is still uncommon due to the following reservations:

(i) Based on the information generated so far, ZSM have been less studied under field conditions with rare multilocation testing. Besides this, very limited information is available on the application methodology and delivery methods of ZSM under field conditions. As the results obtained under in vitro environments or under greenhouse conditions may divert from actual field conditions, therefore, special attention and emphasis should be made on testing under natural field conditions to evaluate the potential of ZSM-based technologies for sustainable crop production. Further research is needed to evaluate the influence of other plant growth promoting microbes (PGPM) such as IAA producers, ACC deaminase producers, phosphate solubilizers, and $N_2$ fixers on the availability of Zn in the soil. Additionally, further understanding of synergistic and antagonistic interactions between ZSM and other PGPM and determination of the optimal conditions for ZSM activity is also needed. Interaction between efficient plant species and ZSM would also be useful yet still unexplored. More specifically, understanding the plant-ZSM interaction specific mechanisms under environmental variables and controls needs to be outlined for potential crops. Additionally, the impact assessment of ZSM on different crops grown under the diverse agroecological conditions and geographical locations needs to be assessed. The role of ZSM in increased availability of other nutrients under the influence of soil pH variation and content of other nutrients (e.g., P, N, Fe, K, etc.) also needs to be studied in detail.

(ii) Limited knowledge and awareness among the farmers regarding the application and benefits of ZSM-based biofertilizers in crop production is one of the biggest hurdles for its practical field application. A major section of the farming community is unaware of the Zn biofertilizers and their merits and demerits in enhancing crop yields. Perhaps, they are also unaware of the negative impacts of continuous application of inorganic zinc fertilizers on the ecosystem functions.

(iii) At present, limited attention is given by the scientific community toward development of Zn-based biofertilizer technologies. Specifically, deficiency in technology with respect to carrier suitability and product formulations to enhance the shelf life of the Zn-based biofertilizer is one of the biggest challenges.

(iv) Unavailability of suitable ZSM strains due to lack of their availability in culture collections or microbial banks is yet another hurdle.

## 11.7 Conclusions

Zinc mobilization in soils and into plants using ZSM has emerged as an important tool to enhance Zn bioavailability. Judicious application of ZSM is remarkably an efficient, environmentally sound, and low-cost strategy to enhance Zn concentrations in the edible parts of agricultural crops. Microbial inoculation has shown immense potential to enrich plants with Zn micronutrient, with and without fertilizer sources. Due to lots of ambiguities and dilemma in getting success in enriching seed grains with Zn by breeding or agronomic strategies, biofortification using ZSM should be considered as one of the best approaches with quick effects for short-term adoption. In the future, there is a need to strengthen microbial research programs on the development and field evaluations of ZSM-mediated biofortification methods at critical plant growth stages for enhancing Zn uptake efficiency and elevating Zn accrual in food grains. Studying the bioavailability of Zn inside grain, resulting from ZSM applications in single or consortium mode, will be an interesting aspect to investigate. Significant progress has been made in the ZSM-based Zn fortification of crops, and it has convincingly proven that micronutrition malnutrition can be effectively tackled with ZSM-based management of food crop systems.

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# Endophytic Phytobiomes as Defense Elicitors: Current Insights and Future Prospects

12

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**Abstract**

The endophytes are microbial organisms inhabiting within the plant body either intercellularly or intracellularly. Earlier these endophytes were considered either as pathogenic or having no significant role in the plant's physiology and metabolism, but with the advancement of research and technology, the critical roles played by them are emerging. These endophytes form a symbiotic relationship with their host where in exchange of nutrient and habitat, they provide the plant protection against various stresses both biotic and abiotic. They provide the protection or resistance through either direct mechanisms or indirectly by eliciting various pathways within their host plants against the stresses. The endophytes help the host plant to acclimatize under harsh conditions by eliciting the defense-related genes, which in response triggers the concerned pathways for the synthesis of secondary metabolites for plant defense. A better understanding of the mechanisms and the role of endophytes in stress management will help in designing defense strategies to cope with the stresses and to improve integrated strategies for stress management in agriculture. The chapter thus explores the various mechanisms with the endophytes eliciting both biotic and abiotic defense responses in their host plants under stress conditions and their future application in agriculture and crop sciences.

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## 12.1 Introduction

The plants in their natural habitat face various biotic and abiotic stresses, which they successfully cope with through their internal defense mechanisms (Gimenez et al. 2018; Mousavi et al. 2019). The cultivated or domesticated crops are more prone to damage compared to their wild relatives. With the ever-increasing food crisis and global target of achieving Sustainable Development Goals 2030 of zero hunger and for the conservation of nature, researchers are now looking for biological, safe alternatives for the management of various environmental stresses along with increasing the productivity of the crop plants without contaminating the environment with chemicals (Bengtsson et al. 2018). The microbial use has emerged as a key player in achieving these goals. Since the past few decades, our understanding of soil microflora has increased tremendously, and now the soil is considered as an equivalent to a living entity (Barman et al. 2019; Harman and Uphoff 2019). These understandings and relevant research have paved a new pathway of sustainable organic agriculture practices. In recent times, the role of rhizospheric bacteria has been largely explored, and they have been widely used for acclimatization of both biotic and abiotic stresses in the crop plants (Timmusk et al. 2017). Studies carried out during the last decade have highlighted that the plants are ubiquitously inhabited by microbes, which live within the host plant without causing any disease symptoms (Bacon 2018; Marsberg et al. 2017). These microbes are beneficial to their host plants and help them to cope with both biotic and abiotic stresses (Mishra et al. 2018a; Rho et al. 2018).

### 12.1.1 Plant Endophytes

The microorganisms living intercellularly or intracellularly within the host plants are termed as plant endophytes. The endophytes are microorganisms that inhabit and colonize within the plant tissues and share a symbiotic relationship with its host plant and do not manifest any disease symptoms in them (Bacon 2018). They are ubiquitous in distribution belonging to a varied group of microorganisms ranging from bacteria and fungi, including actinomycetes (Rho et al. 2018). Some commonly reported genera of bacterial and fungal plant endophytes include *Bacillus* sp., *Colletotrichum* sp., *Phomopsis* sp., etc. These microorganisms have been isolated from all parts of plants including leaves, stems, and roots and even from the floral tissue (Strobel 2018; Frank et al. 2017; Nissinen et al. 2012). The plant endophytes share a complex and multifaceted association with their host plants, where they have been reported to have a positive effect on their host. To adjust and to

survive within the host tissues, the microbes develop a mutualistic relationship where both the host and inhabitant are benefited (Jia et al. 2016; Wani et al. 2015).

These microbes not only help the plant to respond and acclimatize during the biotic and abiotic stresses, but they also help to ameliorate the stress through triggering various pathways in the plant and regulating the responses (Mishra et al. 2018a; Pandey et al. 2018). They protect and prepare the host plants for pathogen attacks, environmental stress, and against herbivores as well. They have been reported to trigger secondary metabolite pathways in the plants, which leads to the production of various defense-related compounds in the host (Singh and Gaur 2017). Several endophytes are themselves involved in the production of commercially important secondary metabolites known to be produced by their host plants. For instance, the consortium-based application of *Pseudomonas fluorescens* and *Bacillus amyloliquefaciens* enhances the withanolide content in *Withania somnifera* under biotic stress condition (Mishra et al. 2018b), an endophyte, namely, *Phoma medicaginis* isolated from *Taxus wallichiana* var. *mairei* individually producing paclitaxel (Zaiyou et al. 2017). These metabolites are also valuable for humans commercially. These features make the endophytes a desirable choice as a source of commercially or medicinally valuable compounds that are used as drugs.

Some endophytes act as a biocontrol agent owing to their antimicrobial or mycoparasitic activities. The biocontrol activity is showing endophytes that either attack the pathogens directly through their antagonistic activity or indirectly through antibiosis by the production of various antimicrobial compounds like fengycin, iturin, etc. (Zouari et al. 2016; Brader et al. 2014). The endophytic microbes are known to produce various cellulosic and chitinolytic enzymes that degrade and disintegrate the cell wall of the pathogen (Naik 2019; Abdel-Rahim and Abo-Elyousr 2018) (Fig. 12.1).

### 12.1.2 Endophytic Microbes Interaction with Host Plants

The substantive evidence on plant-associated endophytes was found in the fossilized plant tissues which revealed that endophyte–host interactions may have evolved from the time of very existence of higher plants on the planet (Strobel 2003), signifying an essential part of plant evolution (Mendes et al. 2013; Philippot et al. 2013). In the past two decades, the researchers have made great efforts to assess the role of endophytes and their interaction with host plants. The ability of endophytes to colonize in the internal plant tissues has made them very crucial for the commercial as well as societal agricultural practices. The close mutualistic association of endophytes with the plant is an essential part of their existence or survival, and several advanced studies demonstrate that the host plants are dependent on the endophytes for several fundamental activities (Hardoim et al. 2015; Potshangbam et al. 2017). For instance, symbiotic relationship of rhizobia and the leguminous crop is the well-studied endophytic association; the bacterial endosymbiont fulfills the scarcity of nitrogen, whereas the host ensures the favorable condition for endophyte (Santoyo et al. 2016).

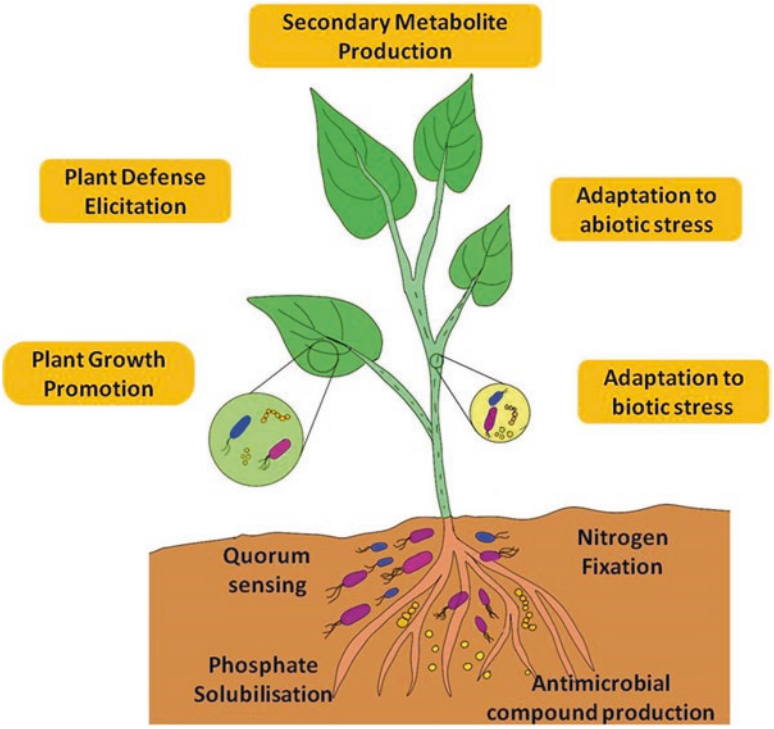


**Fig. 12.1** Role of endophytes on plant growth promotion and stress management

Furthermore, several endophytes can convert the complex form of minerals and macro- and micronutrients into simplex form and avail to the host plant for the growth and development (Ma et al. 2011; Gaiero et al. 2013). The observations of two decades have projected that the endophytes migrated from the rhizospheric or seed-born microbial communities, and molecular tools based on advanced findings have revealed that these microbes are highly versatile and possess several functional genes that govern the several novel beneficial applications to the host plant (Ali et al. 2014). For example, the highly anti-cancerous compound Taxol was synthesized by endophytes of *Taxus baccata* (yew plant) which was not possible without endosymbiotic association (Somjaipeng et al. 2015). However, recognition of these specialized designated genes for the plant-microbe association is still the subject of investigation. To maintain the healthy symbiosis, endophytes maintain the optimum conditions for the host plant by protecting the host plants against several invaders, pre-immunizing against various biotic and abiotic stresses, and producing or inducing the host plant for the synthesis of metabolites to promote the growth and yield (Singh and Gaur 2016, 2017; Mishra et al. 2018a, b).

Moreover, an overwhelming number of studies highlight the considerably ponderability of microbial association with plants in seed germination, seedlings vigor, cellular development, and nutrient availability, ameliorate the biotic and abiotic

stress resistance ability of the plant, and improve the synthesis of therapeutically important secondary metabolites and their productivity (Naveed et al. 2014; Forchetti et al. 2010; Khan et al. 2012). The perception of "plant symbionts" has re-modulated the conception of "plant microbiome," and therefore the current era emphasizes to investigate the coevolution of plants and their symbionts at the different environment and key factors and conditions, which govern the symbiosis between both partners (Turner et al. 2013). The upregulation of plant gene expression after the treatment of endophytes is one of the strongest evidence about the impact of endophytes on host plant (Mishra et al. 2018a, b; Berendsen et al. 2015). Furthermore, the modern technology-based studies, i.e., next-generation sequencing (NGS), metagenomics, metatranscriptomics, genome sequencing, and comparative genomics, may facilitate the much deeper knowledge about the multidimensional interactions between endophytes and their plant hosts.

## 12.2 Different Modes of Plant-Microbe Interaction

Endosymbionts are considered as a "second genome of plants" because they are very closely associated with host plants and independently play a very crucial role to accomplish the several definite tasks assigned by the host. Instead of this, host plants ensure the favorable condition of associated microbes by providing the required carbon and nutrients to proliferate competitively in a complex interactive environment. These microbial interactions encompasses the complete host plants as well as specific organ or region, i.e., roots, shoots, flowers, seeds, and leaves or rhizospheric, epiphytic, and endophytic (Turner et al. 2013; Rout and Southworth 2013; Wang et al. 2017; Malviya et al. 2019; Solanki et al. 2019). Other than this, the host plant also possesses an array of biological information that describes the association of endosymbionts including functional genes, transcripts, proteins, and several biologically active metabolites (Busby et al. 2017). Presently, several studies elaborated the significance of plant-associated microbiota on plant growth and development (Brussaard 2012). However, the highly focused investigation is still needed for the enumeration of potent plant-associated microflora.

Plants are closely associated with numerous range of microbiota both below and above the ground for their mutual benefits. This microbiome is characterized on the basis of their colonization and can be classified as rhizospheric (closely associated with the root), phyllospheric (associated with aerial part of the plant), and endophytic (inside the plant tissues) at their natural habitats (Truyens et al. 2015; Bakker et al. 2014). Out of them, the plant possesses microflora which are organ-specific. These endosymbiotic microbial loads are highly inconsistent because of the variation in the genome, environment, climate, and plant development. Several conditions, such as the activation of the innate immune system, can also be intervening in the modulation of the associated microbial load of the host plant.

To diagnose the impact of several factors on plant microbiomes, this chapter also summarizes the diversity of plant microbiome based on different compartments individually. These compartments are the major reservoir of plant microbiome and primarily known as rhizosphere, endosphere, and phyllosphere.

### 12.2.1 Rhizosphere

Out of the several natural habitats, soil system is the great reservoir of microbial load and exhibited about $10^8$–$10^9$ CFU per gram of the soil (Chaparro et al. 2014). The rhizosphere is the most dynamic region which is extremely affected by the plant secretomes such as root exudates, mucilages, sloughed cells, and rhizodeposition (Spence et al. 2014). Plants regularly secrete several bioactive secondary metabolites as a rich source of carbohydrates, proteins, lipids, phenolic compounds, and organic acids, which can efficiently modulate the microbial dynamics (Andreote et al. 2014). Furthermore, the plant litters are also a good source of several organic molecules (i.e., amino acids, nucleic acids, and saccharides), cellulose, hemicelluloses, lignin, and polyphenolic compounds (Osono 2007, McGuire and Treseder 2010, Talbot and Treseder 2012). The microbial load of the rhizospheric region is completely different from the normal soil communities because of the availability of root secretomes and plant secondary metabolites that refurbished the conditions and nutrient availability. Molecules represent the root secretomes that play an essential role in the quorum sensing and may be also able to instigate the metabolic activity of soil-inhabiting microbiomes (Vandenkoornhuyse et al. 2015), even though few of them are secreted under special circumstances to magnetize the specific microbial community such as malic acid secreted by the plant to attract the *Bacillus* sp. (Rudrappa et al. 2008); rhizodeposition of plant litter especially lignin and other polyphenols mainly conserved the subkingdom Basidiomycota (Baldrian 2006). Furthermore, some plant secretomes also have growth-limiting abilities (Martiny 2016). On the one hand, the higher abundance of microbiome significantly induces the microbial load in a per gram of soil and can reach up to the $10^{11}$, which can alter the environment of the rhizosphere. For example, deficiency of oxygen, as it is continuously utilized by the microbial biomass, can also lead to the alteration in the pH, the concentration of microbial enzymatic activity (Classen et al. 2015), and the organic contents present in the rhizosphere. On the other hand, the higher abundance of microbial diversity at the rhizosphere represents the higher genetic information which might be useful to diagnose several unrevealed metabolic pathways. The modulation in microbial diversity also explores the unresolved consequence of lifestyle, genetic adaptability, interaction with other communities, coevolution, and existence with organisms in the similar climatic conditions (Philippot et al. 2010; Berendsen et al. 2012). The mutualistic association between plants and associated microorganisms can also lead to several genetic re-modulation, horizontal gene transformation (HGT), etc. It follows the selection of highly appropriate microbial community under the influence of native environmental factors, and consequently the host plant communicates through the sharing of genetic information and possesses novel metabolic adaptability. The mutualistic association with the plant could be accomplished because of the availability of huge microbial diversity at rhizospheric as well as phyllospheric regions. Furthermore, the richness of highly transmitted genetic materials such as viruses, plasmids, and genes at the rhizospheric region can also facilitate an opportunity to exchange several unrevealed information for the plant-microbe interaction.

### 12.2.2 Phyllosphere

The phyllosphere can be considered as the second most primitive zone after the rhizosphere for plant-microbe interaction as it is the aerial part of the plant including leaves where the intensity of microbial diversity was found to be higher (Vorholt 2012; Copeland et al. 2015). Microbes associated at the phyllospheric region of the plant are also able to confer the health and wealth of the plant through the induction of mineralization of nutrients, biosynthesis of several phytohormones, and activation of the innate immune response of the host plants against several pathogenic invaders (Cappelletti et al. 2016). Carbon sequestration done by them is an important task which maintains the balance of the environment (Bulgarelli et al. 2013; Bringel and Couée 2015). Furthermore, they also perform the considerable role in the survival of plants under extreme conditions, i.e., nutrient-limiting conditions, high and low pH, unfavorable temperature, less humidity, UV, etc. (Whipps et al. 2008). Out of various microbes, bacterial communities are the most prominent colonizers at the phyllospheric region, although they are drifted severally. Because of the close attachment with several environmental factors, the microbial load at the phyllosphere is fluctuating very drastically even in same species of plants, under similar environmental condition, as well as at same developmental stage (Rastogi et al. 2013; Knief et al. 2012). Furthermore, the diversity of phyllospheric microflora also depends on the several key factors, i.e., leaf area, leaf secretome, the intensity of light and UV, airflow rate, moisture content, etc. For example, most of the pigments producing bacterial communities efficiently colonize at phyllospheric region because of the ability of higher tolerance toward the UV radiations. The origin of microbes is the next most considerable factor that imprinted the great versatility in microbial diversity at the phyllosphere. Other factors, especially air, wind, and water, are the important sources of microbial cells that are also able to alter the microbial dynamics at the phyllosphere (Bullgarelli et al. 2013). As due to the high airflow, several spore-forming microbes could colonize on the aerial part of the plant that is found far from the plant origin (Bullgarelli et al. 2013). Furthermore, the fluctuations in phytomicrobiome mostly rely on the available carbon substrates and nutrients on the aerial parts especially on the leaf surface including amino acids, glucose, xylose, volatiles, etc. (Lindow and Brandl 2003). Bacterial communities colonizing at the phyllosphere are also well studied for their biofilm formation to maintain the heterogeneity of the microbial population and for adherence under unfavorable circumstances. Among several bacterial communities, Proteobacteria, Actinobacteria, Bacteroidetes, and Firmicutes are the most common colonizers and contribute a decisive role which leads to the novel competency of the host plant (Redford et al. 2010; Vorholt 2012). Whereas, the consequence of the close associations and beneficiary assignments, the other microbes also acquire the several evolutionary pieces of evidence which describe the different modes of plant-microbe association in a time-dependent manner. Due to several constitutive and constructive applications, the knowledge about the longtime evolutionary relationship and association between the plant and microbiota will possibly open the new sights for the development of novel applications in the field of sustainable agricultural practices.

### 12.2.3 Endosphere

Every plant presents on earth at different habitats having their specific endophytes, and it assumed that the presence of endophytes in the inner tissues of plants is necessary for their existence (Rosenblueth and Martínez-Romero 2006). In summary, the host plants carry several closely associated endophytes at the same time under in vitro condition. However, the role of endophytes in plant growth promotion and molecular, biochemical, and physiological cross talk between the plant and endophytes to perceive the mechanisms during symbiotic association is still a matter of concern. Some findings reveal that endophytes can promote growth as well as reduce the disease incidence in the host plant. For example, the endophytic actinomycetes, namely, *Streptomyces diastaticus* SP2, *S. fradiae* SP4, *S. olivochromogenes* SP5, *S. collinus* SP8, *S. ossamyceticus* SP10, and *S. griseus* SP12 of different medicinal plants, were efficiently able to enhance the productivity and reduce the disease incidence by triggering the systemic resistance and mitigation of oxidative stress in *Cicer arietinum* against *S. rolfsii* (Singh and Gaur 2016, 2017). Endophytes isolated from sugarcane roots significantly enhance the level of amino acids (Ferrara et al. 2012), while mutant endophytic strain suppresses the pathogenicity of *Diatraea saccharalis* by the production of CRY1 Ac7protein (Quecine et al. 2014). However, out of several attempts on endophytes individually, few reports are also advocated about the aptitude of different endophytic communities to govern more than one function simultaneously. For example, the consortium mode application of endophytic bacterial isolates (*Bacillus amyloliquefaciens* and *Pseudomonas fluorescens*) is not only able to detoxify the excessive generation of ROS and RNS by the activation of defense-responsive genes and systemic resistance-related genes in *Withania somnifera* (L.) Dunal under *Alternaria alternata* stress (Mishra et al. 2018a) but also modulates the expression level of intermediate genes of withanolide biosynthetic pathway (MEV and MEP pathway) under biotic stress condition (Mishra et al. 2018b).

Still, limited information subjected to endophytic diversity creates discrepancies to understand the ecology of plant-associated microbes. In search of the origin of endophytes, several efforts are performed by researchers to track the path of GFP-tagged endophytes (Rouws et al. 2010; Compant et al. 2005; Germaine et al. 2004) or by GUS staining (James et al. 2002; Compant et al. 2005), and they advocated that endophytes are migrated through the rhizospheric or rhizoplane regions mainly, whereas few of them are transmitted *via* seeds (Hallmann et al. 1997; Saikkonen et al. 1998; Mitter et al. 2013) and entered through the opening during the formation of root hairs or the emergence of lateral root. However, very few of them access in the plant *via* phyllosphere region and entered by stomatal openings, wounds, and hydathodes. Moreover, various microbes possess the ability to secrete several plant cell wall lytic enzymes, i.e., cellulases, xylanases, pectinases, endoglucanases, etc., which enable the bacterial entry in plant internal tissues. The origin of endophytes is useful to understand the direct association with the plant and its proliferation and enumeration inside the inert plant tissues. Recently, it was also studied that specific endophytic communities can cross talk with plants, and strategies followed for their survival are evidence that their genome organization facilitates the survival and transmission.

Recently, few researchers have recognized that the endophytic microbes articulate the molecular signals through the horizontal and vertical transmissions. The majority of recognized plant endophytes is horizontally transmitted and produces a potential conflict of fitness where antagonistic coevolution of functional trait expression possibly arises between the plant and microbial symbionts (Wani et al. 2015). Vertically transmitted endophytes confer the increased host benefits over those horizontally transmitted that are significantly dependent on plant density (van Overbeek and Saikkonen 2016). Furthermore, several studies on the effect of habitat and environmental factors on genetic variation of microbes are noticed that microbes living freely in the water, soil, and air will require an array of genes to develop resistance against adverse environmental stresses, having larger genetic information, whereas microbes residing in irrevocable conditions, i.e., endosymbiotic condition, severally required less molecular adaptation, having comparatively small genome size (Andreote et al. 2012). Similarly, Mitter et al. (2013) revealed a great genetic drift in the diversity of endophytes, and they concluded that the endophytes are a group of different microbial communities which migrated from different habitats. Those which originate from variable ecosystems have a larger genome size, whereas the rest of them which originate from stable environments contained smaller genome size.

Out of the plant-microbe interaction, for the interpretation of endophytes as a phytoendobiome is still needed much deeper knowledge. However, many researchers noticed that in spite of host fitness-based genetic information, endosymbionts also possesses several clusters of functional genes that significantly evolved in the establishment, persistence, and proliferation of the host plant (Bulgarelli et al. 2012; Lundberg et al. 2012). Furthermore, it should also be noted that although the richness of endophytes mostly relies on the population dynamics of rhizospheric microbiota, few groups of microbial communities, i.e., Actinobacteria and Proteobacteria, immanently maintained their presence as an endophyte which annunciates about the specific selection-based criteria of the plant. The role of plant innate immunity, including recognition of microbe-associated molecular patterns (MAMPs), is the possible reason behind the microbial selection.

## 12.3 Cross Talk Between the Plant and Endophytes During Interaction

There are several crucial steps involved for a successful colonization of endosymbiont during plant-microbe interaction including the entry of endophyte in the inner tissue of the plant, the recognition of the plant, and finally the cross talk through the signaling molecules between the host plant and endophytes plants for colonization (Rosenblueth and Martínez-Romero 2006; Compant et al. 2010; Brader et al. 2014). Plant secretomes (exudates are very rich with water and nutrients and work as biomolecules) are facilitating the movement of the entire variant microflora toward the rhizospheric or phyllospheric region. And after that, host plant designates a few of the beneficial microbes as endophytes and allows to colonize and proliferate inside

the plant tissue for growth and development. Flavonoids are the fascinating chemoattractants that perform a decisive role in the plant-endophyte interaction, including various plant secretomes as biomolecules. Flavonoids are well known for the accomplishment of meaningful association with legumes by rhizobia (Arora and Mishra 2016). Furthermore, flavonoids are also involved in the compatible association of non-rhizobial endophytes with the host plant, and these metabolites also affect the colonization of *Serratia* sp. EDA2 and *Azorhizobium caulinodans* ORS571 in the root of rice and wheat (Webster et al. 1998; Balachandar et al. 2006). Lipo-chitooligosaccharides (LCO) are reported as Nod factors, inducing the common symbiotic pathway (CSP) in arbuscular mycorrhizal associations as well as rhizobia-legume associations (Gough and Cullimore, 2011). As per the recent study, a beneficial plant endophyte *Mucor* sp. is associated with *Arabidopsis thaliana* because of the secretion of strigolactone (Rozpądek et al. 2018). Moreover, strigolactone also triggers the biosynthesis and secretion of chitin oligomers that induce the signaling pathways for the symbiotic association between the plant and endosymbiont (López-Ráez et al. 2017). Moreover, a superfamily of plant cell wall proteins, i.e., arabinogalactan (ABO) glycosylated members of the hydroxyproline-rich glycoprotein (HRGP), works as a receptor and signaling molecules participants to accomplish the symbiotic association during plant-microbe interaction (Nguema-Ona et al. 2013). Furthermore, some other basic plant biostimulants such as sugars, amino acids, organic acids, phenolic compounds, and other secondary metabolites provide the signal to endophytes for the mutualistic association (Chagas et al. 2017). However, the strategies followed by the plant to distinguish the plant beneficially and plant pathogenic microbes are still a matter of investigation. However, several studies favor the role of plant innate immune system in the identification of plant growth-promoting microorganisms and their colonization in inert plant tissues (Fesel and Zuccaro 2016). The current evidence on plant gene expression suggests that endophytes perform an essential role in the expression level of plant genes (Mishra et al. 2018b; Singh and Gaur 2017). However, the stimulation of defense-related signaling molecules such as ethylene (ET)/jasmonic acid (JA)/salicylic acid (SA) against different stresses relies on the defense strategies of plant endophytes (Singh and Gaur 2017). In the recent investigation, Mishra et al. (2018a) revealed that the consortium of bacterial endophytes, namely, *B. amyloliquefaciens* and *P. fluorescens*, suppresses the pathogenicity of *Alternaria alternata* in *W. somnifera* by the induction of SA and JA signaling-mediated induced systemic resistance (ISR) (Fig. 12.2). Furthermore, it also modulates the expression level of intermediate genes of MVA and MEP pathways (Mishra et al. 2018b). In contrast to the above findings, several plant defense-related genes were downregulated during the colonization of rhizobia and arbuscular mycorrhizal fungi (AMF) (Fouad et al. 2014; Benhiba et al. 2015; Sarkar et al. 2016). However, the stimulation of plant signaling molecules (SA/JA/ET) after the colonization of plant mutualistic partners controls the overwhelming actions of microbes (Plett and Martin 2018). It was also observed that several hormone pathways related to miRNA transcripts were induced for the proper colonization of endophytes (Formey et al. 2014). For example, the host plant adjourns the GA signaling pathway and induces the expression level of

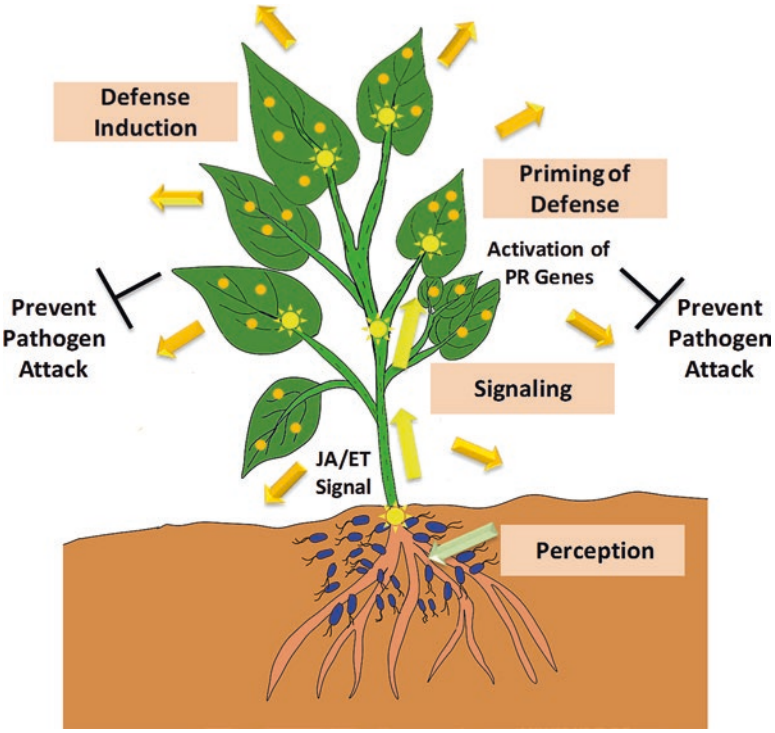


**Fig. 12.2** Induced systemic resistance (ISR) in plants

miRNA – E4D3Z3Y01BW0TQ, to avoid the repressive action against mutualistic associations of AMF with the plant (Wu et al. 2016). The delay in the expression of plant defense-related genes might play a crucial role in the establishment of a mutualistic association with endophytes (Plett and Martin 2018). Several advanced techniques such as genomics- and metagenomics-based findings imprinted the novel insights subjected to the behavior of endophytes during the colonization in the endophytic regions of the plant. As per the findings of Hardoim et al. (2015), the genes involved in catabolic activities in endophytes are much more dominant during the invasion of phytopathogens as compared to the anabolic pathway-related genes (higher copy number in the genome) which are having multifunctional roles. The genes, namely, nitrogenase and ribulose bisphosphate carboxylase/oxygenase (RuBisCO) in combination, work as an indicator for endophytes for symbiotic nitrogen fixation-based association (Karpinets et al. 2014). The lateral gene transformation also performs a crucial role in acquiring several key features for the colonization of endophytes (Tisserant et al. 2013). For instance, several plant beneficiary bacterial endophytes possess the gene mannitol dehydrogenase through lateral gene transformation that not only significantly suppresses the proliferation of fungal phytopathogens but also provides an opportunity to colonize inside the plant (Wu et al. 2011). The existence and positions of several other genes designated for specific

applications such as amino acid synthesis, iron transportation, hemolysin, and hemagglutinin also provide clues regarding the plant-microbe interaction as well as modulation in host and endophytes lifestyles (Taghavi et al. 2010; Xu et al. 2016; Shidore et al. 2010). Besides, the recent soil and plant metagenomics studies reveal that endophytes also depend on the host plants for several activities. Therefore, they reduce their genome size during evolution as a result of adaptation (Sessitsch et al. 2012; Brewer et al. 2016; Hottes et al. 2013). On the other hand, the species- and genotypic-based variations of the host also modulate the diversity of endophytes (Rodríguez-Blanco et al. 2015; Ding and Melcher 2016). Few endophytes modify themselves as per the response of the local environment (biotic and abiotic stresses), developmental stage, and genetic variation of the host (Bacon et al. 2008). For instance, an asymptomatic endophyte *Ramularia collo-cygni* can reside inside the host plant during the developmental stage; however, at the growing stage, it modulates itself as a necrotrophic pathogen (Walters et al. 2008). In the same way, *Fusarium verticillioides* is able to proliferate as an endophyte or pathogen in maize plant (Oren et al. 2003). However, key factors behind the transition of endophytes to phytopathogen are not much studied. Therefore, for better understanding, several advanced tools and techniques based on comparative analyses are needed that can provide novel insights about the specific circumstances which transform the endophyte into a pathogen.

The exact mechanism followed by the microbes to reside inside the plant tissue is still a case of an investigation. However, few studies demonstrate that the infiltration of endophytes in the inner plant tissue is possibly through the inactivation of the primary defense system of the host plant which leads the selection of endophytes by recognition of microbe-associated molecular patterns (MAMPs) (Newman et al. 2013; Cord-Landwehr et al. 2016). These MAMPs include several receptor molecules such as flagellin (Flg), elongation factor TU (EF-Tu), peptidoglycan (PGN), lipopolysaccharides (LPS), bacterial cold shock protein, several plant ROS scavenging molecules (SOD, GPx, APx, etc.), β-glycan (GE), β-glucans, oligopeptides (Pep-13), xylanase (EIX), and chitin (Newman et al. 2013; Shimizu et al. 2010), which are enucleated by the pattern recognition receptors (PRRs; present on the surface of plant cells). For example, the defensive reaction of the host plant is triggered by the recognition of chitin-specific receptors (PR-3; chitin oligomers) (Sanchez-Vallet et al. 2015). Furthermore, endophytes also synthesize several unknown MAMPs to defend themselves from the innate immune system of the plant and are non-detectable by PRRs (Vandenkoornhuyse et al. 2015; Cord-Landwehr et al. 2016). For example, *Burkholderia phytofirmans* (as an endophyte) introduces flagellin (FLS2) that is different from phytopathogens (i.e., *Pseudomonas aeruginosa or Xanthomonas campestris*) (Trda et al. 2015). Moreover, endophytes also produce several ROS/RNS scavenging molecules (such as superoxide dismutases, catalases, peroxidases, alkyl hydroperoxide reductases, and glutathione S-transferases) to protect themselves against nitro-oxidative bursts (Zeidler et al. 2004). As a part of these, endophytes are also able to modulate the host immune system by the formation of protein secretion systems (SSs). For instance, several pathogens deliver the effector proteins though the high stimulation of type III secretion system (T3SS) and type IV

secretion system (T4SS) (Green and Mecsas 2016; Liu et al. 2017). However, endosymbionts communicate with host plants by least stimulation of T3SS and T4SS except few rhizobial and non-photosynthetic *Bradyrhizobium* strains where T3SS plays a decisive role in legumes for nodulation and rice for colonization, respectively (Okazaki et al. 2013; Ausmees et al. 2004; Piromyou et al. 2015). Similarly, type VI secretion systems (T6SSs) are present in several commensal plant symbionts for antimicrobial activity (Reinhold-Hurek and Hurek 2011; Bernal et al. 2018).

## 12.4 Role of Endophytes on ISR Elicitation

The new sustainable agricultural practices lead to the search for effective bioinoculants for the amelioration of productivity and health issues of plants. In this context, the effective bioinoculant is not only able to promote the growth and productivity of plants by the secretion of several phytohormones and other plant growth-promoting substances but is also involved in eliciting different defense responses in both abiotic (i.e., drought, salinity, heavy metals, extreme temperature, radiation, etc.) and biotic (such as insects, mites, aphids, nematodes, bacteria, fungi, viruses, etc.) stresses through different mode of actions. Such ability to suppress the infection of different plant invaders (through the secretion of antibiotics and antimicrobial substance) is termed as antagonism or biocontrol. It includes other specific mechanisms such as antibiosis, competition, etc. Alternatively, microbes can induce the innate immune system of the host plants through the synthesis of various secondary metabolites and in a way that the endophytes elicit the resistance of the plant toward several biotic and abiotic stresses. The microbe-mediated induction of systemic resistance of the plant is known as induced systemic resistance (ISR) (Fig. 12.2). Moreover, the resistance develops due to the chemical inducers or pathogenicity of necrotrophic pathogens is termed as systemic acquired resistance (SAR) (Fig. 12.3). Both pathways not only differ by the elicitors but also accomplished it by different signal transduction pathways. The ISR is activated through the signaling pathway of using jasmonic acid or ethylene (Matilla et al. 2010), whereas the stimulation of salicylic acid is responsible for the elicitation of SAR. In a few cases, jasmonic acid or ethylene also performs a decisive role in the activation of SAR. ISR cannot be distinguished based on SAR-based signal transduction pathway because some current findings have demonstrated that endophytes and other plant-associated beneficial microbes can induce the ISR which is completely dependent on the salicylic acid (van Loon et al. 2006).

Furthermore, bacterial endophytes are involved in the amelioration of various stress responses and are known to initiate responses like those of the rhizosphere-associated bacteria (Ryan et al. 2008). The mechanisms involved in eliciting abiotic defense responses have been extensively reviewed by Khan et al. (2015), and more recently by Lata et al. (2018). The endophytes have been reported to play a significant role in the amelioration of diverse abiotic stresses. The mechanisms involved in biotic stress defense responses have been thoroughly studied by Kloepper and Ryu (2006) and Busby et al. (2016), which involve the role of both bacterial and fungal plant endophytes against the invading plant pathogens.

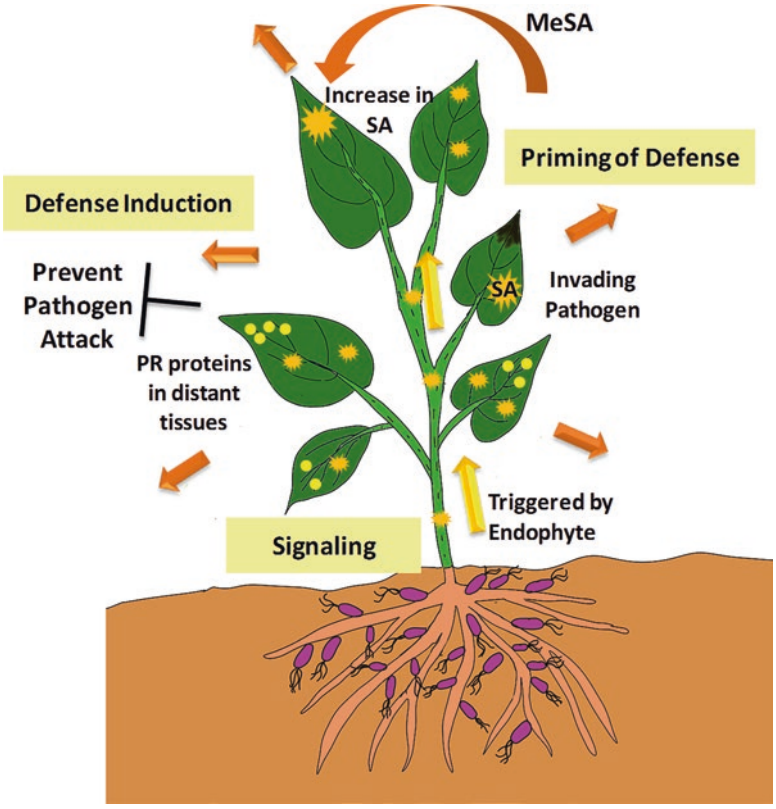


**Fig. 12.3** Systemic acquired resistance (SAR) in plants

## 12.5 Role of Endophytes in Biotic Stresses

The plants in their natural habitat face several biotic stresses which include the attack of various macroscopic organisms including insects, mites, aphids, nematodes, etc. as well as various microscopic pathogens like bacteria, fungi, viruses, etc. It has been reported that the priming or treatment of plants with the endophytes has resulted in a decrease in damage caused by invading pathogens and pests (Romero et al. 2018; Pappas et al. 2018). The mechanisms related to the antimicrobial activity of potent endophytes have been studied severally (Mishra et al. 2018a; Singh and Gaur 2016, 2017). Many reports demonstrate that endophytes suppress the pathogenicity of the plant by direct antagonism (by the synthesis of several antimicrobial substances including antibiotics) or through the modulation in the severity of the host defense system (via ISR) against phytopathogens (Singh and Gaur 2017; Mishra et al. 2018a).

Furthermore, endosymbionts induce the stimulation of JA, SA, and ET (ISR and SAR pathway signaling molecules), which are severally known for the stress responses against phytopathogens (Khare et al. 2016).

Moreover, the application of endophytes also regulates the gene expression of host plants which leads to the enhanced physiological performance, higher expression in defense-related pathways, synthesis of antimicrobials, etc. (Mishra et al. 2018b). Phytohormones also play a decisive role in the plant defense system. For instance, gibberellin ($GA_3$) production by endophytes pre-immunizes the host plant toward the phytopathogens as well as insects (Waqas et al. 2015). Furthermore, seed coating with endophytic actinobacterial strains not only improves the strength and physiology of chickpea plants but also upregulates the expression level of defense-related genes against *S. rolfsii* (Singh and Gaur 2016). Endosymbionts are well known to synthesize several volatile organic compounds (VOCs) which have significant antimicrobial activity against fungal, bacterial, and viral phytopathogens and nematodes. Endophytes are also able to synthesize phytoalexins (low molecular weight antimicrobial molecules) that induce the production of phyto-stimulants in host plants. In a study, Gupta et al. (2017) reported that chitinolytic bacterial strains, namely, *Chitiniphilus* sp. and *Streptomyces* sp., inhibit the pathogenicity of *Meloidogyne incognita* (nematode) and induce the synthesis of secondary metabolites in *Bacopa monnieri.* Furthermore, findings of several workers reveal that the pretreatment of endophytes improves the cellulose content and lamina density, which reduces the attack by herbivores including leaf-cutting ants (Van Bael et al. 2012; Estrada et al. 2013). In this way, the endophytes not only develop resistance against plant invaders through ameliorating the host defense system but also stimulate several antimicrobial substances that have an antagonistic approach toward the phytopathogens.

### 12.5.1 Bacterial Endophytes

The niche provided by the host plants provides a safe harbor for the bacterial strains that can colonize *in planta* without causing diseases in their host plants (Bacon 2018). The bacterial endophytes are widely reported to inhabit within the intercellular and intracellular cells within the host tissue (Liu et al. 2017; Hardoim et al. 2015; Thomas and Sekhar 2014) (Table 12.1). Several endophytes have not only been isolated from the leaves, stems, and roots of the plants but also the seeds (White et al. 2018; Ambrose et al. 2018; Truyens et al. 2015). The endophyte isolation has been carried out from different plants like crop plants (Contreras-Cornejo et al. 2018; Abd-Allah et al. 2017; Ahmad et al. 2015), forest trees (Yao et al. 2017; Somjaipeng et al. 2015), and medicinally important plants (Mishra et al. 2018a; Singh and Gaur 2016) as well as from grasses (White et al. 2018).

The recent studies have shown that the endophytic bacteria play a critical role in eliciting defense responses in host plants against the invading pathogens through both direct and indirect mechanisms (Strobel 2018; Lata et al. 2018; Singh and Gaur 2017). The mechanisms of eliciting defense by endophytes are reported to be similar to that of the rhizospheric bacteria (Brader et al. 2014). The prior introduction or priming of plants through the treatment of roots or seed treatments prevents the host

**Table 12.1** Endophytes defense mechanism against the phytopathogens

| S.no. | Endophytes | Sources | Pathogens | Crops | Defence responses | References |
|---|---|---|---|---|---|---|
| *Bacterial endophytes* | | | | | | |
| 1. | *Bacillus amyloliquefaciens* (GB03) *and Microbacterium imperiale* (MAIIF2a) | Cassava *(Manihot esculenta)* | *Fusarium solani* | Cassava *(Manihot esculenta)* | ISR | Freitas et al. (2019) |
| 2. | *Streptomyces* and *Pseudomonas* | Fruit trees | *Agrobacterium tumefaciens* | Honggengansutao and Okinawa | ISR and SAR | Li et al. (2019) |
| 3. | *Piriformospora indica* | *Anthurium andraeanum* | *Ralstonia solanacearum* | *Anthurium andraeanum* | ISR | Lin et al. (2019) |
| 4. | Endophytic bacteria strain REB01 | Rice seed | *Rhizoctonia solani* | Rice | ISR | Mao et al. (2019) |
| 5. | *Bacillus*, *Paenibacillus*, *Pediococcus*, *Lactococcus*, and *Pantoea* | Seeds of diverse cucurbits | Fungal and oomycete pathogens including powdery mildew | Cucurbits and vegetables | ISR | Khalaf and Raizada (2018) |
| 6. | *B.amyloliquefaciens* and *P. florescence* | *W. somnifera* and *R. serpentina* | *Alternaria alternata* | *Withania somnifera* | SAR and ISR | Mishra et al. (2018a, b) |
| 7. | *Pseudomonas protegens* Pf-5 and *Pseudomonas* sp. G22 | *Triticum aestivum* | *Magnaporthe oryzae B157 and Rhizoctonia solani* | Rice, sorghum, and wheat | ISR | Patel and Archana (2018) |
| 8. | *Actinobacteria* | Medicinal plants | *S. rolfsii* | *Chickpea* | ISR | Singh and Gaur (2017) |
| 9. | *Pseudozyma churashimaensis* | *Pepper* | *Xanthomonas axonopodis*, *Cucumber mosaic virus*, *Pepper mottle virus*, *Pepper mild mottle virus* | *Pepper* | ISR | Lee et al. (2017) |
| 10. | *Streptomyces* sp. | *Medicinal plants* | *S. rolfsii* | *Chickpea* | ISR | Singh and Gaur (2016) |
| 11. | *Rhodococcus* sp. | Plant leaf | *Ceratocystis fimbriata* | *Sweet potato* | ISR | Hong et al. (2016) |

| | | | | | | |
|---|---|---|---|---|---|---|
| 12. | *Pantoea eucalyptii* | *Solanum lycopersici* | *Botrytis cinerea* | *Arabidopsis thaliana* | ISR | Romero et al. (2016) |
| 13. | *Pseudomonas fluorescens* PICF7 | Olive roots | *Verticillium dahliae* | *Arabidopsis thaliana* | ISR | Maldonado-González et al. (2015) |
| 14. | *B. amyloliquefaciens* | *Ginkgo biloba* | *Phytophthora infestans* | *Pepper* | ISR | Yang et al. (2015) |
| 15. | *Bacillus pumilus* | *Cucumis sativus* | *Ralstonia solanacearum* | *Piper nigrum* | ISR | Yi et al. (2013) |
| 16. | *B. amyloliquefaciens*, *B. pumilus* | *Solanum lycopersicum* | *Xanthomonas vesicatoria* | *Solanum lycopersicum* | ISR | Lanna-Filho et al. (2013) |
| 17. | *Azospirillum* sp. | *Oryza sativa* | *Magnaporthe oryzae* | *Oryza sativa* | SAR | Yasuda et al. (2009) |
| 18. | *Streptomyces* sp. | *Triticum aestivum* | *Erwinia carotovora* | *Arabidopsis thaliana* | SAR | Conn et al. (2008) |
| 19. | *Actinobacteria* | *Triticum aestivum* | *Fusarium oxysporum* | *Arabidopsis thaliana* | SAR | Conn et al. (2008) |
| 20. | *Pseudomonas fluorescence* | *Triticum aestivum* | *Fusarium* wilt | *Dianthus caryophyllus* | ISR | Van et al. (1991) |
| *Fungal endophytes* | | | | | | |
| 1. | *Beauveria bassiana* | *Vitis vinifera* L. | *Planococcus ficus* | *Vitis vinifera* L. | ISR | Moloinyane and Nchu (2019) |
| 2. | *Aspergillus fumigatus* SG-17 | *M. laxiflora* root | Oxidative stress | *M. laxiflora* | ISR and antioxidant ROS scavenging | Qin et al. (2019) |
| 3. | *Fusarium solani* | *Solanum lycopersicum* | *Tetranychus urticae* | *Solanum lycopersicum* | Indirect tomato defense by the production of volatiles | Pappas et al. (2018) |
| 4. | *Trichoderma atroviride* | *Zea mays* | *Spodoptera frugiperda* | *Zea mays* | ISR | Contreras-Cornejo et al. (2018) |

(continued)

**Table 12.1** (continued)

| S.no. | Endophytes | Sources | Pathogens | Crops | Defence responses | References |
|---|---|---|---|---|---|---|
| 5. | *Rhexocercosporidium* sp. and *F. solani* | *Sophora tonkinensis* | *Pathogenic F. solani* | *Panax notoginseng* | Production of secondary metabolites | Yao et al. (2017) |
| 6. | *Epichloë* sp. | *Achnatherum robustum* | *Aphids* | *Achnatherum robustum* | Alkaloid Production | Shymanovich et al. (2015) |
| 7. | *Phomopsis* sp. | *Fallopia japonica* | *Puccinia polygoni-amphibii* var. *tovariae* | *Fallopia japonica* | *Suppressive interaction* | Kurose et al. (2012) |
| 8. | *Trichoderma* spp. | *Arabidopsis thaliana* | *Botrytis cinerea* | *Arabidopsis thaliana* | ISR | Contreras-Cornejo et al. (2011) |
| 9. | *Fusarium solani* | *Solanum lycopersicum* | *Septoria lycopersici* | *Solanum lycopersicum* | ISR | Kavroulakis et al. (2007) |
| 10. | *Fusarium oxysporum* strain 162 | *Solanum lycopersicum* | *Meloidogyne incognita* | *Solanum lycopersicum* | ISR | El-Fattah Adnan Dababat and Alexander Sikora (2007) |
| 11. | *Fusarium oxysporum* | *Solanum lycopersicum* | *Radopholus similis* | *Musa acuminata* | ISR | Vu et al. (2006) |
| 12. | *Piriformospora indica* | *Prosopis juliflora* | *Rhynchosporium secalis* | *Hordeum vulgare* | ISR | Waller et al. (2005) |

plant from diseases caused by bacterial and fungal phytopathogens (Mishra et al. 2018a; Singh and Gaur 2017). The effects of priming of plants with endophytes have been reported to act as a defense against various insects and pest as well (Vidal and Jaber 2015). The bacterial endophytes are reported to activate induced systemic resistance (ISR) similar to that of the rhizospheric bacteria (Berendsen et al. 2018; Shoresh et al. 2010). ISR is a plant defense pathway that is triggered by jasmonic acid or ethylene-based pathways. It is similar in response to systemic acquired resistance (SAR), which is salicylic acid-dependent pathway for the defense against the invading phytopathogen in the host plants (Shoresh et al. 2010). The SAR is activated when a pathogen invades which in turn activates the first line of defense in the host plant and elicits hypersensitive reaction to restrict the spreading and propagation of the pathogen further into the plant cells. The hypersensitive reaction acts suicidal response causing the death of its cells where the pathogen invaded, resulting in restricting the spread of the pathogen through the formation of localized necrotic zones (Gao et al. 2015). The ISR, on the other hand, primes the plants' second line of defenses and thus does not cause visible symptoms like those formed during SAR (Pieterse et al. 2014).

The bacterial endophytes not only trigger defense responses in the host; they are also involved in direct antagonistic action against the invading pathogens through the secretion of various enzymes and antimicrobial compounds. The enzyme produced by the endophytes includes fungal cell wall degrading chitinase and cellulose or for bacterial pathogen β-glucanases (Khare et al. 2018). The endophytes also are a rich repository of various antimicrobial compounds which are used as antibiotics and are known to be antifungal. Some endophytes are belonging to genera *Bacillus*, *Pseudomonas*, and *Burkholderia* that are known to produce a wide range of antimicrobial compounds against the invading pathogens (Martinez-Klimova et al. 2017; Gouda et al. 2016).

Antibacterial compounds like munumbicins A–D (Hasegawa et al. 2006); kakadumycins (Castillo et al. 2003); coronamycins, a peptide antibiotic (Ezra et al. 2004); and dimethyl novobiocins (Kurosawa et al. 2006) have been reported from endophytic strain of *Streptomyces* spp. compounds like pumilacidin, an antifungal compound (Melo et al. 2009); and antifungal iturin A, fengycin, and bacillomycin (Gond et al. 2015) are reported to be produced by *Bacillus* sp. Among the *Pseudomonas*, antifungal volatile organic compounds (Hernández-León et al. 2015) are reported to be produced along with compounds like ecomycins B and C (Miller et al. 1998) and pseudomycins (Harrison et al. 1991).

### 12.5.2 Fungal Endophytes

Several fungal strains have been reported as endophytes (Table 12.1). These endophytes are known for their hyperparasitic or mycoparasitic activity against the invading fungal phytopathogens. Endophytic *Trichoderma* sp. is known to parasitize the phytopathogen *Rhizoctonia solani* and other phytopathogens by coiling

around the pathogen and producing chitinase enzyme leading to the disintegration of the fungal cell wall (Grosch et al. 2006; Chen et al. 2016)

The endophytic fungal isolates have been isolated from several medicinal plants such as *Paraconiothyrium* from *Taxus baccata* (Somjaipeng et al. 2015); *Bacillus amyloliquefaciens* and *Pseudomonas fluorescens* from *W. somnifera* and *R. serpentina* (Mishra et al. 2018a); bacterial strains from *Ginkgo biloba* (Yang et al. 2015); *Trichoderma gamsii* from *Panax notoginseng* (Chen et al. 2016) as well as commercially important plants, i.e., *Trichoderma atroviride* from maize (Contreras-Cornejo et al. 2018); *Pestalotiopsis* sp. from rice (Cord-Landwehr et al. 2016); and *Pseudomonas* sp. from *Solanum lycopersicum* (Subramanian et al. 2015), and these endophytes have been extensively utilized owing to their ability of production of secondary metabolites. Studies have revealed that these fungal endophytes mimic the secondary metabolites produced by their host plant, thus providing an additional layer of defense to their host plant against the invading phytopathogens, pest, and herbivores. However, the exact mechanism involved in the mimicking the production of the secondary metabolites of host plants by the endophytes is still not clear. The fungal endophytes are known to produce a more diverse group of compounds as compared to the native soil fungi (Nisa et al. 2015). Endophytic *Trichoderma harzianum* isolated from *Ilex cornuta* has been reported to produce antimicrobial compound trichodermin against phytopathogens like *Alternaria solani* and *R. solani* (Shentu et al. 2010). Cycloepoxylactone and cycloepoxytriol B are produced endophytic fungus *Phomopsis* sp. isolated from *Laurus azorica* (Hussain et al. 2009). Pestalachloride A isolated from the endophytic fungus *Pestalotiopsis adusta* inhabiting stem of an unknown Chinese tree is reported to be antagonistic against the phytopathogens like *Fusarium culmorum*, *Gibberella zeae* (anamorph *F. graminearum*), and *Verticillium albo-atrum* (Li et al. 2008).

The fungal endophytes like their bacterial counterparts are known to induce ISR mechanism in their host plants to elicit defense responses. The ISR elicited by the fungal endophytes may also be linked to the expression of pathogenesis-related genes. PR genes PR5 and PR7 in tomato plant roots are triggered by the endophytic fungal isolate *Fusarium solani* against the tomato foliar pathogen *Septoria lycopersici* (Kavroulakis et al. 2007). Nonpathogenic strains of *Fusarium oxysporum* are known to elicit ISR in banana plants against *Radopholus similis* (Vu et al. 2006). Endophytic *Trichoderma spp.* have been reported to protect *Arabidopsis thaliana* against *Botrytis cinerea* through ISR (Contreras-Cornejo et al. 2011).

Other mechanisms used by the endophytic fungal isolates include eliciting higher lignin deposition and enhanced activity of both antioxidant and defense enzymes. It has been reported in *Citrullus lanatus* and *Cucumis sativus* primed with the nonpathogenic mutant of *Colletotrichum magna* against the phytopathogens *Colletotrichum orbiculare* and *Fusarium oxysporum* (Redman et al. 1999). In *Cicer arietinum* colonization by endophytic fungi, *Piriformospora indica* has shown enhanced production of the antioxidant enzyme against the phytopathogen *Botrytis cinerea* (Narayan et al. 2017). *Trichoderma* T22 has shown to enhance the expression of the defense enzyme phenylalanine ammonia lyase (PAL) which is involved in the lignifications of the host plant's cell wall in maize plants (Shoresh et al. 2010).

Nematodes are also identified as one of the major agricultural pathogens that lead to severe crop loss annually. Stirling (2017) defined the biological control of nematodes as "reduction in nematode damage by organisms antagonistic to nematodes through the regulation of nematode populations and/or a reduction in the capacity of nematodes to cause damage, which occurs naturally or is accomplished through the manipulation of the environment or by the mass introduction of antagonists" (Stirling 2017). Although nematologists have been extensively researching biological control of nematodes since the 1920s till date, only a few biological control agents have been reported, but some fungal endophytes have been reported to be antagonistic to the invading nematodes. *Clonostachys rosea* has been reported to be a parasitic nematode fungus through the production of fungal proteases (Iqbal et al. 2018).

## 12.6 Role of Endophytes in Abiotic Stresses

Plants were grown under the surroundings of inanimate components of environments (i.e., light, water, carbon, nutrients, etc.) which are associated with climatic, edaphic, and physiographic factors. Categorically the extreme conditions of these factors (i.e., drought, salinity, temperature, heavy metals, poor soil nutrient, and oxidative burst) considerably affect the plant growth and cause abiotic stress. Endophytes successfully ameliorate the negative impact of these abiotic factors by the implementation of several mechanisms. The interaction of endosymbionts with the plant is very crucial not only for the induction of ISR against biotic stresses but also in terms of adaptation and tolerance of both participants against abiotic stresses. The endosymbiotic-mediated abiotic stress responses of the plant are termed as *induced systemic tolerance (IST)*.

In past decades, the emerging role of endophytes for the alleviation of plant abiotic stresses has attained a great response (Nadeem et al. 2014; Souza et al. 2014). Endophytes possess several fundamental metabolic and genetic abilities that significantly perform a vital role in the amelioration of abiotic stresses in the host plant (Hardoim et al. 2015). Several plant-associated beneficial microbes including *Pseudomonas* sp. (Otieno et al. 2015), *Bacillus* sp. (Vardharajula et al. 2011), *Rhizobium* sp. (Remans et al. 2008), *Bradyrhizobium* sp. (Aung et al. 2013), *Streptomycetes* sp. (Naylor et al. 2017), *Trichoderma* sp. (Mishra et al. 2019; Ahmad et al. 2015), *Methylobacterium* sp. (Meena et al. 2012), and *Cyanobacteria* sp. (Singh et al. 2011) are reported for the mitigation of abiotic stresses in the plant. Currently, Mishra et al. (2019) have reported the efficacy of impulsive micromanager *Trichoderma reesei* MTCC5659 on $CO_2$ stress amelioration in drought-resistant and sensitive rice cultivars because of the upregulation of dehydrin (*dhy*), glutathione S-transferases (*gst*), universal stress protein (*usp*), late embryogenesis protein (*lea*), and no epical meristematic (*nam*) genes along with several fundamental processes (i.e., photosynthesis, transpiration, respiration, stomatal conductance, and water-use efficiency). The association of endophytes leads to several modifications in the host plant, i.e., level of phytohormones, defense-related proteins, synthesis of

antioxidants, exopolysaccharide, and enzymes which ensure the tolerance of the host plant against drought stress condition (Hubbard et al. 2014). For example, the application of *Trichoderma reesei* not only enhances the germination of *Cicer arietinum* in diesel fuel-spiked soil but also elevates the dehydrogenase activity and microbial dynamics of rhizospheric soil (Mishra and Nautiyal 2009).

Soil salinity is also a very effective abiotic factor and causes substantive harm to plant growth and productivity. The bacterial endophytes such as *Pseudomonas* sp. and *Bacillus subtilis* (BERA 17) are reported to produce the higher content of ACC deaminase and IAA which confirms the growth, osmo-regularity, photosynthetic rate, and productivity of tomato and chickpea plant under high salinity condition of soil, respectively (Win et al. 2018; Abd-Allah et al. 2017). Likewise, *Burkholderia phytofirmans* strain PsJN ameliorates the salinity stress in *Arabidopsis thaliana* (Pinedo et al. 2015). Similarly, the application of *Streptomyces* sp. confers the NaCl tolerance and growth in tomato plant (Palaniyandi et al. 2014). Besides, these several toxic metalloids (arsenic, cadmium, lead, etc.) and other hazardous compounds are regularly deposited in the soil because of several anthropogenic activities and industrial effluents. They not only affect the growth and productivity of plants but also limit the nutritional value of the soil.

Furthermore, several findings demonstrate that the traces of heavy metals are accruing in the crops and vegetable from the contaminated irrigating water or growing soil (Sharma et al. 2006; Dwivedi et al. 2010; Awasthi et al. 2017). These toxic metalloids are nondegradable; therefore, they can never be eliminated from the environment completely. However, studies revealed that few beneficial microbes efficiently reduce the toxicity through the biotransformation of toxic metalloids in less or nontoxic forms (Lakshmanan et al. 2016; Singh and Gaur 2016). For instance, the endophytic bacterial strains isolated from the *Betula celtiberica* have the great potential to improve the phytoremediation of As (Mesa et al. 2017).

Similarly, *Salix caprea* plant is well known regarding their potential to support heavy metal phytoextraction. Different actinobacterial strains of *Salix caprea* can synthesize the Zn and Cd mobilizing metabolites and therefore enhance the allocation of metals in the plant (Kuffner et al. 2010). On the other hand, the treatment of *Trichoderma* not only reduces the As deposition in chickpea plant but also enhances the nutritional value of the plant yield (Tripathi et al. 2013). Likewise, arsenic (As)-resistant strain *Staphylococcus arlettae* reduces the As uptake in Indian mustard (*Brassica juncea*) along with plant growth-promoting abilities (Srivastava et al. 2015).

Apart of that, endosymbionts also able to trigger the innate immunity of plants through the secretion of extracellular secondary metabolites, and consequently several defense-related activities have been taking place in each and every plant cell to reduce the negative impact of stress conditions. Similar to other microscopic endosymbionts, mycorrhiza also played a decisive role in the plant growth regulation and abiotic stress amelioration. The fine threads like networking of mycorrhizal hyphae are beneficial in the nutrient uptake management by plant roots. An endophytic mycorrhiza, namely, *Piriformospora indica*, ameliorates the level of antioxidants which subsequently improves the NaCl and drought tolerance ability of barley

(Baltruschat et al. 2008) and Chinese cabbage (Sun et al. 2010). On the one hand, endophytes triggered the systemic resistance for the survival of the plant under unfavorable environmental conditions, while on the other they improve the health and yielding attributes of the host plant by phytohormones stimulation, synthesis of phyto-inducers and antimicrobial substances, production of chelating ions, and fixation of N and other factors. All of these traits of endosymbionts make their presence necessary for the amelioration of abiotic stresses and thus prove themselves as an excellent option for crop management strategies.

## 12.7 Future Outlook

Endophytes remain untapped, not widely explored the community of microbes residing within the host plants. A few decades ago, they were thought not to affect their host plants. But in the recent years, the extensive research done on the role of endophytes in regulating various physiological aspects in its host plants have brought to light their critical role and have opened up new avenues for their utilization of various sectors including in pharmaceutical industries, mediating various plant protection strategies and abiotic stress amelioration. The unique ability of the endophytes to mimic the secondary metabolite synthesis pathways of their host plants makes them ideal candidates for the source of several medicinally and pharmaceutically important drugs which earlier could only be obtained through harvesting the host plants. This technique is turning in the conservation of several important plant species that are being threatened to extinction due to overexploitation by human interventions. Thus, these endophytes serve as an economically attractive solution for obtaining various valuable drugs and medicines without harming the environment. However, how and why the endophytes develop these abilities to mimic their host remains the question of further research.

In the past decade, the development of science and technologies through the facilitation of various "omics" studies has opened doors of new possibilities to understand these microbes better and to utilize them for the betterment of human society. The omics studies like transcriptomics, proteomics, and metabolomics help to elucidate the various pathways involved and to understand better the mechanisms that they employ during the tripartite interaction of the endophyte and host and the invading pathogen (Laur et al. 2018). The studies have revealed that these endophytes that are involved in inducing disease resistance in their host plants have a wide variety of tools at their disposal (Mishra et al. 2018a, b; Singh and Gaur 2017). The myriad of metabolites like bacillomycin, phenazines, iturin, fengycin, etc., produced by these endophytes are found to have antimicrobial properties, which they use against the invading pathogens. These compounds in the future could be identified and isolated for the development of pathogen-specific drugs for agricultural purposes (Zouari et al. 2016; Brader et al. 2014). The endophytes are also known to produce fungal cell wall degrading enzymes that contribute to their antagonistic ability against various invading phytopathogens which can be employed in designing targeted, integrated disease management strategies (Mishra et al. 2018a; Singh and Gaur 2017).

These strategies will not only be effective in controlling the diseases but also play a critical role in promoting and achieving the goal of sustainable agriculture by replacing the use of synthetic chemicals. However, how different endophytic communities coordinate among one another in the microenvironment and coexist remains largely unknown. The better understanding of these aspects will further help in designing more precise disease management strategies (Card et al. 2016).

The endophytes are also known to regulate the physiology of the host plants to adapt better and to acclimatize with the surrounding environment. It has been found that the root endophytes help in drought resistance in their plants enhance the expression and activity of a vacuolar H+ -pumping pyrophosphatase (Vigani et al. 2018). Such endophytes can be widely used in abiotic stress mitigation in a cost-effective manner. Extensive research on their potential can unveil untapped aspects of their ability to combat the various constraints faced in the agriculture sector in cost-effective, eco-friendly, and environmentally sustainable manner.

## 12.8 Conclusion

As discussed in the different contents of the chapter, endophytes serve as great alternatives for the growth and development of host plants, including the enhancement of production of the novel active phyto-compounds. Moreover, several strains of endosymbionts (i.e., bacteria, actinomycetes, fungi, etc.) are performing a decisive role in the induction of systemic resistance in plants, thus leading the suppression of disease severity in the host plant. On the one hand, these findings on endophytic research describe the antagonistic mechanisms of microbes, whereas on the other hand it also illustrates the host responses during the signal transduction pathways that govern the disease protection. The induction of systemic resistance of plants is interconnected with plant growth and development. However, the determination of specific microbial traits and gene pools that ignite the systemic resistance-related signaling in the host plant is still the case of an investigation.

Furthermore, the understandings about the selection criteria followed by the host plant for the elicitation of systemic resistance and plant beneficial strategies in stress conditions also remain to be answered. Several researchers are encouraged to explore the diversity and applications for the endophytes for the upliftment of plant growth and yield under different stress conditions. However, the multifarious performance of endophytes and the way of communication with the host plant still need higher attention. Moreover, the endophytes also represent an incredible source of novel bioactive natural products. Several genes obtained from endophytes have great potentiality, which might be helpful in the development of biotic and abiotic resistant varieties and can also help to improve the understanding of the behavior and mechanisms of microbes during the interaction. Furthermore, currently, just 1–2% of plant species are studied for endophytic association (Strobel and Daisy 2003), and the rest of plant diversity remains unexplored (Strobel 2018). It is now needed to explore the remaining plant-associated microbial diversity at metabolomics as well as the genomic level to detect the biochemical and physiological

aspects of endophytes. Moreover, the information regarding the secondary metabolites and novel bioactive compounds obtained from endophytes that can be facilitated as a cure of several problems are not available in any database.

By resolving these issues, we can exploit the endophytes as an emerging tool in agriculture, medical, and phytopharmaceuticals. In the upcoming scenario, the endophytes may revolutionize the agricultural practices in terms of plant growth and stress management. Furthermore, plant-based drugs can also be modified through the intervention of endophytes-derived bioactive molecules in the near future which can be useful for the amelioration of not only yield and growth of the plants for societal benefits but also the pre-immunization of host plants against several invaders. Similarly, the endophytes-treated plant varieties can also be introduced shortly that develop the plant resistance against phytopathogens. The application of endophytes can also be a better option for the preservation and conservation of indigenous plant species at their natural habitats. Moreover, the development of next-generation bio-formulations based on endophytes still needed more attention and much deeper knowledge on the following threatening issues: (i) genetic and molecular cross talk between both partners, (ii) important factors for the stable and healthy association between the plant and endophytes, and (iii) mode of the interaction.

Conclusively, endophytes are a very valuable biological tool, which can be explored in upcoming days to achieve the objectives of disease management, environmental sustainability, and novel drug development. There is a need to explore the molecular and genetic information of the plant and endophytes during the establishment of a plant-microbe association to obtain the higher benefits from this incredible event.

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# Role of Biotechnology in the Exploration of Soil and Plant Microbiomes

13

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**Abstract**

The agricultural production security is a major challenge under global climatic changes as the agricultural production does not solely depend upon the abiotic factors like temperature and moisture but is also equally affected by the biotic factor present in rhizospheric zones known as soil-plant microbiomes. The importance of soil-plant microbiomes in agricultural production has been revealed by reviews and literatures. Rhizospheric microbiomes hold great ability to bestow the crops sustainable and to encourage the application of bio-formulation carrying potential microbiomes, which introduces additional or optional approaches to develop agricultural practices and finally enhances crop productivity. In current chapter, a brief outline of functional activities of rhizospheric microbiomes within their habitat have been focused and majorly on available modern approaches of biotechnological techniques and allied sciences used in identifications, exploration, and taxonomy in soil-plant microbiomes. Further, to conclude the theme of microbiomes exploration and identification, an endeavor has been made to cover some of the highly used tools of bioinformatics.

**Keywords**

Agriculture · Biotechnology · Identification · Microbiomes · Sequencing

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## 13.1 Introduction

Intense agricultural practices have consequences in the enhanced agriculture production of crops like wheat, rice, and maize (Zhang et al. 2011; Deb et al. 2015; Kumar et al. 2019). Higher production of rice and wheat ensured food security for continuously growing world population under changing climatic conditions (Tilman 1999; West et al. 2014). Besides food security, there has been noticed an increasing awareness of undesirable environmental threats arising from the extensive application of modern agriculture techniques. Some of the highlighted impacts of intense agriculture are greenhouse gas emission, nutrient leaching, increase soil degradation, and worst part of modern agriculture techniques which is based on the incorporation of synthetic fertilizers and pesticides that deteriorate the ecosystem gradually (Matson et al. 1997; Kumar et al. 2018; Sabarwal et al. 2018). However, along with the maintenance of food security, it is also essential to the standardization of the modern agriculture practicing protocols and techniques, which stabilized the standard of soil quality through the inclusion of microbes which provide valuable ecosystem services. The presence of all soil nutrients in farmland is not only a single parameter for the healthy soil, but abundance of viable entities like microbes which perform various functions in the rhizosphere in order to maintain its fertility and soil sustainability is also equally important (Bhattacharyya et al. 2016; Singh 2015; Mosttafiz et al. 2012).

Soil is a heterogeneous complex system on earth, which provides residence to great diverse organisms ranging from unicellular to multicellular organisms. It has been concluded in the literature that single gram of soil contains an abundance of viable entities (bacteria) close to $10^5$–$10^6$ different taxonomic groups with huge microbial diversity (Griffiths et al. 2016). The soil microbiome is a collection of microorganism population inhabiting in the animals, plant, rhizosphere, or food which includes fungi, bacteria, archaea, and viruses and other genera (Kurokawa et al. 2007; Smith et al. 2013; Coleman-Derr et al. 2016). Mainly, bacteria and fungi are core regulators of various ecosystem processes and play crucial roles in nutrient cycling (Bardgett et al. 2008). Microorganisms have a diverse responsibility in the soil like the decomposition of various compounds and transformation of nonsoluble nutrients to a soluble form and fixation of different nutrients, resulting in the promotion of plant growth (Bender et al. 2016).

Soil microbiomes are highly abundant in rhizospheric zones and equally diverse in nature (Blazewicz et al. 2013). They perform various beneficial functions in the soil, and their worthy functions need to be assessed, which demand their accurate identification. From the beginning of microbiology, identification of microbial strains was a very tedious and time-taking process as identification of microbes was purely based on the physical and morphological appearance. Microbial characterization based on the morphology was not at all precise and lacked accuracy. In the beginning year of microbiomes identification, in 18th century, the microbiologist of that era has started microbial characterization based on metabolic pathways. Further, soil microbiomes especially unicellular entity like bacteria showed enormous diversity about their metabolisms and cell structures, and hence, identification based on

morphological and biochemical means becomes worthless (Gevers et al. 2005; Achtman and Wagner 2008).

In spite of deficiency in a consistent species-level classification, the well-timed categorization and identification of microorganisms remain to be significant in various sciences related to environmental and soil monitoring or clinical diagnosis (Liu et al. 2010). Especially, the current advances of molecular biology covering modern techniques in the field of genomics and proteomics which are potential to put forward attractive and easy alternatives over conventional microbiological procedures, which are used for the exploration, characterization, and identification of microbiomes (Hugenholtz, and Tyson 2008; Martínez-Porchas and Vargas-Albores 2017). From the last three decades, modern sciences like genomics and proteomics with the inclusion of computational science and informatics science have greatly strengthened and provide quick multidimensional data for quick microbial identification. The sequencing technologies coupled with bioinformatics has not only revealed rapid identification of microbiomes (Martínez-Porchas and Vargas-Albores 2017; Marshall et al. 2018) but also made possible rapid gene annotation of microbiomes within a short period of times (Liu et al. 2007; Logue et al. 2008).

Soil microbiomes communities and their related functions principally conclude the productivity of agriculture crops (Van Der Heijden et al. 2008; Dias et al. 2015; Kumar and Verma 2019). The present compositions of the rhizospheric microbiomes are the most indispensable factors in determining the bacterial and fungal association linked to plant roots (Boe et al. 2000; Berendsen et al. 2018), and hence the identification of microbiomes irrespective of origin is essential for the better understanding of their functions. Molecular identification is highly accurate and hardly creates cumbersome and also makes possible manipulation of microbial treasure for the better uses for human society. In the current chapter, the tools and techniques related to the soil microbiomes exploration and identification in the biotechnology era have been covered comprehensively. The current chapter also covered the putative role of bioinformatics to decipher the molecular taxonomy and phylogenetic studies.

### 13.1.1 Soil Microbiomes Interaction with Plant

In the last two decades, several milestones have been covered with respect to microbiome identification of different origin and the phrase "plant microbiome" or "rhizospheric microbiomes" has gained considerable attention, as rhizospheric microbiome affects the soil fertility in multiple ways. Soil microbes provide the protection to the plant against plant pathogens and promote goodness to the plant and finally enhance the crop productivity. The plant microbiome holds the dissimilar functional gene pool, linked from viruses, prokaryotes, and eukaryotes and also coupled with various residents of a plant host (Rout and Southworth 2013). The hot spot location of microbiomes and plant interaction is the rhizosphere which is always under the inclination of plant roots via accumulation of exudates, mucilages,

and sloughed cells (Uren 2001; Bais et al. 2006; Moe 2013). Hence, plant roots are potential to manipulate the soil and to inhabit microbiomes. Further, the rhizosphere microbiomes can promote plant growth through the secretion of regulatory compounds like siderophore, hormone production as indole acetic acid, gibberellic acid, and stress regulation through 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity (Shi et al. 2011; Philippot et al. 2013; Spence et al. 2014; Sarkar et al. 2018).

The rhizospheric microbiomes have inverse or direct (beneficial) influence on plant growth promotion and wellness. Rhizospheric microbiomes directly affected the attack of pathogens and mutualistic benefits through the decomposition of complex compounds and nutrient solubilization like phosphates, potassium, and other nutrient cycling (Glick 1995); discharge of plant growth hormones like auxin and gibberellins (Narula et al. 2006; Ortíz-Castro et al. 2008; Ali et al. 2009; Mishra et al. 2009); anti-pathogens activities (Kloepper et al. 2004); and stimulation and induction of the plant immune system through induced systemic resistance (Ramamoorthy et al. 2001; Vessey 2003; Rudrappa et al. 2010). The recent literature substantiates that plant host and developmental stage have a noteworthy influence on determining of the rhizospheric microbiome (Peiffer et al. 2013; Chaparro et al. 2014; del Carmen Orozco-Mosqueda et al. 2018).

## 13.2 Microbial Identification

There are diverse microorganisms which exhibit plant growth-promoting (PGP) activities and help in the crop growth and productions (Meena et al. 2017; Sahoo et al. 2019). Their application has lead to the use of microorganisms in agriculture, which has further needed the identification of these organisms having PGP activities. This has given a separate branch of study called systematics or taxonomy. Systematics or taxonomy consists of classification, nomenclature, and identification, where identification is the practical application of the taxonomy. Classification can be considered as an art, but the identification is purely a science (Cowan 1965). There are several methods for identification of microbes ranging from morphological studies to molecular studies (Zhang et al. 2010a; Sahoo et al. 2019). First of all, it is very important to isolate organisms from the pool of the microbes, and this purified single colony should be identified for accurate nomenclature with molecular-based methods (Colombo et al. 2009; Armstrong 2007; Wagner and Haider 2012).

Initially, the organisms were identified based on pathogenicity, whether they are pathogenic or nonpathogenic. Nungester (1963) started microbe identification based on fewer objectives: (a) susceptibility to the antimicrobial drug, (b) based on prognostic value, (c) potential danger to the people in contact to the infected person, and (d) source of infection, etc. Initially, these objectives were used for clinical microbiologist later amended and further utilized for the normal identification irrespective of the field. Before this, the identification involves a comparison of an unknown organism to known organism and eventually giving the name to the former. After that, more progressive methods (Cowan 1965) were used which aim to

determine a few fundamental characteristics, so that the isolates can be placed in the same genera (Drahos 1991; Armstrong 2007; Colombo et al. 2009; Wagner and Haider 2012). Some of the modern techniques used in microbiome identification have been discussed in a further section.

### 13.2.1 Methods of Microbiomes Identification

The methods of identification of microbe can be categorized in (a) classical or traditional and (b) modern methods based on instrument and technique used (Fig. 13.1). Traditional methods rely on the phenotypical characterization of the microbe, which can be observed by the naked eye, including morphological and biochemical analysis. *Psychrobacter* spp., a nonmotile bacteria associated with freshwater fish spoilage, were characterized phenotypically (García-López et al. 2004). Morphological characteristics involve the morphology, shape, size, opacity, color, and transparency of the colony. These characters can be observed after incubating the microbe for a certain period on the solid agar plate. The primary distinguishing characteristics are whether it can grow in an aerobic or anaerobic environment. However, these phenotypic characterizations are not sound very much scientific and sensitive at the level of strain differentiation (Sagan et al. 1994; Collazo et al. 2005; Vernière et al. 1998). Figure 13.1 has briefly described the most common methods generally used in the microbial characterization.

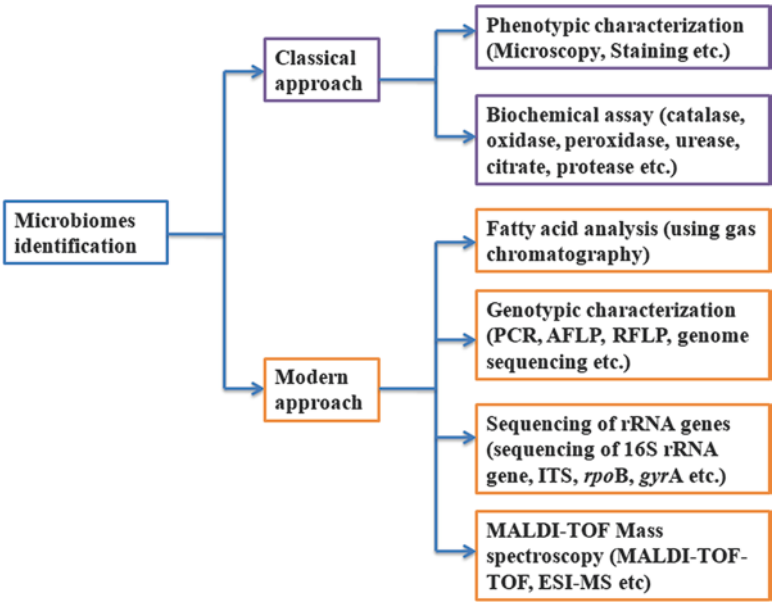


**Fig. 13.1** Methods used to characterize genotypic and phenotypic characteristics and requirement of the characterization

Initially, microscopy was developed mainly for the phenotypical characterization. The phenotypical characterization includes the shape of the microbe whether it is a bacillus, coccus, spiral, or some other shapes and the study of the mobility of the microbe by identifying the flagella whether it is mono-, bi-, or multi-flagellated. Microbes can also be analyzed through the staining pattern of the bacteria, whether it is gram-negative or gram-positive. The presence of spore, capsule, inclusion body, and many more things can be detected. Some of the bacteria have a specific pigmentation and fluorescence, which can be utilized as a characteristic for identification (Giana et al. 2003). There is a variety of staining, i.e., gram staining, endospore staining, and Ziehl-Neelsen staining used for characterization (Hatamoto et al. 2007; Hugon et al. 2017). Besides the light and compound microscopy, there are several other microscopes, which have been proven advantageous in the characterization of the microbes, for example, confocal microscopy is being used to see the organelle of the microbe; besides this, for the greater resolution, SEM (scanning electron microscopy) and TEM (transmission electron microscopy) are being utilized (Hatamoto et al. 2007; Golding et al. 2016).

A variety of biochemical tests like catalase, oxidase, peroxidase, urease, citrate, protease, and others specify the organism at the genus and species level. In *Enterobacteriaceae* and *Pseudomonas* spp., identification of carbapenemase types using a biochemical test is one of the examples (Dortet et al. 2012). Similarly, the nitrate reduction test can be utilized in the identification of *Mycobacteria* (Virtanen 1960). Dichotomous identification is also one of the methods, where the whole kingdom is divided into two parts based on characteristics; as we choose multiple characters, it goes narrower (Noguerola and Blanch 2008). Traditional methods are widely used but have major drawbacks. It is only applicable to an organism which can be cultured in vitro, and there are various microbes which do not fit to a particular genus because of the unique biochemical properties. The modern methods mostly rely on the molecular biology, which can detect similarities and dissimilarities even at the molecular level, no need to be dependent on live culture, and can reveal minute differences between organisms which can escape in classical methods.

### 13.2.2 Molecular Techniques for Microbiomes Identification

Conventional methods of identification are labor-intensive and time-consuming and are often not enough to differentiate the species of the same genus (Verma et al. 2019). These limits are especially important for the medical diagnostic point of view. Rapid and proper identification of the microbe is essential in medical science. Therefore, there is the requirement of the alternating methods for quick identification. Nowadays, molecular taxonomy relies on the 16S ribosomal RNA gene sequencing and several PCR-based methods. The 16S ribosomal RNA gene sequencing has led to microbe identification at species and subspecies level (Adekambi and Drancourt 2004; Chen et al. 2000). These PCR-based sequencing methods are highly sensitive and reproducible. These sequencing methods also

reduce the time and labor as it requires the genomic DNA of the microbe and PCR reaction.

Similarly, microarray-based methods use the probe specific to the organism. In the microarray, there are a number of probes (16S rRNA probes) from different organisms, so from a single microarray, it can identify several microorganisms in a microbiome as each probe corresponds to a specific microorganism (Peplies et al. 2003). The serology-based method also works on the same principle, but here we use the antigen-based enzyme-linked immunosorbent assay (ELISA) plate, and each antigen corresponds to the specific microbe. Besides the simple PCR, there are some recent advancements such as amplified fragment-length polymorphism (AFLP), repetitive element polymerase chain reaction, riboprinting, and multiplex PCR which have been proven useful in the identification (Emerson et al. 2008; Ricke et al. 2018).

### 13.2.3 Selection of Target Gene for Sequencing

Many gene targets have been identified in archaea, bacteria, fungi, and viruses. The gene sequence should have been conserved in the same species of bacteria or fungi. In bacterial identification, mostly 16S rRNA gene (16S rDNA) of ~1500 bp sequence is used for the identification of the species (Nocker et al. 2007). The 16S rDNA has conserved region as well as various regions (Fig. 13.2). In earlier studies, the scientist uses the whole 16S rDNA sequence for the identification. Nowadays, people are using the variable region only for the identification because they are more prone to the mutation and this region of DNA form only loops in the secondary structure of 16S ribosomal subunit (Fig. 13.2b). Some of the bacteria have multiple copies of the gene, while some microorganisms like *Bacillus cereus* and *Bacillus anthracis* have the same sequences. The multiple copies can make the interpretation difficult if every copy has a variation in base sequence. But, in clinical samples, the multiple copies are helpful in the amplification. Because of the difficulties in the interpretation in these cases, sometimes the sequence of the β subunit of the RNA polymerase II (*rpo B*) is used for the identification in place of 16S rDNA (Volokhov et al. 2012).

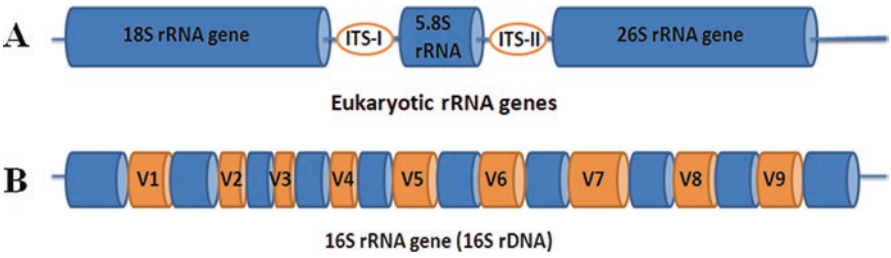


**Fig. 13.2** (**a**) Schematic representation of ITS regions in fungi (eukaryotes) and (**b**) schematic representation of variable regions (orange) and conserved region (blue) present in 16S rRNA genes in bacteria

The *rpoB* gene has been proven useful in the identification of fast-growing bacteria like mycobacteria, i.e., *Mycobacterium chelonae* and *Mycobacterium abscessus*. These two species have indistinguishable 16S rRNA gene sequences but exhibit 13% sequence divergence with *rpoB* (Adékambi and Drancourt 2004; Poretsky et al. 2014; Nasiri et al. 2017). There are some other gene targets, which are the better option to distinguish the differences between species. These genes include tuf (elongation factor), *gyr* A (gyrase), gyr B, sod A (superoxide dismutase), HSP (heat-shock protein), etc. In the case of fungi, the scientist uses the internal transcribed region (ITS-I and ITS-II) sequence (Fig. 13.2a). These variable regions lie between 18S, 5.8S, and 26S rRNA genes. The variation between the ITS regions can be utilized for the identification of the fungi, such as *Saccharomyces*, *Candida*, *Trichosporon*, *Cryptococcus*, and *Aspergillus* (Chen et al. 2000; Ciardo et al. 2006). The ITS regions also have some limitations; because of that, the two variable domains D1 and D2 near the 5′ end of 28S ribosomal RNA genes (Dagar et al. 2011), elongation factor α (e.g., *Fusarium* sp.), and β-tabulin (e.g., *Phaeoacremonium* sp.) are being used as an alternative gene target (Stielow et al. 2015).

### 13.2.4 Gel-Based Identification and Characterization

Protein profiling is one of the recent methods for identification (Karlsson et al. 2018). For the protein profiling, first lyse the cell and then separate on the SDS-PAGE (polyacrylamide gel electrophoresis). Bacteria can be identify on the basis of the migration pattern of the protein on the gel, which can be compared with the reference. The 2DE (two-dimensional gel electrophoresis) is the fusion of the isoelectric focusing (IEF) and SDS-PAGE. In the isoelectric focusing, the proteins are first separated on the basis of the isoelectric point (charge). Then, the SDS-PAGE was performed to separate the protein on the basis of molecular weight. It means that the protein is first separated on the basis of charge and then on the basis of molecular weight. The 2DE map of different organisms can be stored, and a database can be prepared, which can be used as a reference and compared with test organisms (Malmström et al. 2002). The major drawback of this method is that it is labor-extensive and time-consuming and requires an ample amount of the protein. Proteome profile of organisms can be compared with the existing databases, and based on the comparison, the microorganism can be identified. Redmond and his colleague analyzed the exosporium of *Bacillus anthracis* spores by isolating the proteins from the outer casing of the spore using SDS-PAGE and analyzed the isolated protein. The team identified several proteins associated with the exosporium of *B. anthracis* (Redmond et al. 2004). The introduction of MALDI-TOF-MS greatly enhances this method. Using this method, the proteome of many microbes has been made available (Nouwens et al. 2000; Peng et al. 2005; Pieper et al. 2006).

### 13.2.5 Fingerprinting-Based Technology

Fingerprinting is the most widely used technology in the genomics for bacterial identification. The repetitive PCR, amplified fragment-length polymorphism (AFLP), random amplification of polymorphic DNA (RAPD), and multiplex PCR are widely used methods. These techniques utilize the PCR machine to amplify multiple copies of the DNA fragment using the defined set of primers, and then differences in these amplified fragments are utilized for the analysis and differentiation among the species of similar ecology (Lam et al. 2015). Similarly, restriction fragment-length polymorphism (RFLP) utilized a restriction enzyme. These methods are used to take advantage of the DNA polymorphism in the related organism, on the basis of which the organism can be differentiated in the mixed samples (Versalovic et al. 1994; Cocconcelli et al. 1995; Lin et al. 1996).

### 13.2.6 Microarray-Based Technology

Microarray is another technique to identify microbes at the species level and also impart the functional structure of a given microbial community (Bai et al. 2017; Thissen et al. 2019). The basic principle of this technique is to utilize specific probes which are spotted on a solid platform. This is called chip, which is further hybridized with fluorescently labeled DNA or RNA molecules from the microbial population. This genetic material (DNA & RNA) only hybridized with specific probe present on the specialized chip which can be detected. In the case of bacterial identification, several interactions of "polychip" have been used that utilize the small subunit of the ribosomal genes (Liu et al. 2001; Wilson et al. 2002). These chips are useful in the identification of a broad group of environmental bacteria (Loy et al. 2002).

### 13.2.7 Mass Spectrometry-Based Microbiomes Identification

Mass spectrometry is another latest next-generation tool for quick microbiome identification and classification. Matrix-assisted laser desorption ionization-time of flight mass spectroscopy (MALDI-TOF-MS) and electrospray ionization mass spectrometry (ESI-MS) have revolutionized the clinical identification of microorganism in a minute at the species level (Cherkaoui et al. 2010). Thomson (1899) first discovered this method to measure the mass to charge (m/z) ratio for the electron. Further, the mass spectrometry application was expended in physical and chemical characterization, including biological samples. In end of 19th century, the development of the soft ionization processes has made this useful for the large biological molecules (Fenn et al. 1989). MALDI-TOF is widely used in microbial identification. The bacterial cell can directly be utilized for the analysis. It can produce

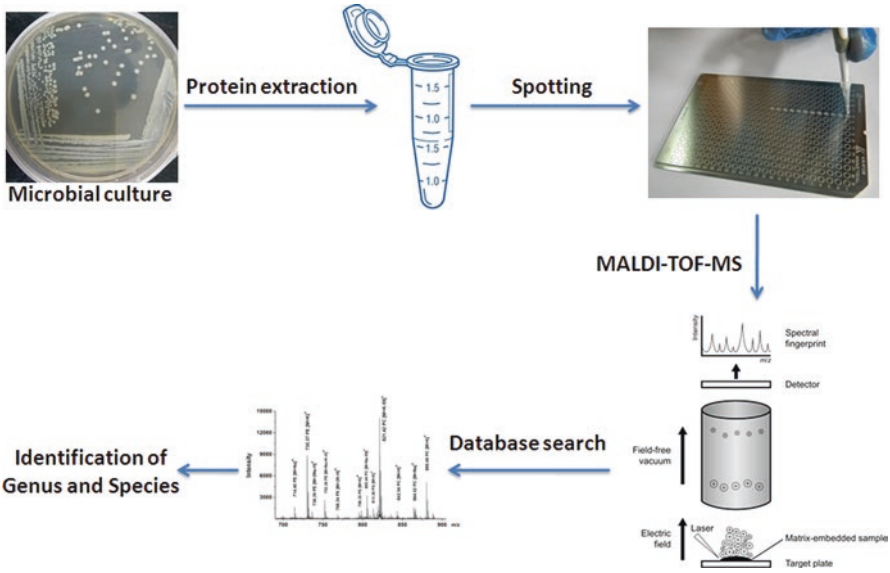


**Fig. 13.3** Flowchart of microbial identification using MALDI-TOF-MS. The microbe was purified, and protein extraction was done from the whole cell lysate. The cell lysate was processed and mixed with the matrix, and the spot was done on the MS plate, which was further put inside the MALDI-TOF instrument. The spectra are analyzed on the database for the identification

the reproducible spectral pattern, which contains the information that can be used for the identification and characterization of the microbe. The basic procedures of the proteome analysis of the bacterial cell have been summarized in Fig. 13.3. For the ionization of the microbial sample, specific matrix has been used, which helps in the sample ionization. There are different types of matrix available for different types of sample. After the ionization of the microbe, particles are allowed to migrate in an electric field, and because of the field, each ionized particle acquires a path which gets detected by the analyzer. Finally, the analyzer gives spectra based on m/z ratio of the particle, which can be compared with the spectra of the known databases using an automated program (Dingle and Butler-Wu 2013). The most commonly used mass spectroscopy for the identification of microorganisms is MALDI-TOF (Krásný et al. 2013). Cell lysate can be utilized for protein profiling using MS. Using protein profiles to differentiate bacteria was first introduced by Cain et al. (1994).

### 13.2.8 Metagenomics

Metagenomics is derived from two major developments in biology. First is the development of next-generation DNA-sequencing technologies which has greatly enhanced capabilities for sequencing large meta-data sets, and second is the emerging appreciation for the importance of complex microbial communities in diverse environmental condition (Handelsman 2005; Petrosino et al. 2009). Metagenomics

is the genome analysis of a population of microorganisms. It doesn't need the culturing of the organism. The genomic DNA can be isolated directly from the soil samples or the defined environment and cloned in the desired vector or plasmid. The idea of cloning DNA samples directly from the environment was first discovered by Pace et al. (1986), and then the next advance is the construction of the metagenomic DNA library of the isolated DNA from the mixture of the microbes and the library vector (Schmidt et al. 1991). Further, the clones can be analyzed or screened for the phylogenetic markers likes 16S ribosomal RNA genes, RecA, gyrA, rpoB, etc., or can be screened for specific traits like enzyme production and expression of specific genes (Stein et al. 1996).

Metagenomics is a tool that can provide an abundance of knowledge to understand the microbial population. It has further improved our understanding of many of the exotic and familiar habitats that are attracting the attention of microbial ecologists, including deep sea, hot springs, temperate, desert, and cold soils; frozen Antarctic lakes, plant rhizospheres, and phyllosphere; and fungi-lichen symbioses (Yin et al. 2018).

## 13.3 Bioinformatic Scope in Microbiome Identification

The modernistic approach of microbial identification is based on the application of modern technology, along with bioinformatics tools. Bioinformatics is an interdisciplinary field of science in which algorithms and software tools can be developed for a better understanding of biological data to accelerate and enhance biological research. In the recent past, the explosion in capabilities of high-throughput omics technologies, system biology, and deep-sequencing platforms is generating a huge amount of data which require computational approaches to manage and analyze (Jiang et al. 2013). There are several computational tools and techniques that are used to analyze the hunks of biological data more accurately and efficiently by automated processes. Bioinformatics played an important role in various fields, i.e., in sequencing and annotation of microbial genomes; it has also aided in the sequencing of observed mutations in the various organisms. These tools have proven useful in the comparative analysis of the microorganisms and understanding of the evolutionary aspect based on molecular biology (Zhang et al. 2010b). Hence, computational biology can be considered as a field of data science for solving problems in biology, medicine, and agriculture.

### 13.3.1 Next-Generation Sequencing of Microbes

Next-generation sequencing (NGS), a newly emerged technique, is being used a lot in various field of science. This technology has tremendous advantages over the Sanger sequencing method. The development in this technique has led to the high-throughput sequencing, which has further boost the several findings which were earlier seemed to be laborious and time-consuming by traditional methods. Millions

of molecules can be sequenced simultaneously producing a huge amount of data. This technology is highly parallelized in such a way that it can analyze a huge amount of data and can give valuable information. Several algorithms have been written for data analysis. The NGS technology is high throughput, which is quick and economic enough to be considered as a tool for bacterial identification and characterization (Loman et al. 2012; Stahl and Lundeberg 2012; Srivastava et al. 2019a, b). Several algorithms have been written and assembled in a group, which further used as a tool for NGS data analysis. Short sequencing reads have limited power to resolve large repetitive regions, even within small microbial genomes (Chain et al. 2009; Nagarajan and Pop 2013). Generally, short-read technologies enable to resolve microbial genomes up to the high-quality draft standard (Treangen and Salzberg 2012), which sound applicable for understanding gene-coding potential, strain typing, or pan-genome analysis (Roberts et al. 2013). Draft genomes are the assemblies of fragmented short genomes which may contain incorrect gene calls, misassembled regions, and other artifacts. Fragmented assemblies are often attributed to repetitive DNA regions (such as rRNA operons) which are enormous in microbial genomes and present the greatest technical challenge to the assembly process, especially when the repetitive region is longer than the read lengths (Treangen and Salzberg 2012; Brown et al. 2014). Finished genome sequences are high quality by definition, represent more accurate genomic information, and can be adopted for model organisms and industrially important microbes (Fraser et al. 2002; Thomma et al. 2016; Boulund et al. 2018). The accurate analysis of NGS data revealed important clues in the quest for the treatment of various life-threatening diseases, improved crop varieties etc. There are several computational methods to analyze NGS data.

### 13.3.2 Data Cleaning and Preprocessing

The very first step is the cleaning or preprocessing of sequences of microbes retrieved from various sequencers. These sequences include the adapter and primers which can occur at both the ends of NGS reads. These adapter and primer sequences can hinder the correct alignment and mapping; because of that, it is important to remove these sequences. These ligated adapter sequences required the deletion from the sequence as the adapter can interfere with the original mapping of the reads and influence SNP calling and other downstream analyses. Various programs/applications are available for cleaning raw data by trimming low-quality reads and adapter contamination removal. Before analysis, these preliminary quality control checks can be applied. There are several open sources as well as commercial tools of data cleaning and processing. In Table 13.1, we have listed some of the online tools available.

**Table 13.1** Tools of data cleaning and processing

| Tools | Link |
|---|---|
| FastQC (Andrews 2010) | http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc/ |
| Fastx toolkit | http://hannonlab.cshl.edu/fastx_toolkit/download.html |
| Galaxy (Giardine et al. 2005) | https://usegalaxy.org/ |
| Trimmomatic (Bolger et al. 2014) | http://www.usadellab.org/cms/?page=trimmomatic |
| CLC Bio (Workbench 2010) | Commercial |

### 13.3.3 Genome Assembly

With the advent of many sequencing techniques, there is a massive overflow of genomic data in less time at low cost. Genome assembly is the process of making the original DNA sequence from a large number of short DNA sequences by putting all the sequences together. These sequences are aligned in such a way that the overlapping sequences read in contiguous sequences (contigs). This is called the de novo assembly, where there is no requirement of the reference genome. The most efficient assemblers for short-read sequences are typically those that employ de Bruijn graphs to produce an assembly (Compeau et al. 2011). Different algorithms are being used for the genome assembly. This algorithm takes all the pieces of DNA sequences, aligns them to one another, and searches for the overlapping sequences. These overlapping reads are merged with two paired reads, and assembly continues. Genome assembly is a difficult computational problem, as many genomes contain large numbers of repeat sequences. One of the first and most widely used de Bruijn graph assembler is the open-source program Velvet (Zerbino and Birney 2008). With further development to improve the resolution of repeats and scaffolding using paired-end and longer reads (Zerbino et al. 2009), Velvet remains one of the most used (and cited) assembler for bacterial genomes, being best suited to Illumina sequence reads (Velvet is included as the default assembler in the IlluminaMiSeq analysis suite).

There are two approaches to the assembly. First is de novo where no reference sequence is taken, and second is reference-guided where a reference genome is taken. Different types of software and algorithms are available for both the approaches of assembly. De novo approach relies on the fact that reads need to be assembled to generate a contiguous sequence either by overlap/layout/consensus graph (e.g., Celera Assembler, Arachne, CAP, and PCAP) or de Bruijn graph (e.g., Euler, Velvet, ABySS, AllPaths, SOAPdenovo, CLC Bio). Reference-guided assembly includes the use of reference genome to assemble reads into contigs. There are some assemblers which take reference genome as template to arrange reads helping in generating quick and accurate assembly (e.g., Velvet, DNASTAR's Lasergene Genomics Suite). This approach helps in identification of insertions and deletions.

### 13.3.4 Assembly Quality Assessment

After the genome assembly, it is very important to check the quality of the assembly. The maximum and minimum length of contigs/scaffolds, its length distribution, the total length of the assembly, etc., are the important criteria for the quality assessment. N50 and L50 are the important criteria as they represent the quality and size of the contigs or scaffolds in the assembly. There are several assembly tools available such as ABySS, Mimicking Intelligent Read Assembly (MIRA), CLC Bio Workbench, etc. The de novo assembly algorithm of CLC Genomics Workbench performs comprehensive support for a variety of data formats, including both short and long reads and mixing of paired reads (both insert size and orientation).

### 13.3.5 Gene Identification Tools

Gene prediction is one of the most important steps in understanding the sequenced genome of a species. At the computational point of view, gene prediction or gene findings are the process of identifying the regions of genomic DNA that encode genes. It includes protein-coding genes as well as RNA genes, but it may also include prediction of other functional elements, i.e., regulatory regions. At present, with comprehensive genome sequence and powerful computational resources available to the scientific communities, and gene finding has been redefined as a largely computational problem. The advances in bioinformatics research ensured the possibility to predict the function of a gene based on its sequence alone, and some of the popular tools have been listed in Table 13.2.

**Table 13.2** Bioinformatics tools to predict the function of a gene based on its sequence

| Name | Description | URL link |
|---|---|---|
| FRAMED | Find genes and frameshift in G+C rich prokaryotic sequences | https://omictools.com/framed-tool |
| GENIUS | Linking ORFs in complete genomes to protein 3D structures | http://genius.cbrc.jp/summary_linking.html |
| GENEID | Program to predict genes, exons, splice sites, and other signals along a DNA sequence | http://genome.crg.es/software/geneid/ |
| GENEPARSER | Parse a DNA sequence into introns and exons | https://bio.tools/GeneParser |
| GeneMark.hmm | Gene prediction program for prokaryotes and eukaryotes | http://exon.gatech.edu/GeneMark/ |
| GeneTack | Prediction of genes with frameshifts in prokaryotic genomes | http://83.149.211.146:23194/~ivan/cgi-bin/GeneTack/cgi/print_page.cgi?fn=home.html&title=Home |
| GLIMMER | Finding genes in microbial DNA | http://www.cbcb.umd.edu/software/glimmer/glimmer2.jun01.shtml |

## 13.4 Conclusion

The classical identification of microbiomes was purely based on biochemical, which is insufficient to deal with the identification issue of diverse rhizospheric microbiomes. Hence, molecular level identification of rhizospheric microbiomes is essential and is covered only under the umbrella of biotechnology and its allied sciences. The advancement in the biotechnology tools has certainly played a crucial role in the accurate identification and phylogenic mapping of the diverse rhizospheric microbiomes as rhizospheric microbiomes have potential to increase crop productions. In last few decades, the application of biotechnology in a combination of with computational sciences like bioinformatics has refined the microbiomes phylogenic position. Advanced technologies like MALDI-TOF-MS, metagenomic studies of various environmental samples, nucleotides sequencing coupled with streams such as bioinformatics, molecular modeling, and docking have certainly led to several innovations and crucial progress in biological sciences. Further, biotechnology and bioinformatics helped in deciphering the structural and functional genes, which are involved in regulations of several physiological mechanisms of higher and lower organisms, including plant microbiomes.

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# Plant-Parasitic Nematode Management by Phytobiomes and Application of Fly Ash

14

Gufran Ahmad, Mohammad Haris, Adnan Shakeel, Abrar Ahmad Khan, and Asgar Ali

**Abstract**

Plant-parasitic nematode causes enormous damages to various crops. To manage this tiny pest through ecofriendly approaches is the need of the hour. Application of biocontrol agents in the management of phytopathogenic nematodes has been a promising tool. There are a wide range of biocontrol agents including fungi and rhizobacteria which can protect the crops from pathogens/pest attack and also promote the yield attributes. It also improves the germination of the seed, helps in the development of root, and much more important thing, it increases the water utilization rate in plants. Moreover, some of the particulate air pollutants are also recommended for the management of phytonematodes. Among the particulate matter, fly ash was found to be most resistant to nematodes. Application of fly ash improves the growth of many crop plants when amended in the soil at low levels due to the presence of almost all the macro- and micronutrients. Many of the researchers observed that fly ash suppress the nematode population or disease caused by nematode in roots of plants, and this is due to the presence of many heavy metals in it.

**Keywords**

Biocontrol · Fly ash · Fungi · PGPR · Phytonematode

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## 14.1 Introduction

Plant-parasitic nematodes (PPN) cause enormous losses and therefore warranted to be managed with safe approaches (Bernard et al. 2017). Among the different stages of life cycle of root-knot nematodes, the second stage of juveniles (J2) penetrates and sucks the nutrients from host plants. (Gheysen and Mitchum 2011). Due to the high impact of PPN, many of the researches have been focused specially in the two groups of PPN like cyst nematodes (*Heterodera* and *Globodera* sp.) and root-knot nematodes (*Meloidogyne* sp.). The life cycle of cyst nematode completes in approximately 30 days of *Heterodera* sp. and 60 days of *Globodera* sp. in optimal situation and has six stages such as the egg, four stages of juvenile (J1, J2, J3, and J4), and lastly adult stage (Williamson and Gleason 2003). Out of four, the second stage causes the infection to the root system of the plants. After detecting the host root, it penetrates intracellularly with the help of cell wall-degrading enzymes secreted by the esophageal glands of the nematodes and migrates toward the vascular bundles. Here nematodes stimulate the formation of a metabolically active and sophisticated nematode feeding site (NFS), which is known as "syncytium" or "transfer cell" through secretion and injecting of enzymes and proteins inside the host cell via stylet (Baum et al. 2007). The main transcriptomic, metabolic, morphological, and physiological changes occur through the formation of NFS, like surrounding cell walls partially split-up, nuclei enlargement, organelles of cytoplasm increase in density, accumulation of endoplasmic reticulum, etc. (Hussey et al. 2002; Siddique and Grundler 2018). A female adult becomes swollen and sedentary and remains adjoining to the roots, and posterior extremity of the body comes outside from the ruptured cells of the root. The males maintain their vermiform form and leave the roots to fertilize. Mature female absorbs the nutrients from nurse cells (giant cell) to support the metabolic activities of the nematode and also helps in egg production. After completing the life cycle, wall of the female body becomes thick and makes a tough brown leather bag which is known as "cyst." The cysts serve as initial protection of around 200–500 eggs against adverse conditions until egg hatching takes place in favorable environmental conditions (Jasmer et al. 2003). In contrast, the duration of life cycle varies from several days to several months based on some factors like humidity, temperature, presence of the convenient host, and the species of RKN. The second stage of juvenile (J2) detects and penetrates inside the root of the host through signals from the host and the penetrating action of the stylet. The young one moves intercellularly within the tissues of the root and ultimately reaches to the vascular bundle. Thus, the nematode absorbs some secretions from the esophageal gland in the formation of 5–7 multinucleated and metabolically active nutritious or feeding cells known as "giant cells" that are the consequence of cytokinetic mitosis (Mhatre et al. 2015; Siddique and Grundler 2018). Male nematodes migrate towards the female for the copulation, on the other hand, apple-shaped swollen females remain sedentary within the roots, and eggs produce in the gelatinous matrix. Nematodes target the roots system of the host plant and cause the infection leading to intermittent supply of water and nutrient. Due to severe infestation, the plant growth and yield are significantly reduced.

Plant-parasitic nematodes recognized as hidden enemies are responsible for the heavy losses in various crops and horticultural. Moreover, PPN is responsible for approximately $ 157 billion yield loss in the world; a yield loss of $ 40.3 million is from India, which shows a 12.3% average loss of yield (Abad et al. 2008; Singh et al. 2015).

## 14.2 Management Strategies of Nematodes

Due to parasitic organisms, the management of plant disease has become a daunting task in the present scenario for researchers in sustainable agriculture. Approximately 4000 organisms which are parasitic have been identified and mostly present in the major biomes. Organisms as plant-parasitic absorb many nutrient content and water through the vascular tissues from their host plants (Press and Phoenix 2005). In the parasitic organisms, the management of nematodes is more difficult due to the habitat and their inhabitation and mode of parasitism (Gillet et al. 2017). In plants, nematodes attack mostly underground parts and cause a serious loss of yield. Many species of nematodes like *Meloidogyne* sp., *Heterodera* sp., *Globodera* sp., and *Pratylenchus* sp. are considered as the most important species from the economic point of view due to the damage and infection level to wide range of host.

Nematicides of chemical origin are responsible for the enhancement of agricultural capacity in terms of increased food and production of fiber. On the other hand, the application of chemically manufactured nematicides has resulted in environmental risk and also the impact on human health (Aktar et al. 2009). The use of synthetic nematicides is being banned because of their dangerous nature, which is harmful to untargeted organisms, a threat to environmental protection, and a cause of public health problems (Schneider et al. 2003). Therefore, it is the need of the hour to search an alternative that must be ecologically safe. Many ecological approaches that promote plant growth-promoting rhizobacteria (PGPR) can act as effective biological control of nematodes, as well as an agent that promotes plant growth and yield of various crops. Strains of rhizobacteria use organic compounds, sugars, and amino acids which are released from the roots of the plant for their growth and energy. In mutualism, strains of rhizobacteria fabricate different substances to improve plant growth and biological control activities in support of the host plant (Karthik et al. 2017). The above facts show that the chapter focused on the positive PGPR correlation for the biological control of nematodes and the enhancement of agricultural productivity. Among the different environment-friendly approaches, PGPR strain can act as effective biological control of the nematode, as well as play an important role in the plant growth-promoting agent for the growth of plants and yield improvement.

Several options are available here to limit the damage, such as eco-friendly use of some industrial waste, biological control, resistant cultivars, intercropping, deep plowing, crop rotation, and nematicides. However, the use of intercropping, crop rotation, and deep plowing is not beneficial in case of cyst formation, gelatinous matrix, various survival adaptations, and survival in the soil without presence of

host. The chemical method has been very effective for controlling nematodes, but farmers avoid this method due to the expensive cost, environmental problems, and high risks of health, while the continued growth of resistant cultivars on the same plot of land leads to the reducing resistance capacity because of the continuous development of virulent pathogens. Removing the nematode stress through the most effective and efficient methods is biological control, which is also an aim to save the crops (Timper 2014). Biological control is defined as "a deficiency of the nematode population, which is accomplished by the activity of living organisms except for the nematode resistant host plant, which occurs through naturally or changes in the environment or the establishment of antagonists" (Tian et al. 2007). The main objective of biological control is reduction of nematode populations by enhancing the number of natural enemies which is present in the soil. The soil is the collection of microflora, which is majorly diverse in structure and activity. In the rhizosphere, microbial flora acts as a front line which performs defense mechanism against various pathogens and can be utilized as biocontrol agents (Mendes et al. 2013). Use of such kind of biological organisms is able to maintain ecological balance and is responsible for the pure environment. After the establishment, the biological agent is active in the soil for a long time and also leads to the concept of "oppressive soil," where microorganisms populate in the soil naturally suppressing the PPN population (Trudgill et al. 2000). Biological control of nematodes is also achieved from bacterial and fungal antagonists. Fungal antagonists to nematodes consist of a variety of microorganisms including endoparasitic fungi, toxin-producing fungi, nematode-trapping fungi, parasites, and vesicular-arbuscular mycorrhiza which feed on the sedentary nematodes, females, and eggs. (Tranier et al. 2014), while bacterial antagonists are made up of mainly three groups such as epiphytic, endophytic, and endoparasitic bacteria. Bacteria reach biological control by the mechanisms such as antibiotics, competition, and parasitism (Abd-Elgawad and Kabeil 2012).

## 14.3 Plant Growth-Promoting Rhizobacteria (PGPR)

The roots of the surrounding plant from the "rhizosphere" soil include many bacterial species, which promote plant growth regulators, improve the development of plants, and increase the availability of nutrients. Such bacteria are called PGPR (Fig. 14.1; Table 14.1). PGPR contain a large group of free-living bacteria that colonizes in rhizomes and contributes to the development of plant growth rather than improving the yield of agricultural crops (Kumar et al. 2016). In soil, diverse microorganisms such as *Rhizobium* sp., *Xanthomonas* sp., *Arthrobacter* sp., *Bacillus* sp., *Bradyrhizobium* sp., *Enterobacter* sp., *Frankia* sp., *Klebsiella* sp., *Proteus* sp., *Flavobacterium* sp., *Microbacterium* sp., *Pseudomonas* sp., *Serratia* sp., *Acinetobacter* sp., *Azospirillum* sp., *Alcaligenes* sp., *Agrobacterium* sp., *Azotobacter* sp., *Burkholderia* sp., *Cellulosimicrobium* sp., and *Erwinia* sp. are the common constituents of rhizosphere and make complete colony in the rhizosphere (Bhattacharyya and Jha 2012; Tailor and Joshi 2014; Karthik et al. 2016; Teymouri

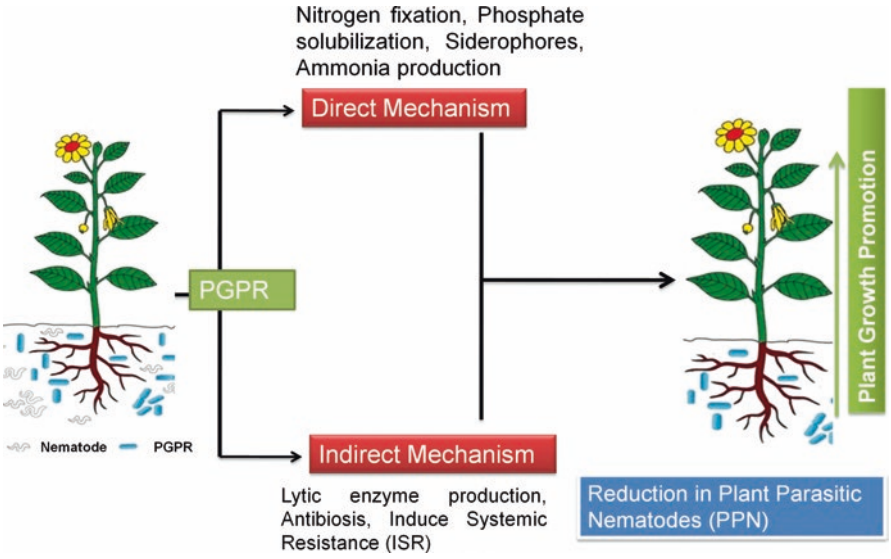


**Fig. 14.1** PGPR help in plant growth and nematode control

et al. 2016). There are widely known commercial rhizosphere-colonized microorganisms among rhizobacterial genera like *Bacillus*, *Azospirillum*, *and Pseudomonas*. Use of rhizospheric bacteria play an important role in the biofertilizer, phytostimulation, biocontrol, and phytoremediation, but it depends on their formation of a colony in the rhizosphere. Microorganisms present in the rhizosphere have the efficiency to improve the plant growth through the production of multiple plant growth substances and also responsible for the inhibition of phytonematodes. To reduce the damage caused by plant-parasitic nematodes, PGPR also known as a potential agent (Tabatabaei and Saeedizadeh 2017; Rashad et al. 2015). Species of *Pseudomonas* and *Bacillus* belong to those bacterial groups which form endospore and are majorly antagonist to PPNs in the rhizosphere. Many *Bacillus* strains can inhibit nematodes and promote plant growth. Furthermore, it has been reported that *Bacillus* sp. was also directly antagonist toward the PPNs like *Meloidogyne*, *Heterodera*, and *Rotylenchulus* (Siddiqui and Mahmood 1999; Kokalis-Burelle et al. 2002; Li et al. 2005). Strains of *Pseudomonas* in the rhizosphere also show pathogenic mechanism against PPNs (Kerry 2000; Siddiqui et al. 2005). Some studies have been conducted to evaluate the mechanism involved in the decreasing populations of PPN during the interaction of *Pseudomonas* and PPNs by the production of induced systemic resistance (ISR) and antibiotics. (Siddiqui and Shaukat 2003c). These two major antagonists and many other rhizobacteria were also reported as PPN antagonists, including members of such kind of genera like *Agrobacterium*, *Arthrobacter*, *Alcaligenes*, *Azospirillum*, *Beijerinckia*, *Bradyrhizobium*, *Clostridium*, *Comamonas*, *Corynebacterium*, *Desulfovibrio*, *Gluconobacter*, *Flavobacterium*, *Phyllobacterium*, *Sphingobacterium*, *Serratia*, *Streptomyces*, *Variovorax*, *Actinomycetes*, *Aureobacterium*, *Azotobacter*, *Bacillus*,

**Table 14.1** Effect of PGPR inoculation on plant-parasitic nematode biocontrol

| Country | PGPR strains | Crops | Plant-parasitic nematodes | Mechanism of action | References |
|---|---|---|---|---|---|
| Egypt | *Bacillus subtilis*, *B. pumilus*, and *Pseudomonas fluorescens* | Cowpea | *Meloidogyne incognita* | Antibiosis, phytohormone and enzymes production | Abd-El-Khair et al. (2019) |
| Egypt | *Bacillus amyloliquefaciens*, *Lysinibacillus sphaericus* | – | *Meloidogyne incognita* | Phytohormone production | Abdel-Salam et al. (2018) |
| Turkey | *Paenibacillus castaneae* | Tomato | *Meloidogyne incognita* | Antibiosis | Cetintas et al. (2018) |
| USA | *Bacillus* sp. | Cotton and soybean | *Heterodera glycine*, *Meloidogyne incognita* | Siderophore formation | Xiang et al. (2017) |
| India | *Pseudomonas fluorescens* | Okra | *Meloidogyne incognita* | Antibiosis | Veronika and Khan (2015) |
| India | *Pseudomonas fluorescens* and *B. subtilis* | Rice | *Meloidogyne graminicola* | Antibiosis | Priya (2015) |
| China | *Bacillus subtilis* | Tomato | *Meloidogyne incognita* | Production of lytic enzymes | Wei et al. (2014) |
| China | *Bacillus cereus*, *Bacillus subtilis*, *Serratia* sp. | *Trichosanthes kirilowii* | *Meloidogyne incognita* | Production of lytic enzymes | Jiang et al. (2014) |
| Pakistan | *Bacillus spp.*, *Azotobacter sp.* | Tomato | *Meloidogyne incognita* | Nitrogen fixation | Anwarul Haq et al. (2011) |
| China | *Bacillus* sp. | In vitro | *Meloidogyne incognita* | Production of lytic enzymes | Wei et al. (2010) |
| India | *Methylobacterium fujisawaense* | – | *Meloidogyne incognita* | Antibiosis | Prabhu et al. (2009) |
| Pakistan | PGPR-H4, PGPR-K315, PGPR-Ax | Mash bean | *Meloidogyne incognita* | Antibiosis | Mahmood et al. (2009) |
| Pakistan | *Pseudomonas aeruginosa* | Mung bean | *Meloidogyne javanica* | Auxin, nematicidal activity, induce resistance | Ahmed et al. (2009) |
| Pakistan | *Bacillus cereus* | Cowpea, mash bean | *Meloidogyne javanica* | Phosphate solubilization | Dawar et al. (2008) |
| Pakistan | *P. variotii* | Banana | *Meloidogyne javanica* | Antibiosis- | Zarina (2007) |
| Pakistan | *B. circulane* | Guar | *Helicotylenchus indicus* | Phosphate solubilization | Khan et al. (2012) |
| Pakistan | *B. circulane* | Guar | *Helicotylenchus indicus* | Phosphate solubilization | Khan et al. (2012) |

| | | | | | |
|---|---|---|---|---|---|
| India | *Bacillus* sp. | Tomato | *Meloidogyne* spp. | Phosphate solubilization and nitrogen fixation | Siddiqui et al. (2005) |
| India | *B. thuringiensis* | Pigeon pea | *M. incognita* | Antibiosis | Dhawan et al. (2004) |
| Pakistan | *P. fluorescens* | In vitro | *Meloidogyne javanica* | Auxin, nematicidal activity, induce resistance | Hamid et al. (2003) |
| Pakistan | *P. fluorescens* | Soybean | *M. javanica* | Root colonization and acts as biocontrol | Siddiqui and Shaukat (2003a, b) |
| India | *Pseudomonas* spp. and *P. fluorescens* | Mustard, soybean, tomato | *Rotylenchulus reniformis* | Auxin, nematicidal activity, induce resistance | Asghar et al. (2002) |
| India | *Pseudomonas* spp. and *P. fluorescens* | Mustard, soybean, tomato | *Rotylenchulus reniformis* | Auxin, nematicidal activity, induce resistance | Niknam and Dhawan (2002) |
| USA | *Paenibacillus macerans* and *Bacillus amyloliquefaciens* | Pepper | *Meloidogyne incognita* | Phytohormone production, induced systemic resistance | Kokalis-Burelle et al. (2000) |

*Burkholderia*, *Chromobacterium*, *Clavibacter, Curtobacterium*, *Desulfovibrio*, *Enterobacter*, *Hydrogenophaga*, *Klebsiella*, *Methylobacterium*, *Pseudomonas*, *Rhizobium*, *and Stenotrotrophomonas* (Tian et al. 2007; Siddiqui and Mahmood 1999; Wani 2015). Utilization of PGPR is beneficial in attaining substantial improvement in both plant growth and nematode suppression. Therefore, PGPR play an important role in the improvement of a sustainable agricultural system, being one of the key components in integrated nematode management (Mhatre et al. 2018; Abd-Elgawad and Kabeil 2012). Many of the PGPR strains like *Bacillus firmus* T11, *Bacillus aryabhattai* A08, *Paenibacillus barcinonensis* A10, *Paenibacillus alvei* T30, and *Bacillus cereus* N10w has to show the reduction in several galls. The greenhouse experiment conducted on carrots, treated with strain T30, caused a significant reduction in gall index and egg mass index as compared to non-treated control set. It was observed that the treatment with *B. aryabhattai* A08 gave significant results on tomato, reducing the gall index and egg mass index compared to the control set. It is concluded that the bacterial strains *P. alvei* T30 and *B. aryabhattai* A08 have potential as biological control agents of *M. incognita* on carrots and tomatoes, respectively (Viljoen et al. 2019).

Application of PGPR can improve the plant growth and yield related characterics with or without nematode infestations. PGPR can suspend the nematode activity and register significant enhancement in the plant health through nitrogen fixation, phosphate solubilization, siderophore production, lytic enzyme production, antibiosis, ISR, etc.

## 14.4 Mechanism of PGPR in Nematode Suppression

### 14.4.1 Direct Antagonism

Rhizobacteria plant-parasite exhibits a different mode of action in the rhizosphere to suppress nematodes. The mechanism for suppression of nematodes can be classified mainly into two major heads. In direct, enzymes are antagonistic to nematodes, toxic substances and other metabolic products regulate the nematode behavior, indirect effect, by changing root variation and motivating the production of repellents by the host which adversely affects. The recognition of the host, changes in the development of the nematode feeding site, and the sex ratio within the root system induce growth of plants, competing for essential nutrients and inducing systemic resistance (Siddiqui and Mahmood 1999; El-Nagdi and Youssef 2004; Singh et al. 2019).

#### 14.4.1.1 Antibiosis

Microorganisms produce antibiotics, which are the organic compounds of low molecular weight. It plays a beneficial role in biocontrol of many pests through parasitism and competition (Raguchander et al. 2011). Most of the rhizobacteria work against PPN through the production of toxins, enzymes, and metabolic by-products. This helps in the survival, development, reproduction, and hatching of

the nematodes (Siddiqui and Mahmood 1999). Furthermore, it is believed that *P. fluorescens* secretes secondary metabolites such as 2-4-diacetylphloroglucinol which reduces the cyst nematode population (Siddiqui and Shaukat 2003c), while some rhizobacteria were found to secrete compounds such as hydrogen cyanamide, which destroys detrimental organisms in the rhizosphere and is beneficial in creating a favorable environment for better development of the plants (Tian et al. 2007). Rose et al. (2012) also observed that the presence of *P. fluorescence* is able to decrease the growth rate of nematodes and is found to be most effective when used in combination with neem cake. In the study of PGPR, three genera, namely, *Azospirillium*, *Rhizobium*, and *Azotobacter* and mycorrhizal genus *Glomus* sp. have been reported to reduce galling in roots which is caused by the *M. javanica* in chickpea (Siddiqui and Mahmood 2001).

#### 14.4.1.2 Production of Lytic Enzymes

The growth and development by the action of enzymes is another mechanism of PGPR through the production of some enzymes like chitinases, phenylalanine ammonia lyase, lipases, proteases, peroxidase, dehydrogenase, β-glucanase, phosphatases, etc. *Corynebacterium paurometabolous* produced chitinase and hydrogen sulfide, which are responsible for the inhibition of nematode egg hatching (Jamal et al. 2018; Patel et al. 2018; Mena and Pimentel 2002). Isolation of three bacteria *Pseudomonas* sp., *Stenotrophomonas maltophilia*, and *Bacillus mycoides* proven to be nematicidal reducing 56–74% population of Tricodorid infesting potato crop. In addition, these bacteria were a specialty for the oxidation of phenol and antifungal activity with the production of HCN and hydrolytic enzymes (Insunza et al. 2002). With the isolation of 16 potential PGPR from grapevine roots, 7 isolates, namely, *S. marcescens*, *C. acidovorans*, *A. piechaudii*, *S. plymuthica*, *Pantoea agglomerans*, *Sphingobacterium spiritivorum*, and *B. mycoides*, have been proved to suspend the reproduction of *Meloidogyne ethiopica*. Secondary metabolites of all the strains exhibit significant hatching prohibition of *M. ethiopica,* and from the isolates, *P. megatorium* and *P. putida* are found the most effective (Aballay et al. 2013).

#### 14.4.1.3 Induced Systemic Resistance (ISR)

The induced resistance plant has increased defense capacity, which is achieved after proper stimulation against the parasite and broad-spectrum diseases. The increase in defense response due to the inducing agent causing the infection through pathogen is called induced systemic resistance (ISR) or systemic acquired resistance (SAR) (Van-Loon 2000). This induced resistance produces non-specific defense against various types of pathogens, such as fungus, bacteria, viruses, nematodes, and insects (Beneduzi et al. 2012). Several organic molecules are found to be associated with systemic resistance such as polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), peroxidase (PO), lipoxygenase (LOX), superoxide dismutase (SOD), chitinase, catalase (CAT), ascorbate peroxidase (APX), proteinase, and β-1,3-glucanase (Pokhare et al. 2012; Mhatre et al. 2017). Such kinds of enzyme begin the stimulation of resistance through the production of phenolic and phytoalexin

compounds (Viswanathan et al. 2003). Various studies observed that rhizobacteria inhibit nematode severity through inducing the plant systemic resistance (Ramamoorthy et al. 2001; Pieterse et al. 2002). This induced resistance is obtained through the mechanical and physical strength of the cell wall by the callose deposition, accumulation of phenolic compounds, and thickening of the cell wall. The synthesis of some biochemical compounds like phytoalexin, PR proteins, siderophores, salicylic acid (SA), lipopolysaccharides (LPSs), PO, jasmonic acid (JA), chitinase, and some other secondary metabolites that regulate the reaction provides the protections (Siddiqui and Mahmood 1999; Ramamoorthy et al. 2001). Another study showed that infections caused by the potato cyst nematodes and root-knot nematodes were reduced through induced systemic resistance by *Rhizobium etli* (Hallmann et al. 2001). *R. etli* have lipopolysaccharides that act as the inducing agent which is helpful in the systemic resistance and is found to play an important role in reducing the penetration and recognition of *Meloidogyne incognita* and *Globodera pallida* (Reitz et al. 2000; Mahdy et al. 2001). According to Meena et al. (2012), the maximum activity of enzymes is recorded in the low nematode population on tomato when treated by the consortium of PGPR formulation (*P. fluorescens*, Pf128+ *B. subtilis*, Bbv 57). Xiang et al. (2017) reported that 613 *Bacillus* strains caused mortality to *H. glycines* in vitro, and, specifically, *B. velezensis* strain Bve2 consistently reduced *H. glycines* cyst population density at 60 DAP in the greenhouse, microplot, and field trials. The *B. mojavensis* strain Bmo3 suppressed *H. glycines* cyst and total *H. glycines* population density under greenhouse conditions, and *B. safensis* strain Bsa27 and Mixture 1 (Bve2 + Bal13) reduced *H. glycines* cyst population density at 60 days after planting in the field trials. Moreover, Aljaafri et al. (2017) found that a harpin elicitor can function to turn on plant defenses in *H. glycines* in soybean and *M. incognita* and *R. reniformis* in cotton in susceptible cultivars.

### 14.4.2 Indirect Effects

PGPR promote the growth of plants by the phytohormone production like auxins, cytokines, ethylene, and abscisic acid. Moreover, siderophore improves the mineral uptake in the plants.

#### 14.4.2.1 Phytohormone Production

Most rhizobacterial strains are capable of producing substances that promote growth and development of plant. PGPR produce some plant growth regulators like auxins, cytokines, gibberellic acid, abscisic acid, ethylene, polyamines, brassinosteroids, jasmonates, salicylic acid, strigolactones, and other compounds that help in the regulation of plant growth (Mhatre et al. 2018; Gopalakrishnan et al. 2015). It is believed that PGPR produce phytohormones and plays an important role in promoting plant growth and interaction between plant and bacteria. Microbial phytohormones responsible for the enhancement in the growth of the plant by the stimulation of cell division, cell elongation, and expansion of tissue indicate

beneficial effects on the growth and yield of plants (Karthik et al. 2016; Khan et al. 2009). Indole acetic acid improves adventitious and lateral roots, which leads to increased nutrient and mineral uptake (Arora et al. 2013; Shaikh and Saraf 2016). It has been suggested that phytohormones formed by PGPR may inhibit the deadly effects of different environmental stresses. For example, *Streptomyces* strains produce phytohormones, which can improve the growth of eggplants through the suppression in the galls and egg masses of the nematode (Rashad et al. 2015). Similarly, Ruanpanun et al. (2010) reported that nematicidal activities of phytohormones generate *Streptomyces* sp. Therefore, any direct effects of bacteria on the production of phytohormones affect the efficiency of phytostimulation.

#### 14.4.2.2 Nitrogen Fixation

Nitrogen is one of the important macronutrients, which is beneficial for the growth and development of the plant, also involved in photosynthesis and protein synthesis and acts as a nitrogenous base in nucleic acids. Due to the regular loss of nitrogen in the soil, agricultural land has a limited amount of nitrogen. However, the plants cannot directly use atmospheric nitrogen. In this situation, PGPR perform an important role in the fixation of nitrogen and nutrients supplementation. These microorganisms are categorized into two separate groups, such as symbiotic and free-living nitrogen-fixing microorganisms (Mhatre et al. 2018; Gopalakrishnan et al. 2015). PGPR play an important function in nitrogen fixation which shows a vital contribution in sustainable agriculture. Aggangan et al. (2013) found that inoculation of nitrogen-fixing bacteria significantly improves the growth of banana through inhibition of the population of *Radopholus similis* and *Meloidogyne incognita*. Similarly, El-Hadad et al. (2011) also suggest improvement in the growth of plants and nematicidal activity by the nitrogen-fixing microorganism, *Paenibacillus polymyxa*. El-Sayed et al. (2014) found the biocontrol activity of nematodes with the help of nitrogen-fixing PGPR strains. Some genera of PGPR such as *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium*, *Frankia*, and *Sinorhizobium* are responsible for the fixation of atmospheric nitrogen which is utilized by the plants and beneficial for plant growth (Zahran 2001; Vessey 2003; Arora et al. 2012; Gaby and Buckley 2012; Dash et al. 2017).

#### 14.4.2.3 Phosphate and Potassium Solubilization

After nitrogen, phosphate is another major macronutrient which can promote plant growth. Phosphate plays a pivotal role in plant growth, such as the composition of nucleic acid, protein synthesis, cell division, growth of new tissues, and association with energy transformation complex (Gopalakrishnan et al. 2015; Oves et al. 2013). PGPR also work in the conversion of unavailable forms of phosphorus from the soil in available form through the production of organic acids, chelation, and acidity (Mhatre et al. 2018; Gulati et al. 2010) and influence nutrient availability and growth of host plants. Several genera of PGPR such as *Bacillus*, *Burkholderia*, *Erwinia*, *Microbacterium*, *Rhizobium*, *Serratia*, *Arthrobacter*, *Beijerinckia*, *Enterobacter*, *Flavobacterium*, *Pseudomonas*, and *Rhodococcus* are already reported as solubilizers of phosphate (Zaidi et al. 2009; Sharma et al. 2013; Ahemad et al. 2009;

Oves et al. 2013). Guang-Can et al. (2008) reported that strain of bacteria such as *B. megaterium*, *B. caryophylli*, *B. cereus*, *P. cichorii*, and *P. syringae* perform such kind of activity like solubilizations of phosphate and efficiency of mineralization, thus enhancing phosphate bioavailability. Inoculation of solubilizing phosphate by the strain of *Mesorhizobium mediterraneum* improved the growth of barley and chickpea (Peix et al. 2001). El-Hadad et al. (2011) observed that inoculation of phosphate-solubilizing bacteria (*B. megaterium*) improved the length of shoot, dry weight of shoot, dry weight of root, and amount of N, P, K in the plant of tomato and decreased the population of *M. incognita* in the rhizosphere. Potassium is another important plant nutrient after phosphate and nitrogen which perform various functions in plants. In the growth of plants, potassium is responsible for the physiological and biochemical functions (Zhang and Kong 2014). However, the maximum amount of potassium nutrient in soil containing orthoclase, feldspar, mica, muscovite, biotite, and illite in the form of potassium, not easily utilized by the plants. Just as bacteria dissolve phosphate, few rhizospheric microorganisms dissolve insoluble form of potassium making it accessible to the plants for their growth and development. PGPR solubilize potassium through various mechanisms like chelation, organic acid, acidolysis, complex lysis, and reduction (Meena et al. 2016). Several genera of microbes like *Acidithiobacillus ferrooxidans*, *B. edaphicus*, *B. mucilaginosus*, *Burkholderia*, *Paenibacillus* sp., and *Pseudomonas* sp. are responsible for the solubilized potassium (Han and Lee 2006).

#### 14.4.2.4 Production of Siderophores and Ammonia

Iron is another necessary plant nutrient element that is beneficial for the living organisms which performs many biological functions like photosynthesis, respiratory, electron transport, and cofactors for many enzymes, etc. (Aguado-Santacruz et al. 2012). Siderophores are iron-chelating agent which make the iron accessible to the plants. PGPR have the ability to develop some specific mechanisms like chelating the insoluble form of iron through the siderophores due to the production of low molecular weight (Dell'mour et al. 2012). Diverse groups of PGPR include *Aeromonas*, *Azadirachta*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Pseudomonas*, *Rhizobium*, *Serratia*, and *Streptomyces* sp. beneficial for the production of the siderophore that transports iron in the cells of plants for growth (Cornelis 2010; Sujatha and Ammani 2013). Ruanpanun et al. (2010) reported that the production of siderophores inhibit the activity of nematodes by the *Streptomyces* sp. Similarly, El-Sayed et al. (2014) also reported that the *Bacillus* sp., *Enterobacter* sp., and *Pseudomonas* sp. improve multifarious growth of plants as well as nematicidal activity.

Plant growth promotes various substances by the strains of PGPR which directly affect the growth of host plant through the stimulation in cell division, development of tissue, and physio- and biochemical functions. However, these PGPR strains provide additional support to the host plant to cope with nematode infection. However, such strain of PGPR provides an additional endorsement to the host plant to cope up with the infection of nematode (Mhatre et al. 2018).

## 14.5 Fly Ash

Fly ash (FA) is one of the important types of particulate waste that is generated by the combustion of coal in thermal power plants for the generation of the increasing demand for electricity (Ahmaruzzaman 2010). With increasing demand of electricity in India, the US Energy Information Administration Department (EIAD) noted that the use of energy consumption growth would exceed and leave behind the countries like China, the USA, and Russia until the end of 2035 because 76.4% of electricity-generating power plants are coal-based (Singh et al. 2018). Production of FA between 2010 and 2011 increased to about 130 million tons, which was about 85.7% higher than 1996 to 1997. The FA utilization rate also increased from 9.63% in 1996 to 1997 to 54.53% in 2010 to 2011, an increase of 466% over 15 years (Singh and Gupta 2014). Atmospheric pollution due to FA has found to be harmful and affects the human health if the FA directly discharges from power plant chimneys into the atmosphere without treatment (Bartoňová 2015; Ding et al. 2015). In thermal power plants, the characteristics of FA depend on the type of coal and the burning conditions. The appearance of fly ash in the form of fine particle and the average diameter is less than 20 μm, bulk density is 0.54–0.86 g/cm$^3$, and specific surface area ranges between 300 and 500 m$^2$/kg (Lanzerstorfer 2018; Yao et al. 2015). There are some essential elements, including P, K, Mg, Zn, Fe, Mn, and others except N are found in FA. FA can improve the texture of the soil, making it more fertile and increasing the growth and yield of many crops (Parab et al. 2012; Kalra et al. 2000; Yeledhalli et al. 2010; Garg et al. 2005; Dhadse et al. 2008; Dermatas and Meng 2003). Besides this, the important oxide components, i.e., $SiO_2$, $Al2O_3$, CaO, MgO, $Na_2O$, and $TiO_2$, are also found in FA (Ahmaruzzaman 2010; Bartoňová 2015; Adriano et al. 1980). FA was found to reduce the gaseous air pollutant like $SO_2$ and VOCs after some modifications (Izquierdo and Rubio 2008; Zhou et al. 2015). Therefore, FA has a great concern to many researchers worldwide.

### 14.5.1 Management of Nematode by Fly Ash

Application of fly ash for the management of plant-parasitic nematodes improves the soil health. Khan (1989) reported that the soil application of fly ash inhibits the root penetration of juveniles and reduce the root-knot disease intensity caused by *M. incognita* on tomato. Similarly, according to Singh (1989), galling and the production of egg mass of *M. incognita* on lentil were found to be reduced. The higher concentration of fly ash suppressed the *M. javanica* in soybean and at 100% fly ash amendment exhibited no gallings and eggmasses of M. javanica (Singh 1993). Singh et al. (1994) studied that all the morphometric parameters of *M. javanica* decreased in pea plants grown in fly ash amended soil. According to Khan et al. (1997), the application of fly ash in soil (20–100%) adversely affected the root invasion by the larvae and decreased the disease intensity (gall and egg mass/root system) of root-knot nematodes, on tomato. Khan and Ghadirpour (1999) observed that fly ash reduced the disease severity caused by *M. incognita* on tomato,

eggplant, and chili. Joshi et al. (2000) observed the effect of organic amendment and fly ash on the root-knot disease of tomato, and the galling index was reduced greatly. Tararmum et al. (2001) observed the effect of different levels of fly ash (0, 25, 50, 75, and 100%) on hatching, penetration, and development of *M. javanica* on chickpea. Hatching and penetration were greatly suppressed. At 50% onward, no J2 developed to the mature female stage. Similarly, Iram (2010) evaluated the different levels of fly ash amended soil in the management of root-knot nematode and observed all the levels of fly ash significantly inhibit the reproduction of nematodes. Iram (2006) studied the response of root-knot nematode, *M incognita*, to fly ash on pepper (*Capsicum annuum* L.). All the fly ash levels reduced the hatching and suppressed the development of juveniles but increased the mortality rate. Root penetration was inversely proportional to fly ash ratios. The highest increase in plant growth was observed at 20% level. Tanweer et al. (2007) observed the effect of fly ash amended soil (0, 10, 20, 30, 40 & 50%) on the development of root-knot nematode, *M. incognita*, on ivy gourd (*Coccinia cordifolia*). A number of galls and egg masses per plant were decreased in 10–50% fly ash levels as compared to control. According to Singh et al. (2011), root galling increased at 25 and 50%, but decreased at the 75 and 100% fly ash levels. Gradual suppression in egg mass production and fecundity were observed at all levels of fly ash. A pot experiment was conducted in a glasshouse to evaluate the effect of *Cassia tora* leaf extract on the growth and yield characteristics of tomato and on the reproduction of root-knot nematodes in fly ash amended soil. Data on plant growth, leaf area, yield characteristics, and root-knot and egg mass indices on tomato grown in different concentrations (0, 20, 30, and 50%) of fly ash amended soil with leaf extract of *Cassia tora* were recorded. The results indicated that the plant growth and yield were enhanced and the nematode population was reduced in the 20% fly ash treatment. Among all the treatments, 50% fly ash amended soil effectively reduced the galling and nematode population (Azam et al. 2013). Ahmad and Khan (2016) evaluated the effect of fly ash on hatching, mortality, and penetration of *M. incognita* in pumpkin roots. All the levels of fly ash were found toxic and reduced the hatching of juveniles, increased the mortality rate, and inhibited the penetration of juveniles. The maximum inhibition was observed at 50% level of fly ash. The development of juveniles of *Meloidogyne incognita* in the roots of pumpkin was significantly suppressed by all fly ash and soil mixtures. The $J_2$ developed to $J_3/J_4$ stages at all levels of fly ash amendment, but their number was less than in control and decreased with the increase of the fly ash up to 40% soil mixture. At the end of the first week, neither premature nor mature females were found. During the second week, $J_2$ developed to older stages. However, while premature females occurred in all roots, only a few mature females occurred in control and at the 5–10% levels of fly ash and none at larger proportions of the amendment. During the third week, the juveniles that had penetrated into the roots developed further. However, numbers of premature females were significantly suppressed by all proportions of fly ash, while mature females were significantly suppressed at 5–10% of this amendment and were still absent at larger proportions. After 4 weeks, all the $J_3/J_4$ had developed further, but premature females were still significantly less than in control at all proportions of

the amendment, and a few mature females were observed only up to 20% of the amendment, with none at all at greater proportions (Ahmad et al. 2017).

### 14.5.2 Fly Ash and Phytobiome Consortia in the Management of Nematodes

Many researchers observed that fly ash in combination with PGPR and fungi caused a significant reduction in the reproduction of many plant-parasitic nematodes. Phytoparasitic nematodes usually cause physical damage to the roots, which may allow secondary infection by other pathogens (Sitaramaiah and Pathak 1993). Endoparasitic nematodes, *Meloidogyne* spp., create a wound in roots, allowing the other pathogens to become established (Siddiqui et al. 2012). There are many synergistic effects of fungi, bacteria, and nematode interactions are also investigated (Stansbury et al. 2001; Rubio-Cabetas et al. 2001; Partridge 2008; Mallesh et al. 2009). According to Khan and Siddiqui (2017), the galling of root and multiplication in the nematode population were decreased in the plant when inoculated with *R. solanacearum* or *P. vexans*. Moreover, inoculation of both *R. solanacearum* and *P. vexans* together caused a greater reduction in a number of galls and population of nematodes than inoculated singly with either *R. solanacearum* or *P. vexans*. Inoculation of *R. solanacearum* with *P. vexans* before *M. incognita* caused a maximum reduction in galling and nematode multiplication.

Glasshouse experiments were conducted twice to assess the ash amendments (0, 20, and 40% with soil), *Pseudomonas striata*, and a root-nodule bacterium *Rhizobium* sp. on the reproduction of root-knot nematode *Meloidogyne incognita* and the growth and transpiration of pea. 10%–50% levels of fly ash amended soil caused decreased egg masses and galls of *M. incognita* in carrot, while 50% fly ash amended soil completely inhibits the egg masses and galls formation (Haris et al. 2018). *Rhizobium* sp. was found to be much more effective and reduced the galling and nematode multiplication in comparison to *P. striata*. Moreover, the use of both organisms together also had a greater adverse effect on galling and nematode multiplication than caused by either of them alone. The 40% fly ash mixed soil with both *Rhizobium* sp. and *P. striata* showed the highest reduction in galling and nematode multiplication (Siddiqui and Singh 2005).

## 14.6 Conclusions and Future Prospects

Following conclusions from the present article can be drawn:

1. Various plant-parasitic nematodes such as *Meloidogyne*, *Heterodera*, *Trichodorus*, etc. are responsible for causing a greater loss in crop production.
2. Fly ash can be used in the management of plant-parasitic nematodes leading to enhanced plant growth and yield attributes.

3. An important rhizosphere component, PGPR also play an important role in the reduction of plant-parasitic nematodes infestations in various crops.
4. The most effective module has been found to be the consortium of root biome and fly ash which can be used in the management of plant-parasitic nematodes.
5. Application of these consortia not only improves the soil health but also enhances the crop productivity in a significant manner.
6. However, the presence of some heavy metals in the fly ash is the main problem which needs to be reckoned before its application in the field as they may be a source of soil pollution.

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# Phytobiome Engineering and Its Impact on Next-Generation Agriculture

15

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**Abstract**

Phytobiomes exist in plant biome and consist of diverse microbial communities. In the recent past, a new field emphasizing on the characterization of the plant-associated microbiome, referred to as the phytobiome, is seen as a solution toward the green agriculture methods as these phytobiomes can be easily manipulated for the betterment of agricultural practices in a eco-friendly method of crop production. Microbiome engineering plays a fundamental role in plant's requirements of nutrients and disease management, and it helps in maintaining the soil conditions for agricultural production. This chapter discusses about the influential role of microbiome on the plant and related environment. This chapter focuses on the role of quorum sensing and signaling and its application in the eco-friendly method of farming that is sustainable agriculture.

**Keywords**

Phytobiomes · Eco-friendly · Quorum sensing · Sustainable agriculture

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## 15.1 Introduction

In the recent past, a new field emphasizing on the characterization of the plant-associated microbiome is referred to as the phytobiome. These phytobiomes can impart its role in the production of enhanced yield and disease-resistant and ecologically adapted crops, along with preserving the natural ecosystem. Phytobiome consists of all living organisms in, on, or around plants (e.g., microorganisms, animals, other plants) and the environment (i.e., soil, air, water, and climate). The phytomicrobiomes are vibrant in composition when evaluated with the atmosphere in which they thrive (Hawkes and Connor 2017). The plant microflora is composed of a diversity of microbes concomitant within a typical habitat representing an ecological balance (Jasim et al. 2014).

Soil microorganisms play fundamental roles in achieving food demand as per increasing global demand, and it helps in maintaining the soil quality (Kumari et al. 2017; Majeed et al. 2015; Rosier et al. 2018). This method has further made us comprehend that microbes could momentously influence host improvement and evolutionary dynamics of plant populations. Overall, possibilities of a new green revolution rely on the ecological management and engineering of this microbiome and its practical applications in an eco-friendly manner (Gupta and Dikshit 2010; Patil and Solanki 2016).

The phytobiome complex communicates within its network (consisting microflora, environment, and the host plant) through various mechanisms like nutrient recycling, competition for nutrients, antagonism, and chemical signals. These chemical signal molecules are responsible for the maintenance of ecological balance in the soil community. There is a need for understanding these signal systems for its developing new strategies of breeding, to combat different stresses (biotic and abiotic) in the agricultural system. Recently the researchers are focusing on new horizon of metagenomics to get highly efficient molecular database of phytobiomes and other omics technologies to get a better understanding of plant microbiome and its communication within the plant system and with the surroundings as well. Therefore, there is an urgent need of interdisciplinary, system approach for the study of phytobiome's dynamics and interactions.

## 15.2 Interdependency of Plant and Microbiome: Novel Opportunity

The mutual interaction of microbiome and plant in the rhizosphere has led nutrient and disease management in plants. The plant directly or indirectly influences the microbes around its rhizosphere; this potential of plant provides us a novel opportunity toward understanding their interaction and microbiome engineering. The role of these rhizospheric microorganisms becomes important for ecologists and researchers as it can benefit plant health, leading us toward sustainable methods of farming. The microbiome helps plants in nutrient enhancement, disease resistance, and resistance toward different biotic and abiotic stresses, ozone depletion and

increasing carbon-dioxide level is one of them (Bacon et al. 2015; Kasim et al. 2016). The application of microbiome on main agricultural crops like wheat, maize, rice, and barley is extensively studied during the last two decades (Chen et al. 2012; Moshynets et al. 2019; Mücke et al. 2019; Rankl et al. 2016; Sivakumar et al. 2018; Yuan et al. 2010) (Table 15.1). The microbes (bacteria, fungus, viruses, and nematodes) and rhizosphere have great impact on attaining sustainable agriculture goals as new techniques like high-throughput analysis, genome banking, metagenomics, proteomics, and micro-engineering assist crop production methods (Bais et al. 2006; Doornbos et al. 2012; Finkel et al. 2017).

### 15.2.1 Role of the Plant

Recently, researchers and scientists are focusing on two major aspects to promote potential and helpful microbiome utilization near rhizosphere so as to get improved agricultural products (Bakker et al. 2012). The first aspect relies on manipulating the specific microbial diversity as per the requirement of the crop plant; it helps in nutrient uptake and provides disease resistance for the plant. Another aspect utilizes the plant's ability to attract specific microbiome for maintaining the promotion of plant growth and susceptibility toward adverse ecological conditions. In the nearest future, direct manipulation of the soil microbiome would lead us to attain green agriculture goal (Kumari et al. 2019a; Pandin et al. 2017; Rognes et al. 2016).

### 15.2.2 Role of the Microbiome: Bacterial Microbiome

For decades, rhizobia were believed to be the only nitrogen-fixing population of legume nodules. However, other bacteria, which are not typical rhizobia, are habitually perceived within nodules obtained from soil, thus exposing the existence of a phytomicrobiome where the interaction among the individuals not only is complex but also likely affects the behavior and fitness of the host plant (Borriss 2015; Igual et al. 2001; Kashyap et al. 2018; Lemanceau et al. 2017). It is also noted that the incredibly diverse population of bacteria residing within nodules induce neither nodulation nor nitrogen fixation. This community exists within the nodule, albeit outnumbered by nitrogen-fixing rhizobia. This phytomicrobiome has the potential to enhance plant survival, particularly under environmental stress conditions. This knowledge has paved the way for research in bringing out strategies to formulate bio-inoculants utilizing these rhizobia (Albareda et al. 2006; Sang et al. 2018).

### 15.2.3 Fungal Microbiome

A variety of fungal microbiomes have been reported in plants and soil atmosphere. Several reports have demonstrated the role of fungal association with plants like *Alternaria, Fusarium* spp. *Acremonium*, *Cladosporium*, *Epicoccum* (Bailey et al.

**Table 15.1** Phytobiomes and their interaction with majors crops triggering beneficial effects

| Phytobiomes | Occurrence | Targeted gene/plant | Sequencing methods/platform | Significance | References |
|---|---|---|---|---|---|
| **Maize (*Zea maize*)** | | | | | |
| *Frigoribacterium* sp., *Microbacterium* sp., *Pantoea* sp., *Sphingomonas* sp., *Bacillus* sp., *Paenibacillus* sp. | Associated with kernel of maize rhizosphere | ACC deaminase, IAA pathway | Sanger sequencing | Antifungal | Rijavec et al. (2007) |
| *Alcaligenes, Bacillus, Erwinia, Methylobacterium, Microbacterium, Tsukamurella, and Rhodococcus* | Enhanced colonization in roots exposed to high-salinity conditions | ACC deaminase, IAA, *acdS* gene | Sanger sequencing | | Rosenblueth et al. (2012), Bouffaud et al. (2018) |
| *Pantoea agglomerans* | Enhanced colonization in roots exposed to high-salinity conditions | Type 2 (*PIP2-1*) gene | 16 s RNA | Salt tolerance | Gond et al. (2015) |
| *Burkholderia phytofirmans* | Enhanced colonization in roots exposed to high-salinity conditions | ACC deaminase, IAA | 16 s RNA | Salt tolerance | Santoyo et al. (2016) |
| *Azospirillum lipoferum* CRT 1, *A. brasilense* | Positively affects metabolome (sugar metabolism) | PGP, phytohormone production, IAA pathway | 16 s RNA | PGP | Rozier et al. (2017), Steenhoudt and Vanderleyden (2000) |
| *P. thivervalensis*, *Serratia marcescens* | Enhanced colonization in rhizosphere under the fertilized condition | PGP, phytohormone production, IAA pathway | 16 s RNA | PGP, phytohormone production | Shahzad et al. (2013) |

| | | | | | |
|---|---|---|---|---|---|
| *Pseudomonas putida* KT2440 | | Aminotransferase | 16S and ITS2, in vitro expression technology (IVET), rRNA gene amplicon analysis | Capacity to remove xenobiotics, improved soil fitness | Ramos-González et al. (2005) |
| *Pseudomonas aeruginosa Pseudomonas monteilii*, *Enterobacter asburiae* | Enhanced colonization in roots exposed to drought conditions | Indole acetic acid, siderophore, phosphate solubilization, hydrolytic enzymes | Biochemical and 16S rDNA gene sequencing | Antifungal, under drought stress | Sandhya et al. (2017) |
| **Rice (*Oryza sativa*)** | | | | | |
| *Azospirillum* spp., *Pseudomonas sp.* | Enhanced colonization in roots, increased nitrogen assimilation | Indole acetic acid, siderophore, phosphate solubilization, hydrolytic enzymes | 16 s RNA | PGP, and biofertilizer | Gond et al. (2015) |
| *Bacillus amyloliquefaciens* | Improve metabolism in abiotic stress condition | Proline, sugar, glycine metabolism | 3-D QT-PCR | Improved saline drought and stress tolerance | Tiwari et al. (2017) |
| *Bacillus subtilis* | Improve metabolism | Malic acid pathway | OSMS | PGP, phytohormone production | Rekha et al. (2017) |

(continued)

**Table 15.1** (continued)

| Phytobiomes | Occurrence | Targeted gene/plant | Sequencing methods/platform | Significance | References |
|---|---|---|---|---|---|
| *Acinetobacter* sp., *Curtobacterium citreum*, *Curtobacterium* sp., *Microbacterium* sp., *Paenibacillus* sp., *Pantoea agglomerans*, *Pantoea ananatis*, *Pantoea* sp., *Pseudomonas* sp., *Rhizobium larrymoorei*, *Sphingomonas* sp., and *Staphylococcus cohnii* | Improve metabolism in abiotic stress condition | Indole acetic acid, siderophore, phosphate solubilization, hydrolytic enzymes | 3-D QT-PCR, high-throughput methods | PGP, phytohormone production, $PO_4$ solubilization, and antifungal metabolites production | Ruiza et al. (2011) |
| *Agromyces mediolanus*, *Curtobacterium citreum*, *Curtobacterium herbarum*, *Curtobacterium* sp., *Flavobacterium johnsoniae* | Enhanced colonization in roots, increased nitrogen assimilation | Indole acetic acid, siderophore, phosphate solubilization, hydrolytic enzymes | Sanger sequencing | PGP, mitigating of biotic and abiotic stresses | Hardoim et al. (2012) |
| **Wheat *(Triticum aestivum)*** | | | | | |
| Bacillus sp., *Paenibacillus* sp., and *Pantoea* sp. | $PO_4$ solubilization, PGP, IAA antifungal compound and siderophore | | Sanger sequencing | | Díaz Herrera et al. (2016) |
| **Indian mustard and pumpkin** | | | | | |
| *Enterobacter cloacae, Pseudomonas sp., Bacillus sp.* | Phytohormone production, nutrient enrichment | PGP, phytohormone production, IAA pathway | | Enhanced plant growth and reduced metal contamination, Cr (VI) content | Ahemad and Kibret (2014) |

| | | | | | |
|---|---|---|---|---|---|
| **Soybean *(Glycine max)*** | | | | | |
| *Pseudomonas fluorescens japonicum CB 1809 and USDA110, Azospirillum sp., Bacillus pumilus, Rhizobium japonicum, Azotobacter chroococcum, and Azospirillum brasilense* | | | | More nodulation, increased growth and seed protein, water stress tolerance, drought resistance | Aung et al. (2013), Masciarelli et al. (2014), Naseri et al. (2013), Zahedi and Abbasi (2015) |
| *Bradyrhizobium japonicum, Bacillus thuringiensis* | The highest and most consistent increase in nodulation, shoot dry weight and root fresh weight, and root volume | | | | Mishra et al. (2009) |
| *B. japonicum, A. brasilense* | Indole acetic acid, siderophore, phosphate solubilization, hydrolytic enzymes | | | Seed germination, nodule formation, and early development of soybean seedlings | Cassan et al. (2009) |
| **Barley *(Hordeum vulgare)*** | | | | | |
| *Bacillus amyloliquefaciens, Bacillus megaterium M3 and MIX (Bacillus subtilis OSU142, B. megaterium M3), Azospirillum brasilense Sp245* | Improve metabolism | Biofilm formation | – | Increased yield, growth of seedlings, salinity tolerance | Kasim et al. (2016) |

(continued)

**Table 15.1** (continued)

| Phytobiomes | Occurrence | Targeted gene/plant | Sequencing methods/platform | Significance | References |
|---|---|---|---|---|---|
| **Gram *(Cicer arietinum* L.)** | | | | | |
| *Pseudomonas fluorescens, Mesorhizobium* sp. | Enhanced colonization in roots | ACC deaminase, IAA | 16 s RNA | The maximum increase in nodule number, dry matter, and nutrient content | Verma et al. (2012) |
| *Bacillus* sp., *Mesorhizobium* sp., | – | – | – | Increase in plant P uptake | Wani et al. (2007) |
| *Pseudomonas jessenii*, *Mesorhizobium ciceri* | Rhizospheric nodulation | PGP, phytohormone production, IAA pathway | – | Increased nodule size, weight, N contents in shoot and seed yield | Valverde et al. (2006) |
| *Pseudomonas sp., Mesorhizobium sp.* | Rhizospheric nodulation | PGP, phytohormone production | – | Enhancement of nodulation and stimulation of plant growth | Malik and Sindhu (2011) |

2006; Baker 1988; Chaparro et al. 2011; Mohammad et al. 2012; Mohsenzadeh and Shahrokhi 2014), *Penicillium* spp. Eurotiomycetes, Dothideomycetes, Leotiomycetes, Sordariomycetes, Tremellomycetes, *Aureobasidium, Cladosporium, Chaetomium* (Almeida et al. 2007; Cook 1993; Harman et al. 2004; Jetiyanon et al. 2003), *Fusarium* and its teleomorphs, *Microdochium, Stemphylium,* and *Xylaria* (Yadav et al. 2015).

Many fungi form vesicular-arbuscular mycorrhizal (VAM) relationship with plants. These fungi provide nutrient absorption in root and help as a carbohydrate source for the fungi in return (Harman et al. 2004). So it is possible to have two-thirds of all plants form a relationship with VAM fungi in their root systems (Tilman et al. 2002). Some VAM fungal relationships with plants depend on fungi to provide sufficient levels of phosphorus (Svenningsen et al. 2018). Phosphorus absorption is not the only benefit derived by the plant from the mycorrhizal relationship, but also VAM fungi protect plant roots from pathogens.

### 15.2.4 Other Insects

Along with symbiotic and asymbiotic PGPR and diversity of fungal microbiomes, there is a special class of organisms like insects which affects plant's aboveground parts like leaves and stems. These may act as causative agents of different plant diseases. Meanwhile some can be beneficial for the plants. Some insect's behavior helps plants in its defense mechanism, as they may feed on some harmful bacteria or fungal strains and, therefore, the plants are indirectly benefitted. Such insects include caterpillar, aphids, and grasshoppers. Recently Hannula et al. (2019) studied the behavior of insects in maintaining plant-soil interactions. They hypothesized that plant-mediated changes in soil microbiome would affect the microbiome of caterpillars feeding on plants that grow later in these soils, through modifications of the microbiome of their host plants. After analyzing the data, they found that insect microbiome relied on soil microbiome and those effects of plants on soil microbiome. This mutual interaction of plant and soil microbiome affects the aboveground insects which later on feeds the other plants (Hannula et al. 2019).

## 15.3 Role of the Environment

### 15.3.1 Mechanism of Communication

#### 15.3.1.1 Plant-Microbe Signaling

Plant-microbe interacts through chemical signal system produced as exudates and the microorganisms in the soil. Plants root exudates attract the microbiome, which is often called rhizodeposition. The rhizodeposition mainly consists of carbon compounds, mucilage, soluble root exudates, and some organic carbon both volatile and nonvolatile (Bais et al. 2006; Doornbos et al. 2012; Finkel et al. 2017; Hartmann et al. 2009). These compounds help the host plant to attract a diversity of bacteria,

including both prokaryotes and eukaryotes (Doornbos et al. 2012). Plant secrets amino acid and carbohydrate as exudates through roots which attract a variety of bacteria in the rhizosphere as compared to bulk soil (Kumari et al. 2019b); besides, a number of chemical signals triggered by bacteria and plants interact to communicate. Mark et al. (2005) characterized expression of some genes responsible for the mutualistic association of *Pseudomonas* strain in the soil with beetroot plant, and It induced more colonization of *Pseudomonas aeruginosa* strain PAO1in the host plant rhizosphere. This condition was caused due to some exudates secreted from the host plant (Mark et al. 2005). Similarly, Han et al. (2016) reported that two isolates from banana (*B. subtilis* N11) and cucumber rhizosphere (*B. amyloliquefaciens* SQR9) which were reciprocally inoculated in same plants indicated more enrichment of microbiome colonization among cucumber and banana plant-soil interaction zone. Chemotaxis and biofilm formation were reported as the reason of this increased interaction with reciprocal rhizospheric soil and plant environment. Plants root exudates trigger signals which led to communication among the microbes and neighboring plants in the rhizospheric zone. Some plants secrete a chemical (mucilage) which acts as biocontrol agent and prevents the growth of pathogenic microbes around the meristamic zone of root tip and elongating cells (Walker et al. 2003). A compound canavanine, which mostly resembles arginine, is exudates from certain leguminous roots. Canavanine has toxic effects on native bacteria around the host plants, but a different strain of bacteria in the same rhizosphere can detoxify this compound. In this way, a specific strain of bacteria is considered beneficial for the rhizospheric colonization among the legumes (Cai et al. 2009). The signals produced by phytobiomes are known as quorum sensing signals (QSS). Transcriptosome and proteome of host plant play active role responding to the signals produced by microbiomes. These responses are brought about by specific changes in the plant protein in response to structure and specific concentration of quorum sensing molecules. Quorum sensing and biofilm formation are considered key feature of microbiome engineering as it provides the agronomist a platform to manipulate the microbiome as per the requirement of modern agricultural method in a sustainable manner. Table 15.2 shows a recent advancement in the field of microbiome engineering and quorum sensing (QS) molecular studies in different plants and agricultural crops.

#### 15.3.1.2 Quorum Sensing and Biofilm Formation

Cell-to-cell communication between bacteria is mediated via diffusible chemical signals, known as quorum sensing (Abisado et al. 2018). During the process of swarming, virulence, and biofilm formation, regulation of microbial genes is through the quorum sensing molecules. The complex extracellular matrix of exopolysaccharides and proteins is mainly involved in the formation of biofilm (Gond et al. 2015; Rosier et al. 2018). This embedded matrix of compounds helps the bacterial population in the adherence on or inside plant tissue and cells (Kashyap et al. 2019; Rankl et al. 2016). It is reported that in axenic conditions, *Bacillus amyloliquefaciens* sp. plantarum FZB42 transposon mutagenesis leads to gene expression related to bacterial swarming, biofilm formation, root colonization, and plant

**Table 15.2** Quorum-sensing-mediated communication mechanism of phytobiome (special reference to staple crops)

| Bacteria | Occurrence | Effect | Method of analysis | References |
|---|---|---|---|---|
| *Pantoea stewartii* | Maize rhizosphere | The causative agent of Stewart's wilt | Transcriptome EsaR regulon, whole genome sequencing | Ramachandran et al. (2014), Tan et al. (2015) |
| *Burkholderia* sp. | Maize rhizosphere | PGP and antifungal activity | Whole genome sequencing | Abisado et al. (2018) |
| *Pseudomonas aylmerense* sp. | Maize rhizosphere | Xenobiotic degrader and PGP | 16S rRNA sequence analysis, DNA fingerprinting, BLAST | Tchagang et al. (2018) |
| *Pseudomonas aeruginosa* PGPR2 | Maize rhizosphere | PGP and disease resistance activity | INSeq technology | Sivakumar et al. (2018) |
| *Xanthomonas oryzae* pv. *oryzae* (Xoo) | Rice rhizosphere | Causes bacterial blight disease in rice through its flagellin | Solexa/Illumina sequencing | Yu et al. (2014) |
| *Xanthomonas oryzae* pv. *oryzae* (Xoo) | Rice rhizosphere | XA13, COPT1, and COPT5 proteins are linked to causing copper redistribution in rice plant | qRT-PCR | Yuan et al. (2010) |
| *Xanthomonas oryzae* pv. *oryzae (Xoo* | *Rice rhizosphere* | *Set of proteins involved in causing virulence in rice* | *TALEs target genes were identified, causing virulence in rice through* in silico *methods* | Mücke et al. (2019) |
| *Magnaporthe oryzae (fungus)* | *Rice rhizosphere,* | *Causes rice blast,* | *Avirulence genes identified,* | Yoshida et al. (2009) |
| *Bacillus, Pseudomonas* spp. *(AHLs) in wheat* | *wheat rhizosphere* | *improves growth and resistance in wheat* | *qRT-PCR* | Moshynets et al. (2019) |
| *Burkholderia glumae* | *Rice rhizosphere* | *Causes blight of rice* | *luxI homolog, tofI, and luxR homolog, tofR by DNA sequencing* | Chen et al. (2012) |
| *Pseudomonas* ultra-performance liquid chromatography (UPLC)'s spp. | *Rice, wheat, maize* | *Responsible for PGP* | *Acylated homoserine lactone was identified* | Venturi (2006) |

(continued)

**Table 15.2** (continued)

| Bacteria | Occurrence | Effect | Method of analysis | References |
|---|---|---|---|---|
| *PGPRs of barley* | *Barley* | *Responsible for lateral root formation, increased K uptake* | *AHLs were identified* | Rankl et al. (2016) |
| *PGPRs of barley* | *Barley* | *Responsible for lateral root formation, increased K uptake* | AHLs identified by UPLC | Götz et al. (2007) |
| *Acidovorax radicis N35* | *Primed to barley seeds* | *Responsible for PGP* | *AHL compound detection through pcr + biosensor strain* | Han et al. (2016) |
| *Paenibacillus, Pantoea,* and *Pseudomonas* spp. | *Primed to barley seeds* | *Phytohormones stimulation* | *Illumina Miseq* | Yang et al. (2017) |
| *Paenibacillus, Pantoea,* and *Pseudomonas* spp. | *Primed to barley seeds* | *PGP improved mineral nutrition and induced resistance against the fungal pathogen Blumeria graminis* | *Ion Torrent (gyrB gene)* | Rahman et al. (2018) |

*PGP* plant growth promotion, AHL *N*-acyl homoserine lactone, *PCR* polymerase chain reaction, *UPLC* ultra-performance liquid chromatography, *TALE* transcription activator-like effector

growth promotion (Budiharjo et al. 2014). The bacterial strain which is involved in film formation is quite more tolerant to antimicrobial compounds; they are different from other nitrogen-fixing bacteria (free living) phenotypically and physiologically as well (Kavamura and de Melo 2014). Free-living diazotroph *Azospirillum brasilense* found in the wheat rhizosphere promotes growth through biofilm formation in the root zone (Aßmus et al. 1995). It is suggested that root exudates are initiator and manipulator molecules for the biological and physiological interactions between microorganisms and the host plant root system (Bais et al. 2004). Bais et al. reported about the role of biofilm formation on biocontrol in an experiment carried out on *Arabidopsis*. He observed that when *Bacillus subtilis* mutant strain was inoculated with *Arabidopsis*, there was no biofilm formation and *Arabidopsis* was infected with *Pseudomonas syringae*, whereas when another mutant *B. subtilis* subtilis strain 6051 was inoculated it was able to form biofilm and triggered the biocontrol on to *P. syringae*, *B. subtilis* strain 6051 produced some specific antimicrobial compound (a lipopeptide) and surfactin which were lethal to *P. syringae*. It could be concluded that biofilm formation is an essential factor in the biocontrol activity of phytobiomes (Bais et al. 2004a).

#### 15.3.1.3 Phytobiome Engineering

According to the United Nation's recent report, the global population will overpass the data of more than nine billion by twenty-second century (Kumari et al. 2017). To meet the ever-increasing demand for food, there must be more agricultural yield per unit area of available agricultural land. The role of plant microbiome has been now seen as an effective way to enhance the global food production, in an eco-friendly manner. This practice would need the engineering of phytobiomes. Upon effective way of manipulation, these phytobiomes may encounter the problems arose due to excessive use of inorganic fertilizers. The phytobiome engineering is creating an opportunity to enhance the nutritional requirement of plants in a sustainable manner, beside that they are also enabling the host plant to combat the physiological stresses like heat, salinity, and other stresses (Abhinandan et al. 2018; Bouffaud et al. 2018). Thus, the significance of evolving approaches that involve engineering of phytobiomes for plant's growth promotion, enhanced nutrition availability, biocontrol, and disease resistance is currently acknowledged (Kashyap et al. 2018; Kumari et al. 2019a; Pandin et al. 2017; Rosier et al. 2018).

The richness of microbial diversity in the rhizospheric soil is greatly enhanced and altered with the crop rotation technique of agriculture, which allows the phytobiome manipulation. Altered microbial diversity in the root zone enables the plant to avail better nutrients and more disease resistance as well (Cook 1993; Doornbos et al. 2012; Finkel et al. 2017). Researchers observed that the altered microbial strains affected the cultivars especially corn (Foo et al. 2017; Pham et al. 2017; Rekha et al. 2017; Rozier et al. 2017). The other way of manipulating the microbial community is the co-inoculating with other strains; this strategy is found to be useful in plant disease management also (Khezri et al. 2011; Pandin et al. 2017; Sumi et al. 2015). Further, the growth management of inoculated microbiome is crucial as the reduction of time in its exploration would directly affect the colonizing capacity around the root zone; co-inoculation of beneficial strains could be a crucial factor in achieving the proper exploration of inoculated phytobiomes. The co-inoculation of specific strain is also vital as they may secret antibiotics and disease-resistant compounds (Larran et al. 2016; Rafique et al. 2015; Sudha et al. 2016; Tao et al. 2014). Some microorganisms like *Rhizobium* spp. have the capacity to enhance the nutritional availability of the soil, and ultimately, the plant gets its benefit. Rhizobium spp. are reported to have the potential of enhancing nitrogen contents in soil, especially in leguminous plants (Bach et al. 2016; Han et al. 2016; Manjunath et al. 2016; Timmusk et al. 2014). Some inoculants have the potential to improve the phosphate level in the soil; these inoculations could be incorporated with rock phosphates (Chakdar et al. 2018; Geetha and Joshi 2013). Although this manipulation has excellent possibilities for sustainable methods of attaining desired yield goals, few things may be considered as limiting factors. The maintenance of the desired density of inoculated strain is a point of consideration, as they start decreasing by their density with time. Another significant consent is as follows: are these engineered phytobiomes free from other chemicals like their metabolites and antibiotic compounds? So these methods of manipulating phytobiomes must assure their

ethical adaptability from the human health point of view (Amundson et al. 2015; Morawicki and Díaz González 2018).

Table 15.1 describes the mutual association and beneficial activities of the microbiome and the target plant. Scientists are trying to establish the best delivery system for enabling the PGPR and other microbes to communicate in more important perspectives. Researchers are nowadays focusing on the development of better carrier molecules and multi-strain inoculation environment. Another focused area is the formulation of low-cost yet sustainable encapsulation systems and a better transition of it from laboratory to the farmer's field. These technologies have to be implemented soon for the development of transgenic crops (Bargaz et al. 2018; Kafle et al. 2019; Ribeiro et al. 2018; Rosier et al. 2018; de Souza et al. 2015).

Several PGPR inoculums products are currently in the market globally (Kumari et al. 2019b). The main problem in fungal microbiome engineering is actually due to difficulties in culturing them in the absence of their host/obligate symbionts. In this condition, inoculums production gets limited, though several global companies are launching AM inoculums products, useful in forestry, agriculture, and horticulture. New developments like in vitro monoxenic root organ cultures have enabled the scientists to establish AM fungal culture successfully. Molecular identification and different in silico lab techniques are enabling us to understand the communication among them (Hunter et al. 2017; Jacoby et al. 2017; de Souza et al. 2015).

## 15.4 Its Application on Sustainable Agriculture

### 15.4.1 Soil Health

Recent advances in the field of microbial identification and its metabolite interaction (signals) attracted the researchers toward its practical applications for sustainable and integrated approaches to agriculture. This microbiome has potential to increase plant nutrient uptake (Abhinandan et al. 2018; Santoyo et al. 2016) and enhance plant growth (Aung et al. 2013; Baltrus 2017; Díaz Herrera et al. 2016; Zahedi and Abbasi 2015). Benefits of these living microorganisms are vibrant and potentially self-sustaining, which ultimately reduce the need for repeated applications, pests attack, and pathogens that evolve resistance to the treatments (Lucas 2011). Phytobiomes help the host plant by increasing their growth (enhances the phytohormone biosynthesis, nutrient availability) directly or indirectly (Brown and Saa 2015; du Jardin 2015). They also enable the plant against different biotic and abiotic stress. Different microorganism-based bioformulations are available in the agricultural market. These microbiomes are engineered or co-inoculated PGPRs like *Serratia*, *Variovorax*, and *Azotobacter* species. Endophytic bacteria and fungal formulations are beneficial for the plant (Nakkeeran et al. 2006; Barea 2015; Bishnoi 2015; FAO 2016; Le Mire et al. 2016).

### 15.4.2 Disease Control

Consistent use of inorganic fertilizer and pesticides has led to dangerous conditions of soil due to its residual persistence, which raises food safety concerns among the consumers (Ahemad and Kibret 2014; Anand et al. 2016). Recently PGPRs and other microflora have been proven to be better biocontrol agents as they help the plant in fighting against pathogens and by electing antagonistism (Beattie 2006). These microorganisms are designed now with advanced systems like proteomics and high-throughput data analysis methods. These methods are economically acceptable, and as per the host's requirement, they are easily degradable. These formulations are a better solution to integrated pest management (IPM) programs. Being safe to use, ecologically adaptable, and easy to handle, it is an excellent opportunity for a new green agricultural method of farming. This microbiome increases the availability of nutrients and effects positively on the plant hormone biosynthesis. They produce siderophores enabling the plant to fight against the harmful pathogens (Kumari et al. 2016; Yang et al. 2009; Haghighi et al. 2011; Anand et al. 2016; Le Mire et al. 2016; Rubin et al. 2017). A variety of bacteria, specially *Bacillus*, are reported to possess the growth-promoting activities along with disease resistance in agricultural and medicinal plants (Kashyap et al. 2019; Kumari et al. 2017). Nanomaterial-based biocontrol strain engineering is another fascinating area on which researchers are working to synthesize nanoparticle or nanofiber-based sensor tool and formulation development with minimal impact on natural rhizosphere (Kashyap et al. 2019; Kumari et al. 2019b).

## 15.5 Challenges of Phytobiome Engineering

The modern technologies like high-throughput sequence analysis methods have influenced the characterization methods of plant microbiome. These technologies have an impact on promoting high-level research on plant health in a stressed condition.

Another technological advancement that took place recently is metagenomic analysis. This technology is enabling the identification of a functional group of microbes based on their metabolic potential. This technology helps us in the study of different types of secretions from plant root (exudates) and their interaction toward plant microbiome and surroundings.

Besides these developments, there are some challenges in the field of phytobiome engineering, and this may be due to a large number of genomic data and diversity of data as well. The influence of environment is also an important factor that controls the changes in the microbial diversity in plant biome. The biggest limitation of micro-engineering-based agricultural advancement is its ethical acceptance. The farmer is properly not well educated about the costiveness of this recent

agricultural facility. Another drawback is this engineered product is not easily available for commercialization.

## 15.6 Future Prospects

Presently, diverse research tactics are being addressed to discover the aspect of microbiome engineering parameters while averting the presence of pathogens. Upon getting fruitful rhizosphere, new advantageous mechanistic approaches can lead to new advancement in its engineering. By exploiting the useful microbial services, we could enhance rhizobial performance or persistence. It would decrease the demand of agrochemical fertilizers and pesticides in our agricultural system. A combination of all of these approaches can increase our understanding of how to enrich the effectiveness and tenacity of bacteria in the rhizosphere to finally improve plant health and agroecosystem productivity.

## 15.7 Conclusion

It is evident that all plants can acquire a variety of microorganisms throughout all phases of their lifetime. Phytobiome characterizes plant growth and evolutionary change, and thus, a thorough understanding of the relationship among plant and their associated microbes could facilitate the engineering of these communities. Microbial inoculants could be a boon to the agricultural sector if implemented on following aspects: (i) to increase the scientific/technical aspects of inoculum production; (ii) to produce precise normative for the preparation of inoculum, either from seed or on soil, or the plant on which inoculum is to experiment; (iii) to reduce the unpredictability of the field results; and (iv) to increase knowledge and propagation by elaborating its usefullness and limitations to the society

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