THE PLANT MICROBIOME IN SUSTAINABLE AGRICULTURE

EDITED BY
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Preface

Global agriculture is facing a major challenge of ensuring sustainable and healthy food production for an ever exploding human population, while seeking to reduce adverse effects on the ecosystem. Recent reports indicate that factors like soil health (nutrients, water, and pH), vulnerability to diseases and pests, agronomic practices and climate change affect crop growth and yield. These factors are the prime cause of crop failure and decline in average yields.

The quest to harness the potential of useful microbes from different ecological niches have shown interesting outcomes in that microbiome plays an important role in growth and development of other living communities. Plants depend on their microbiome for multifarious life supporting activities including nutrient acquisition and augmentation of the defense system towards biotic and abiotic stresses. However, the process of crop domestication may have negative associations with the composition and function of the host associated microbiota, thereby limiting their advantageous effects on crop health and development. With major emphasis on agriculture, characterizing the plant microbiome and its function could be applied for better crop designing and management to grow crops in resource limited environments, and protect them from intruding pathogens. Unfortunately, at present, most of the breeding programs across the globe have not taken microbial action into account. Therefore, a deeper understanding of the interrelationships of the soil–plant–microorganism system is essential for improving the efficacy and potential applications of plant growth promoting inocula for achieving sustainable food security and development.

Many of the laboratories working on plant growth-promoting rhizobacteria (PGPR) have reported that cocktails of useful bacteria in the form of synthetic communities are better than the single inoculants which face competition from other microorganisms and which could be killed or suppressed under suboptimal conditions. These findings clearly establish the importance of the microbial community in the well-being of crop plants. Now the researchers are focusing towards deciphering the microbial communities using next generation molecular approaches, and they are dominating the conventional methods. Modern molecular tools are utilized to recover the microbial information's links with different ecological niches. This information can be used to establish and maintain plant and human health, and finally to achieve comprehensive information of the plant microbiome that can be helped to improve agricultural production. In the past two decades, the plant microbiome has gained interest and crop performance is increasingly being recognized as the result of multipartite interactions. The huge gene pool of the microorganisms living in

close association as endosymbionts and surface colonizers extends to the host genome and contributes to its phenotype. The studies clearly indicate that the totality of this genetic information in the form of the hologenome may allow adaptation of crops to diverse environmental conditions and interactions.

The present book provides a comprehensive review and compiled information on different aspects of plant microbe research with reference to its scope in the agriculture system which can be transformed by a complete understanding and application of the specific microbiota in a holistic manner. In the book, the chapters are contributed by active researchers having expertise in the domain. Following an introduction to the specificities of microbiome research, modern tools and techniques to understand the plant microbiome are described. The updated information on the microbiome of different crops and cropping systems, followed by functional ecology and its potential for abiotic and biotic stress management, crop health and nutrient fortification, has been presented in different chapters. As they are of particular relevance for the future of agriculture in a sustainable manner, the biotechnological and molecular aspects of the translational microbiome are thoroughly covered across the book. Lastly, the relevance of the microbial community as the reservoir of novel genes and metabolites and as the key to green and clean agriculture have been discussed. This book will stimulate the readers to understand this complex subject in a lucid manner. It provides a path to researchers to address some of the contemporary issues before the scientific community, towards development of environmentally friendly and sustainable agriculture to meet the needs of our universe. With great pleasure, the editors acknowledge the efforts and contributions of expert authors, which were crucial for the quality of information provided.

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Dr. Alok Kumar Srivastava is a dedicated scientist in the area of molecular microbiology, presently working as Principal Scientist, ICAR-National Bureau of Agriculturally Important Microorganisms, India. He has made an outstanding and pioneering contribution in the area of Molecular Plant Pathology, contributed to structural, functional and comparative genomics of agriculturally important microorganisms, pathogens, development of molecular diagnostic tools and biological control of important diseases. He successfully sequenced the whole genome of 15 AIMs, deciphered microbial communities of Leh, mangrove soil of Andaman, landfill sites, and saline soils through metagenomics. Dr. Srivastava is Ph.D. from Banaras Hindu University, India, and completed his post doc at Otto Warburg Centre of Biotechnology with Prof Ilan Chet, at The Hebrew University of Jerusalem, Israel. He has also visited several countries including Hungary, France, The Netherlands, and Norway. He worked as a visiting research scientist in the Department of Plant Sciences, McGill University, Canada in the year 2010. He has 31 years of research experience in the area of biological control of fungal pathogens and plant growth-promoting rhizobacteria (PGPRs) and has supervised eight PhD students. He has successfully completed several externally funded research projects from DST, DBT and other agencies of the Indian Government. He has published about than 130 research papers in journals of international repute, several review articles, edited three books, and has more than 2473 citations to his credit (H index-25, I_{10} index 55). He is also associated with the capacity building program in the

area of microbiology and has organized/participated in many training programs as a resource expert. Currently, he is associated with one of the mega network projects, "Application of Microorganisms in Agriculture and Allied Sectors (AMAAS)" of ICAR, India and is performing whole genome sequencing of important microorganisms. He is also providing molecular identification services for the microorganisms to be registered with the Central Insecticide Board and Registration Committee (CIB&RC) Govt. of India. His group is focusing on the management of abiotic stresses and soil health through *Bacillus* and other predominant genera as PGPR, its capabilities related to soil fertility and plant nutrition mobilization, the production of bacterial phytohormones and solubilization of mineral phosphates, allowing them to inhabit diverse niches in agro-ecosystems. For further details of this work visit https://scholar.google.co.in/citations?user=De5ciSsAAAAJ&hl=en

Dr. Prem Lal Kashyap had his agricultural education from Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya (CSKHPKV), Palampur and Punjab Agricultural University (PAU), Ludhiana (Punjab). Presently, he is working as Scientist (Plant Pathology) at ICAR –Indian Institute of Wheat & Barley Research, Karnal and has nine years of research experience. He is a dedicated researcher in the area of plant pathology, and has made an outstanding and pioneering contribution in the area of phytopathogenomics, biomarker development, biocontrol and integrated disease management. He identified five new chemical molecules for the management of wheat diseases and pests; reported five new races of yellow rust of wheat and contributed in development of "GEHOON DOCTOR" mobile app. He decoded whole genome of several agriculturally important microorganisms, developed biomarkers for early and precise diagnosis of plant pathogens (*Alternaria, Fusarium, Colletotrichum, Tilletia indica, Urocystis agropyri, Puccinia triticina etc.*) and developed a formulation 'Biogrow' available for commercialization by AgriInovate (ICAR) India. He has three wheat varieties to his credit and has filed two patents. Dr. Kashyap has contributed immensely in resource generation through externally funded projects from DBT and other agencies of Indian Government. He also created a large pool of trained human resource in the area of plant pathology that has enabled successful product development following his approach. He has more than 100 publications to his credit, including 65 peer reviewed research papers, 3 books, 22 book chapters, 24 popular articles and 6 technical manuals. He has visited several countries including USA, Kenya, Mexico, Bangladesh and Bolivia for academic pursuits. For significant research contribution in the field of agricultural sciences, Dr. Kashyap has been recognized with several prestigious awards including NAAS Associateship-2019, NAAS Young Scientist Award, Dr. Basant Ram Young Scientist Award, Prof. Abrar Mustafa Memorial Award, M. K. Patel Memorial Young Scientist Award and Prof. Mahatim Singh Memorial Award.

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1

Plant Microbiome

Past, Present and Future

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1.1 Introduction

The evolution on earth have revealed the association of microbes to a plant and its specific tissue or organs (Compant et al. 2019; Hardoim et al. 2015; Reinhold-Hurek et al. 2015; Spinler et al. 2019; Uroz et al. 2019; Vorholt 2012). Plant microbiome encloses all associated plant microbes, whether phyllospheric, rhizospheric, or endospheric (all microbial genomes) (Brader et al. 2017; Lemanceau et al. 2017). Revealing of plant microbiome functionality has led to knowledge about plant microbe interactions, which are advantageous for plant growth and its production. The demographic, environmental, climatic, and man-made conditions have made crop production very challenging (Asl 2017; Kanianska 2016; Templeton and Scherr 1999). Microorganisms have been shown very advantageous in sustainable crop production. They have shown potential as biofertilizers, biopesticides, and growth enhancers (Bhardwaj et al. 2014; Cheng et al. 2017; Gopalakrishnan et al. 2015; Goswami et al. 2019; Kashyap et al. 2017b; Kushwaha et al. 2019a; Marrone 2019; Mendes et al. 2013; Mitter et al. 2016; Singh et al. 2019b). They have proven good alternatives compared with chemical products as excessive use of these can affect the harmony and microbiota of plant, which may lead to disintegration of soil fertility and quality (Lemaire et al. 2014; Marenya and Barrett 2009; Vorholt et al. 2017). There are number of inoculants proposed by the researchers, but with limited success in the field (Müller et al. 2016; Souza et al. 2015). Manipulation of the plant microbiome has the potential to improve crop production, reduce plant diseases (Andrews 1992; Bloemberg and Lugtenberg 2001; Hegazi et al. 2019; Singh et al. 2019a) and greenhouse gas emission and chemical inputs (Adesemoye et al. 2009; Cheng et al. 2017; Marrone 2019; Singh et al. 2010). In addition to this, it is important for nutrient cycling (the global biogeochemical cycle) also (Philippot et al. 2009).

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Almost all the plants host a microbial community, including bacteria, archaea, fungi, and blue green algae. The rhizospheric microbiome includes microbes associated with the roots along with the soil. The phyllospheric microbiome includes the plant arial surface microbes, whereas the endospheric microbiome include all microbes associated with the internal tissue termed endophytes. The rhizosphere is very rich in a soil-derived microbial community influenced by plant mucilage and root exudates (Kent and Triplett 2002). The phyllosphere is nutrient poor and subject to extreme temperature, radiation, and moisture (Kushwaha et al. 2019b; Vorholt 2012). The rhizospheric and phyllospheric microbes are closely associated with the plant surface and termed epiphytes, whereas the microbes associated with the internal organs and tissues are termed endophytes. The enrichment of microorganisms in the plant is not random, but a targeted process (Berg et al. 2017). The attraction of microbes to the root by the nutrients and the secondary metabolites has been studied. Chemoattractants and repellants have also been studied in the past few years (Feng et al. 2018; Oku et al. 2014; Pinedo et al. 2015). Although the structure of plant microbiomes is well studied, there are many knowledge gaps because of the plant species-specific components; targeted studies have been performed on crops and model plants such as *Arabidopsis*. The gaps are especially related to plants in natural ecosystems and their relationship to plant health.

So much application and the increasing complexity of the microbiomes have led to the development of many techniques in the field of identification, as well as taxonomy. For the identification of microbes, several classical methods had been used previously which included phenotypical as well as biochemical methods. These methods were only effective with the culturable microbes, so it was mandatory to develop the technology (Jesumirhewe et al. 2016). Nowadays, there are many more sequencing technologies which have been further improved. Besides 16S ribosomal RNA and internal transcribed spacer (ITS) sequencing, the use of MALDI-TOF-MS (matrix assisted laser desorption ionization-time of flight-mass spectroscopy) have proven to be useful in microbe identification (Adekambi and Drancourt 2004; Chen et al. 2000; Kashyap et al. 2016; Nouwens et al. 2000; Peng et al. 2005; Pieper et al. 2006; Rai et al. 2016; Sharma et al. 2015). Several polymerase chain reaction (PCR) based technologies such as repetitive PCR, amplified fragment length polymorphism (AFLP), random amplification of polymorphic DNA (RAPD), and multiplex PCR have been improved (Kashyap et al. 2016; Rai et al. 2015, 2016; Srivastava et al. 2014). The NGS (next generation sequencing) and multi-omics (genomics, metagenomics, transcriptomics, and proteomics) technologies allow much deeper insight into the structure of plant-associated microbial communities and its interaction with the ecosystem which support and often extends the current body of knowledge (Berg et al. 2015; Jansson and Baker 2016). In addition to this, these tools also reveal the functional dynamics and plant–microbe interaction as well as new PGP (plant growth promoting) traits.

In brief this chapter includes several approaches for studying the plant microbiome, the past and present tools for the identification of the microbiome and their advancement. In addition to these we have discussed the different microbes present in different plant parts. This chapter gives an overview of the application of the microbes in agriculture and the allied sectors. Figure 1.1 describes the pictorial representation of the structure of the plant microbiome, its biotic (plant microbe interaction) and abiotic factors which govern the structure and composition, its study using classical and modern tools and its application in the field of agriculture and other allied sectors.

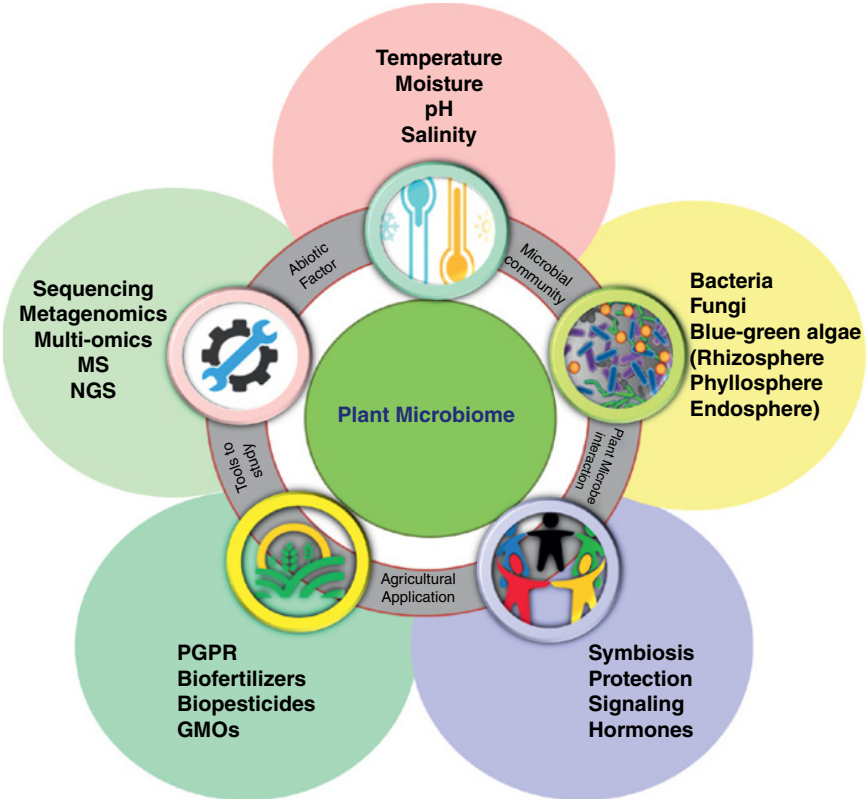

Figure 1.1 Plant microbiome structure, function, application, and their study using modern tools. The abiotic factors affect the microbiome which includes mainly bacteria, fungi, blue-green algae and several other microbes. They interact with the plant environment by means of different methods. Because of the interaction, they develop some characteristics which benefit each other. There are several tools to study all these components. The figure depicts the basics of plant microbiome components and their study. MS: mass-spectroscopy; NGS: next generation sequencing; GMO: genetically modified organism; PGPR: plant growth-promoting rhizobacteria.

1.2 Plant Microbiome

Microbes evolved on Earth approximately 3.5 billion years ago and eventually occupied every habitable environment in the planet's biosphere (Margulis 1981). Although microorganisms are known to be responsible for key functions on Earth, such as nutrient and biogeochemical cycling, and determining the health and disease state of the planet's plant and animal inhabitants, more than millions of microbes thought to exist have yet to be discovered. The plant microbiome includes only a fraction of the existing microbiome, which can be further divided on the basis of the localization and association to the plant part (Sanchez-Canizares et al. 2017). On the basis of localization, the microbiome may be divided into rhizospheric, phyllospheric, and endospheric. Figure 1.2 explains the components of the plant microbiome, and their interaction.

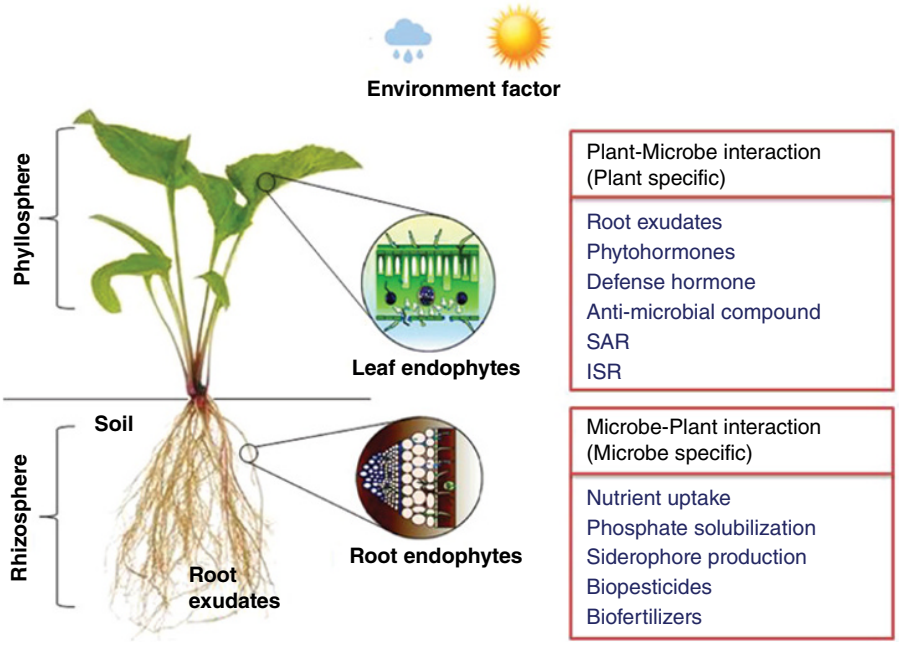

Figure 1.2 Components of the plant microbiome and its interrelations. SAR: systemic acquired resistance; ISR: Induced systemic resistance

1.2.1 Rhizospheric Microbiome

The rhizospheric microbiome is generally influenced by the deposition of mucilage secreted by root exudates and sloughed cells. The root exudates contain a number of organic acids, sugar, amino acids, fatty acids, vitamins, growth factors, hormones, and secondary metabolites. These compounds particularly decide the fate of the rhizospheric microbiome. The rhizospheric microbiome is of particular interest because of the plant growth promoting characteristics (Cai et al. 2017; Goswami et al. 2019; Guo et al. 2019; Pereira et al. 2019; Sharma et al. 2019; Singh et al. 2014; Solanki et al. 2012; Srivastava et al. 2013). They are generally described as plant growth promoting rhizobacteria (PGPR) and they act through a variety of mechanisms. They efficiently colonize the rhizosphere and stimulate plant growth through direct or indirect mechanisms (Lugtenberg and Kamilova 2009; Solanki et al. 2014, 2015). They possess general plant growth promoting properties such as mineralization and solubilization of phosphate, and siderephore formation, and they increase the bioavailability of inorganic phosphorus and iron (Cai et al. 2017; Guo et al. 2019; Hegazi et al. 2019; Pereira et al. 2019; Singh et al. 2019a). Because of these properties they are of interest to use as inoculants for plant growth promotion (Lugtenberg and Kamilova 2009). The rhizobacteria include the N_2 fixing free living *Azotobacter*, symbiont *Rhizobium* spp., *Bacillus* sp., *Pseudomonas* sp., etc.

1.2.2 Phyllospheric Microbiome

The phyllosphere is basically the aerial part of the plant which is poor in nutrients. There are a number of abiotic factors which influence the phyllospheric microbiome such as fluctuation in the temperature, moisture, radiation, wind, and precipitation; because of this it is much more dynamic compared with the rhizospheric microbiome (Kembel et al. 2014; Lindow 1996; Thapa and Prasanna 2018). Leaves also secrete some organic acids, sugar, and phytohormones through the stomata, hairs and veins which attract microbe for colonization on the leaf surface. The leaf surface colonizes $10^7/cm^2$ microbes (Lindow and Brandl 2003). The use of both traditional, culturing-based taxonomy and modern tools has illustrated that the diversity among bacterial members is mainly restricted to *Actinobacteria, Proteobacteria, Firmicutes, Bacteroidetes,* and less frequently, cyanobacteria; oomycetous communities are common inhabitants, besides fungi. The phyllospheric and rhizospheric microbes are also known as epiphytes.

1.2.3 Endospheric Microbiome

The microbiome inhabiting intra or inter-cellular plant tissues is defined as the endophytic microbiome and the microorganisms are known as endophytes. Some of the endophytes dedicate all their life cycle or part of the life cycle to the host plant. The term endophyte refers to a complex set of interactions, including the interactions between the different microbes that comprise the endophyte community and the host-plant defense mechanism to prevent these fungal species from becoming pathogens (Xu et al. 2016). They have the ability to colonize the inside of the tissue of different plant parts like healthy leaves, petioles, stems, twigs, bark, roots, fruit, flowers, and seeds without causing any harmful effect or infection in their host plant (Varma et al. 2012; Wassermann et al. 2019; White et al. 2019). Endophytic fungi belong to many different ecological and phylogenetic groups in a highly diverse environment. Endophytic microbes mostly belong to the *Ralstonia*, *Burkholderia*, *Pseudomonas*, *Staphylococcus*, *Mesorhizobium*, *Propionibacterium*, *Dyella*, *Bacillus,* and Ascomycetes; the rest are very rare (Kushwaha et al. 2019a, 2019b; Rosa et al. 2010). Some endophytes are listed in Table 1.1 along with their isolated plant parts. Apparently, more than one million different endophytic fungal strains inhabiting about 300,000 various plant species have been reported already (Wassermann et al. 2019; White et al. 2019). This amount of diversity is because of the presence of several endophytic fungi in an individual plant species, which can colonize the inside of the plant tissue. To mediate the plant–endophyte interaction, they produce a number of bioactive metabolites or secondary metabolites (Xu et al. 2016). These secondary metabolites have several applications in medicine, agriculture, and industry. In addition, these fungal endophytic metabolites have many roles in plant growth promotion by enhancing phosphate solubilization, siderophore production and other PGP traits, and they help in enhancing the production of the host plant (Taghavi et al. 2009; Varma et al. 2012; Wassermann et al. 2019; White et al. 2019). Many bioactive metabolites are originated from microbial organisms; fungi are the core important groups of eukaryotic organisms that have the wide capacity to produce numerous metabolites. Several bioactive compounds, including antifungal and antibacterial agents, have been isolated from fungi (Suryanarayanan et al. 2009).

Table 1.1 Some endophytic fungi isolated from different parts of the plants.

Host plants				
Common name	**Scientific names**	**Isolation parts**	**Frequent taxonomic groups**	**References**
Orange	*Citrus* spp.	Leaves and seeds	*Colletotrichum gloeosporioides, Guignardia citricarpa* and *Cladosporium* sp.	Zhang et al. (2013); Jalgaonwala et al. (2011)
Rice	*Oryza sativa* L.	Leaves, seeds, and roots	*Chaetomium globosum*, *Penicillium chrysogenum*, *Fusarium oxysporum* and *Cladosporium cladosporioides*	Wang et al. (2016); Walitang et al. (2017)
Sugarcane	*Saccharum* spp.	Leaves	Ascomycota phylum	Dong et al. (2018)
Wheat	*Triticum aestivum* L.	Leaves, stems, glumes, and grains	*Alternaria alternata, Cladosporium herbarum, Epicoccum nigrum*, *Cryptococcus* sp., *Rhodotorula rubra, Penicillium* sp., and *Fusarium graminearum*	Pagé et al. (2019), Conn and Franco (2004); Jalgaonwala et al. (2011)
Banana	*Musa acuminata Colla*	Leaves	*Xylaria* sp., *Colletotrichum musae* and *Cordana musae*	Gamez et al. (2019); Jalgaonwala et al. (2011)
Common bean	*Phaseolus vulgaris* L.	Leaves	*Colletotrichum, Hannaella, Cochliobolus,* and *Phomopsis*.	López-López et al. (2010)
Cowpea	*Vigna unguiculata* (L.) Walp.	Root nodules and seeds	*Aspergillus* spp., *Penicillium* spp. and *Fusarium* spp.	Leite et al. (2017)
Maize	*Zea mays* L.	Roots, leaves, and stems	*Alternaria alternata* and *Aureobasidium pullulans* var. *melanigerum*	Abedinzadeh et al. (2019); Jalgaonwala et al. (2011)

1.2.4 The Effect of Abiotic and Biotic Factors on the Plant Microbiome

The microbial composition associated with the plant may vary depending on several factors (Figure 1.2). The abiotic factors like temperature, moisture, soil pH, fertility, salinity, exudes, soil structure and soil organic matter affect the below ground microbiota (Fierer 2017). Whereas the environmental conditions, climate, pathogens, and human practices affect both the below and above ground microbiota (Hardoim et al. 2015; Hartmann et al. 2009). The plant species and genotype as well as the rhizodeposition and exudates also affect the recruitment of the microbes. It has been seen in many findings that the different plant microbiomes differ from one another, even though they are in the same soil conditions (Aleklett et al. 2015; Chaparro et al. 2014; Samad et al. 2017).

Beside these abiotic factors, the interaction between plants and microbes also influences the microbiome. This plant–microbe interaction is highly complex and dynamic. The plant

defense system also affects the composition of the plant microbiome. It has been reported that a mutation in *Arabidopsis thaliana* deficient in the systemic acquired resistance (SAR) shows a difference in rhizospheric bacterial community as compared to the wild type plant (Doornbos et al. 2011; Hein et al. 2008). The salicylic acid (SA) mediated defense reduces the phyllospheric microbiome while the plant deficient in jasmonate mediated defense shows an increase in epiphytic diversity (Kniskern et al. 2007). Several other examples also show that the plant defense system also influence the microbe community, and SAR is responsible in controlling the bacterial population (Cheng et al. 2017; Egamberdieva et al. 2017). Some chemicals are produced by plant-like flavonoids and trigger diverse responses in rhizobia, and strigolactones induce hyphal branching in mycorrhizal fungi, etc. Plants also produce some antimicrobial compounds like phenolics, terpenoids, and alkaloids which also control the microbial population.

1.3 Approaches to Studying the Plant Microbiome

1.3.1 Classical Approaches

There are several approaches for studying the plant microbiome. The classical approaches based on culture isolation and purification of the microbes from the microbiome on different nutrient media and growth conditions depend on the target organism. Then these purified microbes are identified biochemically or phenotypically. This method has many drawbacks because only culturable microbes can be identified by this method and only 1% of the microbial population are culturable (Newcombe et al. 2018; Sarhan et al. 2019). The requirement of the pure culture misses the majority of the microbial population. To overcome this problem, several PCR-based sequencing technologies have been developed. For prokaryotic bacteria, 16S ribosomal gene sequencing is used, whereas for eukaryotic fungi the ITS and intraspecific ITS sequencing is utilized for identification (Chen et al. 2000; Ciardo et al. 2006; Nocker et al. 2007). To reduce the complexity in identification, several other targets have been chosen for the PCR amplification, for example *rpoB, gyrA, gyrB*, *sod*A, and *hsp* gene sequencing are utilized to differentiate the closely related species like *Mycobacterium chelonae* and *Mycobacterium abscessus*. The ITS regions also have some limitations, and because of that, the two variable domains D1, D2 near the 5′ end of the 28S ribosomal RNA genes, elongation factor α (e.g. *Fusarium* sp.), and β-tabulin (e.g. *Phaeoacremonium* sp.) are being used as alternative gene targets (Kashyap et al. 2017a; Stielow et al. 2015; Dagar et al. 2011).

1.3.2 Modern Approaches

Fingerprinting technology is also widely used in microbiome identification. Repetitive PCR, RAPD, and multiplex PCR are widely used methods (Cocconcelli et al. 1995; Kashyap et al. 2016; Lin et al. 1996; Rai et al. 2016). These methods utilize PCR along with the specific set of primers. The change in the sequence or the pattern can be utilized in identification. The restriction fragment length polymorphism (RFLP) is another method that utilizes restriction enzymes. 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 (Cocconcelli et al. 1995; Lin et al. 1996; Versalovic et al. 1994). Microarray-based technology has also been developed for microbe identification which has several probes on the surface of the chip immobilized on the silica plate (Liu et al. 2001; Sharma et al. 2017; Wilson et al. 2002). Depending on the probe, it may be a protein or DNA microarray. Protein microarray utilizes specific antibody (protein) against the microbe while DNA microarray utilizes the specific marker DNA as a probe (Liu et al. 2001; Wilson et al. 2002).

Protein profiling is one of the recent methods for identification (Emerson et al. 2008). Microbes can be identified 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 iso-electric focusing (IEF) and SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis), where proteins are first separated on the basis of the iso-electric point (charge) 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 (Malmstrom et al. 2002). The major drawback of this method is that it is a labor extensive and time consuming and requires ample amount of the protein. Proteome profiles of organisms can be compared with the existing databases and on the basis of the comparison the microorganism can be identified. This method is greatly enhanced by the introduction of MALDI-TOF-MS. Using this method the proteome of many microbes has been made available (Nouwens et al. 2000; Peng et al. 2005; Pieper et al. 2006).

Recently, metagenomics development has made the study of the microbiome an easy task. This has been possible because of the development of NGS technology and powerful databases. Metagenomics is the genome analysis of the population of microorganisms present in a microbiome. This doesn't include the culturing of the microbes and purification. The genomic DNA can be isolated directly from the rhizosphere or the plant part and a library can be made which can further be utilized for sequencing. Similarly, the transcriptomics and proteomics have also proven useful in microbiome studies (Handelsman 2005; Jiao et al. 2019; Loman et al. 2012; Petrosino et al. 2009; Stahl and Lundeberg 2012).

1.3.3 Plant Microbiome Studies: Limitations and Future Directions

Development of the single molecule sequencing and the MS (mass spectroscopy) technologies over the last two decades have made it possible to study microbiomes of a variety of complex ecosystems. In the beginning of the year 2000, Banfield and co-workers used the combination of the sequencing with MS approaches to study microbiomes of low microbial complexity (Ram et al. 2005). Subsequently, microbiomes of varying diversity and complexity have been investigated using either advance sequencing or MS or both (Wilmes et al. 2015). Because of the regularly improving technologies, the expectation of greater insights into the microbial communities of the previously studied communities has increased.

Knowledge of the molecular function of the microbiome is still a big challenge (Sergaki et al. 2018). The biggest obstacle is bioinformatic and computational analysis. Several other challenges also exist, such as the extraction of the biomolecules from the highly diverse and complex samples; assembly of the complete genome rather than sequence fragments directly from the complex ecosystem; high throughput MS technology for metaproteomics and

metabolomics; the requirement of high speed computational systems for *de novo* assembly of large metagenomes and metatranscriptomes; sufficient storage; and the development of algorithms and mathematical tools for data interpretation to provide meaningful biological insights. We expect that positive initiatives will enable new technologies in the future that do not currently exist, such as high resolution ion mobility separation of peptides and metabolites; high speed and high storage systems; and improved databases and algorithms (Jansson and Baker 2016). These technologies with computational improvements will be vital for deciphering the role of microbes in the natural habitat and determining the role of the complex interplay between the members of microbial communities and ecosystems.

1.4 Microbiome and Agriculture in Past and Current Scenarios

In the last few decades, microbiome research has customized the insight on the complexity of microbial community structure. Presently, microbiome research is at the initial stages of starting to understand the set-up of microbial complex communities, the inter-genera and intra-generic dependencies, and extended to the biotic as well as abiotic factors (del Pilar Martínez-Diz et al. 2019; Rodriguez et al. 2019; Sanchez-Canizares et al. 2017). The enhancing demand for alternative experimental protocols, as well as the development of new tools has given a new dimension to the comprehension of the dynamics that exit within the microbiomes and their interaction with host organisms (Goodrich et al. 2017).

There have been numerous attempts to understand the complexity of plant microbiomes and soil (Cui et al. 2019; Goswami et al. 2019; Lundberg et al. 2012; Rodriguez et al. 2019), prompting new innovations for the next green revolution with sustainable crop production (Jez et al. 2016). In the last decade, a number of reports have been published (Figure 1.3) which show extensive research in the rhizospheric microbiome as well as the endospheric

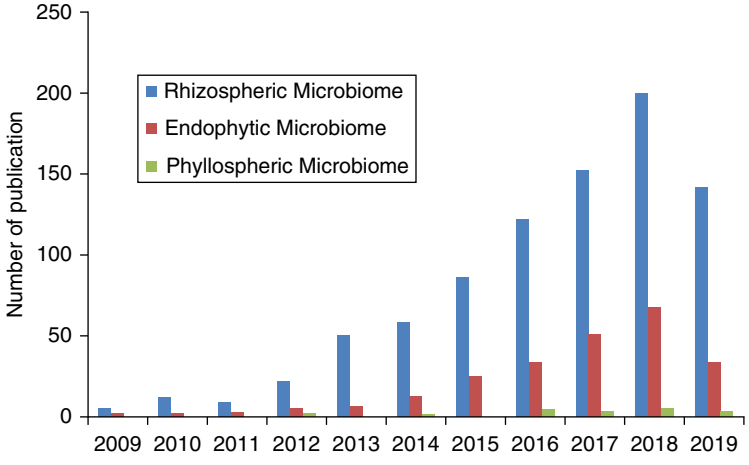

Figure 1.3 Graphical representation of the publications on different microbiomes in the last decade. *Source:* The data have been extracted from the NCBI-Pubmed. (www.ncbi.nlm.nih.gov/pubmed).

microbiome, but very little information about the phyllospheric microbiome. Comprehension of the extended potential of microbiomes, demands the innovation of new analytical strategies to gain higher crop production through the higher application of microbial communities with maximum efficiency (Bashiardes et al. 2018). The significance of maintaining a diverse microbiome and well-balanced, diversity in the rhizophere is important in crop production. Microbiome applications, however, have been implemented on farms with the aim of improving crop production via maintenance of soil health, fertility and nutrient availability to the plant through various solubilization mechanisms (Ab Rahman et al. 2018). Pertaining to this, the prime challenge is the delivery of laboratory generated innovations to the field and further, working out the structure of the rhizospheric microbiome (Schlaeppi and Bulgarelli 2015; Sergaki et al. 2018). It essentially involves correlating the dynamics of the microbial community with functioning of the microbiome (Sanchez-Canizares et al. 2017).

Plant disease control is important for the production of biomaterials, consumable resources and food. According to estimates, the most important international issue in recent times is that global food production must be increased by 70 percent until 2050, to meet the growing concern for global food security (Ingram 2011; Keinan and Clark 2012; Valdes 2019). Presently, the current food production system is responsible for the loss of 60 percent of global terrestrial biodiversity, along with 25 percent of global greenhouse gas emissions (Ab Rahman et al. 2018). The expanding world population demands an efficient management system and control of diseases in crop production, as crop protection plays a vital role in protecting crop productivity against competition from pathogens (Oerke and Dehne 2004).

Beneficial biocontrol of microbiomes is one of the few options that shows the ability to control disease, and can provide benefits by competing with pathogens or by directly antagonizing plant pathogens by release of antimicrobial chemical compounds (Chen et al. 2019; Choudhary et al. 2007; Kepler et al. 2017; Mansfield 2000). The localized infection with phyto-pathogens can lead to SAR, but the biopriming of beneficial non-pathogenic rhizobacteria to plants for induced systemic resistance (ISR) against pathogens is also practiced (Cheng et al. 2017; Egamberdieva et al. 2017). Further, biopriming offers a superior facilitation to plants that are capable of responding more quickly and strongly to pathogen attack. For instance, organic particles primed with *Gliocladium virens* (KA 2301) and *Trichoderma harzianum* (KA 159.2), were found efficient in inhibiting *Phytophthora cinnamomi* in avocado roots when used as surface mulch (Costa et al. 2000; Singh et al. 2019b). Similarly, disease severity and stem lesion length of *Phytophthora capsici* root and crown rot of bell pepper was noticeably reduced and further, the total microbial population and biocontrol activity was increased when delivering soil was mixed with compost holding chitosan, crab shell waste along with citrus pulp and cane molasses (del Carmen Orozco-Mosqueda et al. 2018; Kim et al. 1997).

1.4.1 Plant Growth Promoting Activities by Microbiome

The microbiome is capable of various functional traits like nitrogen fixation; solubilization; and mineralization of zinc (Zn), iron (Fe), potassium (K), phosphorus (P); and production

of siderophores and hydrocyanic acid (HCN); and hormone production tagged microbiomes with the entitled plant growth promoting microorganisms (Khan et al. 2018; Meena et al. 2017; Singh et al. 2016). Reports pertaining to microbial metabolic and multi-functional traits have covered the protocols for developing microbiome inoculants for plant growth promotion (PGP), biological control of phyto-pests and pathogens, biodegradation of contaminated soil and degradation of agricultural wastes (Kumar et al. 2018; Singh et al. 2017). Hence, the soils with a high abundance of organisms like N-fixers, P-solubilizers, phytohormone producers and bioremediators are considered high quality soils, and support crop health. The ecofriendly microbiome strain application in agriculture minimized the use of chemical fertilizers. The application of microbiomes, either individually or in consortium, are rich in their specific functional traits for making soils fertile, productive, and sustainable (Bhardwaj et al. 2014; Hunter 2016). There are several successful instances of microbiome application in various crops. Application of plant growth promoting biofertilizer in paddy fields in Vietnam resulted in a significant reduction in usage of synthetic fertilizers by 52 percent (Nguyen et al. 2017). This reduction in fertilizers is not gained within one or two years, but the application of biofertilizers were continuously applied to the paddy fields for over 15 years, and co-inoculation of fertilizers and biofertilizers improved sweet potato yields in Uganda.

Inoculation of arbuscular mycorrhiza in sweet potato cultivar NASPOT 11 with NPK fertilizers enhanced production between 12.8 and 20.1 ton ha^{-1} compared to previous production of 4.5 ton ha^{-1} (Mukhongo et al. 2017). From numerous reports, it has been concluded that inoculation of host-specific microbiomes especially endophytic and rhizospheric microflora, promotes growth and results in a higher yield (Thokchom et al. 2017).

Foeniculum vulgare shows a significant improvement in growth and essential oil content, when treated with bio-fertilizers (El-Azim et al. 2017). The results have revealed and supported that microbial input not only increases plant growth, but is also capable of bringing qualitative and quantitative changes. Application of microbial inoculations having plant growth promoting traits in agriculture have a significant value and do not add greenhouse gas in the environment (Sabir et al. 2012; Sharma and Sharma 2017; Singh et al. 2019b). An experiment was conducted with the objective of assessing the impact of bio-fertilizers on growth of paddy fields and greenhouse gas emission in alluvial soils of Indonesia. The results showed that emission of greenhouse gases such as nitrous oxide (N_2O), methane (CH_4), and carbon dioxide (CO_2) was significantly condensed with inoculation of biofertilizer in the paddy plot and further, it was concluded that biofertilizers improved the paddy production. Simultaneously, chemical fertilizer input was also reduced with 75 percent of the recommended dose, along with a decrease of greenhouse gas emissions (Hadi and Nur 2017).

The application of *Trichoderma*-based bio-fertilizer increased plant growth and productivity up to 12.9 percent. Further, mineral content and antioxidant level of ascorbic acid, β-carotene and lycopene in plants and fruits was increased. Asl (2017) has reported that using nitrogen fixing and P-solubilizing biofertilizers in sesame improved growth promotion, harvest index and nutrient use efficiency. The discussed instances corroborate that the application of specific microbial formulation functions have a high degree of ability to elevate the growth promotion and nutritional health of crop plants.

1.4.2 Biopesticide Potential of the Microbiome

Among the available options, biological control of plant pests and phyto-pathogens appears to be the best options for low cost, eco-friendly, and sustainable management approaches for protecting crops. Nowadays, microbial biocontrol is accepted as an important tool for the control of diseases in plants, and this method is sustainable in agriculture practices (Al-Ani and Albaayit 2018; Azcón-Aguilar and Barea 1997; Mona et al. 2017). A large number of biological controls are reported as options, and a deep comprehension of the complex interaction between plants, the environment, and pathogens is essential for future exploration (Mirzaee et al. 2015).

In terms of plant pathology, biocontrol has been accepted as the co-relationship of various environmental factors used to weaken the non-favorable effects of factors detrimental to the growth of organisms, and increase the growth of useful organisms like crops, beneficial insects, and microorganisms (Al-Ani and Albaayit 2018; Pal and Gardener 2006). The use of natural products and synthetic compounds extracted from plant or modified organisms or gene products are included under biological control (Levy et al. 2018; Pal and Gardener 2006). The prime goal of performing research on biocontrol is to minimize the dependence of agrochemical application and reduce the risks to human health and the environment (Berg 2009). Biocontrol through microbes was developed in the last 30–40 years, when plant pathology research attracted more interest in the use of beneficial microorganisms for the management of plant diseases (Berg 2009; Nihorimbere et al. 2011). The various modes of interactions between the populations are categorized as mutualism, neutralism, protocooperation, predation, commensalism, competition, parasitism, and amensalism (Compant et al. 2005; Kiely et al. 2006). All these biological controlling mechanism/interactions between plants and microbes takes place naturally at the microscopic level (Berg 2009). During the course of the plant life cycle, plants are susceptible to the various environmental challenges of biotic (fungi, oomycetes, nematodes, bacteria and viruses etc.) and abiotic stresses (drought, cold, salinity, flooding etc.). Counter to the pathogenic attack, plants have developed a wide range of mechanisms to counter attack and ward off attackers (Ponce de León and Montesano 2013). Broadly, plant protection mechanisms can be divided into passive defenses; non-host resistance (NHR); physical and chemical barriers (PCB); rapid active defenses (RAD); and delayed active defenses (DAD).

RAD includes alteration in membrane function, initial oxidative damage, cell wall reinforcement, hypersensitive response (HR) and finally in programmed cell death (PCD) (Pieterse et al. 2014), while DAD involves pathogen containment and wound repair, pathogenesis-related (PR) gene expression and SAR (Reichling 2018). Plant protecting signaling molecules includes salicylic acid (SA), and it is accepted as a necessary molecule for defense against biotrophic pathogens and SAR, while jasmonic acid (JA) and ethylene (ET) are involved in defense against necrotrophic pathogens or in beneficial plant microbe interactions. Stimulation and activation of JA and ET are triggers for action through biopriming and ISR (Agrios 2005). The SAR and ISR are higher states of alertness in the plant that have the potential for a quick and stronger response against pathogen attack.

1.4.3 Correlation of Microbiomes and Genetic Engineering as GMO

The World Health Organization (WHO) defined genetic modification as biological practice that manipulates the genetic material of all types of living organisms through the use