Unravelling the Interaction of Plant and Their Phyllosphere Microbiome

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Abstract

The phyllosphere is a type of an ecosystem having economical and ecological values and comprises of several microbial population that are present on the aerial parts of the plant. It is a vibrant environment where inhabitant microorganisms have the ability to change in humidity, temperature and heat during the whole day and night. The interaction between the microorganisms in the phyllosphere influences the growth of plants in natural habitat, the productivity of agricultural crops and the protective of horticultural produce for human consumption. Phyllosphere microbial community will help us to understand a deep knowledge of the phyllosphere microorganisms. The focus of this chapter will be (1) diversity study of phyllosphere microbial community; (2) mechanisms of phyllosphere microbe colonization; (3) understanding of the leaf structure, environmental and ecological parameters for growth and survival colonists; (4) understanding of the influences of biotic and abiotic factors on phyllospheric microbiome; (5) adaptations of microorganisms for establishment in the habitat of phyllosphere; and (6) significance of plant genotypic control of phyllosphere communities and its role in plant protection and plant growth. Futhermore, the insights study of phyllosphere microbiota; structure, function and valuable challenges for future research.

Keywords

Phyllosphere • Microbiome • Plant genotype • Biotic and abiotic factors • Host-microbe associations

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Abbreviations

ARDRA	Amplified ribosomal DNA restriction analysis
EPS	Extracellular polymeric substances
IAA	Indole-3-acetic acid
PCR	Polymerase chain reaction
PMC	Phyllosphere microbial communities
rDNA	Ribosomal DNA
ROS	Reactive oxygen species
RT-PCR	Reverse transcription polymerase chain reaction
VOC	Volatile organic compound

10.1 Introduction

Phyllosphere microbiota comprises of interaction and the relationship between microorganisms, plants and the environment. Plant pathologists and microbiologists have observed the habitat of microorganisms in the aerial part of the plant and called it the phyllosphere, in the 1950s, as few microorganisms improved plant performance while some microorganisms act as pathogens, threatening plant health (Corinn Vacher et al. 2016). The phyllosphere consists of the aerial portions of the plant, the set of photosynthetic leaves that are most common habitats for microorganisms on earth. The phyllosphere consists of different plant parts like the leaves (phylloplane), stem (caulosphere), flowers (anthosphere) and fruits (carposphere), and the presence and composition of the microorganisms in all the parts are significantly different (Junker et al. 2011). The leaf surface of the plant is colonized by microbes like fungi and bacteria from seed, soil, air and water through animal sources (Vorholt 2012).The phyllosphere harbours hundreds of microorganisms which have either beneficial or deleterious effects on the plants (Penuelas et al. 2014).The universal surface area of phyllosphere has been calculated approximately 10^9 km^2, and the presence of bacterial population is approximately 10^{26} cells. The total fungal population estimation of the phyllosphere is estimated to be lower (Lindow et al. 2003). Among all the cellular organisms, bacteria are estimated as the most abundant participant of phyllospheric community with cell density of 10^8 cells/cm^2 of leaf surface (Leveau et al. 2006). The distribution and multiplication of microorganisms are irregular and uneven on phyllosphere due to abiotic and biotic factors and physiological and anatomical behaviour of plant leaf (Remus-Emsermann et al. 2012). Phyllospheric microbiota have an ability to influence and alter both the structure and biochemistry of the plant like leaf functional characters, affect plant growth and affect ecosystem like water and nutrient cycling. Besides these, phyllospheric microorganisms also actively participate in secreting bioactive compounds called secondary metabolites like

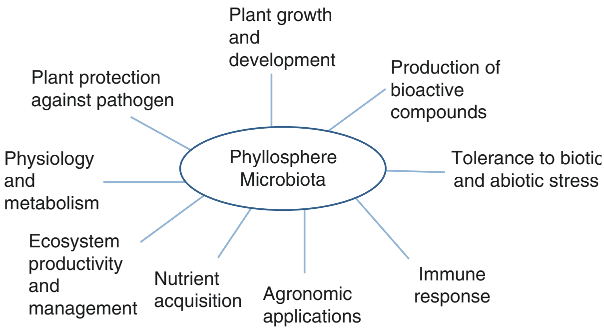

Fig. 10.1 Different roles of phyllosphere microbiodata

polyphenols, terpenoids, alkaloids and organic volatile compounds from the plant due to its locations, inside the leaf tissue or between plant surface and the atmosphere. (Bringel and Couee 2015). The association between the microorganisms and the phyllosphere is temporary due to harsh environmental conditions, and those who resist on phyllosphere are called as "true" occupants which persist, multiply and grow on phyllosphere. Quantitatively the phyllosphere microbiota vary at different developmental and genotypic stages of the plant (Penuelas et al. 2012). Unlike rhizosphere, phyllosphere microorganisms can influence neighbouring environmental ecosystem, and their continued existence is regulated by the plant itself (Barcel et al. 2012). Understanding the mechanism involves microorganisms, plants and the environment for phyllosphere microbiota (Fig. 10.1). This chapter will discuss interaction between the phyllosphere and microorganisms with their structures, functions and mechanisms and applications with important challenges for future research.

10.2 Diversity and Structure of Phyllosphere Microbiota

The microbial phyllosphere is characteristically regarded by 16S and 18S ribosomal RNA (rRNA) gene for bacteria and fungi. The high level of variation in sequences either within the region or in combinations of regions provides taxonomic resolutions that are often comparable with that achieved using whole rRNA gene sequences (Maughan et al. 2012). Earlier the culture-dependent techniques were used (Kuklinsky-Sobral et al. 2004), but currently the advanced molecular tools are being used to improve the technology like polymerase chain reaction (PCR), reverse transcription polymerase chain reaction (RT-PCR), primer designing for amplification of hypervariable regions and amplicon pyrosequencing for amplification of only microbial DNA and analyse the composition of microbial phyllospheric populations (Bulgarelli et al. 2012; Kim et al. 2012).This advancement has opened the door to explore further research for leaf-associate microorganisms like epiphytes and endophytes, by culture-independent methods. Epiphytic microorganisms that display enzyme indicators which were reported in the phyllosphere of tobacco signify that signalling routes may be participating in framing epiphytic microbial communities. The epiphytes also produce exopolysaccharides for adhesion or protection from desiccation (Monier and Lindow 2004) and secrete phytohormones, which enable nutrient secretion from plant tissue and as a result help in relaxation of plant cell wall (Vorholt 2012), while the endophytic microorganism resides within the specific chemical environment of host plant tissue like the leaf, stem, root, etc. and adapt to plant physiology in order to produce plant secondary metabolites (Becon and White 2000). The culture-independent molecular techniques proved the phyllosphere is a composite community of microbes. Bacterial communities at the phylum level, across a large array of farming crops (Fig.10.2) like rice, wheat, lettuce, apple, spinach and naturally occurring trees/plants, are composed of Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria (Bulgarelli et al. 2013). The study of population composition at the genus level recommends that Methylobacterium, Pantoea, Sphingomonas, Pseudomonas, Bacillus Massilia and Arthrobacter steadily occurred as a part of the phyllosphere microbiota through varied types of plant species. There are examples of phyllosphere studies with molecular methods where spatial and temporal discrepancy in microbial population and the other aspects that come this difference show in Table 10.1.

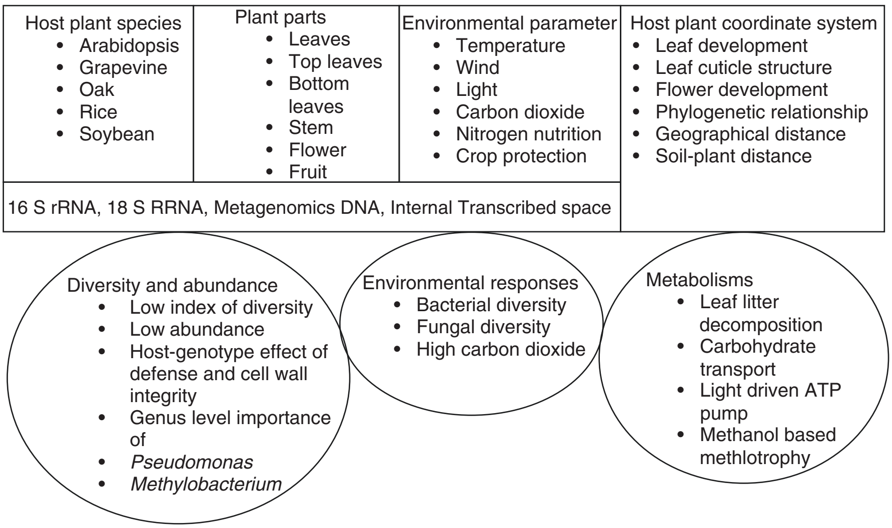

Fig. 10.2 Phyllosphere microbiota biodiversity, metabolism and environmental pliability (Modified and adapted from Bringel and Couee 2015)

Table 10.1 Molecular approaches to study the phyllosphere communities (Rastogi et al. 2013)

Molecular approach	Plant	Major findings	Reference
16S rRNA gene pyrosequencing	Grape	On the surface of leaves and berries from the same grapevine with significantly different bacterial communities	Leveau and Tech (2011)
16S rRNA gene pyrosequencing	Spinach	*Proteobacteria* and *Firmicutes* were the most commonly associated bacteria on field-grown spinach leaves. At genus level, communities were largely composed of *Pseudomonas*	Lopez-Velasco et al. (2011)
16S rRNA gene pyrosequencing	Lettuce	Planting season and irrigation practices (sprinkler/drip) together explained majority of the variation in phyllosphere microbiota composition. *E. coli* O157:H7 inoculation resulted in lower population sizes and induced minor, but lasting changes in microbiota composition	William et al. (2013)
16S rRNA gene pyrosequencing	Pine and other tree	Tree species, not the location, is the major determinant of phyllosphere bacteria community composition	Redford et al. (2010)
16S rRNA gene pyrosequencing, metaproteogenomics	Soyabean, clover, Arabidopsis	Unique metabolic adaptation contributes to the epiphytic fitness of *Sphingomonas* and *Methylobacterium*	Delmotte et al. (2009)
Metaproteogenomics	Rice	Phyllosphere communities were largely composed of *Rhizobium, Methylobacterium* and *Microbacterium*. Several methylotrophic enzymes were assigned to *Methylobacterium,* suggesting their role in the carbon cycle	Knief et al. (2012)
ITS pyrosequencing	Oak	Urban and rural management practices affect fungal communities in the oak phyllosphere	

(continued)

Table 10.1 (continued)

Molecular approach	Plant	Major findings	Reference
ITS pyrosequencing	Beech	Fungal communities showed variation even at the smallest spatial scale of individual leaf surfaces. Plant genotype was identified as a major driver of the fungal community composition	Cordier et al. (2012)
ITS pyrosequencing	Balsam poplar	Plant species was found as the major determinant of fungal community composition	

10.3 Process of Colonization, Recognization and Adhesion in the Phyllosphere

The cell and the spores of microorganisms occupying the surface of the leaf first interact with a cuticle, an exogenous and a hydrophobic waxy layer which defends the plant against dryness and other stresses. The formation of long-chain fatty acids with sterols and terpenoids shows 15% of leaf dry weight (Eglinton and Hamilton 1967). The architecture and composition may vary among plant species and environmental conditions. The shiny or crystal appearance of the leaf, resulting in a dull, powdery bloom form, is due to epicuticular waxes (Yeats and Rose 2013). Some microbial communities on the phyllosphere are either affected by the wax phenotype positively or negatively or not affected like the permanent residents of microorganisms like Pseudomonadaceae, Methylobacteriaceae, Flexibacteriaceae, Flavobacteriaceae, Sphingomonadaceae, Rhizobiaceae, Enterobacteriaceae, etc. The phyllosphere microbiota affected the genetic determinants of cuticle formation (Bodenhausen et al. 2014). It revealed that cuticular wax properties played a specific role for adapted microbial communities.

The endophytic and epiphytic microorganisms of the phyllosphere cross the cuticle as well as participate in the abundance of ozone in the environment (Nakamiya et al. 2009, Nadalig et al. 2014). The *Arthrobacter* genus from the phylum Actinobacteria is able to degrade various organic molecules and remain in the phyllosphere. The other species of *Arthrobacter* degrade pesticides like glyphosate, phenylurea herbicides, malathion and aromatic hydrocarbon like phenol, s-triazines, phenanthrene and chlorophenols (Scheublin and Laveau 2013).Phyllospheric microbiome also degrades organic and aromatic molecules, participates in controlling the atmospheric pollution in industrial and urban areas and also shows significant role in sustainable agricultural environments by atmospheric drift of phytosanitary product. Hence, the epiphytic and endophytic microorganisms can also be envisaged for the beneficial effect on plants and could be used as probiotic agents (Bercel 2012). Adhesion plays role to conflict from separation raindrops or by rainfall and is a condition for the EPS formation comprising of mucus that gives defence from dryness.

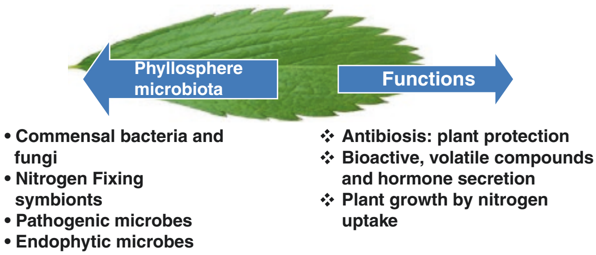

Fig. 10.3 Phyllosphere microbiodata association

Adhesion is effective for leaf expansion and epiphytic colonization. Epiphytic microorganisms can have the capability for aggregate formation and synthesized exopolysaccharide which can improve adhesion and protection from desiccation (Monier and Lindow 2004) and also synthesize and secrete plant hormones, like indole-3-acetic acid (IAA), which enable nutrient secretion from plant tissues and help in relaxation of the plant cell wall. Although the understanding of these adaptive mechanisms remains incomplete (Fig. 10.3).

10.4 Plant Microbe Interaction

10.4.1 Interaction Between Bacteria on Phyllosphere

Most of the bacteria on the leaf surface form large aggregates instead of small groups or single cells. These aggregates are formed at junction of epidermal cells with veins and base of trichomes which are bordered with extracellular polymeric substances. The extracellular polymeric substances maintain moisture around the bacteria and concentrate detoxifying enzymes (Lindow et al. 2003; Baldotto et al. 2008; Monier et al. 2004). The environment of host plant is altered by the bacteria which has been associated with plant pathogenic bacteria, *P. syringae*, which causes diseases in plants related with their epiphytic population on leaves (Stromberg et al. 1999). The epiphytic bacteria present on leaves of plant are swept up into the atmosphere. They have the capability to form precipitation in the clouds by catalysing ice formation (Morris et al. 2014). Methylotrophic bacteria use methanol or methane as a carbon source and are quite rich in the phyllosphere and useful for plant growth like the genus *Methylobacterium* (Abanda-Nkpwatt et al. 2006).The phyllosphere diazotrophic bacteria use atmospheric nitrogen as a source of nitrogen to assess their community structure (Furnkranz et al. 2008). With drought conditions, the nitrogen-fixing bacteria have been raised, suggesting that their involvement may extend the ability of the plant to acclimatize in the environment. The capability of *P. syringae*, a plant pathogenic bacteria, causes disease strongly linked with their epiphytic population size on phyllosphere and may be the subsection of the community that is without symptoms on plant leaves and hence in more close interaction with plant cells (Beattie et al. 1999).

10.4.2 Interaction with Fungi

Filamentous fungi and yeast are massively colonized on the phyllosphere. The density of yeast is 500 cells/cm^2, but it varies from plant to plant species and within the plant (Inacio et al. 2010). Some fungi present in the phyllosphere and act as a biological control due to its ability to outcompete pathogenic microorganisms like *Aureobasidium pullulans* (Cordier et al. 2012). Some fungal pathogens like *Erysiphe*, *Blumeria* and *Podosphaera* elongated their mycelium on the leaf surface, all pervading the cuticle then rupturing the cell wall with particular structure called as appressoria. Once infected, the mycelia grow superficially, covering grey or white coat on the leaves (Glawe 2008). Within the leaf tissue, the endophytic fungal species develop without causing noticeable symptoms, transferred from one adult plant to the next plant, and develop an epiphytic stage before entering into the leaf tissues. (Rodriguez et al. 2009). Some of the endophytic fungi are latent pathogens, for example, palm tree *Iriartea deltoidea*, which produces reactive oxygen species (ROS) by *Diplodia mutila*, an endophytic fungus, and alters it to a pathogen, which confines plantlet persistence to shaded areas (Alverez-Loayza et al. 2011). Some fungi defend the plant from stresses, including herbivores, pathogens and drought. Some fungi are involved in the decomposition of cellulose in senescent leaves and young litter which are then replaced with new colonizers with a higher capacity to decompose lignin that belongs to the higher fungi *Basidiomycota*. The fungi present in riparian ecosystem are termed as aquatic hyphomycetes and play a key role in the functioning of aquatic food webs, as its action enhances the palatability of leaves to invertebrates (Barlocher 2016). In more than 50 plant species, the aquatic hyphomycetes have been found in the phyllosphere (Chauvet et al. 2016). Fungal communities of phyllosphere play a significant role in nutrient cycling and in the functional coupling of aquatic and terrestrial ecosystems. Henceforth, to recognize the procedures of shaping these communities and evaluating their effect to global change is significant.

10.5 Study of the Microbial Community on Leaf

The leaf surface area is a hostile atmosphere for the microbes. The surface of the leaf is affected by relative humidity, sunlight, fluctuating temperature and occurrence and nonoccurrence moisture due to dew and raindrops. On the leaf surface, sometimes microscopic water films occur due to condensation of water vapour which enters into the stomata (Burkhardt and Hunsche 2013). Leaf surfaces are multifaceted microenvironments which show two-dimensional and three-dimensional diverse structures. The dorsal and ventral sides of the leaf surface (Eglinton and Hamilton 1967; Schreiber et al. 2004; Reisberg et al. 2013) are affected by the microbes which live on plant surfaces and the access to nutrients from leaf tissues called as epiphytic microorganism (Bulgarelli et al. 2013), by imparting less or more defence from sunlight (Atamna-Ismaeel et al. 2012) or by showing permission for diffusing in the endosphere of plant (Hirano and Upper

2000; Schreiber et al. 2004). Inside and outside, the live conditions of the leaves are produced by the fluxes of CO_2, O_2 and water vapour resulting from photosynthesis, respiration and evapotranspiration. Many parameters affect the habitat of microbes accomplished by microorganisms on leaves; first, a very thin laminar layer around the leaf, where stomata emitted moisture, can be concealed, thus diminishing the stress of water where epiphytes are revealed. And also, some of the bacteria invade inside the leaf instead of the exposed outside leaf, and to escape the stresses outside of the leaf, they inhabit in sub-stomatal cells or other inner locations (Lindow and Brandl 2003). Many species from the angiosperm group showed the dissimilar structure of leaf both on ventral and the dorsal sides. The ventral side of the leaf consists of a thick cuticle, epidermal cells inside which are tightly occupied sheet of palisade mesophyll cells helpful in optimizing radiance. The transpiration rate and exchange of gases is controlled by the dorsal side part of the leaf which comprise of an epidermal layer with many stomata, beneath which are spongy mesophyll cells loosely arranged (Vacher et al. 2016). The stomata is the site of entry for microorganisms to the inner leaf tissue. Once it enters, it will assemble the interspace between the cells of the spongy mesophyll. As a result, they get nearer to the smaller veins of the leaf where sieve element of the phloem uploads the sugars (Rennie and Turgeon 2009). Plant photoassimilates glucose, fructose and sucrose which are present on leaf surfaces, but fluctuation of day or night alters the changes in the plant metabolite synthesis, which also affects the nutrient availability and growth of epiphytic microorganisms. Among all the metabolites of plant, carbohydrate is the most functional under stress conditions like abiotic and biotic (Trouvelot et al. 2014). Some of the plant metabolites are not directly and freely accessible for epiphytic microorganisms like amino acids, soluble sugars, amines, polyols, VOCs such as isoprenoids, halogenated compounds or alcohols, also salts and plant water. The waxy and lipidic cuticles protect the plant leaf surface which bound metabolite fluxes and water; hence the biochemical exchanges are dependent on different gateways like exudation, leaching, excretion, guttation, infiltration and wounding. All these properties belong to the oligotrophic habitat with limitations in nitrogen and carbon sources. The presence of nutrient containing carbon on the leaves is a main factor of epiphytic colonization. On a well-fertilized plant, the bacterial communities are restricted by nitrogen and carbon accessibility.

10.6 Influence of Biotic and Abiotic Factors on Phyllospheric Microbiome

The microbiome of leaf is affected by stresses like abiotic and biotic factors in determining microbiota composition and dynamics. Phyllospheric microbiome is affected by biological and environmental factors like host genotype, plant development, climate, geographical location and seasonal changes. The phyllosphere is an unlock structure where microbes can penetrate plant leaves by immigration from the other plants, soil, atmosphere, animals and insects. The composition of the phyllosphere microbiome is mainly dependent on the host genotype (de Oliveira Costa et al. 2012; Kim et al. 2012), while the composition of the community is influenced by the geographic location (Rastogi et al. 2012). Some perennial plant communities alter noticeably from 1 year to the next year and have more seasonal changeability, while some perennial plants have season-dependent communities that are more alike from year to year (Jackson and Denney 2010). From the soil-grown plants, phyllosphere communities show similar characteristics to soil communities (Perazzolli et al. 2014) whereas media-grown, sterile plantlets have shown same properties with airborne communities. Birth-, migration- and death-like demographic aspects of the plant can show an important function for the growth of microbes (Vorholt 2012). Overall the plant phyllosphere is very complex and affected by biological and environmental factors on the composition of the population which remains indistinct when all these factors are measured simultaneously. In natural environment, the phyllosphere microbes of seasonal farming plants are mainly affected by temporal population growth, whereas host genotype and spatial division may imitate the exact preference for study of species and the nearby geographic distances between the replicate areas. At the beginning of the summer season, communities' samples strongly resembled the soil population, and, as the season proceeds, the phyllosphere microbiome developed progressively discrete and little wide. Therefore, due to this deviation in population structure, species that is significantly used as biocontrol agents in the beginning of summer season may not be efficient in the end of the summer. To realize that the natural progression in the population is controlled, the development of the microbiome which is useful in biocontrolling farming plantlets will need awareness about population size and shape changes in the continuation of the progressive season (Copeland et al. 2015).

10.7 Establishment of Phyllosphere Habitat by Microbial Adaptations

Adaptation is the ability of the phyllosphere microbiota to withstand stress conditions such as deficient supply of nutrients, production of bioactive compounds like antifungal and antibacterial secondary metabolites and seasonal environmental conditions (Vorholt 2012). Phyllosphere microbiota can be expected to have common strategies to survive these stresses, in addition to species-specific strategies. During photosynthesis, the phyllosphere is exposed to oxygen and the light during daytime. The microbial colonizers prone to reactive oxygen species damage the protein, lipid and nucleic acid. Photolyases, DNA repair caused by ultraviolet light and the production of pigments are being used as protection mechanisms by microbial epiphytic fitness. Catalases and superoxide dismutase enzymes play an active role in reactive oxygen species (ROS) detoxifications (Vorholt 2012). The common environmental factor in phyllosphere is dryness. The secretion of bioactive compounds from microbial masses is helpful to overcome the dryness. Exopolysaccharides are produced by the aggregates, which maintained moisture surrounding the bacteria, and participate in epiphytic fitness. Some phyllosphere bacteria secreted the biosurfactants that increase wettability. To overcome the osmotic stress, the epiphytes like

Pseudomonas spp. and others can defend themselves from the osmotic stress by secreting trehalose or choline or importing plant-derived osmoprotectants or de novo synthesis. Plants have the unique property to produce bioactive compounds which sometimes are antimicrobial in nature and produce antibiotics; hence plant adapted themselves from these pathogenic microorganisms, for example, *P. syringae*, a plant pathogen which is important for evasion of the inhabitant immune reaction and plant reproduction (Stoitsova et al. 2008).

10.8 Impact of Phyllospheric Microorganisms on Plant Growth and Plant Protection

Leaf-associated microorganisms are well known for symbiosis and mutualism relationship with host plant that can influence host plant growth and function, like the production of hormones and growth-promoting nutrients, and also prevent the hosts from infection from disease-producing agents (Innerebner et al. 2011). Under different environmental conditions, phyllosphere microbes have the capability to effect on plant ecosystem and biogeography contribution which effect on plant activities (Fürnkranz et al. 2008), but the discrepancy of bacterial biodiversity in the phyllosphere of the host plants is not well understood. Phyllosphere microbes secrete plant growth regulators, volatile and non-volatile compounds, which may influence the plant growth, morphogenesis and plant immunity. Also they act as phytostimulators, biofertilizers and biopesticides to protect against invading pathogens and for plant growth, development and health. Sometimes plant defence chemicals are degraded by plant foliage-associated bacteria which minimize defence against insect defoliators (Mason et al. 2014). On plant surfaces, the genera *Enterobacter*, *Pseudomonas*, and *Stenotrophomonas* of bacterial symbionts, secreted by the Colorado potato beetle larvae, suppress the anti-herbivore defences and enhance the microbial defence in tomato plant (Chung et al. 2013). The interaction between herbivore masticate insect and its single host plant, *Cardamine cordifolia*, experimental showed that some bacterial strains showed difference on the way of ecologically impacted insect herbivores while some bacterial strain, for example, *Pseudomonas* species, helped host choice by herbivores (Humphrey et al. 2014). Phyllospheric microbiome lives in a sunlight-exposed habitat. The energy formed by photochemical conversion of the sunlight that can organic sources from the host plant could be a beneficial for development in an inadequate amount of nutrient. Study of metagenomic information has showed that in phyllospheric communities, the existence of bacterial rhodopsin genes is there (Bringel and Couee 2015). Retinal-dependent rhodopsin proton pumps which are found in some epiphytic microorganisms stimulated by the wavelength different from the carotenoids and chlorophyll absorption spectra, which participate in photosynthetic processes and production of the plant sugar sources, are finally accessible to epiphytic microorganisms (Atamna-Ismaeel et al. 2012). The light-dependent processes are affected by nitrogen and carbon sources in nutrient regulation and signalling (Moran and Miller 2007). The biosynthetic pathway for the production of plant bioactive molecules in epiphytic bacteria can be affected by the nitrogen and carbon sources and the changes in plant-light interactions and photoassimilate production in the host plant (Sulmon et al. 2011). Manching et al. (2014) have recently discussed universal association between leaf epiphytic bacterial species and plant nitrogen balance in maize crop.

10.9 Phyllospheric Plant–Microorganism–Atmosphere Interactions: Physiological, Ecological and Molecular Studies

The role of physiological, ecological and molecular studies of microbial communities on both sides of the leaves, inside and outside, is likely to affect plant growth and its metabolisms (Lindow et al. 2003; Rastogi et al. 2013), which influence the ecosystem and environmental efficiency eventually. In the phyllosphere, the nitrogen fixation is the key process where nitrogen is added in tropical humid ecosystems (Abril et al. 2005). The tropical rainforest plants, where the formation of phyllospheric populations of nitrogen-fixing microorganisms has been observed but temperate-forest ecosystems have also been observed for the presence of nitrogen-fixing microorganisms, their abundance and diversity vary depending on the availability of water (Pennuelas et al. 2012). Development of plant can also be affected by the foliar microorganisms as they are involved in the production of growth hormones. The external and internal microbiotas can have many other roles, like the indirect defence against pathogens, by the interaction of foliar plant pathogen and commensal bacteria (Vorholt 2012), or secretion and production of different types and quantities of bioactive compounds and emissions of organic volatile compounds (Bulgarelli et al. 2013). Speciation, dispersal, drift and selection are the four processes which shaped the ecological communities. Diversity of the microorganisms that occurs on the leaf surface of plant comes under dispersal and evolutionary diversification. External selection supports those microbes which are mainly adapted to the common situtations like leaf morphology, chemistry and microclimate. The selected microbes then grow and reproduce. The biotic interaction where internal selection is done, like parasitism and competition, controls the shape and structure of the microbial population. Stochastic modification in the population structure is done by drift. The shape and the overall structure of the microbial population are influenced by plant type and structure, the environmental conditions and the population itself. Molecular studies are the best tool, which help to get information about diversity, species richness and analysis of microbial community (Brusseau et al. 1994). Microbial diversity analysis is done by the widely used technique amplified ribosomal DNA restriction analysis (ARDRA), and it is significant to discriminate the species level of the microbes. This technique requires amplification of the 16S and 18S ribosomal DNA (rDNA) region proceeded by restriction enzyme digestion (Heyndrickx et al. 1996). Species richness, occurrence, community structure and diversity in the phyllosphere of the tropical plants are studied by full-length sequencing detail of 16S rDNA, differential carbon-substrate consumption pattern and ARDRA. For example, *Methylobacterium* are classified taxonomically on the basis of 16S rRNA gene sequence data (Tsuji et al. 1990), and the carbon-substrate utilization study of all the species of *Methylobacterium* are well deliberated (Jourand et al. 2004).

10.10 Current Progress and Future Challenge

Microbiological study of phyllosphere is a promising research field at the early stages. The phyllosphere is a best part of the plant to get the basic knowledge of plant structure, growth functioning and environmentalism, especially to perform experiment and visual inspection, and these studies are helpful to have an impact on different aspects of plant like morphological and anatomical condition and physiological process where atmospheric gases participated in phyllosphere functioning. New technologies such as proteogenomics and metaproteogenomics and next-generation sequencing used for community profiling are novel platforms to get knowledge of the shape of microbial population and to explore new objectives for theory of research, escorted by revealing new protein function that is significant for growth, development and survival in the phyllosphere. But still more knowledge is required to know about phyllosphere microorganisms and their interaction in situ and other complementary approaches. Future studies and research work require to explore these dynamic and complex interaction approaches and evaluate their role in the growth of plant and physiology of phyllosphere microorganisms. Ecosystem functioning, the diversity of phyllosphere microbial communities (PMCs) and plant performance have to be deliberated. Hence, the next research needs to understand and identify the correct community parameters of PMCs, ecosystem functions and plant performance. The link between the evolution of PMCs, the dynamics and adaptation mechanism in plants to changing environmental conditions requires further understanding. The effect of environmental change conditions on the plant fitness and diversification of PMCs requires to be explored.

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