



Prospecting the characteristics and significance of the phyllosphere microbiome

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Abstract

Phyllosphere represents the largest global interface of the aerial parts of the plant, comprising mainly stem and leaves, which is inhabited by various groups of microorganisms. Analyses of the spatial abundance of microflora, diversity, and distribution of microbial communities and the influence of abiotic and biotic factors have revealed that this niche is unique. This reflects the impact of both evolutionary and ecological factors, leading to sorting of microbial species, delineation of keystone species or microbial hubs, mediated by inter-kingdom connectivity and networking. Production of hormones, pigments, volatiles, extracellular polysaccharides (EPS), cross-kingdom signals, and quorum sensing are characteristic facets, which promote proliferation and survival in the harsh and inhospitable habitat of the phyllosphere, exposed to radiation and environmental extremes. The use of both traditional morphology-culturing-based taxonomy and modern tools of metagenomics, proteomics, and transcriptomics 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. Despite scanty published work on foliar disease-related aspects, the phyllosphere can provide a model microenvironment, in which the interactions between the pathogen and biocontrol agent can be visualized and modulated. The major aims of the present review are as follows: (i) to elucidate the mechanisms of microbial colonization and decipher the nature of spatial and temporal changes in the abundance and diversity in this niche; (ii) to illustrate the significance of the different taxonomic groups; and (iii) outline future strategies for research on the phyllosphere microbiome.

Keywords Biocontrol · Epiphytes · Endophytes · Leaves · PPFM's · Microbial diversity · Microbial taxa

Introduction

The phyllosphere (Ruinen 1956) is the aerial or the above ground part of the plant which comprises the floral and vegetative foliar parts, in which the dominant part is the leaves, representing a unique habitat for microbial diversity. The terminology of phyllosphere has undergone several transformations during the last two decades. Morris (2002) extended this concept to include both the areas inside and outside the leaf and stated that “the phyllosphere is the micro-environment extending from the leaf surface outward to the outer edge of the boundary layer surrounding the leaf and inward into the leaf tissues.” Doan and Leveau (2015), on

the other hand, compartmentalized the phyllosphere into two intimately connected, yet unique niche, i.e., “phylloplane” and “phylloelma” to include both the leaf surface landscape and the leaf surface waterscape. This niche is most often exposed to the vagaries of environmental fluctuations, including nutrient stress, ultraviolet radiations, and desiccation. Despite such influences of abiotic factors, the phyllosphere is colonized by a plethora of microorganisms, each with its own set of attributes and functionalities. Significant contributions to the global nitrogen and carbon cycles, bioremediation of xenobiotics, plant growth-promoting traits, biocontrol agents against pathogens, etc. are the result of the activities of the members present in this global niche.

The common residents of the phyllosphere include members belonging to the taxonomic groups of bacteria, fungi (including oomycetes), yeasts, algae, which may exist as epiphytes, endophytes, and free-living microorganisms; members of Archaea, protozoa, and nematodes represent the less encountered species (Andrews and Harris 2000; Hirano and

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Upper 2000; Lindow and Leveau 2002; Lindow and Brandl 2003; Leveau 2006; Bodenhausen et al. 2013; Counce et al. 2014; Kembel et al. 2014; Thapa 2017). Organisms with quick and frequent sporulation and yeasts are often observed to colonize this habitat more actively (Andrews and Harris 2000). Filamentous fungi are generally ephemeral dwellers on the leaf surfaces, being present predominantly as spores, and along with many plant pathogenic bacteria and oomycetes, they often represent an important component of the phyllosphere, influencing detrimentally both plant health and vigor (Glawe 2008; Mansfield et al. 2012; Cappelletti et al. 2016).

The phyllosphere has an area of roughly about one billion square kilometers (Morris and Kinkel 2002), in which the number of bacteria may reach up to 10^6 – 10^7 cells per square centimeter of leaf area which makes them the dominant colonizers (Lindow and Brandl 2003; Bailey 2006). This phenomenon can be attributed to the diverse strategies bacteria exhibit and their interactions with the plant, which may be commensalistic, pathogenic, or even mutualistic (Kishore et al. 2005).

Phyllospheric communities have immense importance in aspects related to nitrogen fixation (Jones 1970; Freiberg 1998; Furnkranz et al. 2008), bioremediation of harmful chemicals or pollutants, and or as biocontrol agents against important foliar plant pathogens (Beattie and Lindow 1995; Balint-Kurti et al. 2010; De Marco et al. 2004). As the microbial diversity in the phyllosphere is a reflection of environmental conditions, they can contribute significantly to the global food webs and nutrient linkages. Epiphytic loads in seagrasses were found to be weakly correlated with phosphorus levels, and the effects of nutrient enrichment were localized, but they can be indicators of the changes in the environmental conditions (Frankovich and Fourqurean 1997). Foliar nutrient content and soil nutrients, along with host genotype can modulate the microbial communities in the phyllosphere. In a recent study undertaken, leaf nutrients such as iron, manganese along with chlorophyll and soil organic carbon were observed to modulate the phyllosphere microbiome and community composition (Thapa 2017). Many members of the phyllosphere microflora are known to stimulate plant growth by the production of various plant hormones or through root exudates (Lighthart 1997; Lindow et al. 1998; Beattie and Lindow 1999). Thapa et al. (2017) investigated the influence of host genotype on the phyllosphere microbiome and their interactions with nutrient levels of rhizosphere and leaves in seven Indian genotypes of rice, namely ADT-38, ADT-43, CR-1009, PB-1, PS-5, P-44, and Pusa Basmati 1609. Distinct variation in the plant growth and microbiome-related parameters, including the distribution and abundance of microbial communities (eubacterial, cyanobacterial, and archaeobacterial), analyzed using quantitative PCR (qPCR) method, illustrated the genotypic influence. Delmotte et al.

(2009) undertook the metaproteogenomic analyses of phyllosphere microbial communities belonging to *Arabidopsis thaliana*, soybean, and clover, and identified a number of proteins related to the utilization of methanol as a source of carbon and energy, besides those involved in the transport of carbohydrates. The major drivers of the phyllospheric community diversity and abundance include abiotic factors such as season, water content, leaf age, ultraviolet light, relative humidity, soil and biotic factors such as pathogens, nematodes, or insects, and most importantly, the host genotype and other plants present in the surroundings (Balint-Kurti et al. 2010; Hunter et al. 2010; Humphrey et al. 2014; Agler et al. 2016). This review focuses on the influence of phyllosphere microbiome on the health and vigor of the plant, the strategies used by microorganisms to colonize this niche, the major microbial communities residing, and their interactions. An overview of both cultivation independent and dependent methods utilized in deciphering the structure and abundance of microbial communities in the phyllosphere, and future needs for research in this niche are also discussed.

Strategies for bacterial colonization

Bacterial colonization takes place on the surface of the leaf, and their abundance and diversity are dictated by the characteristics of the leaf habitat, and a number of other host and environmental factors. Their colonization on the leaves is also localized (Fig. 1a, b) and metabolic activities of the bacteria influence their interactions with the leaf (Fig. 1c). Epiphytes generally employ two distinct fitness strategies for their growth and survival on the aerial surfaces of the plant: firstly, tolerance strategy which permits the inhabitants to tolerate direct exposure to environmental stresses on the surface of the leaf mainly UV radiation and low moisture conditions and, secondly, avoidance strategy which allows the epiphytes to colonize sites that do not face these stresses (Beattie and Lindow 1995). Saprophytes typically employ tolerance strategies to survive in the foliar zone, as they cannot survive endophytically. Foliar pathogens, however, can utilize both the strategies to harbor the plants more efficiently.

Sites of colonization in the phyllosphere

Bacterial colonization usually occurs in distinct sites on the leaf surface such as bases of trichomes, stomata, hydathodes, grooves along the veins, epidermal cell junctions, and cuticle depressions (Beattie and Lindow 1999). These microsites present on the surface of leaves provide conducive conditions for the epiphytes to proliferate and flourish (Monier and Lindow 2004). Analyses revealed the presence of large aggregates of bacterial communities that were present at particular sites—mainly stomata, epidermal cell grooves along the veins

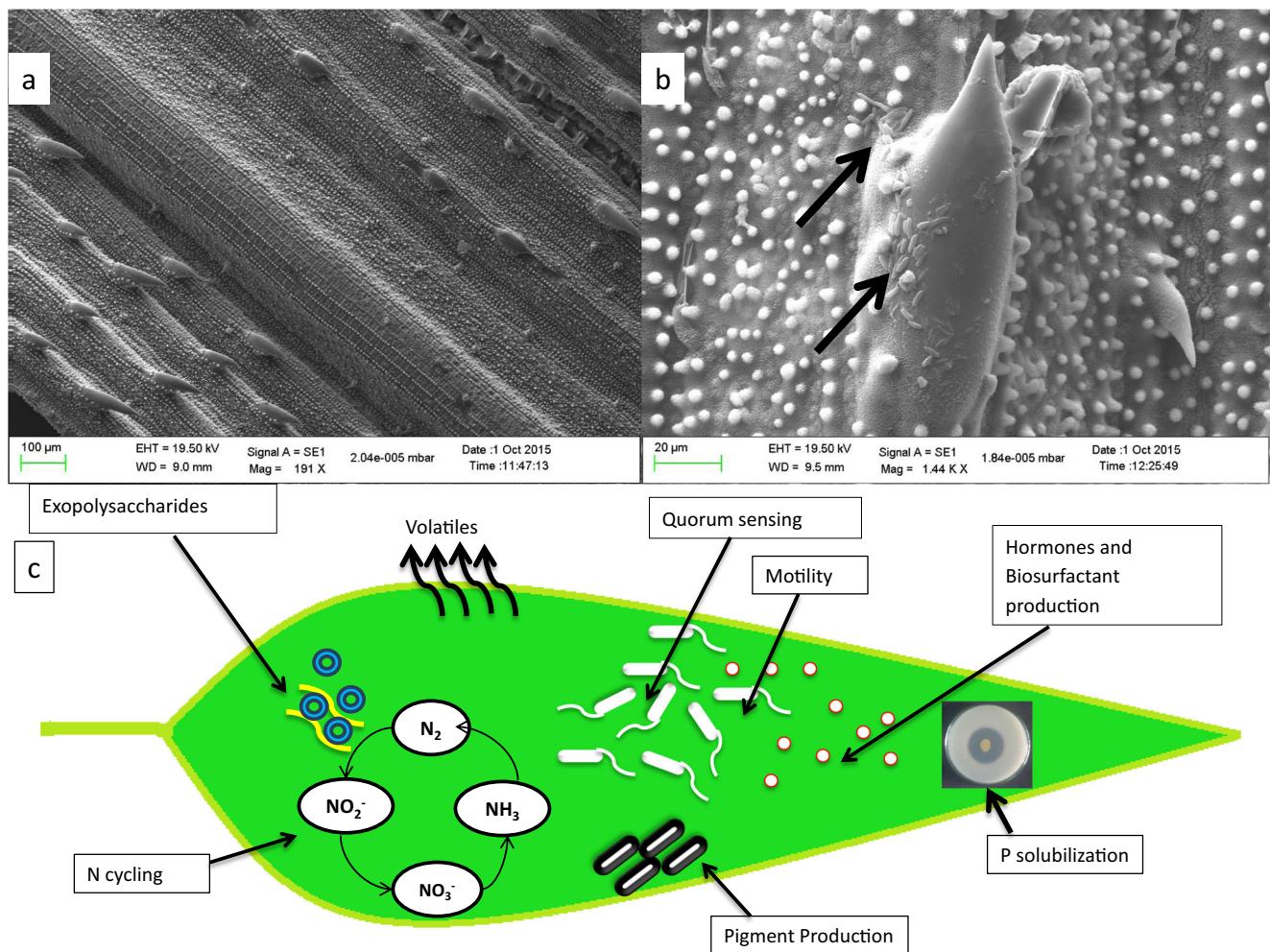


Fig. 1 **a, b** Scanning electron micrographs of rice (*Oryzae sativa*) leaf showing the surface topography and microbial aggregates at the base of the trichome. Arrows indicate the presence of bacteria. **c** Overview of strategies employed for colonization of the leaf, by microorganisms

and at the bases of the trichomes. On the basis of the literature surveyed, the three major anatomical determinants which help in the proliferation of phylloepiphytic communities are the presence of trichomes, active secretory cells and the absence of epicuticular waxes.

The first line of defense which a leaf presents is the cuticle, which is a complex hydrophobic structure composed of epicuticular wax (made up of long chain fatty acids or its derivatives, cutin and polysaccharides). It surrounds the leaf surface as a thin layer and its thickness may vary from plant to plant or species to species. The cuticular components are important drivers of bacterial community structure and significant to both plants and pathogens, in terms of initiating pre-invasion, or infection and immune responses (Aragon et al. 2017).

Trichomes are epidermal appendages that help in controlling the leaf temperature, protection of the leaf against UV light, besides secreting a variety of secondary metabolites which deters herbivores and inhibits pathogen development. The base of these glandular trichomes is the commonly inhabited sites involved in the secretion of chemical

compounds, such as sugars, proteins, oils, secondary metabolites, and mucilage (Olson and Nechols 1995; Ascensao and Pais 1998). This leads to suitable conditions for microbial colonization, as it also helps in the retention of water droplets (Brewer et al. 1991), and influences the epiphytic fitness of the various groups of microorganisms. Baldotto and Olivares (2008) studied the phyllosphere bacterial community of 47 different plant species in a tropical ecosystem, which was found to be dominated by epiphytic bacteria, exhibiting three major distribution patterns—solitary cells, biofilms, or microcolonies. Microscopic studies revealed epidermal cell wall junctions, glandular and non-glandular trichomes, veins, stomata, and epidermal cell wall surface as the preferred sites for colonization, irrespective of plant species. The presence of trichomes on the leaf surface influenced the microbial communities favorably, while epicuticular wax was a negative determinant for their growth. In general, a greater number of culturable bacteria are obtained from broad leaves, as compared grasses or waxy plants, using common microbiological methods (Lindow and Leveau 2002; Morris and Kinkel 2002;

Baldotto and Olivares 2008). Nongkhilaw and Joshi (2014) utilized scanning electron microscopy (SEM) to study the epiphytic bacterial community on the ethnomedicinal plants of Meghalaya (India) namely, *Rubia cordifolia*, *Centella asiatica*, *Potentilla fulgens*, *Acmela oleracea*, and *Houlthynia cordata*. The nutrient availability on the surface of leaf is a factor which determines the epiphytic microbial growth (Lindow and Leveau 2002). Leaching of solutes from the leaves releases a number of organic and inorganic nutrients (Tukey 1970), and due to increasing wettability with age, older leaves exude greater amounts.

Hormones and other chemicals

Several bacteria can increase the nutrient concentrations on the surface of the leaf by the production of plant hormones basically auxins (indole 3-acetic acid, commonly), which even at very low concentrations promotes the loosening of the cell wall and enhances greater levels of release (Fry 1989). Cytokinins are also thought to be involved in the colonization process by bacteria of the genus *Methylobacterium*, as they are involved in plant cell division and expansion, triggering the release of methanol from the plant cell wall, which is a source of C source for their growth (Kutschera 2007).

In certain cases, some compounds particularly biosurfactants, produced by the bacteria increase the wettability of the leaf surface (Georgiou et al. 1992; Knoll and Schreiber 2000), facilitating the solubilization and diffusion of substrates, and increasing its availability to the bacteria. Burch et al. (2016) observed a relatively greater abundance of surfactant-producing bacteria in the phyllosphere, as compared to other environments. Trehalose is a commonly used osmoprotectant responsible for the maintenance and survival of *Pseudomonas syringae* in the phyllosphere (Freeman et al. 2010). This has also been implicated in the behavior of *Pseudomonas aeruginosa* as an opportunistic pathogen, as it promotes the procurement of nitrogen and stimulates its proliferation in the leaf apoplast (Djonovic et al. 2013).

Phytotoxins are commonly secreted by phytopathogenic microbes, which either damage the plant cells directly or contribute towards enhanced bacterial virulence, through prevailing over the host barrier and aggravating the damage due to chlorosis and tissue rot. Syringomycins and syringopeptins represent such toxins which are produced by *Pseudomonas syringae* during plant infection. Being amphipathic in nature, they induce pores in the plasma membrane of plant cells tissue through necrosis (Melotto and Kunkel 2013). The targets of these toxins are directed towards disruption of enzymatic machinery of amino acid biosynthesis or nitrogen metabolism, which lead to accumulation of nitrogen containing intermediates, often utilized as food by the pathogens (Arrebola et al. 2011). Some phytotoxins modulate the metabolic and signaling pathways in the host, facilitating the invasion by pathogen.

Most of these phytotoxic molecules exhibit structural and functional similarity to auxins and other plant hormones. A toxin coronatine, mimicking the plant hormone jasmonic acid isoleucine, is produced by *Pseudomonas syringae*, leading to multiple modes of virulence. This promotes the opening of stomata for bacterial entry, and its proliferation in the apoplast, leading to systemic susceptibility and disease symptoms (Zheng et al. 2012). Other mechanisms involve manipulating plant defenses and metabolism by producing or suppressing different hormones (Robert-Seilaniantz et al. 2011). Certain phytopathogens also release a number of enzymes involved in the breakdown of plant cell wall structural materials, or hydrolysis of the connective tissues between plant cells, thereby providing a source of carbon for the pathogen.

Exopolysaccharides

Bacteria also grow as biofilms, modifying the leaf surface by the production of extracellular polysaccharide (EPS) which helps in the anchorage of cells to the surface and increases the water availability to bacteria. EPS prevents desiccation under limiting moisture conditions, mediated by the retention of water in its highly hygroscopic polysaccharide matrix (Chang et al. 2007). EPS molecules are also linked to epiphytic fitness and survival, including freeze thaw resistance (Wu et al. 2012), osmotic stress tolerance (Freeman et al. 2013), and sustenance of the microbial population (Dunger et al. 2007). Such structured communities, growing as biofilms, are known to operate using diffusible signals or quorum sensing and regulate EPS synthesis. These biofilms are generally not mono-specific and are made up of a complex mixture of bacteria and fungi that also help certain phytopathogens in evading the plant immune response, due to quenching of calcium signaling (Aslam et al. 2008). Xanthan gum, a characteristic EPS produced by the *Xanthomonas* species, is encoded by the gum gene cluster (*gumB* to *gumM*) and the mutant strains (unable to produce EPS) show different phenotypes ranging from altered biofilm formation, impaired survival under oxidative stress during stationary phase, and reduced epiphytic survival on citrus leaves (Dunger et al. 2007; Rigano et al. 2007; Vorhölter et al. 2008).

Pigmentation and UV protection

Solar radiations can be broadly categorized into three wavelength classes: UVA (320–400 nm), UVB (290–320 nm), and UVC (less than 290 nm); out of which the shorter wavelength radiations are potentially harmful. However, this mostly gets filtered by the ozone and oxygen in the atmosphere. UVA radiation contributes more to the total energy and is responsible for the generation of reactive oxygen species including singlet oxygen, superoxide radical, and hydrogen peroxide. UVB contributes to only 5% of total energy and can cause

direct DNA damage by cyclobutane pyrimidine dimers, which can ultimately result in the cessation of DNA replication and transcription. Pigmentation is a possible specialized adaptation strategy in the phyllosphere, which is rarely found in the rhizosphere inhabiting bacteria. Many pigmented bacteria belonging to the genera *Pantoea*, *Clavibacter*, *Xanthomonas*, etc. are present on the leaf surfaces. These are not affected by the harmful effects of solar radiations due to their protective pigmentation (Xanthomonadin) and/or elicitation of antioxidant machinery, including expression of enzymes such as catalase and superoxide dismutase (Rajagopal et al. 1997). Jacobs et al. (2005) evaluated the role of pigmentation in the survival of *Clavibacter michiganensis* and as an effective leaf colonization strategy using pigment-deficient mutants.

Kadivar and Stapleton (2003) investigated the effects of ultra violet radiation on the maize phyllosphere diversity. They found an increased abundance of phyllospheric communities in the samples exposed to UV as compared to control plants, which suggests that the number of tolerant species increased, as compared to the sensitive ones. The phyllosphere of *Tamarix aphylla*, a globally distributed, salt-secreting desert tree, harbors epibionts containing light sensing and protection genes, which protect against the constant exposure to harmful solar radiations. Furthermore, the prevalence of anoxygenic photosystem genes without the copies of RuBisCo suggests that the photosystem apparatus is used for ATP production, rather than for fixing atmospheric carbon, clearly justifying its significance (Finkel et al. 2016).

Quorum sensing

The growth of bacteria modifies the leaf environment, and a succession of microorganisms in this niche leads to the dominance of different sets of microorganisms with the passage of time. Quorum sensing is a phenomenon by which bacteria communicate with one another through small signaling molecules often termed as autoinducers. These are sensed by other bacteria in the vicinity, influencing gene expression, once a threshold number is reached. This results in not only diverse groups of organisms and quantitatively different traits, but also density-dependent behavior leading to modifications in the abundance and diversity of microbial communities in the phyllosphere (Hirano and Upper 1993). Common signaling molecules which are used by phytopathogens are AHLs (acyl homoserine lactone); *Pseudomonas syringae* produces 3-oxo-C6-HSL (homoserine lactone), which regulates motility and EPS production (Quinones et al. 2005).

Motility

Chemotaxis is a major factor which enables the bacteria to swarm across solid surfaces, such as leaves; this is facilitated

by the expression of the flagellar gene and the functioning of surfactant molecules (Burch et al. 2012). Epiphytic pathogens promote their survival employing motility systems to move towards stomata, or other points of entry in the leaves or to the interior of the plant. The epiphytic fitness in *Pseudomonas syringae* is mainly mediated by flagella-driven motility, which helps in improved surface colonization and effective plant virulence (Tans-Kersten et al. 2001). Because of the phenomenon of motility, there often exists a continuum between the external and internal leaf associated populations (Beattie and Lindow 1999).

Methods for analyses of microbial communities

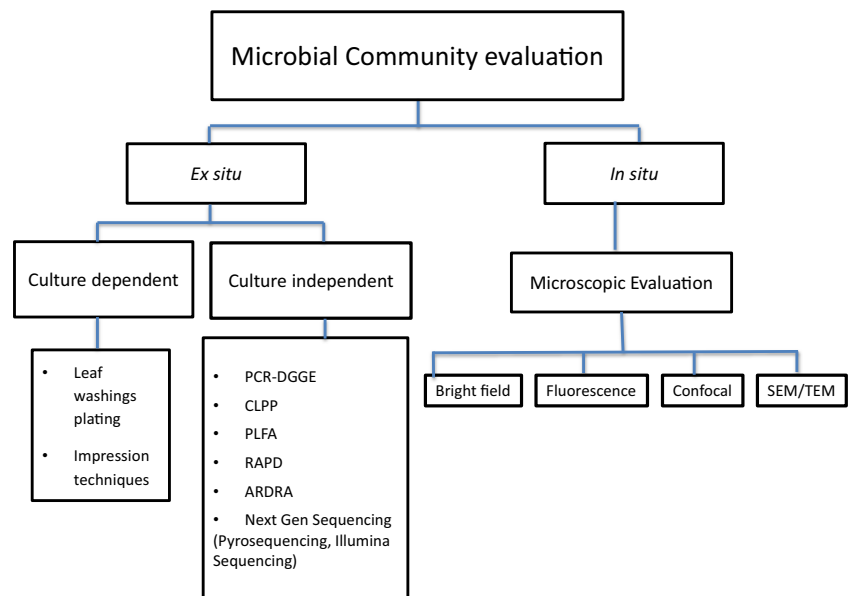
A range of techniques are available for analyzing the diversity and abundance of microflora present on the phyllosphere and evaluating their community dynamics (Fig. 2). Fundamentally, however, two basic approaches are used: firstly, methods in which the microorganisms are viewed on the surface of the leaf directly or indirectly (in situ) and, secondly, those in which the microorganisms are removed from the surface and evaluated (ex situ). The latter approach can itself be categorized into two types that are culture-dependent and culture-independent approaches.

In situ methods

In the in situ method of evaluation, the microorganisms can be viewed as they exist, i.e., still attached to the surface, as against the isolation technique of enumerating microorganisms. This provides information on their spatial distribution on the surface or their interactions with other microorganisms on the surface. Impression techniques mainly involve pressing the surface of the leaf on a suitable growth medium (Bainbridge and Dickinson 1972) or culturing microbes removed using adhesive tapes (Langvad 1980). Such methods provide useful insights on their spatial relationships, but not their relationship with the topography of the surface. This is mainly because the fast growing species can dominate the plate more rapidly and also as these colonies grow, they can merge. This limitation can be circumvented using enrichment media, which allows only specific organisms to grow.

Microscope-based methods are aimed at targeting the numbers of different microbes present, their location and spatial relationships with each other (Hallett et al. 2010). Various microscopic techniques allow the visualization of microorganisms on the surface of leaves in their native state, observations on the distributions and the interactions between microorganisms and the plant surface (Lee and Hyde 2002). Reports are also available, in which leaves are bleached (Daft and Leben

Fig. 2 Methods for evaluating the microbial communities in the phyllosphere



1966) or are cast in varnish (Dickinson et al. 1974) and peeled off from the leaf surface for proper transmission of light and observed using bright field microscopy. A major drawback of microscopy is the difficulty in visualizing small or transparent bodies on the leaf surface. This can be improved by staining or fixing. Combining microscopy with advanced fluorescence techniques such as immuno-labelling and fluorescent in situ hybridization (FISH) can allow identification by providing clear images (Andrews et al. 2002) or images in three dimensions (confocal microscopy). Baldotto and Olivares (2008) evaluated the phylloepiphytic diversity of 47 plant species, using both light and SEM. The latter technique allows visualization of structures of different sizes and location on different planes owing to its high resolution and large depth of focus (Pathan et al. 2008), as observed in Fig. 1a, b, in which images of rice leaf are given from our studies (Thapa et al. 2018).

Ex situ methods

The earliest report on microbes residing in the phyllosphere was generated using the culture-dependent approach. This involved commonly used techniques such as washing the microorganisms from the surface of the plant, dilution-plating and enumeration by the counting the colony-forming units (CFU). However, only a small percent of diversity as low as 0.3–3% was estimated by this method (Wagner et al. 1993). Also, this approach often neglects the major part of the diversity that is responsible for the global functionalities and also eliminates the slow-growing bacteria and those that are unable to grow in routine media (Yashiro et al. 2011). However, culture-based methods are important in exploring the microbial ecology of habitats, both natural as well as those resulting from human interventions. But being extremely biased in their

evaluation by only targeting a particular group of organisms, they do not provide a true representation of the microbial genetic diversity. A substantial number of the microorganisms present in the phyllosphere may exist in the viable but not culturable (VBNC) state. These VBNC organisms include distinct groups of organisms which may be abundant or very active, but remain unknown as they are not amenable to growth/identification using standard culture methods. As non-culturable diversity reflects only the different types of microflora which are refractile to routine culturing methods or growth media or may not be present in sufficient numbers to be cultured, due to selective modulation by the plant, there is a need to use additional methods to supplement this data.

In the past few decades, different culture-independent approaches have been employed by various researchers for deciphering the distribution of diverse microbial populations and community structure analyses in a multitude of environmental conditions. Although these techniques have been more commonly employed for the study of environmental locations such as bulk soil, rhizosphere of plants, and even water samples, the application of these techniques has now been extended for the study of microorganisms present within the phyllosphere, both as epiphytes and endophytes (Table 1).

16S rRNA polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) is used to generate unique genetic fingerprints of the individual bacterial species present within a community in the form of band profile (Yang et al. 2001). Analyses of phyllosphere microflora of seven different plant species using both culture dependent and independent methods illustrated that the culture-independent methods revealed a greater diversity, than with the conventional approaches.

Phospholipid fatty acid analysis (PLFA) is another useful technique, based on the hypothesis that phospholipids are

Table 1 Culture-independent approaches used for microbial community analysis in different plant species

Host species	Method	Dominating species	References
Monocotyledons			
Cultivated rice	16S rRNA, metaproteomics	<i>Actinobacteria</i> , <i>Proteobacteria</i>	Knief et al. (2012)
	Fluorescent nuclear staining technique	<i>Sphingomonas</i> , <i>Acidovorax</i> , <i>Methylobacterium</i> , <i>Pseudomonas</i> , <i>Sinorhizobium</i>	Niwa et al. (2011)
	PCR-DGGE	<i>Proteobacteria</i> , <i>Firmicutes</i> , <i>Planctomycetes</i> , <i>Cyanobacteria</i> , <i>Actinobacteria</i>	Thapa et al. (2018)
Wheat	16S rDNA clone library, PCR-DGGE	γ - <i>Proteobacteria</i>	Gu et al. (2010)
Maize	PCR-DGGE, DNA Hybridization	<i>Sphingomonas</i> , <i>Acinetobacter</i>	Kadivar and Stapleton (2003)
Barley	PCR, T-RFLP, RISA	γ - <i>Proteobacteria</i>	Gravouil (2012)
Seagrasses	16S rRNA-DGGE analysis	<i>Cytophaga</i> , <i>Flavobacteria</i> , <i>Bacteroides</i>	Uku et al. (2007)
Dicotyledons			
Tropical tree species	16S rRNA pyrosequencing	<i>Alphaproteobacteria</i> , <i>Acidobacteria</i> , <i>Gammaproteobacteria</i>	Kim et al. (2012)
Soybean, clover	16S rRNA-DGGE and metaproteomics	<i>Alphaproteobacteria</i> , <i>Sphingomonas</i> sp., <i>Methylobacterium</i>	Delmotte et al. (2009)
Apple	Culture dependent and 16S rRNA analysis	<i>Sphingomonadales</i> , <i>Actinomycetales</i> , <i>Rhizobiales</i> , <i>Pseudomonadales</i> , <i>Burkholderiales</i>	Yashiro et al. (2011)
3 plant species (<i>Amygdalus communis</i> , <i>Citrus paradisi</i> , <i>Nicotiana glauca</i>)	Culture dependent and DGGE	<i>Bacilli</i> , <i>Actinobacteria</i>	Izhaki et al. (2013)
Lettuce	Pyrosequencing and 16S rRNA analysis	<i>Proteobacteria</i> , <i>Firmicutes</i> , <i>Actinobacteria</i>	Williams et al. (2013)
Tomato	16S rRNA analysis and DGGE	<i>Bacillus</i> , <i>Pseudomonadales</i> , <i>Curtobacterium</i> , <i>Sphingomonas</i>	Enya et al. (2007)
<i>Arabidopsis</i>	16S rRNA analysis and Genome wide association studies (GWAS)	<i>Proteobacteria</i> , <i>Bacteroidetes</i> , <i>Actinobacteria</i>	Horton et al. (2014)

present proportionately as a part of the cell biomass and any variation in the fatty acids visualized as differences in the community-level profiles is representative of the taxonomic groups present, referred to commonly as a marker (Lv et al. 2012). Community-level physiological profiling (CLPP) forms an alternative technique to study bacteria based on the catabolic diversity of microorganisms. This approach illustrates the differences in the ability of the microbial community to utilize a wide range of organic substrates varying in structural complexity; evaluated by recording the ability to metabolize a range of individual carbon sources, present in a microtitre plate format. Suda et al. (2009) studied the phyllosphere microbial community of powdery mildew infected cucumber and Japanese spindle using culture dependent and independent approaches. CLPP along with DGGE revealed that the functional diversity, species richness, and evenness were more in infected leaves as compared to the uninfected leaves; this illustrated that specific bacteria are harbored by the plant during the infection. Thapa et al. (2018) utilized PCR-DGGE approach to distinguish and characterize the

phyllosphere microbial communities using bacterial group-specific primers and observed the distinct effect of fertilizer application on the structure of different bacterial communities.

Illumina MiSeq has also been used for microbial profiling lately because of its higher accuracy and greater throughput which provides more comprehensive details of the microbial community profiles. Illumina-based 16S rRNA gene sequencing was used to study bacterial communities associated with leafy green vegetables. Proteobacteria, Bacteroidetes, Actinobacteria, and Firmicutes were the most dominant phyla present in the phyllosphere of rocket salad (*Diplotaxis tenuifolia*) and lettuce (*Lactuca sativa*), irrespective of culture-based or culture-independent analyses (Dees et al. 2015).

Phyllosphere bacterial community of *Wolffia australiana*, a floating macrophyte in paddy soil ecosystem, using Illumina sequencing revealed Proteobacteria and Bacteroidetes as the predominant phyla, although the diversity of the phyllosphere was comparatively lesser than the corresponding water and soil (Xie et al. 2015). High throughput pyrosequencing

analyses of 16S rRNA amplicons obtained from fresh spinach leaves revealed the presence of about 75% unique sequences, which were not present in the existing databases (Lopez-Velasco et al. 2011). Knief et al. (2012) studied the rhizosphere and phyllosphere of rice plants using metagenomic and metaproteomic approaches. Various subsets of proteins were identified in the samples which were specific for survival in the phyllosphere; enzymes involved in one carbon metabolism were detected in both phyllosphere and rhizosphere, but were more prevalent in the phyllosphere, where the genus *Methylobacterium* is commonly found. Effect of elevated levels of ozone on the phyllosphere and rhizosphere communities of rice was studied using next generation 16S rRNA sequencing. They concluded that even though the effect was mild, it did not affect the microbial communities, on the whole (Ueda et al. 2016).

A recent development in this area is the genome wide association study (GWAS) or whole genome association study carried out to investigate the host plant contribution in shaping the leaf microbial community of *Arabidopsis thaliana* using single nucleotide polymorphisms (SNPs) data. Plant loci responsible for defense and cell wall integrity were found to have an upper hand in shaping the bacterial community of the phyllosphere (Horton et al. 2014). Thapa et al. (2017, 2018) observed that the methods of rice cultivation and fertilizer application along with host genotype significantly modulated both the structural (taxonomical) and functional attributes of the rice phyllosphere microbiome.

Major groups of phyllosphere inhabitants

Plants and their associated microbial communities are closely interrelated to each other both ecologically and evolutionarily (Vandenkoornhuysen et al. 2015). Three major groups of microbes are present in the phyllosphere, i.e., bacteria, fungi, and oomycetes, which interact with abiotic factors and host genotype leading to effective plant colonization. Thus, it becomes essential to refer the system as a holobiont where the plants as well as their associated organisms are intimately associated with each other. Plants also sustain keystone species which play a significant role in their structural and functional development (Vorholt 2012). Modulatory effects of host genotype and environmental factors on the nature and abundance of microbial communities emphasize the holobiont concept, illustrated by similarities between the leaf and root microbiome in *Arabidopsis* (Bai et al. 2015). Phyllosphere harbors a plethora of microorganisms which determines the functionality of the plant, whether related to biocontrol against pathogens or epiphytic fitness or even its growth and development (Ludwig-Müller 2015). Agler et al. (2016) discussed the significance of microbial “hubs” representing a small number of taxa which are strongly interconnected and have a pronounced

impact on the type of communities residing in or on the host. They also help in stabilizing specific populations on the individual plants. Different groups of microorganisms inhabiting the phyllosphere are listed below, each having their specific role in this niche.

Bacteria

Bacteria are the most abundant inhabitants of the phyllosphere, which either have positive, negative, or neutral influence on the host plants being colonized. Most common groups of bacteria which are present in the phyllosphere are Proteobacteria (alpha-, beta-, and gammaproteobacteria), Bacteroidetes, Actinobacteria, and Firmicutes. Even within the same group, differences may arise due to the habitat or geographic location or even due to the varietal differences among the same host plants. Survey of literature on the phyllosphere microbial communities has illustrated that Proteobacteria and Firmicutes are the dominant phyla present in the phyllosphere of various plants with *Bacillus* and *Pseudomonas* representing the most dominant genera. Actinobacteria and Bacteroidetes were also present in the majority of cases. Most commonly encountered species were *Bacillus*, *Pantoea*, *Erwinia*, *Pseudomonas*, *Sphingomonas*, *Acinetobacter*, *Xanthomonas*, and *Gluconobacter* (Table 2). Venkatachalam et al. (2016) studied the culturable phyllosphere microbiome of rice, both under controlled conditions and in open fields. More species diversity was found under controlled conditions in pot trials than in fields. Sequence analysis revealed the predominance of *Pantoea*, *Exiguobacterium*, and *Bacillus* genera.

Microorganisms present in the phyllosphere were capable of showing nitrogen fixation and hormone production, which are common plant growth-promoting traits. Interspecific variability is often more than the intraspecific variability; this is often due to the differences in the leaf structure or leaf age, leaf geometry or cuticle structure or trichomes, chemical composition of the volatile substances exuded by the leaf etc. (Redford et al. 2010). Environmental conditions are also known to play an important role in the bacterial community composition with hot and humid conditions being more favorable for the growth of bacteria rather than the cooler and drier conditions. Junker et al. (2011) revealed that the bacterial community on the leaves of *Saponaria officinalis* and *Lotus corniculatus* are the same and thus not host dependent, whereas Redford et al. (2010) reported that the interspecific variability exceeded the intraspecific variability, illustrative of host specificity. Similarly, Laforest-Lapointe et al. (2016) evaluated the microbial diversity from the leaves of trees from five dominant temperate species of angiosperms and gymnosperms. They demonstrated that maximum intra individual variation is the result of the differences between individuals, while the canopy location did not significantly

Table 2 Major genera of bacteria found in different plants

Plants	Genera	References
Cultivated crops		
Rice (<i>Oryza sativa</i>)	<i>Pantoea</i> , <i>Exiguobacterium</i> , <i>Bacillus</i> , <i>Pseudomonas</i> , <i>Enterobacter</i> , <i>Erwinia</i> , <i>Streptomyces</i>	Venkatachalam et al. (2016); Thapa et al. (2018)
Maize (<i>Zea mays</i>)	<i>Acinetobacter</i> , <i>Sphingomonas</i>	Kadivar and Stapleton (2003)
Soybean (<i>Glycine max</i>), clover (<i>Trifolium repens</i>), and <i>Arabidopsis</i>	<i>Sphingomonas</i> , <i>Methylobacterium</i> , <i>Pseudomonas</i>	Delmotte et al. (2009)
Lettuce (<i>Lactuca sativa</i>)	<i>Proteobacteria</i> , <i>Firmicutes</i> , <i>Bacteroidetes</i> , <i>Actinobacteria</i>	Rastogi et al. (2012)
Sugarbeet (<i>Beta vulgaris</i>)	<i>Pseudomonas</i> , <i>Erwinia</i>	Thompson et al. (1993)
Other crops and trees		
<i>Citrus sinensis</i> cv Valencia	<i>Acinetobacter</i> , <i>Bacillus</i> , <i>Enterobacter</i> , <i>Pseudomonas</i>	Yang et al. (2001)
Cottonwood (<i>Populus deltoides</i>)	<i>Proteobacteria</i> , <i>Acidobacteria</i>	Redford and Fierer (2009)
<i>Deschampsia antarctica</i>	<i>Pseudomonas</i> , <i>Psychrobacter</i>	Cid et al. (2017)
Tropical trees (<i>Schizostachyum</i> , <i>Gnetum</i> , <i>Shorea</i> , <i>Arytera</i> , <i>Dyera</i>)	<i>Acidobacteria</i> , <i>Actinobacteria</i> , <i>Alphaproteobacteria</i> , <i>Bacteroidetes</i>	Kim et al. (2012)
Mangrove forests (<i>Avicenia</i> , <i>Laguncularia</i> , <i>Rhizophora</i>)	<i>Bacteroidetes</i> , <i>Actinobacteria</i> , <i>Proteobacteria</i>	Dias et al. (2012)
<i>Bougainvillea</i>	<i>Enterobacter</i> , <i>Pseudomonas</i>	Sangthong et al. (2016)
Grapevine (<i>Vitis</i> sp.)	<i>Pseudomonas</i> , <i>Curtobacterium</i> , <i>Bacillus</i>	Martins et al. (2013)
<i>Tamarix</i>	<i>Actinomycetales</i> , <i>Bacillales</i> , <i>Halomonas</i> , <i>Halobacteria</i>	Finkel et al. (2011)
<i>Wolffia australiana</i>	<i>Proteobacteria</i> , <i>Bacteroidetes</i>	Xie et al. (2015)
<i>Agave tequilana</i>	<i>Proteobacteria</i> , <i>Actinobacteria</i> , <i>Firmicutes</i> , <i>Bacteroidetes</i>	Coleman-Derr et al. (2016)

influence the diversity. They suggested that a critical understanding of the changes in leaf characteristics and local abiotic conditions, as also the host identity is essential for a better understanding of the spatial variation of the phyllosphere microbiome. Bai et al. (2015) revealed a functional overlap between the leaf and root microbiota in *Arabidopsis thaliana* plant. They also suggested that a proportion of leaf colonizing bacteria originated from the unplanted soil and that reciprocal bacterial colonization events may take place between the leaf and the roots. Similarly, Wagner et al. (2016) proposed that the host genotype and age of the plant shape the root and leaf microbiome of *Boechera stricta*, a wild perennial plant.

Vogel et al. (2016) in an interesting study demonstrated that the phyllospheric commensal bacteria have a profound impact on the transcriptome of the plant. They used model bacteria *Sphingomonas melonis* and *Methylobacterium extorquens* and found that the expression of nearly 400 genes may be involved in the plant defense responses. Cid et al. (2017) studied the phyllosphere of *Deschampsia antarctica*, a plant native to the Antarctic at different locations. Bacterial community composition illustrated by the sequencing of DGGE bands revealed significant differences in the in community composition at different locations. Also, the most prominent ones were the Pseudomonadales (*Pseudomonas* and *Psychrobacter*) and Rhizobiales (*Agrobacterium* and *Aurantimonas*) orders, irrespective of the different locations.

Psychrobacter articus, a cold-adapted bacterium, used as a model for psychrophilic proteins, was also present. Thapa et al. (2018) analyzed the diversity of the rice phyllosphere microbiome, as influenced by fertilizer application and the method of rice cultivation (conventional–flooded, direct seeded rice/DSR, and system of rice intensification/SRI) in variety Pusa Basmati 1509. PCR-DGGE analyses using bacterial group-specific primers illustrated that fertilizer application brought about a distinct modulation in the communities belonging to phyla such as Bacteroidetes, Firmicutes, and Planctomyces, besides Proteobacteria. SRI method of cultivation influenced the cyanobacterial population distinctly. The group-specific PCR-DGGE analysis showed significant differences among various treatments, in terms of several diversity indices, except those of Shannon and Pielou indices; SRI-RDF exhibited highest values in terms of Simpson's Index while those under the conventional-control ranked highest in terms of species richness indices of Margalef and Menhinick.

Because of the continuum existing between the outside and inside of the plant surface due to presence of the structures like stomata, hydathodes, and lenticels, there is always a tendency for the epiphytic microflora to become endophytic and vice versa. As the conditions inside the plant surface are more favorable compared to the hostile conditions outside, epiphytes having suitable traits can successfully establish themselves as endophytes, showing no external signs of infection or negative effect on their host (Holliday 1989; Schulz and

Boyle 2006). Having a similar ecological niche to colonize as compared to the phytopathogens, they are the most suitable candidates as potential biocontrol agents (Berg et al. 2005). Such bacteria have been isolated from all compartments of plants, including seeds, which generally colonize the intercellular spaces (Posada and Vega 2005). Several benefits of endophytes such as accelerated seedling emergence, promotion of plant establishment under adverse conditions (Chanway 1997), and enhancement of plant growth through the improved availability of nutrients (Bent and Chanway 1998), capacity for xenobiotic degradation, resistance to heavy metals or antimicrobials particularly in case of heavy metal stress have been reported (Kang et al. 2012; Yamaji et al. 2016).

Methanol, a volatile organic compound, is released from the plant surface as a result of pectin demethylation. Phyllosphere being the largest global interface, the amount of methanol released can be quite significant. Several bacteria belonging to the genus *Methylobacterium* are the most commonly encountered members of the pink pigmented facultative methylotrophs (PPFM), which can utilize methanol as their sole source of carbon and energy (Corpe and Rheem 1989); related proteins involved are shown to be present through metaproteomic analyses (Delmotte et al. 2009). They secrete cytokinins, auxins, and vitamin B₁₂ (Ivanova et al. 2000; Trotsenko et al. 2001; Doronina et al. 2002; Koenig et al. 2002; Omer et al. 2004; Ivanova et al. 2006), which are involved in plant growth and development, seed germination, leading to increase in the yields (Abanda-Nkpwatt et al. 2006; Lee et al. 2006; Ryu et al. 2006; Verginer et al. 2010; Meena et al. 2012). The tolerance of aerobic methylotrophs to heavy metals such as nickel, cadmium, copper, zinc, chromium, mercury, lead, and arsenic is also well established (De Marco et al. 2004; Idris et al. 2006; Dourado et al. 2012). Wellner et al. (2011) investigated the diversity and distribution of bacteria, with special emphasis on *Methylobacterium* spp. from the phyllosphere of host plant species namely *Trifolium repens* and *Cerastium holosteoides* from three geographic locations and land use types (meadows, mown pasture, and pasture) using culture-dependent and culture-independent approaches. Their results indicated that *Methylobacterium* spp. represent the abundant and stable members of the phyllosphere community, and dominate the methylotrophic community.

Fungi and yeasts

The fungal phyllosphere community plays several roles in ecosystem functions, including nutrient cycling in soil (Van Der Heijden et al. 2008) through their interactions with the rhizosphere microbiome (Bell et al. 2014) and sustain ecosystem productivity (Maherali and Klironomos 2007; van der Heijden et al. 2016). It is possible that phyllospheric fungi

can also function similarly in the aerial parts. Phyllospheric fungi can be categorized as epiphytic or endophytic but this can lead to ambiguity, as some epiphytic fungi can actively colonize the internal tissues through stomata or epidermal regions (Viret and Petrini 1994; Fauth et al. 1996; Jumpponen and Jones 2009; Cordier et al. 2012). Filamentous fungi population can range between 10² and 10⁸ CFU per gram leaf, whereas yeast population can range between 10 and 10¹⁰ CFU per gram leaf (Thompson et al. 1993; Inacio et al. 2002). Yeast like fungus *Aureobasidium pullulans* is dominant in the phyllosphere as well on the surface of fruits. Due to its ability to outcompete pathogens, it can be used as a biological control agent (Cordier et al. 2012; Setati et al. 2012). Common fungal genera occurring in the leaves are *Cladosporium*, *Alternaria*, *Penicillium*, *Acremonium*, *Mucor*, and *Aspergillus*, whereas commonly occurring yeasts are *Cryptococcus*, *Sporobolomyces*, and *Rhodotorula* (Table 3). Venkatachalam et al. (2016) isolated *Curvularia lunata* and *Bipolaris ravenelii* from the phyllosphere of rice (*Oryza sativa*); these are known to commonly act as phytopathogens and implicated in a number of foliar diseases (Thaung 2008; Zheng et al. 2013).

Fungi are also subjected to seasonal dynamics (Jumpponen and Jones 2010; Rastogi et al. 2012). Penuelas et al. (2012) highlighted the strong influence of seasonal fluctuations on the species richness and diversity using the T-RFLP profiles from the fungi as well as bacteria on the surface and interior of leaves of *Quercus ilex*. Fungi in the phyllosphere can behave either as saprophytes, phytopathogens or in association with the algae as lichens (Frey-Klett et al. 2011). They have several roles to play in this niche, by inhibiting the development of pathogenic fungi, decomposition of plant exudates, increasing the stress tolerance of the plants, deterring herbivory by the production of alkaloids, and by acting as early decomposers of leaf litter and promotion of nutrient cycling (Cowan 2001). Certain aquatic hyphomycetes are involved in the decomposition of litter in riparian ecosystems, improving the palatability of leaves for invertebrates; hence, play an important role in the functioning of aquatic food webs (Bärlocher 2016). These phyllosphere fungal communities are important in nutrient cycling and in the functional coupling of terrestrial and aquatic ecosystems; hence, it is essential to identify the processes which shape these communities and assess their response to global change.

Cyanobacteria and algae

Algae, including cyanobacteria represent the base of food pyramids in a majority of environments. The abundance and diversity of algae in soil, including the rhizosphere, is well documented (Singh and Bisoyi 1989; Nayak and Prasanna 2007; Prasanna et al. 2009; Lin and Wu 2014), particularly in the paddy fields. Very few reports have described the diversity of cyanobacteria and algae in the phyllosphere of different plants. Most of the published literature on algae or

Table 3 Major groups of fungi found in different plant species

Plant	Genera	References
<i>Quercus macrocarpa</i>	<i>Microsphaeropsis, Alternaria, Epicoccum, Erysiphe, Cladosporium, Mycosphaella, Taphrina</i>	Jumpponen and Jones (2009)
<i>Fagus sylvatica</i>	<i>Lalaria, Taphrina, Aureobasidium, Cryptococcus</i>	Cordier et al. (2012)
Grapevine (<i>Vitis</i> sp.)	<i>Aureobasidium, Epicoccum</i>	Grube et al. (2011)
Sugarbeet (<i>Beta vulgaris</i>)	<i>Cladosporium, Alternaria, Cryptococcus, Sporobolomyces</i>	Thompson et al. (1993)
Lettuce (<i>Lactuca sativa</i>)	<i>Cladosporium, Sporobolomyces</i>	Hunter et al. (2015)
<i>Camellia japonica</i>	<i>Pestalotiopsis, Aureobasidium, Phoma, Ramichloridium, Cladosporium</i>	Osono (2008)
Rice (<i>Oryza sativa</i>)	<i>Curvularia lunata, Bipolaris ravenelli</i>	Venkatachalam et al. (2016)

cyanobacteria reported their abundance in the tropical forests because of the prevailing suitable humidity conditions. Rigonato et al. (2012) studied the cyanobacterial diversity in a well-preserved Brazilian mangrove ecosystem using culture-independent approaches. Results demonstrated that the cyanobacterial diversity is influenced by the ecosystem as well as the plant species. *Nostocales* and *Oscillatoriales* were the most predominant orders present. Venkatachalam et al. (2016) reported the presence of cyanobacteria in the phyllosphere of rice (*Oryza sativa*) for the first time, using culture enrichment as well as culture-independent approaches (PCR-DGGE). Bright field microscopy revealed unicellular as well as filamentous type of morphotypes (*Lyngbya*, *Plectonema*, and *Nostoc*), whereas metagenomic analysis showed almost 4 to 12 phylotypes (distinct species) in samples from various cultivation practices. Kim et al. (2012) found the occurrence of cyanobacteria in the phyllosphere of tropical rainforest of Malaysia. They were consistently present in all the samples, but were not the dominant inhabitants. In a similar study, Lin et al. (2012) analyzed the diversity, with emphasis on the epiphyllous algae in the rain forest in Taiwan. They observed six species of green algae, including *Chloroidium saccharophilum*, *Ettlia pseudoalveolaris*, *Klebsormidium flaccidum*, *Prasiococcus calcarius*, *Rosenvingiella radicans*, and *Trebouxia* sp., and one cyanobacterial species, *Leptolyngbya* sp. These algae have an important role in nutrient cycling due to their role in nitrogen mobilization and also influencing the loss of water by evaporation on the host plant. These are also thought to be involved in the production of photosynthates for the use of heterotrophs, such as bacteria and fungi in the phyllosphere.

Higher organisms

Protozoans are considered as important residents, due to their predation of bacteria and fungi in soil as well as in aquatic habitats. They are commonly present on the soil and other

terrestrial habitats. They are able to encyst rapidly on drying as well as excyst when sufficient moisture is present. Moisture being a limiting factor in the phyllosphere, these organisms have received the least attention, as their presence is generally transient in nature. Mueller and Mueller (1970) reported the occurrence of *Calpoda cucullus* on 88% of the small herbaceous plants as well as on the bark of 98% trees that were surveyed.

Phyllosphere microorganisms as biocontrol agents

Phyllosphere isolates can help in reducing the number of foliar pathogens on the surface of the leaves, and this strategy is ecologically significant as they occupy the same niche as the pathogens. A variety of antibiotics have been implicated in disease control. But most of these antibiotics are identified on the basis of in vitro studies, rather than in vivo studies; hence, the effect is variable (Andrews 1985). In vitro production of antibiotics is dependent on the type of media used for the study. Stockwell et al. (2002) studied the control of fire blight on pear using *Pantoea agglomerans* and reported that an antibiotic deficient mutant of *Pantoea agglomerans* Eh 252 was less effective than the wild-type strain in controlling the disease.

Competition for the nutrients and space offers another means of biological control. The population not adhered properly on the surface are washed away and so the phytopathogens are not able to colonize the surface. Systemic induced response is another way of establishing biocontrol by the inhabitants. This type of resistance helps in making the susceptible plant resistant to subsequent pathogen attack by signaling the defense responses (Kloepper et al. 2004). The responses could be production of defense enzymes, antibiotic production, or lignification of cell walls by the plant to fight the pathogen (van Loon and Glick 2004). A number of systemic

resistance pathways have been known which includes salicylic acid dependent and production of pathogenesis related (PR) proteins (Delaney 1997), salicylic acid independent and production of reactive oxygen species and PR proteins (Bargabus-Larson and Jacobsen 2007), and jasmonic acid and ethylene dependent pathway (Pieterse et al. 1998). Production of compounds like indole acetic acid and N-acyl homoserine lactone (AHL) assist bacteria in the colonization of plant surface (Lindow and Brandl 2003).

Sartori et al. (2015) studied the biocontrol potential of phyllospheric microorganisms from maize against *Exserohilum turcicum*, the causal agent of leaf blight. *Bacillus* and *Pantoea* species were the dominant forms that showed the inhibition of the fungus and were able to tolerate conditions like low water potential and osmotic stress. There was a negative and significant correlation between the growth of the pathogen and the dominance index of the epiphyte. Shrestha et al. (2016) investigated the prospects of biological control of rice associated *Bacillus* against sheath blight and panicle blight of rice, caused by *Rhizoctonia solani* and *Burkholderia glumae*, respectively. A variety of *Bacillus* isolates were observed to inhibit the sclerotial germination of the fungus, which could be attributed to the various antimicrobial secondary metabolites produced by the bacteria. Various gram negative bacteria also show plant protection activity. *Pseudomonas graminis* isolated from the apple phyllosphere showed control against fire blight caused by *Erwinia amylovora* (Mikiciński et al. 2016).

Siderophore production is also reported to be an important biocontrol strategy. Michavila et al. (2017) reported a non-pathogenic strain from the lemon phyllosphere, *Pseudomonas protegens* CS1, which served as a biocontrol against citrus canker. Siderophore pyochelin and

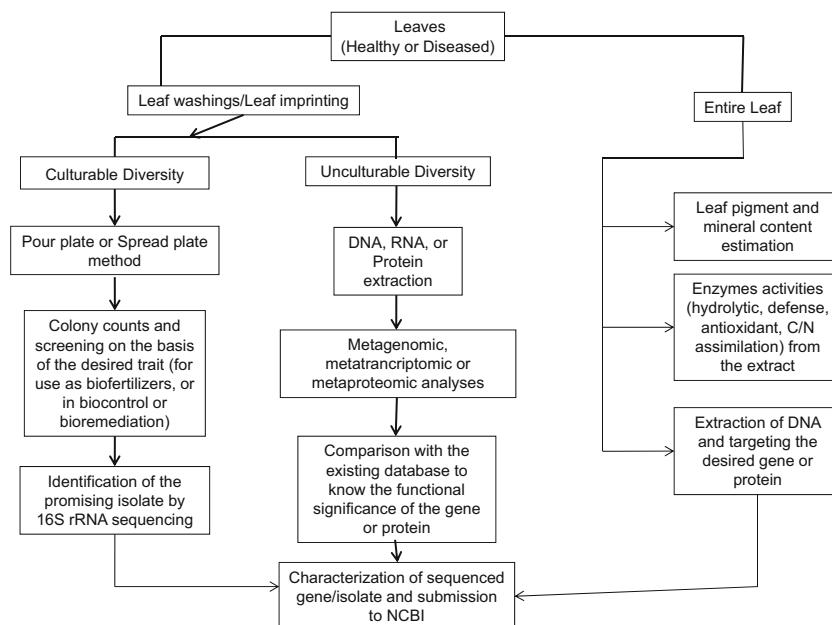
elicited generation of reactive oxygen species (ROS) were responsible for the biocontrol. Harsonowati et al. (2017) investigated the biocontrol of rice blast caused by *Pyricularia oryzae* by indigenous phyllosphere actinomycetes. Isolates mostly belonged to *Streptomyces* genera, whereas others belonged to *Saccharothrix*, *Gordonia*, or *Lentzea*. It was also found that these isolates had non-ribosomal peptide synthetase (NRPS) gene (99.94% isolates) and type 1 polyketide synthase gene (66.67% isolates) in their genome, which is responsible for the production of bioactive compounds.

Apart from bacteria, phyllosphere inhabiting yeast and fungi show biocontrol activities against different phytopathogens. In a study conducted by Kalogiannis et al. (2006), out of the 30 recovered yeasts from the phyllosphere of tomato plant, 9 were able to bring about a reduction in the disease index by >90% against *Botrytis cinerea*, the causal agent of gray mold. Hilber-Bodmer et al. (2017) analyzed the phyllosphere and rhizosphere yeast diversity of apple and found that soil isolated yeast were more potent as compared to those from the phyllosphere, against various filamentous fungi. Common phyllospheric isolates were *Rhodotorula*, *Cryptococcus*, *Pichia*, *Hanseniaspora*, *Debaryomyces*, etc.

Conclusions

The shoot system of the plant represents the biological interface that serves as a common site for carbon assimilation, release of oxygen, and reproductive abilities of the plant. Recent reports suggest a taxonomic and functional overlap with root microbiota, which raises interesting speculations regarding the reciprocal translocation of a

Fig. 3 Outline of proposed flow of activities for phyllosphere microbiome research



subset of the microbiota between these niches. Major taxonomic groups present in this habitat include members of the Proteobacteria, Actinobacteria, Acidobacteria, Planctomyces, Bacteroidetes, oomycetous/filamentous fungi, and cyanobacteria. Commonly encountered genera are *Bacillus*, *Pseudomonas*, *Pantoea*, Enterobacteriaceae members, *Methylobacterium*, Actinobacteria, etc. Among the numerous methods available to enumerate the microbial populations present in the phyllosphere, both culture dependent and independent, each has its own specific sets of merits and demerits. Culture-independent methods such as metagenomics, proteomic signatures, transcriptomics along with bioinformatic tools have illustrated their tremendous potential in linking microbial diversity and abundance to functional facets of the host plant, with the impact of their interactions with the microbiome. Additionally, they are quick, reliable, and present a broader perspective of the diversity and abundance of microbial communities. Figure 3 provides an outline of the activities, which may provide a comprehensive understanding of the phyllosphere microbiome and its significance in biology and its applications in agroecology.

However, there are several gaps in understanding this niche. Future research efforts, therefore, need to focus on the following aspects:

- Nature and diversity of leaf exudates and their interactions with phyllosphere microflora
- Analyses of efficacy of foliar sprays vis a vis natural flora and their implication for effective nutrient cycling and biocontrol
- Deciphering the mode of continuum and distinctness of the rhizosphere and phyllosphere microbiome
- Fine analyses of the spatial and temporal distribution of major and minor/trace nutrients in the leaf and their effects on the abundance and diversity of microflora
- Leaf volatiles forms an important aspect of study providing an in-depth analyses of the gaseous cycles that are present in the atmosphere
- Development of beneficial biocontrol agents from the phyllosphere that could help in the abatement of foliar diseases

Studies of phyllosphere microbiology have provided new insights in the field of microbial ecology and have the potential to provide solutions for the problems related to climate change, carbon sequestration, besides control options against foliar pathogens.

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