Dynamics of Plant Microbiome and Its Effect on the Plant Traits

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Shivali Sharma, Shanu Magotra, Sneha Ganjoo, Tabia Andrabi, Rikita Gupta, Shilpi Sharma, and Jyoti Vakhlu

Abstract

Plants host a plethora of complex microbial communities in and on their surfaces designated as plant microbiome. The plant microbiome symbolizes the collective communities of microbes, their (meta) genomes and their interactions (mutualism-antagonism continuum) in a particular environment. The cross-talk between plant microbiome plays an important function in the performance of plant and is hot topic for research in biology. Plant microbiome endows the plant with resistance to biotic and abiotic factors, promotes plant growth and enriches the soil associated with the plant. The plant trait expression is regulated by the orchestrated effect of plant as well as microbial genes. Therefore, there is an urgent need to explore the diversity and the functionally potential of microbial communities. However, a big challenge in the present scenario is to widen technologies to improve agricultural management, e.g. plant growth promotion, biocontrol and bioremediation. Recent advances in sequencing technologies and multi-omics approaches integrate the studies on plant-microbe interactions, which gives an insight about what's happening in real-time within the cells. Metatranscriptomics and metaproteomics have come up as a holistic approach that give a picture of major metabolic pathways and the plant-associated interactions. These technologies clearly depict which functional microbial communities are dominant in crop plants and under different environmental conditions. The integration of various computational tools helps to decode the functions of proteins, individual signal molecules and gene cascades, with respect to their pathways.

Keywords

Plants · microbes · Microbiome · Plant-microbe interactions · Metagenomics · Plant traits

S. Sharma · S. Magotra · S. Ganjoo · T. Andrabi · R. Gupta · S. Sharma · J. Vakhlu (✉)
Microbiomics Laboratory, School of Biotechnology, University of Jammu, Jammu, Jammu and Kashmir, India

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12.1 Introduction

Both biotic and the abiotic systems cannot function without interacting with their microbiome, be it an animal, plant or our planet earth. Most of the bio-geothermal cycles are run by microbes and this makes life on earth possible (Boundy-Mills 2006). Microbes that are associated with the plant, animal and human systems include bacteria, archaea and fungi. They play numerous roles in maintaining health and growth of organisms, by designing behavioural strategies for avoiding or removing pathogens. Emphasis on studying the microbial function and community structure is a growing area of research (Bahrndorff et al. 2016; Ezenwa et al. 2012; Liu et al. 2012).

The importance of microbes for plants has been recognized hundreds of years ago and is credited to Lorenz Hiltner (1904). He developed many techniques to study plant-microbe interaction by combination of microscopy and analytical tools. His research is considered as a milestone in the field of plant-microbe interaction (Caporaso et al. 2012; Jansson et al. 2012). The cognisance reveals a very close symbiotic relationship between plants and their associated microbiomes. There is diversity in plant microbiomics, both at structural and functional level. Plants dock microbes within specific habitats, which can be classified as rhizosphere, the area around roots (Berendsen et al. 2012); phyllosphere, the area around above-ground parts (Vorholt 2012); and endosphere, the internal tissues (Hardoim et al. 2015).

Lorenz Hiltner in 1904 was the first to observe that soil surrounding the roots of the plants are loaded with microorganisms, as compared to soil distant from the root and called this area the rhizosphere. The rhizosphere is considered to be a complex system, where the interaction between the plant and the microbes occurs, playing a significant role in plant health. The plant and soil microbial community modulates the plant nutrition uptake and growth rate, resistance to environmental stress factors, plant survival and sustenance (Mendes et al. 2013). Therefore, the overall fitness of the plant depends on its associated microbiota, which together forms the plant 'holobiont'. The term holobiont was coined by Lynn Margulis in 1991 in the book *Symbiosis as a Source of Evolutionary Innovation* for the assemblages of different species (eukaryotes and prokaryotes) that form one ecological unit. The associated bacterial and fungal communities within a given ecological niche were then started being called the 'microbiome' in analogy to genome (Richardson 2017).

Although rhizosphere is the extensively studied plant microbial niche till date, in the recent years, the study on the microbiome of phyllosphere is also increasing. The phyllosphere is also termed as pervasive global habitat of diverse microbial communities. It is an interface between the plant parts which are above the ground and the atmosphere. The microbial communities from phyllosphere were found potentially active to influence plant biogeography and ecosystem function. These communities also influence the fitness and function of their hosts in the same way the rhizospheric community does (Kembel et al. 2014). The abundance of phyllospheric communities is estimated to be more than 10^{26} in a billion square km leaf surface area worldwide. These aerial inhabitants represent one of the largest biological interfaces on earth. They caper roles in CO_2 fixation, molecular O_2 release,

nitrogen cycle and primary biomass production (Delmotte et al. 2009). A square cm of leaf is estimated to have 10^6–10^7 inhabitants (Lindow and Brandl 2003).

Microbes not only inhabit outer surfaces, but also live deep inside the cells and tissues. These are termed 'the endophytes'. The first proof of existence of endophytes was given by Victor Galippe in the year 1887. Hallmann and co-workers in the year 1997 gave practical description of endophytes. Endophytes play both helpful and harmful roles for their hosts. They can be both mutualists and antagonists depending on the prevailing conditions (Hardoim et al. 2015). The helpful ones produce numerous secondary metabolites, proteins, enzymes, small RNA, etc. that promote plant growth and help them adapt better to their surroundings (Nair and Padmavathy 2014). Endophytes have been exploited as sources of many antimicrobial compounds. Highly diverse endophytes of medicinal plants have been identified as seed germination helpers and oxidative stress relievers. A large number of plant growth-promoting endophytes are being isolated and widely used to improve quantitative and qualitative yield of plants (Santoyo et al. 2016; Khan et al. 2017).

Microbial community structure changes when we move from rhizosphere to the endosphere. However, the positive impacts obscure the negatives in all areas. There is a wide scope to extensively utilize these microbial communities for the betterment of environment, as a whole. As on today, the presence of microbiome associated with almost all ecological niches is an established fact and emphasis is on the role they play in the niche they reside in (Fig. 12.1).

12.2 Effect of Microbiome on Plant Traits

The plants interact with the environment through its underground part – the roots – and aerial parts, stem, leaves and fruits. All these parts have a vast diversity of associated microflora. Therefore, it becomes important to unravel diversity of microbial communities and how they interact with the plants and regulated plant traits. The diverse microbial community associated with plants, the plant microbiome, shows a similar impact on plants, as gut and skin microbiome have on human health (Berendsen et al. 2012). Any imbalance or disturbances in the microbial species can result in disease outbreaks, in both plants as well as animals. Therefore, there is a need to maintain the healthy state of the host and suppressing the disease-causing pathogens of the host's native microbiome. Broadly, plant traits can be divided into four categories – morphology (leaf and root anatomy, length or shape), reproduction (clonal growth, fruit dimensions, number of seed, reproduction type, etc.), phenology (flowering age and time, leaflets growing time, etc.) and ecology (physical environment conditions, biotic and abiotic stress tolerance, etc.).

The microbiome has been found to have several noticeable deep effects on seedling vigour, seed germination, development of the innate immune system and nutrition (Mendes et al. 2013; Berg et al. 2014a, b; Schikora et al. 2016). By introducing bacteria into plant seeds, plant traits can be enhanced. Mitter and co-workers (2017) proved that in maize seeds, co-inoculation with *Paraburkholderia phytofirmans* Ps JN can improve growth and development as compared with non-treated samples.

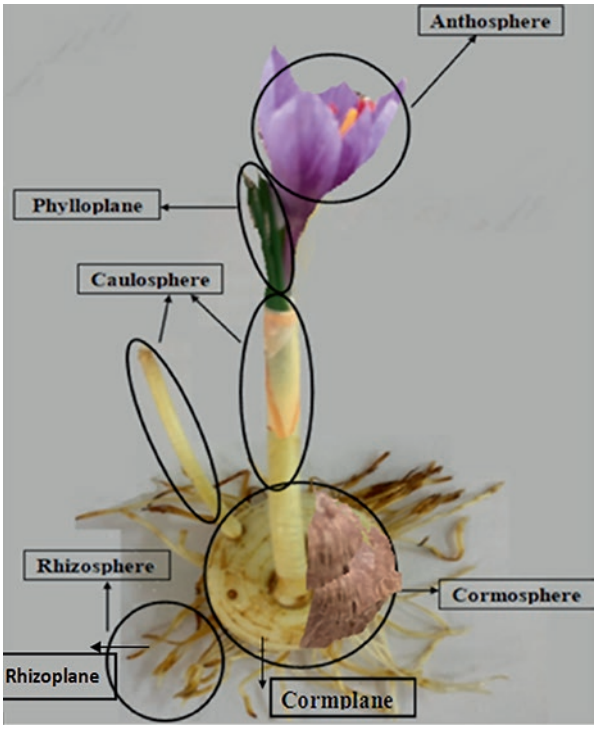

Fig. 12.1 The diagrammatic representation of plant parts where microbial interactions take place

This gives an idea about altering microbiome to modulate the behaviour of host as per the requirement.

Different plant functional traits of leaf and the roots traits have been measured with response to the microbial interactions and manipulations. The plant-associated microbes mediate plant traits either by synthesizing biologically active compounds, which provide novel biochemical competence, and/or by altering the plant existing metabolic pathways (Friesen et al. 2011). In *Brassica rapa*, it was observed that by altering the composition of the below-ground part associated microbial communities, the plant grew smaller in size with lower chlorophyll content and less number of flowers, compared with plant populations that were grown along with complex and diverse soil microbiome (Lau and Lennon 2011). Inoculating strawberry plant with bacteria *Bacillus amylolequifaciens* and *Paraburkholderia fungorum* significantly shows enhanced growth in leaf length/number and shoot/root dry weight (Rahman et al. 2018).

When the roots are young, the rate of nitrate, potassium and phosphate uptake is more. The dry matter and NPK accumulate at a fast pace and thus crop yield will be high. The need is to look for such microbiome that changes plant root trait. Okon and Kapulnik (1986) isolated several *Azospirillum* strains which influence morphological changes in roots in wheat, sorghum, corn and setaria. The age of bacterial inocula and its concentration differentially affect the surface area and length of roots. The number of root hairs, branches of root hair and lateral roots increases by

inoculating bacteria during the first three weeks after germination of seeds. In the later stages of plant development, root biomass also increases (Okon and Kapulnik 1986; Fallik et al. 1994). The root microbiome also modulates cell division and differentiation in the primary root and lateral roots which influence root growth and development (Verbon and Liberman 2016).

The potential of plant-associated microbiome for flowering is also widely exploited. The soil microbiota has a great influence on the flowering phenology and fruit production. This has been proved in a wild relative of *Arabidopsis, Boechera stricta* (Lau and Lennon 2012; Wagner et al. 2014). In *Arabidopsis* also, the microbiome help in retaining functional traits of early flowering that were modified through artificial selection on flowering time (Panke-Buisse et al. 2017). Selected microbes associated with early and late flowering (EF and LF), when inoculated into soils of the novel plant *A. thaliana* hosts, show differences in the flowering times. Microbes associated with late flowering flowered 15–17% later with consequent increase in inflorescence biomass than plants inoculated with EF-linked microbiomes (Panke-Buisse et al. 2015).

The role of different bacteria and arbuscular mycorrhizae fungi (AMF) on clonal reproduction in plants has been widely studied in last few decades. Different species of AMF differentially alter clonal reproduction in many plants. Impacts of AMF on *Prunella vulgaris* show improved growth of ramets and clonal reproduction. AMF shows strong effects which could potentially affect size of population and variation of clonal reproduction in plant communities (Streitwolf-Engel et al. 2001). The symbiotic effects of the AMF are not only observed in colonized roots but also in the above-ground plant compartments, e.g. flowers, leaf and fruits. Studies have shown that AMF in combination with PGPB or alone positively affected fruit and flower size, due to accumulation of higher concentration of anthocyanin, sugars, ascorbic acid that resulted in earlier flowering & fruiting in strawberry. Co-inoculation with the AMF and PGPB shows distinguished results such as increased flower number and more fruit production along with larger size of fruit. In inoculated plants, the sugar concentration, folic and ascorbic acid concentration was also found to be higher compared with uninoculated plants (Lingua et al. 2013; Bona et al. 2015).

The plant growth-promoting bacteria (PGPB) have been known to elevate the nutritional and functional properties of plants like cumin and flax seed oil. The plants when inoculated with the PGPB (*Hymenobacter* sp., *Paenibacillus* sp. and *Streptomyces* sp.) modulate its nutritive properties that are reflected in increasing total polyphenols, flavonoids, caroteniods and essential fatty acid content as well as resulted in greater antioxidant activity, compared with non-treated plant samples (Dimitrijevic et al. 2018). Plants like strawberry are known to exhibit different medicinal properties as it is a good source of natural antioxidants such as secondary metabolites, phenolic compounds and carotenoids and hence show free radical scavenging activities. The plants inoculated with friendly bacterial species enhance the production of total antioxidants compounds thereby enhancing functional properties of plants (Rahman et al. 2018). The characterization of the functional traits of culturable rice microbiome shows the potential for the production of IAA and N

fixation by different microbiome members suggesting their applications as plant growth promoters (Venkatachalam et al. 2016).

The plant microbiome co-operates among themselves and functions together to protect plant from stress. *Curvularia protuberate* and *Fusarium culmorum* show potential against crop heat and salt tolerance enhancement (Kandel 2016). Date palm (*Phoeix dactylifera)* endophytic bacteria *Enterobacter* sp. have been characterized and tested for the ability which help the plants to grow under saline conditions, by producing stress-tolerant enzyme ACC deaminase (Yaish et al. 2015). Mycorrhizae and PGPB help in improving crop productivity under unfavourable environment by increasing uptake of nutrients (e.g. N and P) and increase surface area of roots. As the surface area of roots increases, there is an increase in the availability of nutrients for plant consumption (Nadeem et al. 2014). Proteobacteria are reported to provide protection against nematodes and stress resistance to plants (Allen et al. 2015; Cao et al. 2015).

To maintain healthy ecological conditions, microbes help plants in remediating pollutants from soil as well to sustain healthy ecological balance. *H.cannabinus* maintains a core root microbiome consisting of Enterobacteriaceae, Pseudomonadaceae and Comamonadaceae when it grows in area of metal pollution (Chen et al. 2018). Plants also scavenge air pollutants significantly from atmosphere through their above-ground parts. Plant-associated microbiomes degrade, detoxify and sequester the pollutants to clean the environment (Stevens 2016). The inoculation of AMF in legume tree elicited the phytoremediation of the soil polluted with lead, and rhizoremediation helps maintaining the natural flora and fauna for ecological sustenance (Yang et al. 2016) (Table 12.1).

12.3 Approaches to Study the Plant Microbiome

As humans, plants have also been recently categorized as meta-organisms that harbour a distinct microbiome and live in a symbiotic relationship with its associated microflora (Berg et al. 2013), thus making its study a curious area of exploration.

The study of a plants' microbiome involves two parts: studying the community structure and the community function.

12.3.1 Studying the Community Structure

It includes studying the composition of a plants' microbiome in terms of its microbial diversity and species richness, i.e. who is there?

As already discussed, the plant harbours a diverse range of microbial communities that exist in an interactive relationship with each other and with the plant. It has been challenging to completely define the composition of plant microbiome. However, the advent of various approaches has made this path easy. Cultivation dependent and independent are the two approaches available to determine the makeup of a plant microbiome. Conventionally, evaluation of microbial diversity

Table 12.1 Effect of various bioelicitors on traits of the host plant

Microorganisms	Host plant	Effect on the plant traits	References
Pseudomonas strain	*Fragaria x ananassa var. Selva* (Strawberry)	Increased plant growth and nutrition, increases anthocyanin concentration	Lingua et al. (2013)
Pseudomonas sp., *Bacillus subtilis* and *Pantoea* sp.	*Crocus sativus* L	Increased weight in cormlets	Parray et al. (2013)
Pseudomonas putida, Azospirilium, Azotobacter	Artichoke (Cynara scolymus)	Increase in the radical length, shoot length/ weight, decreased mean time of germination	Jahanian et al. (2012)
Phyllobacterium brassicacearum strain STM196	*Arabidopsis*	Decreased leaf transpiration as a result of enhanced ABA content	Bresson et al. (2013)
Arbuscular Mycorrhizal Fungi (AMF)	*Crocus sativus* L.	Increase in weight and no. of cormlets, plant height	Lone et al. (2016)
Glomus intraradices, *Glomus mosseae*	*Solanum tuberosum* L. (potato)	Higher level of metabolites and mineral nutrition	Shuab et al. (2017)
Species of *Glomus genus*	*Allium cepa* (Onion)	Increase in chlorophyll content, growth morphology, fresh and dry weight of matter	Shuab et al. (2014)
P. putida H-2–3	Soybean	Improved plant growth as a result of gibberellins secretion	Kang et al. (2014)
AMF+Psedomonas strains	Strawberry	Increase in production of flower and fruit, with larger fruit size	Bona et al. (2015)
Rhizobium leguminosarum (LR-30), *Mesorhizobium ciceri* (CR-30 and CR-39), Rhizobium phaseoli (MR-2)	Wheat	Improved plant growth, biomass and drought tolerance index due to IAA produced	Hussain et al. (2014)

from different parts of plant was based on the use of cultivation-/culture-dependent approach that proceeded by initially isolating the microbes from their host plant by cultivating them in vitro on different nutrient media followed by their identification and characterization. It relied upon the difference in colony morphology, size, number of colony forming units (CFUs) and genotype (small subunit rRNA gene/Inter Spacer Transcribed region) for diversity studies. Cultivation-dependent approach could draw valid conclusions regarding the specific microbes but at the same time limited the unravelling of the community diversity due to the fact that just 0.001–1% of the microbial diversity can be cultivated by routine laboratory techniques (Torsvik and Øvreås 2002; Alain and Querellou 2009). The knowledge that about 99% of microbes circumvent this routine laboratory cultivation led to the advent of the cultivation-independent approach for cataloguing the microbes directly from the

source. Now a plethora of culture-independent approaches is available to study the composition and function of plant microbiome. This is done by isolating the DNA, sequencing the reads and analysing them by bioinformatic analysis.

12.3.2 Studying the Community Function

It covers the functional relationship between the plant and its native microbiota that modulates the growth and development of its host plant, i.e. what are they doing there?

Focus of microbiome research has shifted from just studying the community structure to linking the community structure with its function. One way of such study is to analyse the fluctuating plant genetic expression in presence of the variable environmental conditions. This basically includes studying the genome, metabolites, protein sequences, effect of the microbial communities on the plant genotype as well as phenotype.

The methodology for studying the plant microbiome community structure and function includes:

- DNA isolation and purification
- Sequencing and bioinformatic analysis

The methods of DNA isolation from plants differ with the site or the microenvironment. Considering the plant microenvironment, the isolation can be carried out in different manners. The exterior, the interior, above- and below-ground parts of a plant comprise a complex ecosystem, which in the recent years has got evolved in the form of a collective term called the 'phytosphere'. Further, the phytosphere is sub-divided into various small microhabitats like the endorhiza (root), spermosphere (seeds), anthosphere (flower), carposphere (fruit) (Fürnkranz et al. 2012) and cormosphere (Ambardar et al. 2014).

However, a plant has been compartmentalized based on the tissue environment – endosphere (inner tissue) and ectosphere (outer surface) (Ryan et al. 2008). These microhabitats present in the plant phytosphere comprise of a diverse environments in terms of their physical, chemical and biological environment. Such differences require the need to adopt different methods of sampling and nucleic acid (DNA) isolation depending upon the microbial community in focus and the extraction method in use.

DNA isolation majorly can be divided into two parts. The first is from the surface of the plants that acts as the exophytic environment for various microbial inhabitants forming the plant exomicrobiome and the second is the microenvironment inside the plant tissues colonizing a large plethora of microbial endophytes thus forming an endomicrobiome environment.

The exomicrobiome encompasses a vast range of microbes inhabiting the surface or the external region of the plant directly in contact with the external environment.

This includes the phyllosphere (surface of leaf) (Vorholt 2012), rhizoplane (root-soil interface) and rhizospheric soil (McNear Jr. 2013).

The basic differences in the DNA isolation from the exospheric and endospheric part lie in the fact that while targeting the exospheric microflora, we need not surface sterilize the sample which may otherwise lead to loss of the microbial community in focus. The microbial genome extracted from the exosphere is much lower in biomass relative to the total plant genome thereby creating more chances of having higher amounts of plant DNA/RNA in the extraction sample. However while studying about the rhizosphere microbiome, the quality and quantity of the rhizospheric soil can get hampered due to handling procedures among which a large amount of bulk soil can get transferred along, thereby masking the analysis of the rhizospheric community which needs to be taken care of.

Endomicrobiome is the environment composed of the microbes that can be bacteria or fungi which inhabit inside different tissues of the plant such as the interior of roots, leaves, flowers, seeds, etc.; these organisms have been referred to as endophytes (de Bary 1866). A common endophyte isolation protocol begins with the surface sterilization of the plant tissue surfaces to avoid the inclusion of the surface microflora. The efficiency of the protocol relies on the fact that it must exclude microbes residing on the surfaces of host plants such as fungi and bacteria on the lipophilic waxy plant cuticle surface (Müller and Riederer 2005). On the aerial surface, the microorganisms are more in number compared with plant tissues (Lindow and Brandl 2003); this emphasizes on the importance of extraneous DNA removal. Certain methods incorporate the use of aseptic peeling of tissue surfaces for sterilizations which is not possible for every plant tissue. The processes of cell disruption are standardized for various plant microbiomes and are permutations and combinations of thermal, chemical, physical and enzymatic lysis. Physical treatments such as bead-beating homogenization, sonification, vortexing (Steffan et al. 1988; Miller et al. 1999; Niemi et al. 2001; Miller 2001) and thermal shock (Tsai et al. 1991; Moré et al. 1994; Porteous et al. 1997; Orsini and Romano-Spica 2001) destroy the cell structure creating an access to the whole microbial community, including microbes hidden deep within. The physical method requires preliminary crushing and grinding of the material allowing the extraction or the lysis buffer to access the cells properly. Sodium dodecyl sulphate (SDS) is commonly chemical used, which is an anionic detergent, which dissolves the hydrophobic part of cell membranes. Detergents have often been used in combination with chelating agents like EDTA, Chelex 100 (Robe et al. 2003) and different buffers like Tris and sodium phosphate (Krsek and Wellington 1999) along with heat treatment. Cetyltrimethyl-ammonium bromide (CTAB) forms insoluble complexes with denatured proteins, polysaccharides and cell debris (Saano et al. 1995) and is also used for cell lysis. Enzymatic methods involve the digestion of samples by different enzymes affecting the DNA in the least way possible and particularly used in the case of Gram-positive bacteria that hold resistance to physical and chemical isolation methods (Tsai et al. 1991; Tebbe and Vahjen 1993; Zhou et al. 1996; Niemi et al. 2001).

High-quality DNA is extremely important for the accuracy of the followed procedure. The presence of proteins or polysaccharides may reduce the efficiency of

the Taq DNA polymerase, thus compromising the end products to be further analysed, thus making it a necessity to concentrate the DNA and remove any prevailing contaminants (Nunes et al. 2011). Ion-exchange chromatography, agarose, Sephadex gel filtration and PVPP/PVP gel electrophoresis are used for further purification of DNA after preliminary extraction (Cullen and Hirsch 1998). As this method consumes a lot of time, faster alternatives have been developed that include the DNA extraction and purification kits that can process numerous samples and result in a relatively pure DNA within a short span. Cesium chloride density gradient centrifugation has often been used to purify high-quality DNA (Robe et al. 2003). Further purity of the concentrated DNA can be detected using spectrophotometer-based analysis. DNA absorbs UV light maximally at 260 nm and proteins absorb light maximally at 280 nm. This means that DNA absorbs light at 260 nm 1.8 times more strongly than at 280 nm. If there is protein contaminants in the purified DNA, the absorbance at 280 nm increases. In such case, equal volumes of phenol/chloroform can be added for removal of protein contamination. Then the purified DNA is sequenced and analysed bioinformatically.

With the advent of DNA sequencing methods there is immense acceleration in the analysis of community structure and function from different plant parts. In the early 1970s, the sequencing methods started developing. Ray Wu at Cornell University developed location-specific primer extension strategy for determining DNA sequence (Wu and Taylor 1971). Maxam and Gilbert in 1977 developed chemical degradation method for sequencing. The first-generation sequencing method was developed by Sanger and co-workers in 1977. It was based on chain termination methods. First fully automated DNA sequencing method was developed by Dupont Genesis 2000 in 1987 (Prober et al. 1987). Then there was development of high-throughput sequencing technique called next-generation sequencing (NGS). Nowadays, sequencing is easier and faster compared with early times (Pettersson et al. 2009). NGS is widely used as this allows sequencing of millions of sequences simultaneously (Bentley et al. 2008).

The microbiome sequencing can be done by two approaches:

- Gene-targeted/amplicon microbiome sequencing
- Whole microbiome sequencing

Gene-Targeted/Amplicon Microbiome Sequencing

Gene-targeted microbiome sequencing is also called as amplicon sequencing. In this approach PCR amplification of specific target gene is done. Both community structure and function can be studied by this approach. To study community structure, there are phylogenetically important conserved sequences such as 16S rRNA, 18S rRNA, ITS, etc. Different sequence read comes from different organisms, so community structure can be easily studied as low abundant microbes can also be amplified (Kittelmann et al. 2015). Though amplicon sequencing gives good idea about community structure, it suffers limitation of PCR biasness (Pinto and Raskin 2012).

Whole Microbiome Sequencing

Whole microbiome constitutes all the available microorganisms in a particular habitat. Whole metagenomic sequencing (WMS) can be done to sequence whole microbiome, i.e. all the available species diversity at a particular place. It has multiple advantages over amplicon sequencing such as enhanced detection of bacterial species, better detection of diversity, enhanced prediction of genes, etc. (Ranjan et al. 2016).

To study community function, amplification of specific genes is done which are of our interest. For each organism, gene complement can be analysed to reveal different pathways, e.g. carbon fixation, energy generation, etc. (Tyson et al. 2004), or genes of importance to plants for different functions such as ahpC gene (Lee et al. 2014), hsp90 (Erlejman et al. 2014), albumin, actins, tubulins, cyclophilin, glyceraldehyde-3-phosphate dehydrogenase (G3PDH), hypoxantine phosphoribosyltransferase (HRPT), etc. (Thellin et al. 1999).

Apart from whole microbiome, trends of simultaneous assessment of community structure and function are done nowadays. This is called as 'whole metagenomics'. In whole metagenomics, the structure of the microbial community can be unravelled by both gene-targeted metagenomic (GTM) approach and whole metagenome sequencing (WMS).

In gene-targeted metagenomic approach, a DNA pool is selected and cloned and then subjected to sequencing. GTM can be done by a) sequence-driven screening or b) function-driven screening. Sequence-driven screening is done by using 16S or 18S ribosomal RNA gene sequence as genetic markers to identify genome fragments of specific group of organisms. It is independent of expression of cloned genes in foreign hosts but the primers and DNA probes are designed from conserved regions. Function-driven screening is a direct route to discover gene clusters. The gene clusters are related to metabolic roles in microbial communities (Suenaga 2012). But this suffers from disadvantages like the following: some genomes lack sequence relatives which may lead to missing novel genes, ORF lacks sequence homology to known genes and can be identified as hypothetical protein, complete sequence analysis of insert may target neighbouring genes, etc. (Vieites et al. 2008; Suenaga 2012).

With WMS, the majority of available genome can be retrieved and the functional gene composition of microbial communities can also be accessed. It can also help to find rare prokaryotic and eukaryotic sequence groups. The limitation of PCR biases is also overcome by WMS (Salvetti et al. 2016). Once the reads are sequenced, redundant and low-quality reads are filtered using different methods, e.g. EuDetect and DeConseq (Nakamura et al. 2011; Hess et al. 2011). Then the reads are assembled using either de novo assembly or reference-based assembly. De novo assembly can be achieved by using tools Velvet (Zerbino and Birney 2008) or SOAP (Li et al. 2008) which are based on de Bruijn graphs. If the closely related reference genomes are available for metagenome assembly dataset, then reference-based assembly can be used which can be achieved by using assembly softwares, e.g. Newbler, AMOS or MIRA (Chevreux et al. 1999). After this, the DNA sequences are sorted into groups using tools such as MEGAN (Huson et al. 2007), S-GSOM (Chan et al. 2008), IMG/M (Markowitz et al. 2007), PhymmBL (Brady and Salzberg 2009), PCAHIER (Zheng and Wu 2010), MG-RAST (Glass et al. 2010), CARMA (Krause

Table 12.2 Comparison of NGS technologies

Technique	Advantages	Accuracy	Disadvantages	References
Single-molecule real-time sequencing (SMRT or PacBio)	Longest read length (10 to 15 Kb)	87%	Equipments are very expensive	Pollard et al. (2018)
	Highest consensus accuracy			
Ion torrent	Rapid sequencing speed	99.6%	Difficult to enumerate long repeats	Davies (2010)
	Low operating cost			
Illumina	High sequence yield	99.9%	Requires high concentration of DNA	Kozich et al. (2013)
Nanopore	Longest individual reads	92 to 97%	Lower throughput than other machines	Branton et al. (2010)
	Portable			
Roche 454	Long read size	99.9%	Homopolmer errors	Luo et al. (2012)
	Fast		Expensive	
Sequencing by ligation (SOLiD)	Low per base cost	99.9%	Slower than other methods	Huang et al. (2012)
			Palindromic sequences issue	

et al. 2008), SOrt-ITEMS (Monzoorul Haque et al. 2009), MetaCluster (Leung et al. 2011), etc. Then at last, annotation of metagenome is performed by identifying and predicting genes by FragGeneScan (Rho et al. 2010), Metagene (Noguchi et al. 2008), etc. and assigning putative gene functions by using tools such as KEGG (Kanehisa et al. 2004), COG/KOG (Tatusov et al. 2003), PFAM (Finn et al. 2009), etc (Table 12.2).

12.4 Co-relation Between Soil Microbiome and Plant Genotype

Very much like human gut microflora, the plant microbiome system is occupied by diverse microbial community, establishing a strong functional basis to their respective hosts (Berendsen et al. 2012). The plant roots bridge the complex eukaryote and the surrounding soil which is rich in microbial organization. The bacterial community associated with the soil comes in close proximity with the plant roots and modulates plant growth and development by supplying nutrients and providing stress resistance that operates in an interlocked network mode (Vandenkoornhuyse et al. 2015; Berg et al. 2014a, b). There is limited knowledge about how the root exudates construct the specific rhizosphere microbial diversity (Chaparro et al. 2014) and the chemicals released in the exudates acts as the chemotactic signals to influence the microbial community (Badri et al. 2013a).

The association of plant and its microbiome represents the most investigated area of research in the last few years (Berendsen et al. 2012). The plant-microbe interaction is a complex process that involves a vast array of microbes and the different factors influencing this complex ecosystem. The dynamics of microbial rhizosphere communities is shaped by the different factors such as soil type, plant species, plant developmental stage, climate and geographical location. The plant roots are a key determinant of the rhizosphere microbiome, as it is the roots of the plant that interacts with the surrounding soil. In rhizosphere research, early studies showed 'rhizosphere effect' which depicted enhancement of soil microorganisms resulting from biotic and abiotic alterations of the soil with major emphasis on organic exudates from the plant roots within rhizosphere (Raaijmakers et al. 2009). The differences in the microbial communities are explained by differential gene expression even in the related crops grown in same soil, providing an insight for the plant-mediated selection of taxa in the rhizosphere/rhizoplane (Ofek-Lalzar et al. 2014). Plant roots grow in soil which is highly diverse and abundant in microbial communities but only colonizes specific and taxonomically limited root-associated microbiome. It was shown in isogenic mutants of *A. thialiana* that plant's innate immune system also plays a role in selection of bacterial taxa in rhizosphere. It was seen that salicylic acid, a phytohormone, also plays a role in plant defence and to an extent modulates the root colonization by specific bacterial communities (Lebeis et al. 2015). The plant's evolutionary history can also influence root colonization by microbes even when different genotypes of the same plant are grown in the same soil (Manter et al. 2010; Bouffaud et al. 2014). However, in comparison recent studies on 16S rRNA gene amplicon sequencing of *A. thaliana* revealed a weak 'rhizosphere effect'. OTU (operational taxonomic unit) richness shows slight differences in taxonomic composition and community structure in the rhizosphere soil (Bulgarelli et al. 2012; Lundberg et al. 2012).

Rhizosphere microbiome is modulated by plant in a host-dependent way. Each plant species uphold a particular set of rhizosphere microbiome and hence the microbiome modulation is host-dependent (Turner et al. 2013; Ofek-Lalzar et al. 2014) which is clearly a plant host effect and microbial host preference (Badri et al. 2009), demonstrated the participation of ATP-binding cassette (ABC) transporters in regulation of secretion of phytochemicals in the roots thus modulating the production of phenolics and sugars in ABC transporter mutants of *Arabidopsis thaliana* than in the wild type thus resulting in accumulation of more potential microbiome in the rhizosphere. Another study showed that the glucosinolates are the bioactive chemicals that are naturally produced by *Arabidopsis*. In transgenic mutant producing an exogenous form of glucosinolates showed a varied effect on fungal and bacterial composition of rhizosphere, hence establishing the effect of plant genotype on microbiome (Bressan et al. 2009). *Avena strigosa* (oats) is reported to produce triterpenoid saponins known as avenacins, which shows antifungal activity (Maizel et al. 1964). Mutants lacking avenacins demonstrated different culturable composition of root colonizing fungi than the wild type and the mutant variety was seen to be more susceptible to fungal pathogens (Osbourn et al. 1994).

The microbial composition varies not only with the soil source but is influenced by the plant genotype even under controlled greenhouse conditions (Edwards et al. 2015). In addition to soil, seeds that contain genetic blueprint of plants are reservoirs of diverse microbiota. The seed acts as the principal source of microbial inocula in plant. It has been reported that seed carried microorganisms effect process of seed germination and seedling survival (Truyens et al. 2015). The transfer of endophytic bacteria takes place through vertical transmission, from host plant to seed and then to seedlings as reported in case of rice and wheat (Robinson et al. 2016). It is seen that microbial diversity also varies with different developmental stages. The analysis of the microbial community of *Arabidopsis* at the seedling stage was found to be distinct from vegetative, bolting and flowering stages of the plant (Chaparro et al. 2014). In contrast, some studies suggested that the root microbiota are assembled in the early plant life and are independent of the plant developmental stage. There were no significant differences in the structural microbiota of an early flowering *A. alpina* (mutant) compared to the non-flowering wild-type plants at the same age, suggesting the developmental status is not much responsible for the compositional changes of the host plant microbiota (Chaparro et al. 2014; Dombrowski et al. 2017).

Regardless of the complex diversity in natural environment, there is a need to overlook the plant microbial diversity when interpreting the dynamics of the host plant. Genetic manipulation of plants for disease resistance or crop improvement may have unforeseen effects on the rest of the microbial diversity, which might not be physiologically relevant. The role of the microbiome in regard to the plant health, biogeochemical cycles, productivity and crop improvement should be seen as much as the plant itself.

12.5 Microbiome Dynamics vis-a-vis Plant Growth Phases

The microbiome of a plant can be described as the sum of all the microbial associations with plant in various plant compartments. The study of plant microbiome basically involves the study of its rhizosphere and phyllosphere. The rhizosphere of a plant represents the soil-plant interface and the phyllosphere forms the air-plant interface (Berg et al. 2014a, b). In other words, we can say that all the microorganisms associated with the plant in the below-ground parts are included into the rhizosphere where the microbes associated with the above-ground parts of the plant are a member of phyllosphere. Both these zones are of immense importance to the plant because of the diverse microbial richness including bacteria, fungi, archaea and protists. All the microbial activities in these areas directly/indirectly affect the plant because these can be beneficial to the plant or may affect the plant health by acting as plant pathogens. The rhizospheric soils of different plants represent a region of extreme microbial activity which is mainly due to the release of root exudates (Bowen and Rovira 1999). The release of unique cocktail of exudates by different plant species attracts a specific bacterial assemblage. The phyllosphere of a plant is a habitat for a large and complex microbial community. The diversity of microbes associated with the above-ground parts of the plant is of special interest due to the

large and exposed surface area of plant and is often influenced by numerous environmental factors and physio-chemical properties of the plant (Whipps et al. 2008; Rastogi et al. 2013). However, in addition to rhizosphere and phyllosphere, plants can be sub-grouped into even more microenvironments, such as anthosphere (flower), endorhiza (root), spermosphere (seeds) and the carposphere (fruit) (Berg et al. 2014a, b). All these microenvironments are responsible for providing conditions important for microbial life, thereby specifically affecting the host functioning. The plant microbiome is not only specifically affected by the root exudates or the above-ground interactions but the ageing of the plant has been reported to play a major role in this process as well. Many reports have shown that with the changing growth phase of a plant, its microbiome is severely affected.

12.6 Rhizosphere Microbiome

Rhizodeposits, exudates released by the plants in rhizosphere, are known to be important for variations in the diversity of microbial communities (Smalla et al. 2001). These include water-soluble exudates along with complex organic compounds from dead root cells (Bowen and Rovira 1991). These deposits are also important to plant for growth and disease reduction, for example, some of the plant synthesized sugars, i.e. sucrose released by roots in the rhizosphere is then responsible for the production of plant growth-promoting phytohormones. Exudation patterns undergo certain changes in relation to different plant growth stages, e.g. a higher concentration of exudates is seen during the early growth phases which gradually decreases as the plant ages. This change in the plant life cycle and exudation pattern has often been correlated with the type of microbiome diversity as reported by Yang and Crowley (2000).

The release of photosynthates, for example, and the corresponding composition of rhizodeposits in the rhizosphere have been shown to vary throughout the plant's life cycle due to the changes in plant physiology which occur during the course of development (Gransee and Wittenmayer 2000). The carbon allocation in the below-ground parts has been shown to decrease with the increasing age of plant. This spatial and temporal variation of carbon source effects the composition of rhizosphere microbial diversity. Mougel and co-workers in 2006 reported, during reproductive stages in *Medicago truncatula*, that there is a significant decrease in the root/shoot partitioning of carbon. They explained that this decrease occurred as photosynthates were used more in the shoots than in the roots, which resulted in less release of organic compounds in the rhizosphere during reproductive stages over vegetative stages. Considerable carbohydrate changes have also been noticed especially during the flowering stages of most of the plants. During the vegetative phase of plant development there is more release of soluble root exudates, whereas older plants have more organic compounds derived from dead root cells, especially during seed maturation (Eissenstat and Yanai 2002).

In addition to the variations in the exudation pattern based upon plant growth phase, bacterial and fungal diversity differentially use organic compounds. Bacteria

mostly have higher metabolic reactivity compared with fungi. Bacteria use readily available organic compounds, whereas fungi use complex organic compounds for their metabolism as they possess enzymatic activities (De Boer et al. 2005). This could be co-related with the higher bacterial diversity in the rhizosphere during the vegetative phase of plant growth as a result of increased release of soluble root exudates, whereas, during pod maturation, there is decrease in the amount of rhizodeposits and an increase in complex organic compounds which favours the persistence of fungal diversity at that particular phase of plant growth.

Many studies have shown a change in the rhizospheric fungal and bacterial communities based on plant's developmental stages in a wide variety of plants (i.e., *Arabidopsis*, maize, *Medicago*, pea, sugar beet and wheat). Micallef et al. (2009) reported that rhizosphere microbial communities in Arabidopsis varied with plant developmental stages and microbial community diversity was completely different in early stages of plant development as compared to the microbial diversity of the later stages. In another finding, soybean rhizosphere microbial communities were studied for a change in overall pattern with respect to the plant growth phases and it was seen that more complex microbial communities were produced during early reproductive growth stages of the soybean plant as compared to late stages of plant development (Xu et al. 2009). The characterization of the *Arabidopsis thaliana* core microbiome provided a tool to decipher the influence of different plant growth phases on the rhizosphere microbiome (Lundberg et al. 2012). Chaparro and co-workers in 2014 reported that the root exudates composition changes with respect to plant developmental gradient in *Arabidopsis.* During early time points, sugars, sugar alcohol level secretion, amino acid secretion and phenolics concentration were higher and with plant growth the microbiome diversity decreased. Thus metabolites and substrate secretion select particular microbiome at different life cycle stages (Badri et al. 2013; Chaparro et al. 2013). The rhizosphere microbes selected at particular plant growth phase is usually associated with suppress pathogenic microbes by beneficial ones (Mendes et al. 2011), induce systemic resistance against abiotic stress (Selvakumar et al. 2012), help in nutrition uptake (Bolan 1991; Van Der Heijden et al. 2008), increase the plant's innate immunity (Zamioudis and Pieterse 2012), and in overall plant health (Berendsen et al. 2012; Chaparro et al. 2012).

For the better understanding of dynamics of microbial compositions (microbiomes) over the plant life cycle, Edwards and co-workers in 2018 studied root compartments to characterize the root-associated microbiota of *Oryza sativa* over a period of three consecutive growing seasons. They observed that root microbiota was highly dynamic during the early stages of plant growth. There was compositional stabilization of microbiome for the rest of the life cycle and it was observed that microbiota of drought-stressed plants is immature as compared to unstressed plants. Qiao and group in 2017 compared the community structure of the rhizosphere bacteria of two different cotton cultivars of cotton through high-throughput sequencing technology and found the root-associated microbiome varied significantly during different developmental stages (Qiao et al. 2017).

The impact of plant growth phases in deciding the rhizospheric community can be estimated from a study conducted by Dunfield and Germida in 2003, where they

tried to assess the difference in the rhizospheric microbial diversity of field grown genetically modified and wild-type canola. They observed changes in the microbiome structure associated with genetically modified plants but found them to be temporary as this change did not persist into the next field season.

12.7 Phyllosphere Microbiome

Phyllosphere is habitat for microorganisms in the aerial parts of living plants that includes buds, flower, fruits, leaves and stems (Whipps et al. 2008). Though the phyllosphere of most of the plants, unlike rhizosphere, has not been very well studied, but it is important for the plant as it hosts a variety of microbes which play important role in the growth and development of the host plant. For example, disease resistance and plant growth is increased in presence of beneficial microbes. In most of the plants the rhizosphere and phyllosphere communities fail to share common constituents, clearly exhibiting differences in dominance pattern of microbial communities. Microbial communities associated with the above-ground parts of the plant are influenced by both biotic and abiotic factors, along with plant surface topography and chemistry. Among all the above-ground plant parts, leaves are the most preferred habitat for colonization of microbes due to its large surface area globally.

Bacteria are the most dominant species in the phyllosphere when compared to archaea, filamentous fungi and yeast. The bacterial species from the phyllosphere promote plant growth directly as well indirectly. Fungal phyllosphere endophytes may deter herbivores, protect against plant pathogens and increase drought tolerance in the host. There are many reports suggesting that the microbial community associated with the leaves of the plants changes with the ageing of the leaves, for example *Zea mays* L. leaf microbial community structure was studied using molecular and microscopic strategies and it was seen that the diversity changed with plant age (Manching et al. 2017). There are many studies that have reported changing pattern of phyllosphere community in relation to plant age. Osono and Mori in 2005 reported phyllosphere fungi of giant dog wood changes in seasonal and leaf age-dependent manner and also the composition of assemblages of phyllosphere fungi was influenced by phenological patterns of leaf emergence of deciduous trees.

In order to have complete understanding of the plant health and for designing the strategies for the development of plant growth-promoting and disease control bioformulations, knowledge about the microbiome, both rhizosphere and phyllosphere, is very important as this will open new ways for sustainable agricultural practices and meeting the ever increasing food demand. For the efficient study of microbiome of plant, two major approaches have been utilized: culture-dependent and culture-independent.

An enhanced understanding of the factors that influence beneficial microbial behaviour in the phyllosphere and rhizosphere of the plants is of extreme importance in agriculture to enhance productivity and limit environmental impact while maintaining food safety.

12.8 Suppressive Soil Microbiome

Plants have always been greatly influenced by the soil in which they grow. The soil associated with the below-ground parts of most of the plants can be characterized into two major groups: conducive soils and suppressive soils. Conducive soils or non-suppressive soils are those which provide all the necessary biotic and abiotic conditions required by a pathogen to grow and survive in that habitat whereas suppressive soils prevent soil-borne pathogen establishment and also help in disease suppression (Durán et al. 2017). Both the types of soils differ in their microbial composition as well due to the different physical properties. Young and co-workers in Young et al. 1991 studied three conducive and suppressive soils for various physical and chemical properties and found that suppressive soils were slightly alkaline and conducive soils were slightly acidic. Concentrations of calcium and magnesium were 3–15 times more in suppressive soils compared to conducive.

Suppressive soils, limiting the growth of soil-borne pathogen, have been studied worldwide from the last 60 years (Schlatter et al. 2017). Atkinson in 1892 for the first time reported the suppressive soil for *Fusarium* wilt disease of cotton (Atkinson 1892). Baker and Cook in 1974 described disease-suppressive soils as the soils associated with the plants, which do not allow the pathogens to persist and even if it persists the severity of the disease is very less. Based upon this definition, suppressive soils can be considered as best example of microbe-based plant defence. The plant roots release exudates, attracting the soil microbes and supporting selective microbial community in the rhizosphere. As a result of this, the soil becomes a rich source of beneficial microbes with novel antimicrobial compounds and plant protective traits and this becomes the first line of defence against soil-borne pathogens. These microbes associated with the plant provide protection by competition for nutrients, antibiosis and induction of host resistance (Mazzola 2002). In general two types of suppressiveness are observed: general and specific. When the collective microbial community associated with the plant competes for the available nutrients in that particular niche with the pathogens and suppresses the pathogen growth and development then this is called as general suppression. General suppression is more natural and pre-existing property of soil and this type is usually effective against a broad range of soil-borne pathogens. This type of suppression can be further enhanced by the addition of organic matter in the soils, as this will further support the growth and persistence of plant beneficial microbiome, thereby better plant growth and health but it cannot be transferred from field to field and soil to soil. The other major type of suppression is Specific, and this is attributed to the activity of a specific group of microorganism which inhibits the pathogen progression by interfering in the life cycle of the soil-borne pathogen. Specific suppression is highly effective and is transferable in contrast to general suppression. By mixing small amounts of (1–10% w/w) these specific suppressive soils, we can convert the conducive soils into suppressive soils.

Many research groups have been trying to understand that how indigenous microbiomes are capable of disease reduction in the presence of pathogen, susceptible host and favourable environmental conditions. New molecular biology tools in

Table 12.3 Examples of disease-suppressive soils

Pathogen	Crop	Technique	Abundant microbial taxa	References
Gaeumannomyces graminis var. *tritici*	Wheat	16S - DGGE	*Pseudomonas putida*, *Pseudomonas fluorescens*, *Nocardioides oleivorans, Streptomyces bingchengensis*, *Terrabacter*	Chng et al. (2015)
Fusarium oxysporum	Vanilla	16S - Amplicon	Acidobacteria (groups Gp2, Gp1, Gp3, Gp13), Verrucomicrobia, Actinobacteria (Ktedonobacter), Firmicutes	Xiong et al. (2017)
*Rhizoctonia solani*AG3	Potato	16S - Amplicon	Proteobacteria, Bacteroidetes, Actinobacteria, Acidobacteria	Michelsen et al. (2015)
Heteroderaglycines	Soyabean	ITS - DGGE	*Fusarium* spp., *Cladosporium sphaerospermum*, *Aspergillus versicolor*	Song et al. (2016)
Fusariumoxysporum f. sp. *cubense*	Banana	16S - Amplicon	Acidobacteria (Gp4, Gp5), *Chthomonas, Pseudomonas*, *Tumebacillus*,	Shen et al. (2015)

Source: Gómez Expósito et al. (2017)

combination with the traditional approaches have been used to understand the suppressive soil microbiomes (Cha et al. 2016). Techniques such as metagenome, metatranscriptome, metataxonome in combination with bioinformatics approaches such as metaproteome and metabolome have helped in unravelling the spatial and temporal components of general and suppressive soil microbiomes. By the study of these soils, we can identify microbes which confer disease suppressiveness by making a microbial consortia and by transplanting the microbial communities, soil microbiome engineering (soil amendments) or plant-mediated microbiome engineering (exudation patterns) (Gómez Expósito et al. 2017) (Table 12.3).

12.9 Saffron Microbiome

In the past 10 years, we have taken an initiative to study saffron microbiome and its interactions, from the saffron fields in Kashmir and Kishtwar. Our focus has been mostly on the microbiome associated with the underground parts of saffron such as the rhizosphere and cormosphere. Both cultivation-dependent and cultivation-independent methodologies were used for elucidation of structure and function of the saffron microbial community and study their spatial and temporal dynamics.

Fungal pathogens have been isolated from rotten corms to study the effect of saffron associated bacteria on fungal pathogens causing corm rot diseases in saffron. *Fusarium* corm rot is reportedly the most destructive disease in saffron-producing

areas worldwide. Three fungal pathogens, *Fusarium oxysporum, F. solani* and *Penicillium* sp., have been isolated from saffron fields in Kashmir and identified using molecular phylogeny besides conidial morphology. Out of these three pathogens, *F. oxysporum* R1 shows maximum disease incidence and disease severity is also higher and was found to be closely related to *F. oxysporum f.sp. dianthi* based on ITS sequence phylogeny (Gupta and Vakhlu 2015). Since individual strains of plant pathogenic *F. oxysporum* are host specific despite their wide host range, it is important to characterize the strains. Earlier reports on saffron pathogens lack molecular characterization. Characterization of pathogenic strain infecting saffron would therefore facilitate screening for biocontrol bacteria targeted against the disease.

Plant growth-promoting bacteria (PGPB) have been isolated from field soil, rhizosphere and cormosphere and studied for plant growth-promoting properties, namely, protease production; production of amylase, indole acetic acid, ammonia, catalase, siderophore and cellulase; phosphate solubilization; and antifungal activity. These studies included both *in-vitro* tests and pot trials (Ambardar and Vakhlu 2013; Kour 2014). Bacilli spp. namely *B. thuringiensis* DC1*, B. amyloliquefaciens* DC8 and *B. megaterium* VC3 have been characterized from cormosphere and were found to be present in the corm throughout its life cycle (Kour 2014; Kour et al. 2018). *B. methylotrophicus* and three different strains of *B. aryabhattai* have been characterized from bulk soil. *B. aryabhattai* has also been isolated from rhizosphere (Ambardar and Vakhlu 2013). *B. amyloliquefaciens* strain W2 has been found effective against corm rot caused by *F. oxysporum* R1, using well diffusion and dual culture assays and pot trials. It has been shown to decrease the disease incidence in pot assays from 93% to 40% (Gupta and Vakhlu 2015). The genomic DNA of *B. amyloliquefaciens* W2 has been isolated and sequenced using Ion PGM sequencing platform. The draft genome (3.9 Mb) has 65 contigs (3, 997, 511 bp), 4,163 coding sequences, and an average 46.45% GC content and is available at GenBank. Though comparison of 16S rRNA gene of isolated BamW2 showed 99% similarity with that of *B. amyloliquefaciens* subsp. *plantarum* FZB42, the genome sequence comparison revealed only 48.7% homology between W2 and FZB42 strains (Gupta et al. 2014).

In order to understand the interactions between saffron, its pathogen and native biocontrol agent, transcriptome sequencing-based study with W2 strain as biocontrol agent has been undertaken. Plant gene expression and fungal gene expression in presence and absence of biocontrol agent have been studied and compared to understand the effect of pathogen on healthy corm and infected biocontrol-treated corm. The comparison has revealed differential expression profiles of plant genes as well as fungal genes in both the samples (Unpublished data).

Cultivation-independent approach has unravelled a detailed picture of microbial diversity associated with corms and roots of saffron. Two approaches were used for this study, namely, cloning-dependent and high-throughput sequencing. Cloning-dependent approach included cloning of 16S rRNA gene which was amplified from the metagenomic DNA of the sample. This study revealed that the bacterial composition of bulk soil, rhizosphere and cormosphere of saffron is significantly different ($P < 0.05$) from each other. Briefly, in the flowering stage, rhizosphere and cormosphere of saffron have 22 bacterial genera but none of the genus is common. Bulk soil bacterial community comprises of 13 genera with *Acidobacteria* as the dominant one. In

rhizosphere, 8 different genera were identified and *Pseudomonas* was the most dominant. Cormosphere community, dominated by the genus *Pantoea*, comprised of 6 different bacterial genera. This was the first report on bacterial community structure of saffron plant parts using cultivation-independent 16S metagenomic approach (Ambardar et al. 2014). However, cloning-based sequencing has inherent biases and underestimates community diversity (Ambardar et al. 2014). Therefore, high-throughput sequencing approach is preferred for deep analysis of microbial diversity (Tedersoo et al. 2010). Using this approach for sequencing, 23 bacterial genera were revealed from cormosphere (unpublished work). This number was significantly higher than 6 genera found by cloning-dependent technique, though different life cycle stages under investigation were different (Ambardar et al. 2016a).

Similarly ITS gene high-throughput sequencing was used to catalogue fungal diversity from bulk soil, rhizosphere and cormosphere of saffron. The analysis of 454 pyrosequencing data has suggested niche and growth stage-specific nature of saffron mycobiome. Fungal diversity obtained was different between roots and corms and the dominance pattern in the cormosphere varied among two growth stages. Briefly, during flowering stage, Zygomycota and Basidiomycota were dominant fungal phyla in the rhizosphere and cormosphere respectively. However, in the cormosphere the dominance pattern shifted from Basidiomycota to Zygomycota from flowering to dormant growth stage. On the hand, the bulk soil fungi do not seem to follow this dynamics and was dominated by Ascomycota throughout the study. This was the first report on the fungal community structure and its spatial and temporal dynamics in saffron (Ambardar et al. 2016a, b). To compare microbial diversity of saffron cormosphere from different geographical sites, corm samples from Kashmir and Kishtwar have been further studied to unravel their microbiomes. Subsequently, the technique for studying microbial diversity/microbiome was based on whole metagenome sequencing instead of initial gene-targeted sequencing technology as sequencing is getting cheaper day to day. Preliminary analysis of the microbiome of two sites reveals that the microbial diversity of both Kishtwar and Kashmir cormosphere is different (Unpublished work).

12.10 Microbiome Engineering: Future Aspects and Challenges

Microbiome engineering is alteration of microbial compositions to improve host phenotypes and benefit ecosystems (Foo et al. 2017). It can be a valuable tool to improve agricultural production and increase food security in light of climate change and a growing human population. Manipulation of host microbiome for increased health and productivity is the prime goal of microbiome engineering. The plant beneficial functions are thought to be carried out by a few microbial species by their synergistic effects rather than the whole microbiome. These few species, associated with plants, can be used to benefit susceptible plants against biotic stresses and can also help to increase yield by plant growth promotion. It is therefore essential to elucidate microbiome structure and functions as well as their effect on host's performance (Mueller and Sachs 2015).

Host-mediated artificial selection, microbiome transfer and synthetic microbiomes are the methods that have been used to engineer root-associated microbiome. One of the methods for microbiome transfer for plant disease management is transferring disease-suppressive soils, and this method has been used successfully in potato common scab, sugar beet infection and tobacco black root rot (Foo et al. 2017). Microbial species can be introduced into host by plant multi-generation-host-mediated microbiome selection. Inoculation can be provided to bulk soil, rhizosphere, seeds or seedlings. At the tissue level, inoculation can be done by atomization directly into stems, leaves and flowers, or direct injection into tissues or wounds. Host-mediated microbiome selection is an engineering method that selects microbial communities indirectly through the host. This method leverages host traits that evolved to influence microbiomes and was demonstrated first on *Arabidopsis* (Mueller and Sachs 2015; del Carmen Orozco-Mosqueda et al. 2018). Inoculation into bulk soil and rhizosphere includes introduction of plant growth-promoting (PGP) microbes that may change the structure of plant microbial community. Inoculation of microbial species has also been shown to be effective on seeds and seedlings. Tissue atomization is another technique that has been used to modify seed microbiome (Mitter et al. 2017). Direct inoculation of a PGPB into a plant has been demonstrated to help the bacterium to colonize and survive within the plant. For example, the biocontrol agent and PGPB *Arthrobacter agilis* UMCV2 is reported to survive, after being injected to the stem of *Medicago truncatula* plants (Aviles-Garcia et al. 2016).

Microbiome engineering has an advantage over single gene transfer, since, transfer of one or more microbial species leads to a transfer of greater concentration of genetic material and thus provides greater advantage to plants compared to single gene transfer. For example, the multiple direct and indirect growth-promoting activities of PGPB such as *Pseudomonas fluorescens* UM270 or *Arthrobacter agilis* UMCV2 can be more beneficial than the single gene transfer for *cry* gene from *Bacillus thuringiensis* (del Carmen Orozco-Mosqueda et al. 2018).

Commercialization of microbial bioformulations, for enhancing farm productivity, has gained momentum in recent years with the help of start-up companies (Indigo Ag, Chr-Hansen, NewLeafSymbiotics) as well as multinational companies (Bayer Ltd, Nufarm, Monsanto BioAg). Microbial products and bio-pesticides are expected to grow in the global market to an estimated $6.4 billion by 2022 (Singh et al. 2018). However, there are significant challenges along the road ranging from technical to social. Additional studies to understand the interactions and impact of microbial species on the plant's core microbiome are essential. Along with that, more than 95% of microbes are non-cultivable and hence not characterized yet, constraining the ability to harness their potential for agricultural improvements. Performance in field is another challenge, as the reports have shown mixed results. The impact of environmental factors as well as plant's genotype on microbiome diversity poses a challenge for microbiome engineering particularly for phyllosphere. It is therefore important to study the plant-microbiome interaction taking into consideration effects on both plant and microbial participants. Re-isolation of introduced microbial species is another important aspect to ensure its endophytic

capacity. Effect on the core microbiome over several generations and long-term persistence of the engineered microbiome are some studies that should be taken into consideration. Another area of focus for future research is the understanding of how plant's genetic pathways shape/influence microbiome. There is only limited knowledge of how root exudates select microbiome for plant's advantage. Together, the omics studies can help elucidate this plant-microbiome relationship in depth. This knowledge can be further exploited to improve crop production and reduce dependence on chemical fertilizers by microbiome engineering (Bakker et al. 2018).

Climate change is a major challenge and a threat to food security in the present scenario. This threat to food security can also be managed using microbiome engineering with the integration of systemic biology, ecology and evolutionary biology. The impacts of climate change on soil microbial community composition and metabolic diversity can be predicted by ecological studies and can be complimented by evolutionary studies that will predict stability of synthetic microbial communities and microbial mutualisms over the time. Further field experiments can be conducted for evaluation of external factors on microbial community composition and synergistic benefits of inoculations (Hamilton et al. 2016). A stable microbiome which can improve productivity and stress tolerance under diverse environmental conditions and crop stages is highly desirable.

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